Plasmid analysis, comparative genomics and transcriptomics of beer-spoilage lactic acid bacteria emphasizing the role of dissolved carbon dioxide and traditional beer-spoilage markers

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ABSTRACT

Specific isolates of lactic acid bacteria (LAB) are capable of growing in and spoiling beer, and are the cause of product and process contamination, and financial loss for brewers the world over. To date, our understanding of how these contaminants are able to grow in beer is limited to analysis of hop-tolerance mechanisms, with a limited number of putative hop-tolerance genes having been described. In order to demonstrate that these hop-tolerance genes are incomplete descriptors of overall beer-spoilage ability, the transcriptional activity of these genes in two different beer-spoilage related (BSR) LAB isolates, and the prevalence and sequence conservation of hop-tolerance gene *horC* in BSR LAB with varying beer-spoilage ability is examined. This analysis is followed by work demonstrating that the total plasmid profile of a beer-spoilage LAB, and not just plasmids harboring hop-tolerance genes, contributes to the isolate's overall beer-spoilage phenotype and highlights redundancy in potential beer-spoilage mechanisms. The next chapter provides evidence that the presence of dissolved CO_2 (dCO_2) in beer selects for the ability of LAB to spoil packaged beer, and that tolerance to this stress is not correlated with hop-tolerance, indicating that dCO_2 stress is an important part of the total beer environment. This is followed by the presentation and analysis of the genome of the rapid beer-spoiling isolate *Lactobacillus brevis* BSO 464 and subsequent RNA sequencing for this isolate when grown in degassed and gassed beer so as to elucidate which genes are active when grown in beer, and when grown specifically in the presence of dCO_2. Global transcriptome sequencing of this *L. brevis* isolate and *Pediococcus claussenii* ATCC BAA-344^T when each were grown in growth-limiting concentrations of hops was also performed in order to clarify the hop-specific transcriptional response from that of the response when these isolates grow in the total beer environment. Lastly, comparison is made between available genomes of BSR LAB to reveal that the specific brewery environment a BSR LAB is recovered from, influences genetic variability and that comparison within a given LAB species reveals genetic differences that can be exploited as beer-spoilage genetic markers. This comparative analysis reveals that the total plasmid-coding capacity strongly influences individual BSR LAB beer-spoilage phenotype and the environment they are able to grow in. Overall, beer-spoilage ability is shown to be adaptive and acquired incrementally and not solely as a result of the presence of hop-tolerance genes.

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DEDICATION

I dedicate this thesis to both of my parents for all forms of their unconditional support; to all the people whom I have had the opportunity to work with, learn from; and to many an interesting beer drank.

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LIST OF ABBREVIATIONS

85/15……..……85% lager beer, 15% double strenght modified MRS (no Tween 80)

ABC……ATP-binding-cassette

ATCC……..…American Type Culture Colelction

BLAST…….Basic Local Alignment Search Tool

BSO…….....…beer-spoilage organism

BSR……...beer-spoilage related

BSR LAB……...……beer-spoilage related lactic acid bacteria

BU……..…bitterness units

CCC……...……Coors Culture Collection

CDS……..…coding sequence

CFU……..colony forming units

CNRQ……...…calibrated normalized relative quantity

C_q……quantification cycle

dCO_2……...…dissolved carbon dioxide

DE……..…differentially expressed

ddPCR……..……droplet digital PCR

gDNA……..…genomic DNA

GO……..…Gene Ontology

GOI……gene of interest

HGA……...……hop-gradient agar

HGAE……...…hop-gradient agar +5% (v/v) ethanol

KB290……..……..……*Lactobacillus brevis* KB290

L101……..……*Lactobacillus backii* L101

LAB……..……lactic acid bacteria

Lb367……*Lactobacillus brevis* ATCC 367^T

Lb464……..……*Lactobacillus brevis* BSO 464

Lc03……*Lactobacillus casei* CCC B1025

Ls74…….. *Lactobacillus spp.* ATCC 15578

MBC……...…minimum bacteriocidal concentration

MC……Miller Coors

MDR……..……multidrug resistance
MFS……..……Major Facillitator Superfamily
MIC…….……minimum inhibitory concentration
mMRS……modified MRS (no Tween 80)
MRS…….....……de Man, Rogosa, Sharpe media
MRS-B, MRSB……..…de Man, Rogosa, Sharpe broth
MTPX PCR……..multiplex polymerase chain reaction
$NAD(H)^{+}$……...……..…nicotinamide adenine dinucleotide
$NADP(H)^{+}$……..…… nictotinamide adenine dinucleotide phosphate
ncRNA……non-coding RNA
NF……..……normalization factor
NGS……...…next-generation sequencing
noRT……..……..…….……no reverse transcription
NRC PBI……National Research Council Plant Biotechnology Institute
NRQ……..normalized relative quantity
Pc344……..……*Pediococcus claussenii* ATCC BAA-344^{T}
Pc344-NR…….……. *Pediococcus claussenii* ATCC BAA-344^{T}, non-ropy variant
PCN……..…plasmid copy number
PCR……..……polymerase chain reaction
PGAAP…….……Prokaryotic Genome Automated Annotation Pipeline
PMF……proton motive force
PTS……..……phosphotransferase system
QC……...……quality control
qPCR……quantiative PCR
RAST……..…Rapid Annotation using Subsystem Technology
RNAseq……...…RNA sequencing
RND……...…Resistance-Nodulation-Division (transporter)
RT-qPCR……...…reverse transcription quantitative PCR
SDE……...……significant differential expression
TBE……TRIS/Borate/EDTA buffer
VNBC…….……viable but not culturable

Chapter 1: Introduction, literature review, and objectives

1. INTERFACE

This chapter has been adapted from "Investigation of beer-spoilage lactic acid bacteria using omic approaches" authored by Jordyn Bergsveinson and Barry Ziola for inclusion in the upcoming book *Omics in Brewing Microbiology* (edited by Charlie Bamforth and Nick Bokulich; to be published by Caister Academic Press, Poole, United kingdom, in 2016).

1.1. GENERAL OVERVIEW

1.1.1 Introduction to lactic acid bacteria in beer: "The good, the bad and the ugly"

One only has to perform a cursory literature search of lactic acid bacteria (LAB) to be overwhelmed by available information extolling their industrial importance for fields ranging from human health to food production. Ultimately, LAB are a collection of gram-positive, catalase-negative, non-sporulating, non-motile and acid-tolerant organisms that share the capacity to produce lactic acid as a primary product of sugar fermentation while being incredibly heterogeneous in terms of physiological attributes, metabolic and fermentation capabilities, and ability to inhabit diverse niches (**99**). These diverse attributes greatly increase both their presence and utility in the production of multiple food and beverage products, from wine and beer to cheese, dairy, meat and vegetable products (**83**). Further, members of the LAB group that naturally occupy food and beverage niches have been ascribed the generally-regarded-as-safe (GRAS) designation, allowing them to be exploited for improvement and preservation of a wide range of food and beverage products, and for the production of probiotics (**75**). Unfortunately, the unwanted presence or uncontrolled over-growth of these organisms during food or beverage production can occur, and this poses challenges, as well as opportunities, especially to the brewing industry.

Beer is an unexpected environment to support microbial growth given that beer-spoiling bacteria must simultaneously overcome several physiological hurdles, including the antimicrobial action of ethanol and hop-derived bitter acids, low pH, limited available nutrients, and low O_2, with concurrent high CO_2 levels (**47**, **108, 119**). Nonetheless, LAB likely have always been associated

with and/or involved in the production of beer, either as a naturally occurring agent for traditional spontaneous-fermentation styles such as lambic or weisse beers, producing characteristic “sour” flavors via production of lactic or acetic acid (**148**), or as an unseen source of spoilage that resulted in an undesired or poor quality product. Though the participation of LAB in beer-production and spoilage was not appreciated until Louis Pasteur began to isolate these bacterial cells from beer (**131**), their diverse historical role(s) in brewing allows for the general characterization of “the good, the bad, and the ugly” outcomes of LAB involvement.

LAB isolates can indeed be helpful, if not necessary components, of specialized fermentations that produce specific beer styles, both traditional and new (**148**). This is increasingly important to consider in discussions of beer-spoilage-related (BSR) LAB, as the current global beer market is experiencing a significant expansion in the interest and numbers of “local” craft beers. The influx of new companies necessitates that breweries distinguish themselves with consumers through unique products and this need has lead to innovative use of raw materials and production processes, with the inclusion of both fermenting-LAB and non-traditional yeasts. These “helpful” fermenting LAB, however, must have several important attributes, chief among them being the inability to overgrow in beer and to not inhibit normal yeast function. Thus, current industry trends highlight an important context in which to consider brewing-associated LAB, and open up interesting avenues, as well as areas of concern, for how best to investigate and further the role of LAB in the spectrum of modern beer production.

Putting aside the expansion in modern brew styles, the fact remains that since the industrial revolution, global brewing practices increasingly have focused on producing “clean” and consistent brew products, free of bacterial presence and their metabolites (**148**). With the advent of pasteurization and appreciation for hygienic practices during food and beverage production, the average global beer consumer today is likely accustomed to “conventional” or non-sour products, beers that are free from characteristic signs of LAB over-growth. This means the beer should have no cloudy “haze”, no “sour” taste or other unappealing off-flavors such as “buttery” diacetyl, and be free of bacterial sedimentation or exopolysaccharide “slime” (**7**). Such occurrences in most beer products are unexpected, and encapsulate both the bad and the ugly results of unwanted LAB being present. Despite the use of pasteurization by some large

breweries to prevent spoilage, not all breweries employ this method and contamination of product remains an issue when the product reaches dispensaries in restaurants and other vendors. The outcome of these spoilage events causes a loss of consumer and brand confidence when compromised beer is consumed, or significant revenue and time loss to the brewery in the event of batch contamination. As LAB are attributed with causing 60 – 90 % of the brewing spoilage events worldwide (**2, 5**), significant interest has gone into ascertaining how they spoil beer and how this is best controlled. Despite this interest, incidence of BSR LAB contamination remains difficult to delineate due to under-appreciation of how diverse a group they are even though relevant research constantly highlights this diversity.

1.1.2. The promise of omics for BSR LAB research

Recent review articles cover the evolution and current state of understanding of BSR LAB prevalence, genetics (**17, 108, 131**), and research techniques used during investigation (**1, 18**). Though such background knowledge is of critical importance to understanding the current issues facing the brewing field, this chapter is not meant to be exhaustive of all relevant literature history to BSR LAB. Rather, this information is used to highlight the apparent gaps in knowledge and need for the expansion of research methods into omics applications.

The ability for transcriptomics or proteomics to profile, in a rapid and high throughput manner, how a specific microbe grows under defined conditions or provide information on a microbial community's genetics, activities and ecology means that these omic-approaches can effectively balance the interests of academia and industry, and overcome the problem of understanding BSR LAB variability. To date, research into BSR LAB has often failed to provide data of equal value to research investigators and brewers tasked with carrying out detection of contaminating BSR LAB. For example, detailed study of genetic or physiological stress response mechanisms of BSR LAB is of great value to LAB and brewing research writ-large, however, this data alone presents little utility to individual brewers. Further, the targeted analysis of just a few genes, or one physiological stressor in few specific isolates, has provided only minimal and incremental expansion to our current knowledge regarding LAB. Most importantly, findings from targeted-analysis experiments are frequently inconsistent for all BSR LAB, thus curtailing the value of this data from both academic and industry perspectives.

Omics approaches have proven to be a powerful way to investigate LAB genetic and metabolic diversity (**27, 68, 84**), and when applied broadly, produce large amounts of data that can be mined to give statistically relevant genetic or metabolic markers for beer-spoilage that could be effectively screened for within breweries. Secondly, these approaches help distinguish potentially helpful LAB from BSR LAB for use in specialty brews, by correlating limited beer-growth ability with desirable genetic or metabolic traits, without having to develop optimal strains through the use of laborious genetic modification techniques. The *meta* data that is produced from omics approaches thus allows for the conversion of information obtained by broad-scale or community-analysis of BSR LAB to specific application in the brewery.

1.2. General LAB characteristics

Problematic BSR LAB and sour-beer fermenting LAB alike traditionally belong to the *Firmicutes* phyum, order *Lactobacillales,* in the genera *Lactobacillus* and *Pediococcus* (**106**), with *Lactobacillus brevis, Lactobacillus linderii* and *Pediococcus damnosus* being the most commonly encountered bacteria that spoil beer (**7, 90, 106, 130, 144**). Additional LAB species also have been detected with varying frequencies in brewing environments, including *Lactobacillus amylolyticus* (**15**), *Lactobacillus backii* (**16**), *Lactobacillus brevisimilis* (**4**), *Lactobacillus buchneri*, *Lactobacillus casei*, *Lactobacillus coryneformis*, *Lactobacillus curvatus*, *Lactobacillus delbrueckii* (**106**), *Lactobacillus dextrinicus* (**54**), *Lactobacillus fermentum*, *Lactobacillus fructivorans, Lactobacillus linderi* (**106)**, *Lactobacillus malefermentans* (**107**), *Lactobacillus parabuchneri*, (**106**), *Lactobacillus paracollinoides* (**128**), *Lactobacillus plantarum* (**106**, **144**), *Lactobacillus paraplantarum* (**30**), *Lactobacillus paucivorans* (**42**), *Lactobacillus rossiae* (**29**), *Pediococcus claussenii* (**39**), *Pediococcus inopinatus*, *Pediococcus parvulus* (**88**) and *Pediococcus pentosaceous* (**72**).

Despite the reported incidence of certain species within breweries, it must be emphasized that the ability for BSR LAB to grow in and spoil beer is *not a species attribute, but is an isolate-specific capability*, as is the case for most spoilage lactobacilli (**113**). As this phenomenon indicates that there must be a level of genetic specialization in a BSR LAB isolate that allows for the beer-spoilage phenotype, the search for a small number of "detectable" genetic markers has long been

the focus of analysis. However, this narrow scope of investigation fails to appreciate the degree of genetic dissimilarity inherent among BSR LAB given that these isolates belong to the LAB group as a result of shared functional characteristics (i.e., particular metabolic capacities) and not necessarily genetic relatedness among LAB (**127**). Additionally, the use of "common" BSR LAB isolates for the study of genetic differences likely produces results that are non-universal for BSR LAB. Common isolates are those that can grow in routine culture media, and are thus likely consistently over-represented during detection procedures (**32**). Given that not all *Lactobacillus* and *Pediococcus* isolates can grow in these media, those that can likely skew incidence reports of beer-spoilage, and therefore the amount of research interest and available information on BSR LAB.

1.2.1. BSR LAB diversity

To better understand the genetic adaptations that separate BSR LAB from non-spoiling, same-species isolates, and where BSR LAB may have originated from, we must first examine the diversity of species involved. Both *Lactobacillus* and *Pediococcus* genera are comprised of gram-positive and catalase-negative isolates, and share overlapping DNA G+C content (*Lactobacillus*: 32-55% mol *and Pediococcus*: 35-44 mol %). Although these two genera are closely related to each other and to the genus *Leuconostoc*, as demonstrated by 16S rRNA gene sequence analysis, they each have several distinctive features (**114**). *Pediococcus* isolates grow under a range of facultatively aerobic to microaerophillic conditions and are homofermentative in that they do not generate CO_2 when they produce lactic acid from fermentation of glucose **(65 – 67**). Further, pediococci are not capable of reducing nitrate, while some lactobacilli isolates can (**58**, **60**). As well, *Lactobacillus* species are generally anaerobic, although some are aerotolerant and they may be either homofermentative like *Pediococcus,* or heterofermentative and produce lactic acid, CO_2, and ethanol and/or acetic acid as primary end products of fermentation.

Lactobacillus spp. are currently organized into three distinct metabolic or fermentative groups, prior to further phylogenetic arrangement based on genetic relatedness (**60**, **127**). The first fermentation group is that of the obligate homofermentative (OHO) species, which can only ferment hexoses and do so via the Embden-Meyerhof-Parnas (EMP) pathway, largely producing lactic acid as a byproduct (**60, 67**). Those species that are capable of homofermentation, but

during starvation or glucose limitation can degrade pentoses and gluconate via the pentose phosphate pathway (PPP) to produce acetic acid, ethanol and formic acid as byproducts are referred to as facultative heterofermentative (FHE). Finally, the obligate heterofermentive (OHE) group will metabolize pentoses and hexoses solely through the first part of the PPP via the phosphogluconate pathway and produce lactic acid, CO_2, and ethanol or acetic acid (**60, 67, 158**). In the context of brewing, common BSR LAB belong to all three groups, for example, *L. brevis* and *L. linderii* are OHE and *L. plantarum* is FHE. The different metabolic capacities of these isolates therefore may influence not only the style of beer or location in the brewery they are able to grow in as a result of available nutrients, but also in the severity and type of spoilage they cause based on their metabolic byproducts.

There are eight "niche type" environments where lactobacilli are commonly found, including plant or plant-associated fermentation products, sourdough, meat products, dairy products, wine products, human or animal gastro-intestinal (GI) tracts, human or animal non-GI sources, and the general environment (**127**). Notably, breweries or beer products are not included likely due to the close association BSR LAB have with the brewing environment and because these LAB are not necessarily an essential component of beer fermentation or production. Further, many BSR LAB species can be isolated from different environments. For example, *L. brevis* has been isolated from the human GI tract, and *L. lindneri* and *L. plantarum* can be recovered from plant materials and dairy products (**110**), as well as from beer. The ability of different isolates of the same species to occupy multiple niches and exhibit different fermentation types is common for *Lactobacillus* species (**40**). Thus, it is not surprising that BSR LAB isolates occupying the same niche have different genomic features, underscoring the idea that different genetic mechanisms allow for adaptation to a given environment and/or stress (**127**).

As food production industries are principally concerned with LAB adaptation to their specific application (i.e., unique environment), LAB genomics and phylogenetic relationships have received considerable attention (**60**, **110**, **159**). Whole genome sequencing, phylogenomics and other bioinformatic approaches to compare LAB species have resolved questions of group diversity, evolutionary relatedness and provided a wealth of information concerning general genetic composition. Multiple LAB genomes are available publically through the National

Center for Biotechnology database, with 205 of these being *Lactobacillus* isolates and 12 being *Pediococcus* isolates (as of November 20, 2015), and an estimated 80 unreleased, ongoing projects worldwide (**127**). This genomic data is of great general utility, however, with respect to brewing-microbiology, only a small percent of these genomes or projects belong to BSR isolates (**13, 101-102**). The continued sequencing of LAB genomes is essential, as general analysis of genomic content will be more robust and less inclined towards bias if LAB isolates from a variety of different sources are included (**99, 127**). As there is assumed genetic variation between BSR-isolates from beer and non-BSR-isolates, and BSR LAB species isolated from any source, more data must be made available for both BSR- and non-BSR-LAB from multiple isolation sources in order to effectively determine the evolution and distinguishing characteristics of BSR LAB.

1.3. Traditional and emerging methods for BSR LAB detection and identification

1.3.1. Culture-based Methods

Culture methods are still the most commonly used approach for routine detection and identification of BSR LAB in the brewery, for reason of their ease of use, limited need for specialized training, relatively low monetary and space cost, and proven utility. However, culture methods have inherent disadvantages, due to the variable nature of BSR LAB isolates; i.e., differences in their fastidious aerotolerance or nutritional requirements, and the different adaptive states they may exist in when isolated from beer. These factors make the primary isolation of some LAB contaminants via growth quite difficult (**36, 133**). More importantly, there is no single laboratory (agar) medium that effectively screens and supports growth for all possible beer-spoilage LAB (**138**).

de Man Rogosa Sharpe (MRS) medium (**35**), which was designed for the cultivation of LAB, remains the most relied upon media in brewery settings (**108**). There are several descriptions of supplementing MRS medium with varying concentrations of beer expecting that the added beer enables cultivation of beer-adapted (hard-to-culture) organisms and that the nutrients provided by the MRS medium allow for more rapid growth (**53, 64, 133**). Further modifications to beer-supplemented MRS include adding reducing agents to remove oxygen tension in the medium to

facilitate the growth of a wide range of BSR LAB in addition to microaerophillic strains (**94, 139**). Similarly, other developments, such as the Advanced Beer Detection (ABD) medium, seek to reduce medium osmolarity with the goal of isolating hard-to-culture BSR LAB (**133)**.

Often there is need to exclude other non-LAB brewing microorganisms from growing while concurrently enriching the medium to cultivate specific or hard-to-culture LAB isolates. Enrichment culturing prior to plating is a common technique to influence the number and identity of isolates grown and is often critical for the efficiency of downstream molecular detection techniques. Thus, contaminating yeast or gram-negative bacteria are excluded from growing in detection media by the inclusion of cycloheximide and 2-phenylethanol, respectively (**138**). To select for specific or hard to cultivate BSR LAB, enrichment media are typically differentiated based on carbon sources present to exploit differences in substrate utilization between species (**44, 59**). The most general substitution that can be made is removal of glucose in favour of another carbohydrate, so as to limit the growth of very fast-growing LAB, and thereby "level the playing field" to give hard-to-culture isolates, which are out-competed in most standard growth media, a chance to grow (**44**). In addition, some metabolites produced by LAB; such as lactic acid or bacteriocins that have antimicrobial action, may also add to the selectivity of the enrichment cultivation (**91**).

Ultimately, primary cultivation and even use of specialized culture media to detect and identify BSR LAB are not fully effective for the accurate detection of BSR LAB. Nonetheless, culture methods remain an important area of investigation for reason that culturing is often a preceding step to molecular analysis and because culture-based tests traditionally have provided the most information for spoilage incidence reports, which has greatly influenced our current understanding concerning relevant BSR LAB.

1.3.2. Molecular Techniques

Molecular methods typically have higher associated cost and the need for specialized training, and thus have different utility for research or industry interests. Further, molecular methods can often be labor-intensive despite their ascribed benefit of being "rapid", since pre-enrichment culture or isolation is often required before the molecular detection limit can be achieved or to

remove inhibitory molecules found in beer (**7, 138**). However, the allure of molecular techniques for the brewing industry is centered on their increased specificity and sensitivity in detecting and identifying BSR LAB. A recent review (**18**) provides an extensive comparison of methodology concerning microbial community profiling in the brewing industry, and only a general overview of these current community- or microbe-targeted molecular methods for BSR LAB analysis is presented here.

1.3.2.1. rRNA detection

Many molecular techniques that seek to profile the microbes within a community specifically target ribosomal genes (i.e., 16S rRNA gene, 23S rRNA gene, inter-space regions) given the ubiquitous presence of rRNA (i.e., in both viable and non-viable cells) and the conserved nature of these sequences, enabling the ability to distinguish between species and isolates (**1**). As brewers are often solely interested in the presence of viable cells, other genes that increase the discriminatory power of these nucleic-acid techniques, such as elongation-factor genes or other hop-tolerance genes can be and are also targeted (**73**).

1.3.2.1.1. PCR and qPCR

The polymerase chain reaction (PCR) and various adaptations thereof are arguably the most frequently used means of performing targeted-interrogations of the 16S rDNA and other target genes of interest (i.e., hop-tolerance genes) for BSR LAB (**51 – 52, 104, 136 – 137**). These tests give same-day results, require relatively low expertise to run, and have sensitive detection limits, thus presenting an attractive method for brewery use.

Quantitative PCR (qPCR) usage has increased in brewery application because it allows the rapid quantitation of target DNA at an extremely sensitive level (such as from a single-cell) (**18**). The notable drawbacks include the initial instrument and software costs, increased expertise required over conventional PCR, as well as concerns for quantitation accuracy given that the signal output does not discriminate living and non-living cells, and that results are influenced by the target gene copy number. Thus, an appropriately controlled and validated system is critical for drawing accurate conclusions. Since qPCR is not a community-profiling technique, it has limited use in

interrogating mixed-culture fermentations or unknown isolates. Nonetheless, qPCR allows for the accurate monitoring of both the presence and quantity of specific microbial populations in the brewing environment, and has notable advantages over other methods in terms of analysis speed and achievable sensitivity. Reverse-transcription qPCR (RT-qPCR) assays have also been developed which analyze actively-transcribed mRNA content, and thus viable cells, such as for the detection of the BSR LAB hop-tolerance genes, though these assay must still be stringently and appropriately controlled (**14**, **51, 112**).

PCR assays continue to be optimized as the methodology itself evolves. A recent application is droplet digital PCR (ddPCR), which operates on the principle of absolute target quantification without need for internal control genes or excessive reaction replicates. This method recently was used to investigate the copy number of hop-tolerance genes within a brewery setting (**20**). Though ddPCR is limited in the number of targets it can interrogate, and by its cost, it most certainly can be further developed and applied to investigate gene target distribution and abundance within a brewery or contaminated sample (**63**).

1.3.2.2. Other molecular methods

Fluorescent *in-situ* hybridization is a method that targets specific or groups of isolates in a community and operates on the basis of using different fluorescently-labelled probes that hybridize to specific target regions (e.g., 16S rRNA) in a chemically-fixed cell sample, followed by fluorescent microscopy (**18**, **23**). Based on the fluorescent signals observed, populations of different cells can be assessed. However, this method is limited as to how many unique cell populations it can identify and is thus more suited for targeted-analysis. Fluorescent *in-situ* hybridization can also be coupled to Flow Cytometry,which performs automated cell sorting based on fluorescent signals, allowing for the quantitation of different cells within a population. Together, these methods provide a semi-automated means of acquiring quantitative data within a few days for isolates of interest without the need for excessive pre-processing (e.g., DNA extraction). However, expensive equipment is required and the probes needed are often not commercially available (**18**). Therefore, fluorescent *in-situ* hybridization and fluorescent *in-situ* hybridization-Flow Cytometry do not lend well to in-house application for the brewery, yet provide interesting data when performed as an out-sourced procedure or in a research setting.

Thus far, this methodology has been only well developed for characterization of yeast populations, with few reported studies of application to BSR LAB (**89**, **142 – 143**). However, recent work involving cider fermentations showed that Flow Cytometry could distinguish and separate mixed yeast and bacterial cultures based on membrane integrity and esterase activity, and could identify different physiological states resulting from differences in fermentation conditions, thus having interesting implications for beer fermentations (**57**).

Denaturing gradient gel electrophoresis has also been used in the past to allow for robust identification of microbial community members through the 16S rRNA gene for beer-related LAB, though it no longer is frequently utilized (**18**, **85**, **149**). This method uses universal primers to amplify specific DNA sequences in a community, then separates them in a polyacrylamide gel in a gradient of urea and formamide on the basis of differences in GC content (melting temperature), thereby allowing detection of DNA sequence heterogeneity in microbial communities **18**, **93**). Again, this method has limited use within the brewery in that it is technically difficult and requires DNA extraction, and has a detection threshold that is often above the cell concentration found in beer samples (**28**). Further, it requires subsequent processing and sequencing steps following the gel separation to produce accurate identification of the bacteria yielding the resolved bands, making it a laborious process fraught with the inherent errors and biases related to PCR amplification and DNA extraction (**18**, **28**, **34**).

Another very useful method for assaying microbial community diversity is Terminal Restriction Fragment Length Polymorphism. Universal primers targeting the 16S rRNA gene that have been fluorescently labelled are used to amplify this DNA region from a mixed culture. Amplicons are then purified and in separate reactions, digested by one or more restriction enzymes, followed by capillary electrophoresis. The separation of the fluorescently labelled DNA fragments allows for unique patterns to emerge for a given organism (**18**, **80**). This method is flexible in terms of its ability to provide either high throughput data or more targeted analysis of mixed microbial communities, and is relative easy to use with low cost making it a more attractive option for routine use in contaminant surveillance within breweries (**18 – 19**). Further, this method can be adapted to provide greater resolution for specific BSR LAB targets through modification of the target sequences and restriction enzymes used (**20**).

1.3.3. Multilocus sequence typing

The use of multilocus sequence typing has increased in tandem with whole genome sequencing in order to answer many questions of LAB relatedness and evolution (**26**, **45**, **127**). Multilocus sequence typing relies on DNA sequence analysis of conserved housekeeping genes (or other protein coding sequences) to type bacteria (**45**, **82**) and reveal insight into the overall diversity of a species. Multilocus sequence typing has direct appeal to the brewing industry not only because of lower cost and required time compared to whole genome sequencing, but also due to the potential to distinguish same-species isolates recovered from different sources and thereby the potential influence of the beer niche on genetic adaptations. However, in order to effectively develop multilocus sequence typing into a rapid means of screening for BSR *vs* non-BSR LAB, whole genome data provided by deep sequencing needs to be available to inform on specific assay targets. It should be noted that with a substantial increase in the number of sequenced genomes available, multilocus sequence typing will likely be replaced by the application of *in silico* analysis of genome evolution and phyologeny.

1.3.4. Omics

1.3.4.1. Deep sequencing of DNA and mRNA

Genome sequencing, or in the case of microbial community profiling, meta-genome sequencing, provides the entire genome or identity of each organism in the sample under analysis. The amount of genetic information obtained by this technique is exponentially greater than provided by targeted-sequence analysis (i.e., of housekeeping genes or 16S rDNA). Given that a small handful of genes have not yet proven adequate to distinguish between BSR and non-BSR LAB, the wealth of data from deep DNA and mRNA sequencing is critical for better understanding the total genetic character and higher-level metabolic regulation that differentiates these two groups of organisms, and those LAB that may be able to provide helpful fermentation for craft beers. Further, emerging patterns of species- or genus-level genetic content may be solidified and then incorporated into routine brewery-level diagnostic approaches.

Applications of transcriptomics (and/or metatranscriptomics), or the profiling of the genetic

expression (mRNA) within an organism or community, are also by far the most accurate means of determining and studying important genetic pathways for growth in a given condition. Application of metatranscriptomics allows for analysis of interactions between members of a community such as quorum signaling or overall process and stress regulation mechanisms (**18**, **122, 153**). (Meta)transcriptomics builds upon the genetic content analysis of genomics or metagenomics to reveal what genetic content is specifically active and therefore important for growth of a BSR LAB isolate on its own or in a microbial community (**25**). To date, global transcriptomics of the BSR LAB *P. claussenii* ATCC BAA-344^T when grown in beer has revealed interesting insights into not only the cell regulation and important genetic operons, but also the role of plasmids for growth in beer (**103**).

Deep-sequencing applications represent the current interface of academic research and industrial interests in the brewing field because though they are readily applied in a research setting, they do not lend themselved easily to present routine use within the brewery. However these technologies continually decrease in cost, and are currently available for use in clinical settings, thus it is not unreasonable to predict these methods may become part of routine practice in a variety of fields, including the brewery industry. Nonetheless, support of such current academic research by the brewing industry is important given that omics data has the power to delineate specific markers for LAB beer-spoilage ability, which would allow development of better detection methodology for brewery use.

1.3.4.2. Proteomics and Metabolomics

The use of gas chromatography and mass spectrometry to analyze the total protein, metabolite, or volatile compounds in a beer sample or microbial community can reveal insight into the complex process of microbial energy metabolism, quorum sensing, and protein production during fermentation and spoilage (**38**, **100**). Further, these techniques can also be applied to resolve the nature of the proteome of a community, and investigate probiotic and bacteriocin production (**9**). These methods are beginning to be applied with greater frequency to BSR LAB (**12**, **33**, **155**), with notable recent application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to help characterize the microbial community of lambic beer and to distinguish between *L. brevis* isolates of different beer-spoiling virulence (**74**, **123**). These advances aside,

the brewing industry lags behind other LAB-related fields in applying high-throughput protein analysis or sequencing techniques to solve the problem of LAB beer-spoilage as well as help characterize useful LAB (**9**, **92**).

1.4. BSR LAB and the brewing environment

1.4.1. Niche adaptation and horizontal gene transfer

Distinction between differently adapted LAB isolates lies not only with the analysis of the LAB core genomes, but also in the investigation of chromosomal sequences that appear to have originated in another species and mobile genetic elements such as plasmids (**24**). The latter two genetic features are frequently acquired through horizontal gene transfer (HGT) between isolates of same or different species. By comparing recently divergent as well as ecologically distinct genomes, it is revealed that HGT is important for the transfer of sequences or clusters of sequences, and drives the existence of diversification (**62**, **154**). In fact, HGT events are promoted by environmental stress, resulting in faster adaptation or "short-term" evolution in challenging environments (**41**).

For LAB, HGT events mediated by plasmids are important to a variety of industries (**32**). In the brewing industry, conventional genetic markers of beer-spoilage such as the exopolysaccharide gene *gtf*, and the hop-tolerance genes *hitA*, *horA*, and *horC* are all plasmid-encoded and exhibit a very high degree of sequence identity in many different species (**129**, **152**). The existence of these markers suggests not only the occurrence and support of HGT in and by the brewery, but also the importance of investigating other plasmid-harbored genes that demarcate BSR from non-BSR LAB.

Given that the ecological diversity among LAB appears to be driven in general by genome reduction mechanisms, the acquisition of niche-specific genes through the transfer of plasmids is an important area of investigation. Indeed, recent omics-based studies support the notion that plasmids are important for conferring beer-spoilage ability. New genomic data for several *L. brevis* isolates has revealed that an increased number of plasmids may correlate with the ability of isolates to withstand increasingly harsh and specific environments. For example, *L. brevis*

KB290 originally isolated from a traditional Japanese fermented vegetable and also able to grow in simulated gastric and intestinal juices, has nine plasmids ranging in size from 5.8 to 42 Kb (**48**). Similarly, the rapid beer-spoiling isolate *L. brevis* BSO 464 has eight plasmids ranging from 2.3 to 85 Kb (**13**). These two isolates are incapable of growth in the other isolate's niche-environment (J. Bergsveinson, unpublished), indicating that each possesses specific genetics that do not confer immediate cross-resistance to another stressful environment; as such, these isolates have niche-specific tolerance genes. In contrast, the type strain *L. brevis* ATCC 367^T only harbors two plasmids (13 and 35 Kb) (**83**) and is unable to spoil beer and cannot grow in in gastric juices (J. Bergsveinson, unpublished; **48**). This further suggests that increased plasmid-coding capacity likely supports the ability of *L. brevis* strains to infiltrate diverse environments. This idea is supported by a recent study showing that the sequential loss of plasmids from *L. brevis* BSO 464 results in loss of its original beer-spoilage phenotype, indicating that beer-spoilage is mediated by specific plasmid-encoded functions (**13**). Similarly, transcriptomic analysis performed on BSR LAB *P. claussenii* ATCC $BAA\text{-}344^T$ revealed that several significant plasmid-based transcripts were active across its eight plasmids (ranging from 1.8 to 36 Kb) when in the beer environment, notably on the plasmid that harbored the hop-tolerance gene *horA* (**101, 103**). Collectively, these results strongly suggest that specific plasmids encode previously un-described beer-spoilage related functions and that detailed investigation of plasmid genes in relation to growth in niche environments, such as beer or the brewery, will prove useful.

Increased transcriptomic studies, in conjunction with comparative genomics, will most accurately and fully reveal the importance of plasmid-mediated functions for BSR LAB. Once more it is emphasized, that for this data to be of utility to the brewing industry, this analysis must be performed with more frequency on BSR LAB of both same and different species. As the cost of this analysis decreases and bioinformatics tools become more sensitive (**141, 87**), it will be possible to investigate the broad importance of widely conserved plasmid sequences in BSR LAB, as has been done for other niche-adapted organisms (**41**, **96**). Such analysis is reasonably expected to increase the number of species-independent, but beer-spoilage specific genes (and/or their transcripts) that can be screened for during quality control routines in the brewery.

1.4.2. Origin of BSR LAB

Phylogenetics and comparative genomics can help answer questions on the evolutionary development of BSR LAB, however, the answer to how and when these isolates emerged likely lies within the brewery itself. BSR LAB likely occupied this new niche along with the inclusion of hops in beer between the 5th and 9th century. Following genetic adaptation to this specific stress, BSR LAB then adapted further and have since remained tightly linked with the brewing environment (**129, 130, 132**). Indeed, BSR LAB isolates are rarely isolated elsewhere than breweries or beer, though non-BSR LAB isolates of the same species are (**129, 132**). Breweries thus are both the selective environment and the reservoir for their own contaminants.

A recent study has investigated the distribution pattern of LAB species and putative hop-tolerance genes in a brewery producing several different kinds of beer, using LAB-specific terminal restriction fragment length polymorphism and ddPCR, respectively (**20**). The brewery involved produces conventional beer (potential BSR LAB contaminants), sour beer (helpful LAB fermenters or BSR LAB) and coolship beer (BSR LAB and environmental microflora). The LAB-terminal resitrction fragment length polymorphism analysis applied in this study was specifically developed for LAB isolates and was found to more sensitively discriminate between species of the *Lactobacillales* order and most genera of the *Bacillales* order present in mixed culture (**20**). LAB-terminal resitrction fragment length polymorphism methodology also identified organisms from other phyla not previously reported as recovered from beer, likely as a result of the fact the organisms in question are present at low abundance and are never actively selected for during detection (**20**). By applying this technique to analyze the LAB community profile throughout a brewery, it was possible to conclude that the brewery microbiota is likely driven by contact with raw substrates (grains, hops, yeast and beer), with this contact resulting in the profile of LAB present within a given brewery (**20**). For example, they found that wort samples contained a mixture of *L. delbrueckii*, *L. hilgardii, L. sakei, Lactococcus lactis, Leuconostoc mesenteroides, Streptococcus* spp., as well as a *Bacillus* spp. "A", most of which were only rarely detected in other fermenting and bottled beer samples (**20**). Many of these species, while not necessarily found in finished beer, are apparently associated with grain and therefore their detection in wort is unsurprising (**17**).

Interestingly, distinct LAB profiles from specific brewery samples were detected at different sites, presumably as result of potential contact with the sample. For example, sour and coolship beers were dominated by *L. lindneri* and *Pediococcus* spp., though fermenters and barrel surfaces that contacted these sour fermentations around the time of sampling exhibited similar community composition; however, *L. brevis* and *Lactobacillus* sp. were found to be more common on these surfaces then on other surfaces or in the beer. Floor and packaging area surfaces contained a more diverse LAB composition of LAB, with the predominant organisms being *L. brevis*, *L. delbrueckii,* and *L. lindneri,* which were also detected in the sour wort and beer. Perhaps most interesting was the finding that only *Pediococcus* spp. were detected on grain samples, while, *L. brevis, L. lindneri* and *Pediococcus* spp. were recovered from hop pellets. This is noted as to be potentially due to the weak amplification from grain samples as a result of either inhibition of PCR by grain polyphenols or as a function of low LAB populations (**20**). Though the data gathered is of exceptionally high detail, ultimately this work cautions against ascribing raw substrates as causing contamination of all areas or equipment that share similar microbial community compositions, as there are alternative means for microbial transfer within the environment such as fruit flies, or more likely, human activity (**20**).

Given the ubiquitous presence of LAB in and on natural sources such as plants and humans, it is likely that the introduction of specific LAB species into the brewing environment, and their prevalence and distribution throughout, is an outcome of the specific raw materials (grain, hop, water, yeast) and is a further function of a given brewery's specific geographical location; structural history; recipe, processing and production lines; and personnel hygiene. The individual nature of a brewery has been underscored by the analysis of LAB-contamination in Australian breweries wherein specific contamination was found to be associated more with the particular brewery, rather than with specific antimicrobial challenges present by the starting beer sample (ethanol, hops, pH) (**90**). The microbiological quality and hygiene of a brewery thus is apparently dependent more on production practices and sanitation regimes than it is on the beer characteristics (i.e., highly hopped or alcoholic beers) (**90**).

The work presented in **(20)** is a foundational study from which to model further analysis of other breweries. Though it can be restated that the presence of LAB isolates and

prevalence/distribution of them in a brewery will likely be brewery-specific, ultimately an understanding of where bacterial (LAB) contamination is taking place within a given brewery should allow for the identification of specific contamination sources (i.e., raw materials *vs.* personnel) and help to strategize how best to prevent, or treat and recover contaminated product.

1.5 Hop tolerance

1.5.1. Antimicrobial effect of hops

Beer and the brewery are stressful environments *in toto,* though hops are certainly considered the defining stress for microorganism growth. When hops are added to wort and boiled, α-acids are extracted and subsequently transformed into various iso-α-acids, which are the principal bitter components in beer (**124**). These bitter compounds have a demonstrated antimicrobial effect on gram-positive bacteria (LAB), which was originally recognized to be through increasing the permeability of the bacterial cell wall, causing leakage of the cytoplasmic membrane, inhibiting normal cellular processes, and causing changes in the cellular proton ionophore activity and uptake of leucine (**119**, **120, 140**). Ultimately, bitter compounds act as ionophores, which sequester protons within the cell and dissipate the pH gradient on either side of the cellular membrane, thereby reducing the proton motive force (PMF) and all PMF-dependent cellular activities, such as nutrient uptake (**108**, **120**). Further, the strength of the inhibitory effect of hops was found to be dependent on pH and mediated by a proton/divalent cation (K^+, Mn^{2+}) exchange across the bacterial membrane (**120**). Importantly, these same mechanisms were not found to be active against gram-negative isolates, likely as a result of the protection afforded by their outer membrane (**115**). Further investigation found that a transmembrane redox reaction of hop compounds occurred at low pH (such as in beer) and in the presence of Mn^{2+}, and that this redox activity causes cellular oxidative damage (**10**). Therefore, hop-tolerance likely is a multifactorial process where at least two distinct levels of tolerance mechanisms mediate the stress of hops; namely, proton ionophore-maintenaince and oxidative-stress mechanisms (**10**).

1.5.2. Hop-tolerance mechanisms

To combat the intrusion of hop bitter acids into the cell, it has been suggested that hop-tolerant LAB isolates produce higher molecular weight lipoteichoic acids in the cell wall as a response to

hop presence (**11**, **129**, **157**). The change in lipoteichoic acids composition increases the natural barrier function of the cell wall, fortifying it against the damaging invasion of hop bitter acids (**129**). Further, these lipoteichoic acids are purported to act as reservoirs of divalent cations (Mn^{2+}) that can complex with hop bitter compounds as they move across the cell. The competitive binding of lipoteichoic acids and Mn^{2+} limit the extent to which Mn^{2+}-hop bitter compound complexes are formed further reducing the potential deleterious effects of hops against the cell (**11**, **129**, **151**). This layer of defense or resistance is likely passive and of very little energy burden to the cell if established (**129**). Additionally, in a *L. brevis* model, it has been found that intracellular Mn^{2+}-dependent enzymes are induced by the presence of hop-bitter compounds and this induction may help to maintain redox homeostasis and generate energy in response to a loss of PMF and depletion of Mn^{2+} reserves (**12**). As these types of enzymes are responsible for maintaining cellular redox homeostasis, this cellular response is likely targeted at ameliorating the oxidative stress induced by hop bitter acids (**10**, **151**).

1.5.3. Proposed hop-tolerance genes

Potential genetic elements responsible for conferring some level of hop resistance to isolates have historically received a great deal of attention, given these elements would have utility for rapidly screening and distinguishing BSR LAB from non-BSR LAB. Hop-tolerance genes described to date all share the characteristics of being plasmid-located and having gene products associated with the cytoplasmic membrane, working to either remove hop-compounds from the intracellular space or maintain cellular homeostasis (**129**).

The first gene described was *horA*, recovered from a *L. brevis* isolate (**111**) and its product found to act as an ATP-binding cassette (ABC) transporter which transports hop bitter acids out of the cell (**109**). The product of the *horC* gene, also originally described in *L. brevis*, is predicted to function as a PMF-dependent multidrug transporter belonging to the resistance-modulation-cell division (RND) superfamily that can export bitter compounds from the cell (**130, 134**). Interestingly, HorA and HorC can both confer resistance to multiple structurally unrelated drugs (**109, 130**). HorB is often included in some lists of hop-tolerance genes, though its proposed function as the transcriptional regulator of HorC has not been convincingly confirmed (**14**, **70**). The third major hop-tolerance gene described, *hitA,* is suggested to function in the uptake of

divalent cations (Mn^{2+}) following reduction of the intracellular concentration of these cations by hop bitter compounds and thereby helping maintain Mn^{2+}-dependent cellular functions (**61**). There have also been reports of two genes specific to *Pediococcus* spp., *bsrA* and *bsrB*, which presumably also function as multidrug ABC transporters against the action of hop compounds (**52**).

Although the mechanisms of action and prevalence of these genes in LAB have been documented, questions remain as to ability of these hop-tolerance genes to absolutely account for the hop-tolerance and overall ability of a BSR LAB to grow in and spoil beer. For example, there are reported cases of hop-tolerant isolates that do not possess any of the described hop-tolerance genes (**11**, **90**), or isolates that harbor all three hop-tolerance genes and are hop-sensitive (**7**). In addition, the actual functionality and/or transcriptional activity of these genes in response to hop or beer stress have yet to be analyzed broadly and in depth using current molecular methods. One study utilized RT-qPCR to show that a *L. brevis* strain possessing all four genes (*hitA, horA, horB/C)* and a *P. claussenii* strain possessing only *horA* and *bsrA* did not utilize these genes to the same extent during mid-exponential growth in beer (**14**). In fact, only *horC* showed significant expression in beer in *L. brevis*, while its transcriptional regulator *horB* was not similarly expressed, nor were *hitA* and *horA.* Comparatively, *P. claussenii* demonstrated a significant expression of the *horA* gene and to a lesser extent, *bsrA*. Given these two isolates differ in their beer-spoilage virulence; the transcriptional data raises the following questions. Does the possession of more than one hop-tolerance gene correlate with increased hop-tolerance *and* beer-spoilage virulence? When all genes are present, are they utilized or active to different extents and potentially at different times during an isolate's growth? Finally, what is the role of *horB*?

General hypotheses can be posed in response to these questions. First, there is evidence to suggest that the presence of more than one hop-tolerance gene correlates with increased hop-tolerance and potentially increased beer-spoilage ability, however, these studies have not yet delineated whether all genes under analysis are complete, functional and/or active (**129, 132**). Second, some results suggest that HorC is a major contributor to hop-tolerance and is generally correlated with strong beer-spoilage ability, and therefore might be the preferred mechanism of

action (i.e., energy is spent transcribing this gene) in the face of hop- or beer-stress despite the presence of the other hop-tolerance genes (**13**, **105**). Alternatively, hop-tolerance genes may not be necessarily transcriptionally active simultaneously and instead are activated in some sequential fashion, perhaps to reduce the cell's energy burden. As the HorC transporter is dependent on the PMF and apparently active during mid-exponential growth in beer (in *L. brevis*), it could be that HorA and HitA, which act to either reestablish or maintain the PMF through removal of hop compounds or movement of divalent cations, could actually establish optimal conditions for HorC activity and facilitation of growth.

The role of *horB* remains unclear since although it is nearly always found in conjunction with *horC*, it does not exhibit parallel transcription (**14**, **20**). As LAB are prone to frequent and rapid acclimatization to new environments, and often do not carry extraneous genes, it is curious that *horB* would be kept as a non-functioning artifact along with *horC* (**84**, **116**). Therefore, *horB* may not be a *horC*-specific regulator or is not temporally active with *horC*. These possibilities require investigation with different BSR LAB isolates at different stages of growth in beer.

1.5.4. Hop-tolerance genes and the brewing environment

Until a recently, there was no previous analysis of hop-tolerance gene dispersion in the brewery environment (**20**). This study made use of next-generation ddPCR to quantify the abundance of *hitA*, *horA*, *horB* and *horC*, in conjunction with associated microbial community profiles assessed by LAB-terminal resitrction fragment length polymorphis analysis on various brewery surfaces over time.

Firstly, this study determined that areas involved with sour beer production had the highest gene frequencies, specifically *horC* (**20**). This gene was the most abundant gene in general and was found in a nearly equal ratio with that of its putative transcriptional regulator *horB*, enforcing the notion that *horC* is an important and prevalent hop-tolerance gene selected for in the brewing environment. The *hitA* gene had the lowest frequencies throughout the brewery, corroborating a previous report of low detected frequencies in BSR LAB (**52**). Indeed, *hitA* also had a lower correlation with the presence of the other hop-tolerance genes, which shared amongst themselves high degrees of intercorrelation (**20**). *horA* was the only gene correlated with *Pediococcus,*

supporting the previous observation that this gene is the primary known resistance gene for this genus (**52**). Most interesting was the fact that none of the four hops-related genes correlated with *L. brevis*, which is not only considered the most common brewery contaminant species, but has also been shown to be among the LAB most commonly positive for hop-tolerance genes (**52**). However, given that *L. brevis* was only a minor component of sour beer and processing surfaces, this finding is likely particular to the brewery under analysis (**20**).

These results reveal the importance of tracking spoilage genes within the brewery environment in order to understand contamination risks and patterns, especially where more than one beer style is produced. For example, barrel surfaces, fermenters and packaging-line surfaces (that all come into contact with beer) exhibited fairly high levels of hop-tolerance genes with the highest levels associated with surfaces that contacted sour beers and in unsanitary areas such as the packaging-line sink and below the packaging belt (**20**). These findings are highly illuminating for development of brewery best practices, in that equipment for the production of sour or specialty beer must be specifically dedicated and adequately separated from equipment used in conventional brewing. Further, contact with beer is strongly implicated in transmission of hop-tolerance genes and BSR LAB between different areas of the brewery; thus protocol and human activities must limit this transfer. Tracking transmission of hop-tolerance (and other important genes) within the brewing environment is an incredibly worthwhile undertaking given that it will add insight into the role of the brewery (structures, personnel) *vs* raw material contamination, and into the propagation of hop-tolerance genes and BSR LAB within the brewery (**20**).

1.5.5. Utility of BSR LAB hop-tolerance genes

Although questions remain concerning the utility of hop-tolerance genes in predicting beer-spoilage ability, there is no denying these genes are relevant to BSR LAB and the brewing environment. The most notable feature of these genes is that they are not species-specific markers for hop-tolerance. For example, *horA* and *horC* (in addition to their flanking open reading frame (ORF) regions) are found to be well conserved in other BSR LAB isolates such as *L. backii, L. linderii, L. paracollinoides, P. claussenii,* and *P. damnosus,* in addition to *L. brevis* (**69**, **101**, **131**, **134 – 135**). It has even been reported that these two genes are found at rates as high as 94% and 96% of BSR LAB tested and that all strains have at least one of the genes (**129**).

Caution is required, however, since the full gene length of hop-tolerance genes is rarely sequenced in brewery settings; therefore, the sequence similarity, let alone the functional integrity of these genes, is rarely guaranteed.

Though targeting hop-tolerance genes currently remain the strongest discriminatory method to detect intra-species differences in beer-spoilage ability (**43**, **69**, **112**), these genes are still unable to predict the beer-spoilage capacity of the full spectrum of BSR LAB that have been described. Unfortunately, to date, there is little data suggesting alternative hop-tolerance genes or mechanisms in the absence of any of *hitA, horA,* or *horC* (**90**). This lack of compensatory theories is frustrating in light of the physiological variability (hop-tolerance, growth phenotype) among strains that have identical hop-tolerance genes profiles; other uncharacterized hop-tolerance mechanisms must exist (**52**, **55**, **90**). Since the known hop-tolerance functions are ABC transporters or efflux pumps, and since both types of transporter are common within LAB (**76**, **117**), it is short-sighted to not conceive of other similar genes and proteins across the spectrum of BSR LAB that deal with hops directly or indirectly, or that deal with other stresses in beer. Indeed, given the many different stresses in beer, the ability to grow in and mediate the damage of both beer and hops, is likely the result of a synergy of mechanisms and redundant genetic traits. Until more detailed and high-throughput analyses of these processes are conducted, we remain hindered in our capacity to screen for elements that describe true beer-spoilage ability.

1.6. Stress tolerance and adaptation of BSR LAB

There is considerable literature that discusses general and specific stress responses of LAB in a variety of industries. Though stress tolerance can differ among isolates of the same species, LAB are highly adaptable to stressful environments and adaptation to one particular stress often affords LAB increased tolerance and survival to the challenge of another stress, due to the cross-regulation and functions of stress response pathways (**32**, **97**). BSR LAB isolates exemplify complex stress response regulation given that isolates must simultaneously employ tolerance mechanisms to a variety of stresses.

1.6.1. Stress tolerance to ethanol and low pH

Ethanol levels and pH differ among styles of beer worldwide, however, are commonly within the ranges of 0.5–14% (v/v) ethanol and 3.8 – 4.7 pH (**134**). As a consequence, LAB recovered from beer within or outside these ranges are typically well adapted to one or both of these stresses (**129**). Further, most BSR LAB produce either lactic or acetic acid due to their basic fermentation, which naturally lowers the pH of the surrounding environment. Indeed, it has been reported that decreased pH and increased ethanol in beer had little affect on the growth of LAB, and that there is no correlation between these two factors and contamination, though pH values near 4.0 or below had some inhibitory effect on LAB (**90**). Nonetheless, adaptation to the acidity found in beer is necessary, as low pH can interfere with enzymatic reactions, protein folding and other intracellular processes of non-pH tolerant organisms. LAB, and other pH-tolerant organisms are capable of regulating their intracellular pH in face of acidic conditions through means of proton transport across the cellular membrane (often coupled to cation transport) or through proton-translocating ATP synthase (**32**).

Ethanol, like hops, is an antimicrobial component of beer, easily crossing the bacterial membrane and then modifying activity of cytoplasmic processes such as protein folding and inhibiting enzymatic interactions. Ethanol also increases cell membrane permeability through alteration of the polarity of aqueous and hydrophobic regions of the phospholipid membrane, causing leakage of small molecules from the cell and cell death (**71**). Various tolerance mechanisms may combat these effects, such as membrane fortification through an increase in long-chain fatty acids (> 20 carbons) (**150**). Other general stress-response proteins such as the GroES chaperone, heat-shock proteins, and glutathione reductase (**46**, **78**, **118**) confer increased survival during ethanol stress, as well as to other stresses (**32**). For BSR LAB, it has been found that ethanol tolerance does not differ significantly between BSR and non-BSR LAB, and that overall LAB ethanol-tolerance levels were species-conserved, unlike beer-spoilage capacity (**104**). Though BSR LAB adaptation to low pH and ethanol are important, it does not appear that either is necessarily predictive of the ability to tolerate hops, nor ability to spoil beer (**13**, **90**, **104**).

1.6.2. Stress tolerance to low nutrient availability

Following the breakdown of grain starches during malting and mashing processes in brewing, yeasts are used to consume and ferment available nutrients in order to produce ethanol. Yeasts can make use of the majority of sugars present in wort, in addition to using available amino acids as a source of nitrogen, in a sequence usually dependent on both the strain of yeast and conditions used (**81**, **98**). Organic acids (acetic, citric, lactic, malic, pyruvic and succinic acid) are left behind by yeasts as metabolic by-products, in addition to unused compounds such as dextrins, arabinoxylans and β-glucans (**50**). Remaining nutrients in beer following fermentation are typically in low abundance and are often "alternative" sources of carbon that can vary from brew to brew within and between breweries.

LAB naturally have an array of possible mechanisms to perform nutrient uptake into the cell, thus allowing them to inhabit various nutrient-rich or -poor environmental niches. In nutrient-depleted beer, primary nutrient transport via the use of ATP-binding-cassette (ABC) transporters is proposed to allow for advantageous growth (**76**). These transporters typically have high affinity for a given solute and use ATP-hydrolysis for high rate transport. Further, secondary transport mechanisms, not requiring ATP but relying on the electrochemical ion gradient to transport molecules across the membrane, involve uniporters, antiporters and symporters for effective uptake of molecules (**156**). In some cases, this uptake can even contribute to the production of energy through contribution to the PMF gradient (**156**). Lastly, group translocation, a mechanism that chemically modifies a solute that has been internalized can also facilitate the uptake of a range of carbohydrates (**156**).

There is considerable evidence for the importance of each type of transport uptake mechanism for BSR LAB. First, there are a great number of ABC transporters among LAB in general, and the importance of these proteins for BSR LAB are likely not yet fully appreciated beyond hop-tolerance mechanisms (**76**, **117**). Second, recent transcriptomic work of *P. claussenii* ATCC-BAA344^{T} (Pc344) when grown in beer revealed the importance of both secondary transport systems (i.e., the arginine or agmatine deiminase pathways, citrate fermentation) and group translocation such as the phosphotransferase system (PTS) (**103**). Components of all these systems were actually found to be among the top twenty most significantly expressed transcripts

in beer suggesting the critical role of nutrient-acquisition pathways for survival in beer (**103**). Interestingly, the significantly-expressed agmatine deiminase pathway in Pc344 is very similar to the arginine deiminase pathway which is not specific to nutrient-stress, but has been shown to be up-regulated in response to low pH and acid stress, low oxygen concentration, low arginine supply (6 mM) and cell adaptation to arginine in *L. sanfransciscensis* (**31**). Though the ADI operon is not found in Pc344, the similar agmatine deiminase operon was shown to be critically important for survival in the beer environment, and is a major example of the cross-specificity of LAB stress responses. Finally, another example of cross-resistance is the induction of stationary phase in LAB when faced with nutritional starvation, at which point the cells become more resistant to stresses such as heat and acid (**32**, **49**).

1.6.3. Stress tolerance to low O_2 tension and dissolved CO_2

The low oxygen levels in beer selects for microbes capable of micoaerophilic respiration. LAB, specifically *Lactobacillus* and *Pediococcus* isolates, can produce energy in the absence of oxygen (normally an electron acceptor for generation of ATP via oxidative phosphorylation) by using other electron acceptors to regenerate NAD+ or by substrate-level phosphorylation during fermentation for the regeneration of NAD+ (**156**). Fermentation capacities in anaerobic conditions are known to be different across sub-groups, even genera, of LAB, nonetheless, the overall anaerobic nature of BSR LAB facilitates their resistance to the stress of low oxygen.

The presence of dissolved CO_2 (dCO_2) in beer, and its affect on contaminating LAB has only begun to be investigated for its role as a potentially growth-adverse stress of beer. CO_2 has been demonstrated to affect general microbial transcriptional activities and physiology and the ability of microbial spoilage isolates to grow (**37**, **126**). However, it remains to be determined how dCO_2 levels in beer affect the growth of BSR LAB, including what specific tolerance mechanisms or responses are induced. Such information is important, since it is reasonable to expect brewery-adapted BSR LAB must be able to withstand the sudden, additional stress of high dCO_2 as a result of headspace flushing with CO_2 during packaging.

1.6.4. Viable, but not culturable state

A general adaptation to the beer environment by BSR LAB is the modification of both cell size and morphology. Diminished cell size and a notable rounding or shrinking of bacilli or rod-shaped cells (i.e., taking on a coccoid appearance) have been noted for several BSR LAB isolates following beer-adaptation (**3**). This phenomenon has been proposed to be an attempt by the cell to reduce surface area in contact with beer and to help membrane-associated tolerance genes (i.e., hop-tolerance) deploy more efficiently (**129 – 130**). Furthermore, increased time in beer results in induction of a viable, but not culturable (VNBC) state in LAB cells. Such cells are not detectable by routine non-beer culture media on which they would normally grow colonies, but are alive and capable of renewed metabolic activity through continued exposure to routine media (**36**, **95**). Understanding both the conditions inducing the VBNC state in a variety of different BSR LAB and how to retrieve the culturable phenotype more efficiently is of extreme utility to brewers in accurately detecting where BSR LAB exist in their brewery. Investigation into the genetics and transcriptional activity of BSR LAB throughout their VNBC cycle would also increase the understanding and be of upmost importance for a wide range of industries dependent on LAB.

1.6.5. Maintenance of BSR LAB and importance of biofilms

The mapping of BSR LAB and hop-tolerance genes in the brewery has illustrated the risk of cross-contamination between different equipment surfaces, especially in environments where conventional and sour beer types are produced (**20**). Cross contamination of surface areas supports the increase in diversity of the present microbial community, as well as the development of biofilms, thus likely driving spoilage incidence at various production stages (**86**, **146 – 147**, **125**).

The brewing industry has great concern about biofilms given that they can be established not only in the brewery, but also in draft beer dispensing lines outside the brewery, which brewers do usually not monitor, nor control (**145 – 146**). Though biofilms are typically comprised of a variety of microorganisms, they have a known correlation with product-spoiling bacteria and thus require prevention and attention (**146**, **160**). Increased analysis of brewery biofilms would be useful, especially given that the microbial composition of biofilms may be location unique

and thus require specific or adapted control treatments. Though some specific strains of LAB have been shown to be able to form biofilms (**77**), in general, gram-negative bacteria (and yeast and molds) are among the first to colonize surfaces in the brewery, while LAB are opportunistic colonizers that benefit from the multiple interactions within already established communities, especially if the biofilm provides reduced oxygen levels and an acidic environment (**6**, **125**).

Involvement in biofilms also increases the likelihood of acquiring genetic material advantageous for the brewing environment through HGT (**77**, **146**). Evidence that biofilms support the transfer of beer-spoilage virulence genes comes from the finding of plasmid-harbored hop-tolerance genes among many LAB species, with the interspecies nucleotide sequence identities of these genes and surrounding regions being highly conserved at approximately 99% (**129, 134 – 135**). Indeed, a 5.6 kb region that contains *horA* was found to be 100% identical in *L. backii* and *P. inopinatus* strains isolated from the same brewery (**69**), and this same phenomenon is identified in other *horA*$^{+}$ isolates recovered from different sources (**101**; J. Bergsveinson, unpublished data). As such, HGT among LAB in biofilms is believed to be how hop-tolerance genes, and other putative plasmid-mediated beer-resistance elements are spread within breweries (**129**).

1.7. THESIS OBJECTIVES

The goal of my research was to address current limitations in the understanding of BSR LAB within the brewing industry – specifically, the incomplete working model of BSR LAB stress mechanisms, the poor understanding of the role that plasmids play in beer-spoilage ability, and the under-appreciation of the power of next-generation sequencing (NGS) technologies to investigate these questions. The central hypotheses which guided the experimental work for this thesis are: (1) that the genetics of hop-tolerance is variable in BSR LAB, with the few traditional hop-tolerance genes not being adequate predictors of either hop-tolerance or beer-spoilage ability and (2) that the beer-spoilage phenotype is variable and influenced by both the total genetic content of BSR LAB and other physiological stresses found in beer besides hop-stress.

I began by investigating the differences in transcriptional activity and prevalence of known hop-stress tolerance genes in BSR LAB (Chapters 2 and 3). As hop-tolerance genes are known to be located on plasmids, the role that the entire plasmid profile of a BSR LAB has on its beer-

spoilage ability was also examined (Chapter 4) in order to elucidate if other plasmids that do not harbor hop-tolerance genes are also important. In addition, the presence and/or absence of dissolved CO_2 (dCO_2) in beer was explored to determine its affect on BSR LAB's ability to grow in beer, and the relationship of dCO_2 stress with other beer stresses and BSR LAB stress-tolerance mechanisms (Chapter 5). The NGS technology of RNAseq (sequencing of RNA transcripts) was used to profile the transcriptional response of a dCO_2-tolerant BSR LAB organism when grown in beer with or without the presence of dCO_2 (Chapter 6). This technology was also used to determine the transcriptional response of two unique BSR LAB in response to growth limiting concentrations of hops, in hopes of separating and defining the concepts of hop-tolerance and beer-spoilage (Chapter 7). Lastly, large-scale genomic comparisons were made amongst beer spoiling and non-beer-spoiling *Lactobacillus brevis* and other lactic acid bacteria isolates with available genomes, in order to help corroborate suspected beer-spoilage-specific genetic elements and identify the degree of genetic similarity amongst BSR LAB (Chapter 8).

My data *in toto* point to the need for the brewing industry to perform detailed examination of other beer-stresses apart from hops (e.g., dCO_2) on BSR LAB and to detail how these stresses interact and synergistically affect BSR LAB. My results also call attention to the power of NGS technologies to answer questions of (i) what genetic elements must be expressed; i.e., are critical for growth in different beer and brewery conditions and (ii) how prevalent these genes are amongst beer-spoiling and non-beer-spoiling organisms and, thus, how useful they may be for detection approaches as related to microbial quality control in a brewery setting.

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Chapter 2: RT-qPCR analysis of putative beer-spoilage gene expression during growth of *Lactobacillus brevis* BSO 464 and *Pediococcus claussenii* ATCC BAA-344^T in beer.

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2. INTERFACE

This chapter was previously published in *Applied Microbiology and Biotechnology*, 96(2): 461-70, 2012, and is reprinted here with permission. This study was the first to perform comparative transcriptional analysis of hop-tolerance genes in beer-spoiling isolates directly challenged with beer.

Jordyn Bergsveinson and **Vanessa Pittet** are attributed equal authorship, as we contributed equally to the data generation and manuscript preparation; as such this material was also presented in her Ph.D. thesis “Adaptations of lactic acid bacteria for growth in beer” (Vanessa Pittet, 2012: Chapter 4, University of Saskatchewan, Saskatoon, SK).

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work.

2.1. Abstract

Lactic acid bacteria (LAB) contamination of beer presents a continual economic threat to brewers. Interestingly, only certain isolates of LAB can grow in the hostile beer environment (e.g., as studied here, *Lactobacillus brevis* BSO 464 (Lb464) and a non-ropy isolate of *Pediococcus claussenii* ATCC BAA-344^T (Pc344NR)), indicating that significant genetic specialization is required. The genes *hitA*, *horA*, *horB*, *horC* and *bsrA*, which have been proposed to confer beer-spoiling ability to an organism, are suspected of counteracting the antimicrobial effects of hops. However, these genes are not present in the same combination (if at all) across beer-spoiling organisms. As such, we sought to investigate the extent to which these genes participate during Lb464 and Pc344NR mid-logarithmic growth in beer through reverse transcription quantitative PCR (RT-qPCR) analysis. We first determined the optimal reference gene set needed for data normalization and, for each bacterium, established that two genes were needed for accurate assessment of gene expression. Following this, we found that *horA* expression was induced for Pc344NR, but not for Lb464, during growth in beer. Instead, *horC* expression was dramatically increased in Lb464 when growing in beer, whereas no change was detected for the other putative beer-spoilage-related genes. This indicates that HorC may be one of the principle mediators enabling growth of Lb464 in beer, whereas in Pc344NR this may be attributable to HorA. These findings not only reveal that Lb464 and Pc344NR are unique in their beer-specific genetic expression profile, but also indicate that a range of genetic specialization exists among beer-spoilage bacteria.

2.2. Introduction

The unique physical and chemical composition of beer provides an incredibly harsh environment for bacterial growth, in large part due to the antimicrobial effects of ethanol (0.5-14% v/v) and hops compounds (17-55 ppm iso-α-acids) (**31**). Despite these constraints, bacterial contaminants are frequently isolated from beer, where their growth results in an unfavorable sensory profile due to unappealing “off-flavors” and textures (**1**). As such, undetected microbial contaminants anywhere in the brewing process poses a threat to product quality and the economic success of brewers.

Lactic acid bacteria (LAB) are commonly isolated from spoiled beer, with most isolates being members of the *Lactobacillus* and *Pediococcus* genera (**21, 23**). Interestingly, not all members of either genus, nor all members of a species in each genus, are capable of growth in beer. Instead, ability to grow in beer is isolate-specific, and a remarkable amount of variance exists in the genetic, metabolic, and growth characteristics of beer-spoiling organisms (BSOs). This phenomenon is exemplified by the two brewery isolates studied here, *Lactobacillus brevis* BSO 464 (Lb464) (**28**) and a non-ropy variant of *Pediococcus claussenii* ATCC BAA-344^T (Pc344NR) (**8**). These two bacteria represent commonly found brewery contaminants and, interestingly, their ability to spoil beer is quite different, with Lb464 growing in/spoiling beer in roughly a quarter of the time taken by Pc344NR. Lb464 also demonstrates a higher tolerance to the antimicrobial effects of hops compared to Pc344NR (unpublished, Pittet et al.). Furthermore, the parent of the Pc344NR variant studied here has the ability to produce exopolysaccharide or rope (and therefore possibly biofilms), whereas this trait is not found in Lb464. These findings indicate the presence of genetic specialization in beer-spoilage organisms, which enables growth in (i.e., spoilage of) beer, and persistence in the brewery environment.

Only a few select genes have been proposed to play a role in beer-spoilage by LAB, including *hitA* **(14**), *horA* (**25**), *horB, horC* (**30**, and *bsrA* (**11**), all of which are suspected of circumventing the antimicrobial effects of hops. Hops effectively disrupts the bacterial cell's transmembrane pH gradient and proton motive force (PMF) through the action of bitter-acid compounds (typically isomerized α-acids) that function as protonophores (**26, 29**). Thus, genes such as *horA*, which encodes an ATP-binding cassette-type multidrug resistance transporter, are proposed to enable hops resistance by preventing the accumulation of iso-α-acid in the intercellular space by exporting it from the cell (**25**). Akin to *horA*, both *horB* and *horC* are plasmid-localized genes, which when lost, correspond with an inability to resist hops (**30, 32**). HorC has been suggested to be a PMF-dependent multidrug transporter whose expression is under the control of the transcriptional regulator HorB (**17**). Consequently, these two genes may also aid in hops resistance (and therefore beer spoilage) through iso-α-acid export. In contrast, the *hitA* gene has been proposed to play a role in hops resistance via divalent cation transport (**14**).

Although several putative beer-spoilage-related genes have been described, recently it has been shown that only the presence of *horA* definitively correlates with LAB growth in beer, with simultaneous presence of *hitA* and *horC* allowing faster bacterial growth than *horA* alone (**12**). Even more recently, it was shown that the *bsrA* gene is implicated in pediococci growth in beer (**11**). Despite this progress, our knowledge of beer-spoilage-related genes and mechanisms remains limited, as many LAB isolates able to grow in beer have none or variable numbers of the five putative beer-spoilage-related genes. This is exemplified in the comparison of Lb464, which possesses *hitA, horA, horB,* and *horC* (unpublished, Bergsveinson et al.), while Pc344NR contains only *bsrA* and *horA* (**20**). In order to definitively correlate the presence of one or more genes with the capacity to spoil beer, the nature of beer-specific genetic expression in each organism must be determined and then compared.

Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) is the current method of choice for sensitive and specific gene transcript detection (**3**). Appropriate use of this methodology allows for expression analysis (difference in abundance or relative expression levels of a specific transcript) between and within experimental samples. For these comparisons to be meaningful and accurate, the RT-qPCR experiment must first be normalized with internal reference genes. These are genes that exhibit stable expression across experimental conditions such that the transcription level of a gene of interest (GOI) under the same conditions can be appropriately normalized (**7, 35**). Reference genes also serve as endogenous controls for the experimental set-up by controlling for potential error in sample preparation, and difference in the quality and quantity of the cDNA template (**16**). Careful selection of reference genes is critical for appropriate data analysis, as using "unstable" reference genes can drastically skew the determined expression level of a GOI (**5, 34 - 36**). In this study, we first established the optimal set of RT-qPCR internal reference genes from a list of twelve candidates for both Lb464 and Pc344NR, and then performed comparative analysis of the expression level of the five putative beer-spoilage-related genes during growth in beer and a rich broth medium.

2.3. Materials and Methods

2.3.1. Isolates and sample collection

Lb464 was obtained from the Brewing Research International culture collection, while Pc344NR was originally characterized in our lab (**8**) and subsequently deposited in ATCC and DSM culture collections. Both isolates were grown in Man, Rogosa, and Sharpe broth (**4** (MRSB; pH 6.5; Lactobacilli MRS Broth, Difco, BD Diagnostic Systems, Franklin Lakes NJ, USA) and lager beer (5% v/v alcohol, pH 4.2 and approximately 11 hops-bitterness units) in order to compare the gene expression induced during growth in each medium. For growth in MRSB, an overnight MRSB culture was used as inoculum and growth at 30˚C was monitored until mid-logarithmic phase was reached (Lb464 OD_{600} = 0.084; Pc344NR OD_{600} = 0.47). For growth in beer, each isolate was necessarily acclimatized to the environment (**13**) by first inoculating 25 µL of an overnight MRSB culture into 12 mL of 85/15 medium (85% beer/15% modified 2x MRSB (no Tween, pH 5.5)). This culture was grown overnight at 30˚C, and then 100 µL were used to inoculate 12 mL of 100% beer ("first" beer). Cultures were grown at 30˚C for 40 h (Lb464) or 5 days (Pc344NR), at which point 40 mL were used to inoculate 1160 mL of fresh beer at 30˚C ("second" and final beer). Culture growth was monitored by plate counts until mid-logarithmic growth was achieved (Lb464 = 18 h; Pc344NR = 76 h). Both isolates were cultured in each medium in triplicate to create the needed biological replicates for RT-qPCR analysis.

2.3.2. RNA extraction and cDNA synthesis

Cells from 35 mL of Pc344NR or 140 mL of Lb464 in MRSB were centrifuged for 3 min at 10,000 x g. For both bacteria, cells from 1200 mL of beer were first centrifuged for 10 min at 4,000 x g. This was followed by cell resuspension in 35 mL of supernatant and recentrifugation for 3 min at 10,000 x g. Resultant cell pellets were flash-frozen in liquid nitrogen, then stored at -80˚C for several days for Pc344NR, but no longer than 24 hr for Lb464 (Lb464 RNA was noticeably degraded if the cells were stored longer than 24 hr). Pellets were thawed in 1 mL of TRIzol® (Invitrogen, Life Technologies, Carlsbad CA, USA), and bead-beaten with use of a vortex for 1 min (0.1 mm glass beads, BioSpec Products, Bartlesville OK, USA), followed by 1 min on ice. This was repeated for six (Lb464) or five (Pc344NR) cycles. Following phase separation, the aqueous phase was purified using the UltraClean Microbial RNA Isolation Kit (MO BIO, Carlsbad CA, USA), with the inclusion of a 15 min on-column DNase digestion (On-

Spin Column DNase I Kit, MO BIO). A secondary in-solution DNase digestion (Turbo DNA-free, Ambion, Life Technologies, Carlsbad CA, USA) was also performed (20 min at 37˚C with 6 U DNase) on the isolated RNA to ensure that all DNA was removed. Isolated RNA was quantified and assessed for purity with use of the NanoVue Plus Spectrophotometer (GE Healthcare Life Sciences, Baie d'Urfe QC, Canada) and was visualized on agarose gel and evaluated for degradation. Samples with $A_{260/280}$ values ranging from 1.9-2.1 and $A_{260/230}$ values ranging from 1.7-2.1 were taken as acceptable.

RNA was reverse-transcribed into cDNA in a total reaction volume of 20 µL, using 1 µL of GoScript™ Reverse Transcriptase (Promega, Madison WI, USA), 500 ng of RT Primer Mix (Qiagen, Toronto ON, Canada), GoScript™ 5x Reaction Buffer, MgCl2 (1.5 mM) and dNTP's (0.5 mM). As well, no-reverse transcription (noRT) controls were prepared using the same reaction mixture, but with water replacing the reverse transcriptase enzyme. Reactions were carried out in the 2720 Thermal Cycler (Applied Biosystems, Life Technologies, Carlsbad CA, USA) with the following program: 25˚C for 5 min, 42˚C for 60 min, and 70˚C for 15 min. Completed reactions were then stored at -20˚C.

2.3.3. DNA isolation

DNA was isolated from Lb464 and Pc344NR to facilitate the determination of PCR primer efficiency of each gene under study through use of DNA dilution curve analysis. Overnight cultures in MRSB were used to isolate DNA via the UltraClean™ Microbial DNA Isolation Kit (MO BIO, Carlsbad CA, USA) according to the manufacturer's instructions. Prior to bead-beating, heating of the samples at 70˚C for 10 min was performed to increase DNA yield.

2.3.4. Primer design

Twelve candidate reference genes were assayed for expression stability during growth of Lb464 and Pc344NR in beer and MRSB (Table 2.1.). PCR primers were based on our unpublished Lb464 genome sequence, and the published Pc344NR genome sequence (**20**). Primers were designed with the Integrated DNA Technologies Oligo Analyzer 3.1 program

Table 2.1. Description of candidate reference and putative beer-spoilage-related genes

Gene [a]	Product	Pathway and/or Function
16S rRNA	16S ribosomal RNA	Translation
fabD	Malonyl CoA-acyl carrier protein transacylase	Fatty acid biosynthesis
fusA	Translation elongation factor G	Translation
gmk	Guanylate kinase	Nucleotide synthesis
gyrA	DNA gyrase, A subunit	DNA replication
ileS	Isoleucyl-tRNA-synthetase	Translation
ldhA	D-lactate dehydrogenase	Sugar catabolism
pcrA	ATP-dependent DNA helicase	DNA repair and plasmid replication
pfkA	6-phosphofructokinase	Glycolsis/gluconeogenesis
proC	Pyrroline-5-carboxylate reductase	Amino acid biosynthesis
recA	Recombinase	DNA repair and maintenance
rpoB	RNA polymerase	Transcription
blpA	Putative ABC transporter, ATPase component	Putatively hops resistance
bsrA	Putative ABC transporter, ATPase component	Putatively hops resistance
hitA	Putative divalent cation transporter	Putatively hops resistance
horA	ABC-type multidrug transporter	Putatively hops resistance
horB	Transcriptional regulator	Putatively hops resistance
horC	PMF-dependent multidrug transporter	Putatively hops resistance

[a] Candidate reference genes are listed above the dotted line whereas genes of interest are listed below.

(http://idtdna.com/analyzer/Applications/OligoAnalyzer). There were to be no hairpin structures, self primer-dimers, or heterodimer formation, and amplicons were desired to be 100-250 bp in length. All primers were designed to work at 55˚C, with Tm calculated using the following parameters: oligonucleotide primer concentration of 0.3 µM; Na+ concentration of 0 mM; and Mg++ concentration of 2.5 mM. Lastly, primers were considered acceptable if they did not bind non-specifically elsewhere in the genome (as assessed via BLAST). The primer sets for all genes are given in Tables 2.2. and 2.3. for Lb464 and Pc344NR, respectively (Integrated DNA Technologies, Coralville IA, USA).

2.3.5. qPCR

Each qPCR reaction was performed in triplicate using SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules CA, USA) with a total reaction mixture of 15 µL containing 2 µL of template and 0.3 µM of each primer. Samples were then processed according to the following thermal cycling program using the MiniOpticon™ Real-Time PCR system (Bio-Rad, Hercules CA, USA): 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 55˚C for 10 sec, with fluorescence data collection at 55˚C. Melt curve analysis of the PCR amplicons began at 65˚C, increasing in increments of 0.5˚C/5 sec until a final temperature of 95˚C was reached. Execution of the PCR program and data collection was facilitated by Bio-Rad CFX Manager™ software, version 2.1. Quantification cycle (Cq) values were determined using Regression Mode. A replicate within a triplicate set was excluded from analysis if it had a Cq value that directly contributed to a Cq standard deviation greater than 0.02 for the technical replicates.

PCR efficiency (E) for each primer set in each organism (Tables 2.2. and 2.3.) was calculated via the standard curve method. DNA was used instead of cDNA for efficiency testing so that the cDNA made from the low levels of RNA extracted from beer samples could be used exclusively for expression analysis. Ten-fold dilutions of DNA (5.0 ng/µL to 0.5 pg/µL) from each isolate were made and 2 µL of each of the five standards was used as template. The Cq for each DNA standard was plotted against the log of the DNA concentration, and PCR efficiency was calculated by using the slope of the resulting linear regression plot according to Formula 1:

$$E = 10^{(-1/\text{slope})} \qquad (1)$$

Table 2.2. qPCR primers for *L. brevis* BSO 464

Gene	Fwd-primer	Rev-primer	Amplicon size (bp)	Amplification Efficiency (% ± S.D.)
16S rRNA	ACAATGAAGCGAGTGGCG	GTCTCAGTCCCAATGTGGC	248	102.4 ± 7.4
fabD	CAAGACTTATATCGCCAGGAGC	CTCGCAATTAAGCCGCTG	226	99.9 ± 3.0
fusA	CGACGACTGAGCGTATCCT	CCTTCCATTCAGCCGTAGTG	146	102.7 ± 4.1
gmk	GCTCATTGTGCTTTCTGGTC	GTTGTCAACATACTTGGCATACTC	222	100.9 ± 5.3
gyrA	GCGATGAGTGTCATTGTGC	GTAGCGATACGAGAAGTCTTGC	227	101.0 ± 3.2
ileS	GTCCGCTACAAGTCAATGAGTG	CTGGCTTCAGGGTCAGATATG	240	99.6 ± 4.9
ldhA	GAACTCCTGACTCACGACAAC	CACAGCGTATGGCGAGTAAC	221	98.4 ± 4.3
pcrA	CATAACAATGTGATGCCGTGG	GTTGAGACCCAGACATCATTACC	121	102.9 ± 3.6
proC	GCGATGTTGCCGATCAAAG	GAAATCACTGTGGTCTGCCC	114	102.2 ± 3.4
recA	GCACTTGACGTTGCCTTG	CAGTATCTGGCTGCGACAG	228	101.9 ± 4.8
rpoB	GCCACTGTGATCCCTAACC	CTTCAAGGACTCTTCGACACG	239	101.4 ± 3.8
blpA	TCTTACTCAATGGGCGTTCC	GGACACCAACACGTTATCCA	137	108.3 ± 13.2
hitA	ATAATGTTCGCCAGAAGCCG	CTCACCCAATCAACTGACCT	180	109.0 ± 2.4
horA	AGTTCCTTGACCTGTGTGGA	CTGCGAACAACACTTTGGGA	116	109.2 ± 5.5
horB	TCCTCCAGCATACTTACAAGATT	GCATTACATCCATATTGGCACC	153	108.0 ± 19.2
horC	TACTCGTAGTCTGGCTCCTA	GCTAAATCGCATTGGAACCC	107	102.2 ± 5.4

Table 2.3. qPCR primers for *P. claussenii* ATCC BAA-344^T

Gene	Fwd-primer	Rev-primer	NCBI Gene ID	Amplicon size (bp)	Amplification Efficiency (% ± S.D.)
16S rRNA [a]	GTGGCGAACGGGTGAGTAAC	CATCCAGAAGTGATAGCCGAAACC	11648068	137	96.4 ± 4.0
fabD	GACTGAAGGACGTTGCTCAC	CGATCTTTAATCAGGCGTAACC	11648114	105	106.4 ± 16.1
fusA	AACACATGATGGTGCTTCAC	TGATGCTTGACGCCAAACAG	11647464	246	93.9 ± 3.2
gmk	AATGGCGAGGTTAATGGTG	CACATACTGTAGCGGTGTCC	11647215	131	99.7 ± 5.5
gyrA	GGTACGAATGGCACAGGAC	ACTGGTTCACGTTCTGAGC	11647489	204	99.2 ± 4.9
ileS	GGTCATGGTCTTCAGAATCAG	GGTTGAACAACGGCATAGTC	11646975	213	103.6 ± 2.1
ldhA	CTGGATTCTGAGACGCTGG	CATTAGGTGAATATGCTGGGAC	11647154	211	100.4 ± 3.9
pcrA	ATGAGAAGATTGTTGCTGAGG	ACATCGTTACTAATTGGTATTGAGC	11647423	209	111.2 ± 12.1
pfkA	CTTAGTTGCTGGTGACATCC	TGATATGAACCATCGCCACC	11647054	191	104.9 ± 5.4
proC	TTAGTGTCGCTTGTCTCAGG	GAGAACTTCCTGCAAGAGCTG	11648312	252	95.3 ± 5.8
recA	GATCATTGGCACTTGATGAGG	CTCAGCAACAGCATGTAGTG	11646877	118	106.6 ± 19.2
rpoB	GCTTCGTGAGATGTTCAACG	TCGCCAGTTTCGTGGTTGG	11647469	182	97.4 ± 2.8
bsrA	GGAGGACTGGACCATCAG	CTCTCTTCGGTAGCCATCC	11647751	95	98.9 ± 15.4
horA	GGATCATCAACTCAATCGGTC	CCAAAGTGTTGTTCGCAGC	11946351	155	94.1 ± 4.4

[a] This primer pair produced non-specific amplicons

Primers were considered satisfactory if the efficiency was between 90-110%. PCR amplicons for each gene were also visualized on agarose gel to confirm there was no non-specific amplification, nor formation of heterodimer or self-primer dimers.

For gene expression analysis, each cDNA stock was diluted to ~2-3 ng/µL, and 2 µL were used as qPCR template. Each noRT control was diluted by the same factor as the corresponding cDNA sample and included in the first qPCR run to verify that no residual DNA remained. We used the sample maximization experimental setup for each run (**15**), and each biological replicatewas analyzed in triplicate (qPCR technical replicates). Inter-run calibrators were included for Lb464 runs that involved the five genes of interest so that comparisons among gene expression in the same sample could be done. This was not necessary for Pc344NR, as there were only two GOI, and both could be analyzed in the same run.

2.3.6. Reference gene selection and GOI differential expression analysis

All putative reference genes were analyzed for their expression stability during growth in MRSB and beer for both isolates. The method described by Hellemans et al. (2007) was used to calculate the stability measure M of each reference gene. Genes were then ranked according to their stability measures, and those showing the lowest values were chosen as reference genes. The number of reference genes needed for accurate normalization was determined by calculating the pairwise variation between normalization factors (**35**). Specifically, if the addition of another reference gene did not significantly contribute to the normalization factor (cut-off for pairwise variation between normalization factors was 0.15), the number of genes included before the addition was used as the number of required genes for accurate normalization.

Once the appropriate reference genes were determined, the cDNA samples were analyzed for differential expression of the GOI. The methodology and calculations for relative expression analysis were all based on Hellemans et al. (2007), with the mean quantification cycle being used as a reference Cq for error minimization. Because both GOI for Pc344NR were analyzed in the same qPCR run, normalized relative quantities (NRQ) were determined and used to calculate fold change in expression. However, as several GOI were analyzed in different qPCR runs for

Lb464, inter-run calibration was performed to determine calibrated NRQ values (CNRQ) and the corresponding fold change in expression for genes in this isolate.

2.4. Results

2.4.1. RNA isolation

Lb464 and Pc344NR have notably different growth dynamics. Lb464 is capable of reaching its mid-logarithmic growth in beer within 18 hr, whereas Pc344NR requires roughly 3 days to achieve the same growth stage. This observation indirectly supports the hypothesis that these two organisms are relying on distinct mechanisms to establish growth in a beer environment and/or that these mechanisms differ in their efficacy. It should also be noted that both organisms grow in beer at a slower rate and attain a lower final cell concentration than they do in MRSB. As such, for both isolates, there were substantially fewer colony-forming units (CFU) per mL at mid-logarithmic growth in beer (Lb464 = $3.2x10^6$ CFU/mL; Pc344NR = $1.5x10^6$ CFU/mL) than were present at the same growth stage in MRSB (Lb464 = $2.8x10^7$ CFU/mL; Pc344NR = $1.1x10^8$ CFU/mL). This necessitated the preparation of a much larger volume of beer samples (relative to MRSB) for extraction of RNA to ensure that sufficient cells were being processed.

Several methods were tested for RNA extraction before a reliably efficient protocol was found. Various kits were assayed, however, none were capable of efficient cell breakage, particularly for bacteria grown in beer. As such, a combined method of TRIzol and bead-beating was adapted to work with a column-based purification and clean-up step. MO BIO UltraClean Microbial RNA Isolation Kit columns were chosen as they capture total RNA (i.e., no size exclusion), and two DNase digestion steps were included as it was found that just on-column or just in-solution DNase digestion did not remove sufficient amounts of residual DNA (as determined via noRT controls).

Despite altering various parameters of the TRIzol/column-based extraction protocol, the RNA yield from both organisms when grown in beer was always significantly lower than when grown in MRSB. This effect may be the result of having fewer cells at mid-logarithmic growth in beer (compared to MRSB), however, attempts to compensate for this disparity (by harvesting greater

volumes of beer and adjusting the volume of TRIzol) did little to help increase the overall yield of RNA from either bacterium. This may reflect that growth in beer results in cells better able to resist the efforts of extraction protocols, or that elements of beer are inhibitory to the developed RNA extraction method. It has also previously been reported that bacteria cultured with hops are smaller than when grown without hops present (**24, 26**), a phenomenon that may contribute to the difficulty in extracting RNA from cells grown in beer. In any event, the extraction protocol was optimized to the extent that useable amounts of RNA were consistently achieved for each organism when grown in beer (Lb464 = 400-750 ng/50 µL; Pc344NR = 600-1,000 ng/50 µL).

Of further significance was the observation that prior to any optimization of the extraction protocol, less RNA was extracted from Lb464 grown in either medium and the purity values were lower than for Pc344NR grown in comparable conditions. As the extraction procedure produced consistent quality RNA for Pc344NR, and the starting number of cells was roughly equal, it appears that some intrinsic property of Lb464 inhibits efficient RNA extraction. Thus, steps were taken to optimize the initial breaking open of Lb464 cells (trials with altered volumes of TRIzol, and extended bead-beating cycle), such that the RNA recovery from Lb464 in MRSB was increased to a range just slightly lower than for Pc344NR (Lb464 = 7.5-12.5 µg/50 µL; Pc344NR = 11.5-20 µg/50 µL).

Another possible explanation for the disproportionate RNA yield and quality between the two bacteria (when grown in either media) is that Lb464 RNA is more prone to degradation throughout the extraction protocol than is Pc344NR RNA. This hypothesis was supported by the observation that storage of Lb464 pellets (originally flash frozen in liquid nitrogen) at -80°C for longer than 24 hr dramatically diminished the overall quality of extracted RNA, as assessed by both reported $A2_{60/230}$ and $A_{260/280}$ values, and visualization of the RNA after electrophoresis in agarose gels. This RNA instability at -80°C conditions was not observed for Pc344NR, as cell pellets could be stored frozen for several days without noticeable affect on RNA quality.

Ultimately, the developed extraction protocols for both Lb464 and Pc344NR allowed for the reproducible collection of RNA of reasonable concentration and quality. The subsequent synthesis and assessment of cDNA and noRT controls (via RT-qPCR) from these RNA samples

provided us with further confidence that our DNA removal steps were effective and that the cDNA (and thus original isolated RNA) samples were of good quality.

2.4.2 Primer PCR efficiencies and reference gene selection

PCR efficiencies of primers for all candidate reference and putative beer-spoilage-related genes were found to be in the acceptable range of 90-110% (Tables 2.2. and 2.3.). Each standard curve showed a linear regression coefficient of determination (R2) between 0.993 and 1.0. Agarose gel electrophoresis of qPCR amplicons ensured that the correct sized products had been amplified for all primer pairs, except for the Pc344NR 16S rRNA gene primers which gave non-specific amplification, leading to this gene being dropped from the study for this isolate.

The twelve candidate reference genes analyzed for expression stability participate in a wide variety of metabolic and physiological pathways (Table 2.1.). As beer and MRSB are very different growth environments, we included genes representing a broad range of functions so as to increase the likelihood that some of the genes would be stably expressed in both media. We also ensured that genes were associated with different cellular pathways to avoid biasing the stability measures. Included were genes that participate in essential functions for the survival and fitness of the organism during mid-logarithmic growth, such as nucleotide and amino acid synthesis, transcription, translation, and DNA replication. In addition, we included genes involved in fatty acid synthesis, DNA repair and sugar catabolism despite knowing that the expression of these genes may vary substantially depending on the environment. Inclusion of these genes was done firstly to provide a wide range of potential stability measures, and secondly because some of these genes (e.g., *ldhA*) have been found to be good candidates for reference gene normalization in stressful environments (**6, 9**). It is important to note that the *pfkA* gene encoding 6-phosphofructokinase, which participates in glycolysis and gluconeogenesis, is only present in Pc344NR. Lb464 lacks this gene as it is a heterofermentative bacterium that utilizes hexoses by the 6-phosphogluconate pathway, characteristically producing lactic acid, carbon dioxide, ethanol and/or acetic acid as end-products (**18**). The expression stability of the *pfkA* gene in Pc344NR was assayed, as it was believed to contribute to the overall robustness of the candidate reference gene list, however, primers specific for genes belonging to the 6-phosphogluconate pathway in Lb464 were not designed.

Pairwise variations of the normalization factors for the candidate genes indicated that only two reference genes were needed for normalization in both isolates (cut-off was 0.15). Based on the stability measure for each candidate gene, it was determined that *proC* and *rpoB*, and that *gyrA* and *ldhA* were ideal for normalization of Lb464 and Pc344NR GOI- expression data, respectively (Fig. 2.1.). It should be noted that 16S rRNA was among the most stably expressed genes in Lb464, but it was not used as a reference gene (see 2.5. Discussion). To ensure that results were not biased by genes that participate in the same pathway (i.e., co-regulated unstable genes that erroneously appear stable), individual genes were removed from the analysis and stability values were recalculated in a number of variations. No major differences were found regardless of the genes included for analysis, with the least and most stable genes consistently showing the same order and relative stability (i.e., only difference noted was the order of genes ranking in the middle). This provided further confidence that the stability measures were not being biased and that the best reference genes out of the analyzed set were chosen for normalization of our GOI expression data.

2.4.3. Target gene analysis

Following selection of appropriate reference genes for each bacterium, all cDNA samples were analyzed for the abundance of putative beer-spoilage-related gene transcripts. As noted previously, Lb464 contains many of the genes purported to play a role in hops resistance such as *hitA, horA, horB* and *horC*. In contrast, Pc344NR only has a copy of *bsrA* and *horA*, making *horA* the only gene common to both bacteria. Lb464 does not contain a copy of *bsrA*, however, BLAST analysis of the unpublished Lb464 genome using the Pc344NR *bsrA* protein sequence identified a protein with 60% homology. The gene that codes for this protein was termed *blpA* (bsrA-like-protein-A) and included for comparative analysis.

Subsequent normalization of the RT-qPCR data revealed that Lb464 and Pc344NR do not express horA in a similar fashion during mid-logarithmic growth in beer (Table 2.4. and Fig. 2.2.). While there is a negligible difference in the expression of *horA* in Lb464 when grown in MRSB as compared to beer, there is significant beer-specific induction of *horA* in Pc344NR.

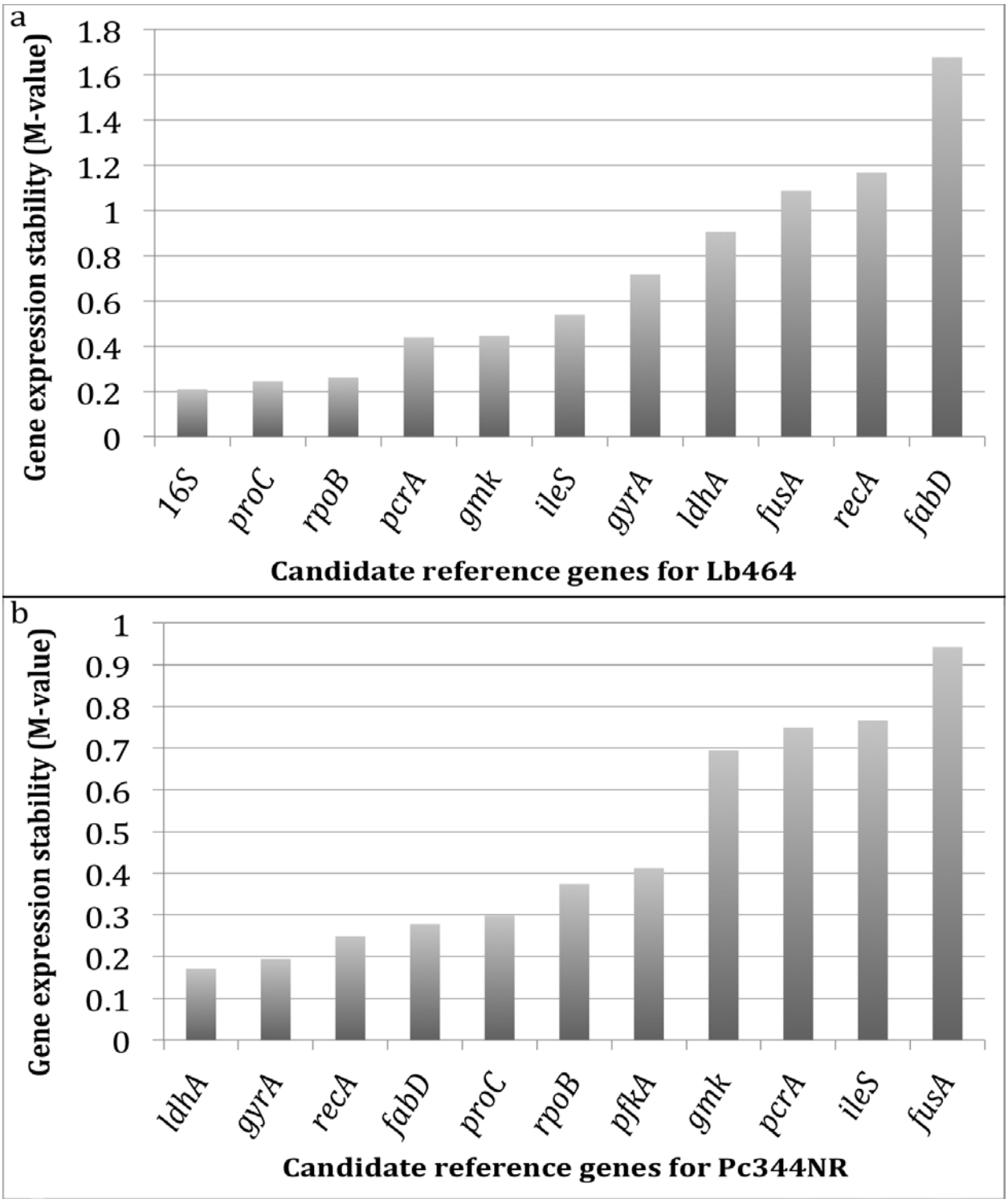

Fig. 2.1. Stability expression measure (M-values) for reference genes in Lb464 (a) and Pc344NR (b) grown in beer and MRSB.

Most stable to least stable expression is from left to right in each panel.

Table 2.4. Transcriptional analysis of reference and putative beer-spoilage-related genes for *L. brevis* BSO 464 and *P. claussenii* ATCC BAA-344^T

gene	Fold Change [a]	
	Lb464	Pc344NR
16S	1.06 ± 0.17	- [b]
fabD	4.83 ± 0.61	1.39 ± 0.16
fusA	0.33 ± 0.06	0.26 ± 0.02
gmk	1.50 ± 0.27	0.47 ± 0.05
gyrA	0.65 ± 0.09	Reference [c]
ileS	0.99 ± 0.33	0.39 ± 0.05
ldhA	2.39 ± 0.33	Reference
pcrA	1.59 ± 0.20	0.36 ± 0.06
pfkA	Not present [d]	0.74 ± 0.07
proC	Reference	1.64 ± 0.14
recA	0.26 ± 0.04	1.43 ± 0.17
rpoB	Reference	0.87 ± 0.11
blpA	0.99 ± 0.23	Not present
bsrA	Not present	1.44 ± 0.16
hitA	1.10 ± 0.20	Not present
horA	1.08 ± 0.26	3.57 ± 0.39
horB	1.08 ± 0.28	Not present
horC	109.8 ± 27.0	Not present

[a] Change in expression in beer relative to in MRSB ± standard deviation. Values less than 1 indicate decreased expression in beer.

[b] Not included in Pc344NR as primers were giving non-specific amplification.

[c] Used as a reference gene for normalization.

[d] Gene is not present in this isolate.

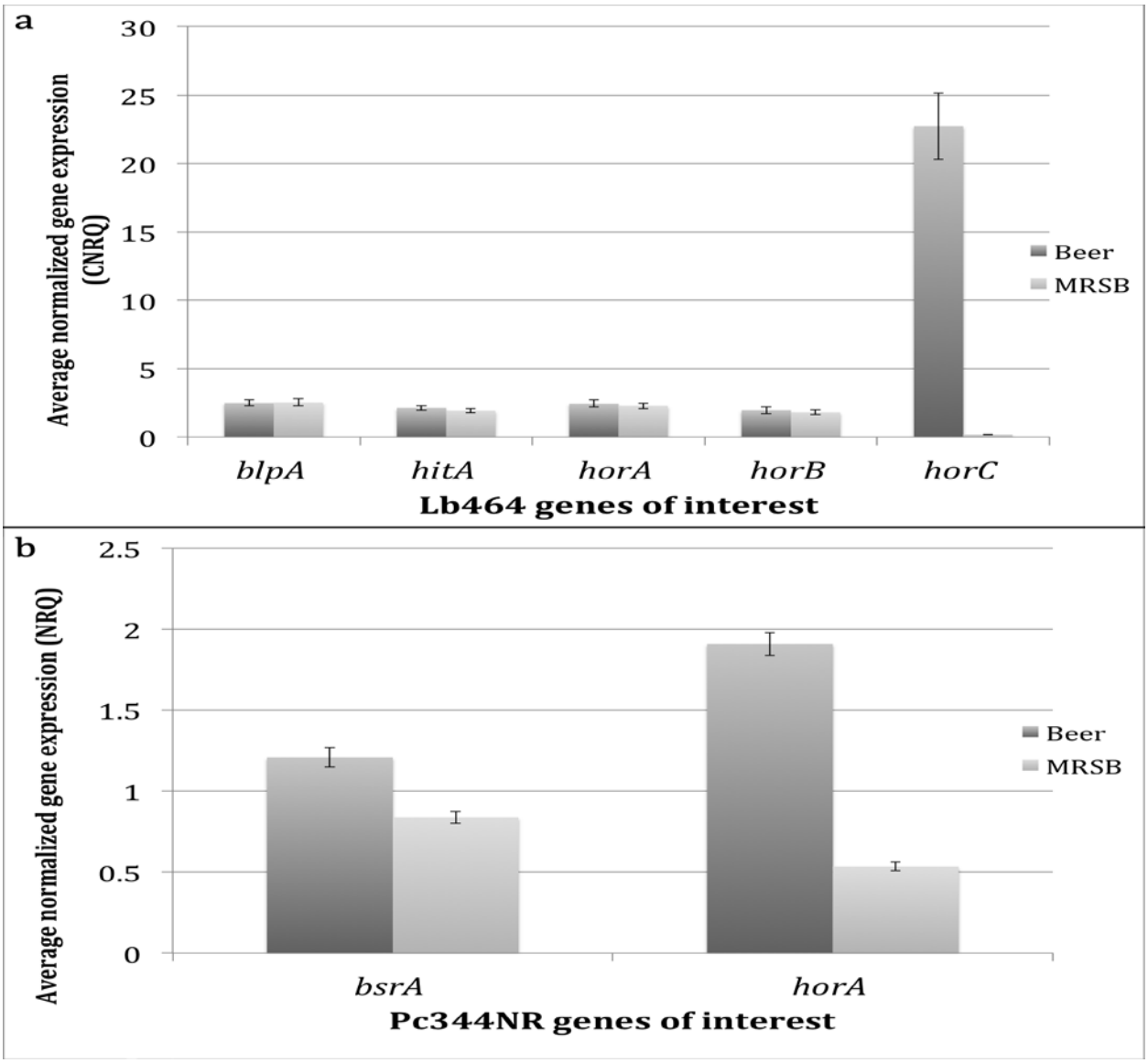

Fig. 2.2. Relative expression levels of putative beer-spoilage-related genes in beer and MRSB during mid-logarithmic growth of Lb464 (a) and Pc344NR (b).

The error bars indicate standard error from three independent experiments. Note the different scales for each plot: plot a shows expression values normalized according to the reference gene normalization factor and inter-run calibrators (CNRQ) so that inter-gene comparisons can be made; plot b is only normalized according to the reference gene normalization factor (NRQ) as these genes were analyzed in the same qPCR run.

The gene with the most notable beer-specific increase in expression is that of *horC* in Lb464 (Table 2.4. and Fig. 2.2.), whereas the other genes assayed show minimal change in expression.

2.5. Discussion

The extent to which beer-spoilage-related genes are expressed during an organism's successful growth in beer has yet to be adequately explored. Despite reports that correlate the presence of *horA, horC, hitA* or *bsrA* with ability to grow in beer, specific transcription of all these genes during growth in beer has not been verified. Consequently, we applied RT-qPCR to determine the expression of these genes in two unique beer-spoiling bacteria. The use of RT-qPCR necessitates the establishment of appropriate reference genes (genes that exhibit stable expression throughout the chosen experimental growth conditions), which are used to normalize expression levels of transcripts for GOI. Not only does this procedure ensure a sufficiently controlled RT-qPCR experiment (e.g., compensation for effects of possible variation in RNA integrity or starting amount of template), it also allows insight into the biological significance of the candidate reference genes and physiology of the bacteria in the growth conditions being studied.

At mid-logarithmic growth in beer and in a non-beer medium, the most stably expressed genes of Lb464 (Fig. 2.1.a) were 16S rRNA (ribosomal RNA), *proC* (amino acid biosynthesis), and *rpoB* (RNA polymerase). Although the 16S rRNA gene was included during Lb464 analysis for comparison against other candidate reference genes, it is known that the 16S rRNA gene does not make a satisfactory reference gene for RT-qPCR analysis of messenger RNA. This is partly due to the high abundance of rRNA in total RNA, which leads to much lower Cq values than for a GOI, making selection of an ideal cDNA dilution for both reference and GOI difficult. Furthermore, it has been shown that rRNA levels tend to correlate with the total amount of RNA, and not necessarily mRNA levels (**19, 35**). Therefore, despite the relative stability of 16S rRNA for Lb464, it was not used for normalizing Lb464 gene expression data. Instead, the stably expressed *proC* and *rpoB* genes were chosen, as it was determined that only two reference genes were needed for normalization of Lb464 GOI expression data.

Interestingly, as demonstrated in Fig. 2.1., there is a dramatic difference among the most stable and least stable candidate reference genes for Lb464 and Pc344NR, suggesting that these beer-spoiling isolates are directing their cellular energy towards different metabolic activities during similar stages of growth. For Pc344NR, it was also found that two reference genes were needed for data normalization; however, unlike Lb464, it was determined that *gyrA* and *ldhA* were the best candidates for normalization of Pc344NR GOI expression data. Furthermore, the stability of these two genes is not at all comparable to what is found in Lb464, further emphasizing the importance of identifying proper reference genes that are specific to the isolate and conditions being analyzed for relative gene expression.

Once a suitable reference gene set was determined for each bacterium, we examined the relative expression levels of five putative beer-spoilage-related genes. Lb464 contains *hitA, horA, horB,* and *horC,* and also contains a protein homologous to *BsrA*, with the encoding gene designated *blpA*. In contrast, Pc344NR contains only *horA* and *bsrA*. Thus, these two bacteria are interesting as they share only one putative beer-spoilage-related gene and exhibit distinct growth rates in beer. Differential expression analysis indicates that Lb464 and Pc344NR have a markedly different profile of beer-spoilage-related gene expression, specifically with respect to *horA* (Table 2.4.). Beer-specific transcription of *horA* in Pc344NR appears to be significantly increased (Fig. 2.2.b), providing further support that for Pc344NR, *horA* is indeed involved in establishing growth in beer (i.e., presumably counteracting the inhibitory effects of hops). The role of *bsrA*, however, is less clear, as it is only marginally induced during growth in beer. Considering this gene has been found mainly in *P. claussenii* and a few select pediococci isolates (**11**), it may not play as large of a role in beer-spoilage as originally anticipated. Comparatively, the increase of *horA* transcription during Lb464 growth in beer is insignificant in comparison to the expression during growth in MRSB (Fig. 2.2.a). This suggests that *horA* is constitutively expressed in Lb464 at some basal level throughout growth in either medium, leading to the conclusion that either HorA activity is not entirely beer-specific, or that induction of *horA* does not occur during mid-logarithmic growth in beer. The same role (or lack thereof) can be proposed for *blpA*, and *horB*, which also show no difference in expression for Lb464 during growth in beer compared to growth in MRSB.

Interestingly, the huge increase in *horC* expression strongly suggests that HorC plays a major role during Lb464 growth in beer. Based on previous studies indicating that HorC is involved in hops resistance (**17, 30**), the efficacy of the HorC-based mechanism of overcoming hops stress is questioned. It could be that *horC* expression compensates for all other hops-resistance mechanisms within Lb464 and thus cellular energy is directed to transcribing *horC* preferentially. Alternatively, it may be that the HorC mechanism is inefficient and therefore *horC* requires strong induction in beer relative to other putative hops-resistance-associated genes present. However, we believe that the dramatic beer-specific increase of *horC* expression and the rapid Lb464 growth rate in beer together strongly indicate *horC* is the principal beer-spoilage-related gene enabling growth in this environment (presumably via hops resistance) for Lb464 (and potentially for other beer-spoilage *L. brevis* isolates as well). This hypothesis is supported by the results of several studies involving *horC* and hops resistance or beer spoilage by *L. brevis*, such as those by Iijima et al. (2006) where a *horA- horC-* variant was transformed with a plasmid containing *horC*. This led to an increase in hops resistance and the ability to grow in beer, both characteristics not present in the isolate transformed with the same plasmid lacking *horC*. In addition, it has been shown by Northern blot analysis that expression of *horC* is increased in another *L. brevis* isolate when grown in the presence of hop extract (**10**). Lastly, reports by Preissler et al. (2010) demonstrate the importance of *horC* in defining beer-spoiling *L. brevis* isolates, as it was shown that isolates containing the *horC* gene had higher iso-α-acid MIC values, in addition to the ability to spoil a range of beer styles (particularly those with high bitterness units).

It is also possible, however, that basal or constitutive expression of *hitA* and *horA* in Lb464 assists in overcoming the stresses in beer (i.e., there is a synergistic effect of transcription of several beer-spoilage-related genes), which is also implied by results from previous studies (**2, 33**). If this is the case, then the combined efficiency of all these mechanisms may be what enables the rapid growth of Lb464 in beer relative to that observed for Pc344NR. Nevertheless, considering the magnitude of *horC* beer-specific expression relative to the induction of the other genes present, it remains likely that Lb464 has preference for the HorC mechanism of action when growing in beer; i.e., to warrant expending the energy required to transcribe *horC* at such high levels. It is unclear, however, how *horC* gene expression is being regulated, as the drastic

increase of its expression when compared to the minimal change in *horB* transcription raises the question of whether HorB is actually regulating *horC* transcription? In this regard, it should be noted that Fuiji et al. (2005) also found that *horB* transcription is not concurrently increased with *horC* transcription under high-hop conditions. Additional investigation therefore is needed, particularly in other LAB, to delineate the role of HorB, if any, in regulating *horC* gene expression.

Considering the striking differences in the beer-specific transcript profile of Lb464 and Pc344NR, it is important to emphasize the expression stability profile of the candidate reference genes, and the necessity of choosing the correct reference gene(s) for transcriptional normalization. When considering the inherent diversity in the metabolic regulation of these organisms, and their genetic variability with regard to beer-spoilage-related genes, it is logical to conclude that their respective mechanisms for overcoming stresses in beer are quite different. This points to further investigation being needed into the general regulation of hops-resistance mechanisms, including determining whether the same pattern of *horC* transcription is consistent throughout other beer-spoiling bacteria carrying this gene. Additionally, the correlation between expression (not only possession) of *horA* and *horC*, incidence of faster growth rate, and increased hops tolerance must be assessed in other LAB. Through careful application of RT-qPCR methodology in pursuing answers to these and related questions, it should be possible to further our understanding of bacterial hops-resistance mechanisms and of beer-spoilage-related genetics.

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Chapter 3: Genetic variability in the hop-tolerance gene *horC* of beer-spoiling lactic acid bacteria

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3. INTERFACE

This chapter has been submitted for review and subsequent publication. This work investigates the prevalence of truncations and mutations in hop-tolerance genes *horB* and *horC* in all *horC*$^{+}$ isolates within our culture collection and presents *in silico* protein function predictions.

Jordyn Bergsveinson was involved with experimental design and validation, designed all primer sequences, performed bioinformatics analysis of protein sequence, function, and phylogeny, and authored the manuscript.

Scott Goerzen is attributed with performing initial screening and gene sequencing of all isolates, and preliminary bioinformatic analysis of nucleotide and protein sequences. He also assisted in performing growth analysis of isolates prior to droplet digital PCR (ddPCR) analysis.

Anna Redekop optimized the ddPCR procedure and helped to prepare materials for ddPCR anlaysis.

Sheree Zoerb helped to prepare materials for and perform ddPCR anlaysis.

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work**.**

3.1 ABSTRACT

horC encodes a membrane transporter predicted to use the cellular proton motive force to export hops from the cell, leading to *horC* being frequently targeted in rapid screening for lactic acid bacteria (LAB) beer-spoilage ability. Recently, a 27-bp *horC* deletion was discovered in a LAB unable to spoil beer. Since LAB vary greatly in beer-spoilage virulence and in presence of hop-tolerance genes, prevalence of this *horC* sequence gap was determined for 27 *horC*$^{+}$ isolates across two LAB genera and seven species to assess how conserved *horC* is and if gene mutations possibly affect HorC function. With a multiplex PCR, 19 (70%) of the isolates were found to contain the 27-bp *horC* gap. Full-length *horC* sequencing was successful for 23 isolates, revealing that eight of the *horC*-gap isolates also contained a 3-bp gap yielding a cysteine excision at residue 297, plus 14 conserved amino acid substitutions, effectively dividing *horC* into three orthologous groups: *horC*-full, *horC*-gap and *horC*-CE297. Conserved *Pediococcus*-specific *horC* point mutations were also found. Four isolates had *horC* genes that could not be sequenced, indicating the presence of *horC* paralogs indistinguishable by the multiplex PCR. The sequence changes detected in the three horC orthologs resulted in different predicted HorC substrate binding affinities. Interestingly, the sequence of *horB*, the putative *horC* transcriptional regulator, was conserved among all 27 isolates, indicating *horB* is essential for *horC* function regardless of *horC* mutations. Droplet digital PCR quantification of *horC* relative to *horB* expression following bacterial growth in medium containing hops revealed that the *horC* ortholog group does not affect *horC* transcription in response to hops and that *horC* transcriptional levels were not proportional to the hops level present. These findings suggest redundancy in LAB hop-tolerance mechanisms and highlight the isolate-specific nature of hop-tolerance. This is also the first evidence for *horB* being a *horC* repressor in the absence of hops. Most importantly, the data show that the conserved nature of putative hop tolerance genes has been over-estimated, and that PCR targeting of short *horC* sequences to indicate beer-spoilage potential is problematical. Increased analysis of how plasmid genes are transferred in breweries and investigations into the role of other plasmid-based genes are both needed to increase robustness of beer-spoilage assessment methods, as the data presented question the dogma that hop-tolerance genes suffice as markers of LAB beer-spoilage ability.

3.2 INTRODUCTION

The central hypothesis that has governed research into beer-spoilage-related lactic acid bacteria (BSR LAB) is that genes conferring hop-tolerance are reliable indicators of overall beer-spoilage ability. This theory has become accepted over time based on a number of general findings and suppositions; namely, (1) hop-stress is an important component of the overall beer-environment (**31, 35**), (2) hop-tolerance genes are non-species specific; i.e., universal (**18, 35 – 36, 38**), and (3) hop-tolerance genes are well-conserved in all isolates and transferred horizontally on plasmids, suggesting they are critical for the hop-tolerance (and beer-spoilage) phenotype (**13**, **19, 31, 37**). However, the premises that these genes are conserved and thus suitable as universal markers of beer-spoilage have not been confirmed. In short, the physiological role and importance of hop-tolerance genes during LAB growth in beer needs to be better defined.

Two recent findings suggest that the conservation of hop-tolerance genes *horB* and *horC* specifically must be re-examined. These genes were originally identified on the 23.4 Kb plasmid pRH45II carried by *L. brevis* ABBC45^{cc}, and on the basis of BLASTP analysis, *horC* was determined to function as a multidrug transporter belonging to the resistance-nodulation-cell division (RND) superfamily, with *horB* being proposed as a transcriptional regulator of *horC* expression (**36, 38**). Recent transcriptional analysis of these genes, however, indicates that while *horC* is expressed during *Lactobacillus brevis* BSO 464 growth in beer, the activity of *horB* is negligible, confounding its putative role as a transcriptional regulator (**3, 36**). Secondly, a *horC* sequence anomaly was recently discovered in a LAB isolate recovered from bottled beer recovered from a ~170 year old shipwreck off the coast of the Åland Islands, Finland. The bacterium involved, *Pediococcus damnosus* VTT-E16, has no demonstrable hop-tolerance and cannot establish growth in modern lager or wheat beer, at any temperature (I. Kajala and B. Bergsveinson; unpublished results). Genome sequencing[1] of the bacterium revealed that the *horC* gene contained a 27-bp gap relative to the original described *horC* sequence from pRH45II, with this nucleotide sequence gap resulting in the omission of nine amino acids from the

[1] Genome sequencing of *P. damnosus* VTT-E16 was performed as part of a research agreement with VTT Technical Research Centre in Finland. Although the sequencing results are not presented here, a brief description of sequencing methodology is provided.

resultant polypeptide. This gave reason to examine just how pervasive are sequence disruptions in both *horC* and its putative transcriptional regulator *horB.*

Determining the degree of conservation of hop-tolerance genes and their overall utility as universal markers is of great importance to the brewing industry as these genes are frequently tested for via PCR or multiplex PCR assays (**13 – 14, 32**), on the assumption of high sequence conservation and thus low false-negative and -positive outcomes. All previously annotated *horC*$^{+}$ isolates within our collection were screened for the 27-bp truncation discovered in *P. damnosus* VTT-E16, and had their full-length *horC* gene sequenced. Various bioinformatics approaches were used to confirm the function and predicted substrate binding capabilities of proteins coded by these sequences. Lastly, droplet digital PCR (ddPCR) was used to assess differences in transcriptional activity of *horB* and altered *horC* genes when the bacteria were grown in the presence of hops.

3.3 MATERIALS and METHODS

3.3.1 Isolates and culturing

Twenty-seven LAB isolates were tested, with 20 belonging to the *Lactobacillus* genus and seven belonging to the *Pediococcus* genus (Table 3.1). All isolates were removed from -80°C storage, inoculated into deMan, Regosa and Sharpe (MRS) broth and incubated at 30°C overnight. Twenty µl of this culture was then passaged into 12 ml of 85/15 medium [85% (v/v) beer and 15% (v/v) double-strength modified MRS (mMRS: MRS without Tween 80)]. These cultures were incubated at 30°C for 48 h, at which point cells were harvested for DNA extraction.

3.3.2 Genome sequencing of *P. damnosus VTT-E16*

DNA of *P. claussenii* VTT-E16 (Table 3.1) was extracted through use of the UltraClean Microbial DNA Isolation Kit (MoBio) according to manufacturer's instructions, with an optional heating step at 70°C for 10 min incorporated prior to bead beating to facilitate efficient cell lysis. DNA quality and quantity was assess via a Qbit Fluorometer (LifeTechnologies) and sequenced at the National Research Council Plant Biotechnology Institute (NRC PBI) in Saskatoon, Saskatchewan, on an Illumina MiSeq platform using paired-end, 250 bp inserts and multiplexed

Table 3.1 Bacterial isolates, beer-spoilage gene profile, and genetic status of the *horC* gene.

Species [a]	Strain [b]	ID #	Recovery Location [c]	*horC*-full [d]	*horC*-gap	*horC*-CE297	*horB* [e]	*horA*	*hitA*
L. amylovorus	Ingledew 12	L19	Fuel Alcohol Plant; Canada		+	+	+	+	+
L. backii	L101	L101	Brewery "Can"; Canada		+		+	-	-
L. brevis	CCC B1202	L01	Brewer "A"; USA		+		+	+	+
	CCC B1203	L02	Brewer "A"; USA		+		+	+	+
	CCC B1206	L04	Brewer "A"; USA		+		+	+	+
	CCC B1204	L05	Brewer "A"; USA		+		+	+	+
	BSO 31	L06	Brewer "?'; UK		+		+	+	+
	CCC 96S1L	L79	Brewer "A"; USA		+	+	+	+	+
	CCC 96S2AL	L80	Brewer "A"; USA		+	+	+	+	+
	MC 9-4s	L94	Brewer "B"; USA	+			+	+	+
	MC 9-4p	L95	Brewer "B"; USA	+			+	+	+
	BSO 464	L103	Brewery "?"; UK	+			+	+	+
L. casei	CCC B1205	L03	Brewer "A"; USA		+		+	+	+
	CCC 95G1L	L76	Brewer "A"; USA		+	+	+	+	+
L. plantarum	BSO 92	L07	Brewery "?"; UK	(+); ND [f]			+	-	-
	ATCC 8041	L70	Corn Silage; unknown		+	+	+	+	+
	CCC 96M2BL	L82	Brewer "A"; USA		+	+	+	+	+

*L.*unspeciated (*L. sakei*)	ATCC 15578	L74	Brewery "C"; Japan		+	+	+	+	+
L. unspeciated *(L. mali)*	ATCC 27304	L68	Wine Must; Japan		(+); ND		+	-	+
L. unspeciated *(L. backii)*	CCC L86	L86	Brewer "A"; USA		+		+	+	+
P. claussenii	CCC B1100	P21	Brewer "A"; USA		+	(+); ND	+	-	-
	CCC B962A	P62	Brewer "A"; USA		+	+	+	+	+
P. damnosus	E6-21	P04	Brewer "D"; USA	(+); ND			+	+	+
	ATCC 29358	P11	Brewing yeast; UK	+			+	-	-
	MC 9-3	P57	Brewer "B"; USA	+			+	+	+
	MC 9-6b	P58	Brewery "E"; USA	+			+	+	+
	VTT-E16	E16	Shipwreck Beer; Finland		+		+	+	?[g]

[a] "*L.*", *Lactobacillu*s; "*P.*", *Pediococcus*. Putative speciation based on 16S rRNA gene sequence is given for unspeciated isolates L68, L74, and L86.

[b] ATCC (American Type Culture Collection), Manassas, VA; BSO (beer spoilage organism), Brewing Research Foundation, Oxoid, UK; CCC (Coors Culture Collection), MillerCoors, Golden CO ; "E" (Molson), Molson Brewery, Toronto, ON; MC (MillerCoors), MillerCoors, Golden CO; VTT, Technical Research Centre, Espo, Finland.

[c] Brewery from where an isolate was recovered or brewer from whom an isolate was obtained from are coded alphabetically; if no letter designation, the source brewery is not known.

[d] Confirmed *horC* ortholog is denoted as +. Note that all *horC*-CE297 orthologs also contain the *horC*-gap.

[e] Hop-tolerance genes are denoted as present (+) or absent (-), according to the BSR MTPX (Table 3.2).

[f] (+) indicates multiplex PCR result/designation; ND indicates that full gene sequence confirmation was not obtained.

[g] Indeterminate result.

in one lane along with four other samples. Read processing, and sequence assembly and analysis were performed using the A5 assembly pipeline and Geneious 7.8. software (**8, 23**).

3.3.3 DNA extraction

Total DNA from each isolate was extracted through use of QuickExtract™ (Epicentre), by taking 10 µL of 85/15 cultures and combining with 100 µL of InstaGene matrix (BioRad), and vortexing for 20 sec. The preparation was then boiled for 10 min, followed by another 20 sec vortex. The sample was centrifuged for 2 min at 10,000 × g. The supernatant containing the DNA as collected and stored at -20°C for downstream use in all polymerase chain reactions (PCR).

3.3.4 PCR amplicon purification and sequencing

All PCR amplicons (16S rRNA gene, *horB, horC*) were purified using a BioBasic EZ-10 Spin Column Purification kit according to the manufacturer's protocol, with the columns spun for 90 sec instead of 60 sec at 10,000 × g to insure the columns were dry. Sanger sequencing of DNA amplicons was performed at the NRC PBI, Saskatoon, Saskatchewan.

3.3.5 16S rRNA gene

A portion of the 16S rRNA gene was PCR amplified using universal 8F and 534R primers (Table 3.2) (**10, 26**). A 15-µl PCR sample reaction volume was used, containing 0.2 U of MyTaq polymerase (Bioline), 1 µM of 5X MyTaq reaction buffer (Bioline), 0.12 µM of primer, and 2 µL of DNA template. PCR amplification was initiated by preheating for at 95°C 1 min, followed by 34 cycles at 95°C for 15 sec, 56°C for 15 sec, and 72°C for 1 min, and a final elongation step at 72°C for 2 min.

3.3.6 *horB* and *horC* screening and full-length gene sequencing

A *horC*-gap multiplex PCR (MTPX PCR) was used to determine whether *horC*+ isolates contain the 27-bp truncation in the gene or not, and to verify the presence of *horB* (Table 3.2). The previously described BSR gene MTPX PCR (**13**) was utilized to characterize the total beer-spoilage gene profile of each isolate by screening for *hitA, horA, horB* and *horC* (Table 3.2).

Table 3.2. PCR primers

PCR	Primer name	Sequence (5 '→ 3')	Amplicon size (bp)
16S rRNA	8F [a]	AGAGTTTGATCCTGGCTCAG	526
	534R [a]	ATTACCGCGGCTGCTGGC	
[b] BSR MTPX	hitA_F	AGCGTAGCAGAAGAACCTAAG	179
	hitA_R	CAATTACCAGGATCCATGTACC	
	horA_F	AAATCTTAACCCTGCCGG	210
	horA_R	GCGGAACGGCGATAAACATA	
	horC_F	CTTGTTGGAGCAATTATTGG	94
	horC_R	CGTTGACAAGTGCTACAGG	
	8F [c]	AGAGTTTGATCCTGGCTCAG	526
	534R [c]	ATTACCGCGGCTGCTGGC	
[d] *horC*-gap MTPX	horB_F	GCCGCGAAAATGATTCAGG	329
	horB_R	GGCAATTCTTGTTCATCAATTAGG	
	horCgp_F	GTGAACGGGTTTCTGTGTAAC	[c] 120 or 93
	horCgp_R	TATTAGCCAAAAGCTCACTACTC	
[e] *horC* full gene	horCfull_F1	ATATGAATAATTGGGTTTCATG	[e] 1046 or 1076
	horCfull_R1	AACATCAGTATAGATCCATATGTAAC	
[f] *horB* full gene	horBfull_F1	ATGTTTGGAGAAAAGGAGGAAC	573
	horBfull_R1	TTAGCTTATCAAACAAAGGATTCC	

[a] Universal primers (**10**, **26**).

[b] Beer-spoilage-related (BSR) gene multiplex (**13**).

[c] 16S rRNA universal primers used as a positive control for the presence of microbial DNA.

[d] Used to detect the presence of the 27-bp truncation and determine if an isolate is *horC*-full or *horC*-gap (Fig. 3.1)

[e] Used for sequencing of the full length *horC* gene; size of the amplicon is dependent on the ortholog amplified.

[f] Used for the sequencing of the full length *horB* gene.

horB and *horC* were screened for in both MTPX PCR assays, albeit amplifying different sections of each gene, for secondary confirmation of gene presence. The *horC*-gap primers (Table 3.2, Fig. 3.1) anneal on either side of the putative 27-bp gap, resulting in a 120-bp amplicon in the absence of this truncation or a 93-bp amplicon if the gap is present. Two previously fully sequenced isolates were used as templates to design the primers, *L. brevis* BSO 464 (Lb464), which has the full-length *horC* gene and is termed *horC*-full and *Lactobacillus sakei* (L74), which has the 27-bp truncation and is termed *horC*-gap.

Primers used for the full length sequencing of the *horC* and *horB* genes (Table 3.2) were designed to encompass the greatest length of the genes to allow for robust genetic and protein analysis. Given the vast majority of isolates in this study have not had their genomes completely sequenced, the flanking regions of *horC* were not known to be conserved, thus designing primers outside the open reading frame of *horC* was not ideal. The *horC*full_F1/R1 primer set thus was designed to produce the longest possible amplicon length (Table 3.2).

The *horC*-gap and BSR MTPX PCRs (Table 3.2) were run using separate PCR amplification programs. *horC*-gap MTPX PCR amplification was initiated by preheating at 95°C for 1 min, followed by 34 cycles at 95°C for 15 sec, 57°C for 15 sec, and 72°C for 1.5 min, and a final elongation step at 72°C for 2 min. The BSR MTPX PCR program was initiated by preheating at 95°C for 5 min, followed by 35 cycles at 94°C for 45 sec, 52°C for 45 sec, and 72°C for 50 sec, and a final elongation step at 72°C for 5 min. For full length sequencing of the *horC* gene, DNA amplification was initiated by preheating at 95°C for 1 min, followed by 34 cycles at 95°C for 15 sec, 52°C for 20 sec, and 72°C for 1.5 min, and a final elongation step at 72°C for 2 min. For *horB* full-length sequencing, primers *horBF*ull_F1/R1 were used (Table 3.2) with the same PCR conditions as amplification of the full *horC gene*, except for the annealing temperature being 52°C for 20 sec. Multiplex PCR products, were visualized on 2% (w/v) agarose gels, by electrophoresing 5-10 μL of sample at 130-140 V for 45 min, alongside a 100 bp ladder (FroggaBio). Gels were then stained in ethidium bromide (0.5 μg/mL) for 15 min and rinsed in deionized water for 5 min. Bands were then visualized using a ChemiDoc™ Imaging system with Image Lab™ software (Bio-Rad). All amplicons were then purified and sequenced.

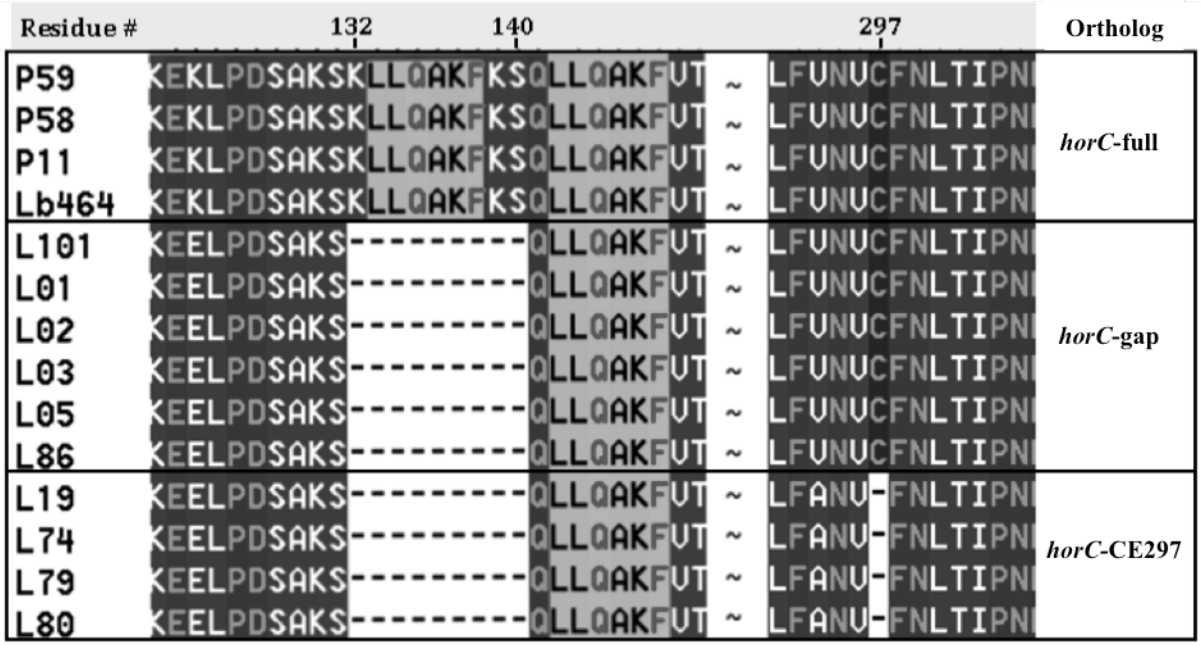

Fig. 3.1. HorC protein sequence alignment for several isolates and general orthologs.

Numbers on the left are isolate identification numbers described in Table 3.1. The nine amino acid gap is depicted between positions 132-140 (KLLQAKFKS), along with the six amino acid (LLQAKF) repeated section located one amino acid after the gap start. Also discovered was a cysteine excision (right) at residue 277, which is consistently found along with 14 other conserved amino acid substitutions across the gene (see Table 3.5).

3.3.7 *hor*C sequence analysis and phylogenic tree creation

Raw *horB* and *horC* gene sequences were assessed for quality on GeneStudio Pro Contig editor (GeneStudio Inc., GA, USA). Sequences were then aligned using Clustal W 1.83 (**24**). Gap additions and ambiguous positions between sequences in the alignment were manually investigated to determine if they were sequence artefacts and edited if necessary.

For the phylogenetic analysis of *horC*, the originally described *horC* sequence (pRH45II; YP_031718.1) was included, in addition to two potential outgroup sequences; a related resistance nodule cell-division (RND) family sequence (*L. brevis* ATCC 367; LVIS_RS20355.1) and a major facilitator superfamily (MFS) sequence (*Lactobacillus rhamnosus* GG; LGG_RS04060) (data not shown). All *horC* and outgroup sequences were aligned by MUSCLE (v3.8.31) supported by the Geneious 7.8 platform (**23**) with default accuracy, and the tree then constructed using the maximum likelihood method via PhyML house in the Geneious suite (**12**).

3.3.8 HorC protein sequence analysis and structure prediction

Nucleotide sequences were translated using the TRANSSEQ feature of the EMBOSS suite of bioinformatic tools (**23**). The resulting protein sequences were then aligned in GeneStudio Alignment editor utilizing Clustal W 1.8 (**24**). Protein structures were predicted using Protter 2D prediction software (**28**) with default settings. RaptorX software was used to predict potential binding sites from an amino acid sequence input based on prevalent predetermined binding motifs that correlate to a bank of small molecules (**22**), and SwissDock server was used to assess the binding capability and molecular affinities of the three HorC orthologs and HorB to three different hop bitter acids: colupulone, humulone and trans-isohumulone (**11**).

3.3.9 Transcriptional assessment via ddPCR

Isolates Lb464 and P58 (*horC*-full), L74 (*horC*-gap) and L101 (*horC*-CE297) were grown overnight in MRS at 30°C, then 10 µl of each was transferred to 8 ml tubes of mMRS pH 5.5 (control tubes). From this same overnight growth in MRS, 20 µl of each cell culture was transferred into 12 ml of mMRS pH 5.5 containing growth-limiting concentrations of hops and incubated at 30°C (see Table 3.3). Preliminary growth analysis of isolates in each growth condition was performed to determine when to isolate cells, as described previously (**4 – 5**).

Table 3.3. Samples for transcriptional analysis

Isolate	Growth Stage [a]	mMRS pH 5.5 h[b]	mMRS + hops BU[c], h
Lb464 (*horC*-full)	mid-log	14	50, 28 75, 56
P58 (*horC*-full)	mid-log	30	8, 50
L101 (*horC*-gap)	mid-log	30	50, 40
L74 (*horC* - CE297)	mid-log	25	30, 50

[a] Stage in bacterial growth when cells were collected for DNA and RNA extraction.

[b] Time when cells were collected.

[c] BU, bitterness units; level of hops challenge that limited isolate growth.

When mid-exponential growth was reached by each isolate in either condition, cells were harvested by centrifugation (5000 × g for 5 min). The cell pellets were then flash frozen in liquid N_2 and stored overnight at -80°C. From every sample, duplicate pellets were prepared for both downstream DNA and RNA extractions.

Total DNA isolation for all cell pellets was done with use of UltraClean Microbial DNA Isolation Kit (MoBio) according to the manufacturer's instructions, however, with an optional heating step at 70°C for 10 min prior to bead beating to facilitate efficient cell lysis. Samples were then digested with 10 U of PmlI restriction enzyne (CAC^GTG; NEBuffer) for 1 h at 37°C and heat inactivated for 20 min at 65°C. Samples were serially diluted and run in a qPCR reaction with universal 16S rDNA primers (Table 3.4) to determine appropriate dilution factor for addition into ddPCR reaction (concentration that gives $C_q \sim 23 – 27$).

Total RNA isolation for all samples was done with use of the PowerMicrobiome RNA Isolation Kit (MoBio) according to the manufacturer's instructions, with 70% ethanol used in place of solution PM4 to prevent small RNA species (5S, tRNA, degraded RNA) from co-precipitating with mRNA and RNA. A 15 min on-column DNase digest was included (DNase I, MoBio). To the 100 µl elutate of total RNA, 1 µl of SUPERase-In™ RNase Inhibitor was added (Ambion). A further DNase treatment was then performed on the elutate using TurboDNase (Ambion), according to the manufacturer's specifications. cDNA and no-reverse-transcriptase (noRT) controls of all RNA samples were performed. The presence of contaminating DNA in each sample was assessed via RT-qPCR as described previously **(3),** using universal primers for the 16S rDNA gene (**25**) (Table 3.4).

The absolute abundance of *horC* and *horB* transcripts in each cDNA and DNA sample was assessed through use of the QX100 Droplet Digital PCR set up and protocol (Bio-Rad). Each 20 µl reaction for ddPCR contained 900 nmol of each forward and reverse primer, 250 nM of each probe, 1× Bio-Rad Droplet PCR Supermix (Table 3.4), and either 4 µl of digested DNA, or 5 µl of cDNA diluted in PCR-grade water to 0.2 ng/µl RNA equivalent. The 20-µl reactions were then pipetted into separate wells of a disposable eight-channel droplet generation cartridge (Bio-Rad) and 70 µl of droplet generation oil (Bio-Rad) loaded into the cartridge oil wells. The

Table 3.4. Primers and probes used for ddPCR transcriptional analysis

Target	Tm °C	Forward Primer 5' → 3'	5' Label [b]; Probe 5' → 3'	Reverse Primer 5' → 3'	Amplicon (bp)
16S rDNA	--	Ec338f [a]; ACTCCTACGGGAGGCAGCAG	--	Ec518r [a]; ATTACCGCGGCTGCTGG	196
horC [b]	58.8	GCTATCCCAAGCACTTCCT	HEX; TCACGAATTAGCGCACAGCAACAA	GGTCGAAACCAAATCCCAAG	124
horB [b,c]	58.8	AGTCGACACAAAATCCTGAATCA	FAM; TCGCGGCCAAGTGATACTTATCCTGA	AGCCTTGATCAATCGTCAGAC	88

[a] Primers Ec338f (**2**) and Ec518r (**27**) were used in RT-qPCR to assess cDNA and noRT quality (**3**).

[b] Both *horC* and *horB* ddPCR probes contained a 3' IowaBlack GQ quencher.

[c] (**7**).

cartridge was then inserted into the QX100 droplet generator (Bio-Rad) to generate droplet-sized water-in-oil emulsions. Emulsion samples were transferred to a 96-well PCR plate (Eppendorf) and after the plate was hot-sealed with foil cover, it was subjected to conventional PCR in the CFX96 Touch Real-Time PCR (Bio-Rad). Thermal cycling conditions consisted of an activation period for 10 min at 95°C, followed by 40 cycles of a denaturation step for 30 sec at 94°C, and an annealing-extension step for 90 sec at 58.8°C, using a ramp rate of 2.5°C/sec for each step, and a final inactivation step of 98°C for 10 min. After PCR amplification, the plate was loaded into the QX100 Droplet Digitial PCR (Bio-Rad) and analyzed for absolute signal quantification of each fluorescence channel in each well. Quantasoft Software v.1.3.2 (Bio-Rad) was used for signal detection and absolute quantification. Two ddPCR reactions were prepared for each biological replicate of all samples, thus N = 4 in all cases.

3.4 RESULTS AND DISCUSSION

3.4.1 Gene profiles of *horC*+ isolates

All 27 putative *horC*+ isolates had their identity confirmed via sequencing a portion of their 16S rRNA gene, and were screened for their total hop-tolerance genes profile and for the described 27-bp truncation in the *horC* gene. Table 3.1 shows that 19 of the 27 *horC*$^{+}$ isolates (70%) also tested positive for the 27-bp gap (denoted as *horC*-gap) according to the screening MTPX PCR (Table 3.2). Concurrently, all 27 isolates were positive for the presence of *horB*, indicating that this gene is acquired alongside *horC*, regardless of the completeness of *horC* (Table 3.1).

To elucidate the effect of the *horC*-gap on predicted structure and function and to determine if other mutations were prevalent within the *horC* gene, the full-length *horC* gene was sequenced for all of the isolates. Full sequencing of *horC* was unsuccessful in four cases, despite positive MTPX PCR results, suggesting that these isolates may contain a closely related paralog that confounds efficient primer annealing during full-length gene sequencing. Indeed, when the amplicon of primers *horC*full_F1 and *horC*full_R1 for these problematic isolates are visualized on agarose gels, there are unexpected bands of varying size, whereas in non-problematic isolates there is only one clean band (data not shown). Thus, the previously described *horC* primers of the BSR MTPX PCR falsely revealed the presence of *horC* in 11% (4 of 27) of the isolates in this study. This finding underscores the issues inherent to PCR screening when using small

amplicons – specifically, a positive PCR result does not guarantee the presence of the full-length and/or functional gene. Though the chances of either aberrant outcome is largely ignored within brewing-related literature, it is reasonable to expect that this chance for error is further confounded for LAB isolates, which are known to be inherently genetically variable and have a highly adaptable nature made possible by genetic mutations, gene loss and genome rearrangement (**34, 37**).

Ultimately, full-gene sequencing results revealed that *horC* is not as highly conserved as previously believed, as there were further sequence anomalies uncovered apart from the 27-bp truncation originally screened for. Eight of the 18 isolates confirmed to possess the 27-bp gap (i.e., one of the 19 *horC*-gap isolates detected through MTPX PCR proved to be a paralog), also possessed an in-frame 3-bp deletion from base pairs 890 – 892 that encodes a cysteine residue upon translation (isolates with this 3-bp excision are called *horC*-CE297). All eight *horC*-CE297 isolates also contain 14 other conserved mutations in *horC* which correspond to specific amino acid substitutions in HorC (Table 3.5 and Fig. 3.1 and 3.3), effectively splitting the *horC* sequence into three separate ortholog groups: *horC*-full (original described sequence), *horC*-gap (discovered in the Finnish shipwreck isolate and prevalent in modern LAB isolates), and *horC*-CE297 (possess both the 27-bp and 3-bp gaps, and other sequence variances). Of further note were five *Pediococcus*-specific single nucleotide polymorphisms (SNPs) throughout the full length (1291 bp) *horC* gene relative to *horC* sequences observed for the lactobacilli isolates; namely, C545T, G869C, T988C, G1018A and G1088C.

Interestingly, the full-length *horB* sequence was completely conserved in all three *horC*-ortholog groups (data not shown). This finding suggests that forces driving mutational changes affect these two genes differently. In particular, it would suggest that the substrate-specificity of *horB* for hops, or its role in regulating *horC*, is advantageous to the cell, hence its high sequence conservation and co-localization with *horC*. In contrast, the *horC* gene, like the genes for other efflux or membrane transporters that provide resistance to damaging compounds, is most likely subject to adaptive mutation to potentially expand substrate specificity and/or adapt to new environmental pressures. Therefore, it is quite conceivable that the three *horC* orthologs, as well as smaller sequence changes, have emerged as a result of different LAB environmental

Table 3.5. Characteristics of the three *horC* orthologs.

	***horC*-full**	***horC*-gap**	***horC*-CE297**	***horB***
Isolates* (source/origin)	L94 (brewery "B"; USA), L95 (brewery "B"; USA), L103 (brewery "?"; UK), P11 (brewing yeast; UK), P57 (beer "B"; USA), P58 (brewery "E"; USA),	VTT-E16 (beer; Finland), L01 (brewer "A"; USA), L02 (brewer "A"; USA), L03 (brewer "A"; USA), L04 (brewer "A"; USA), L05 (brewer "A"; USA), L06 (brewery "?"; UK), L86 (brewer "A"; USA), L101 (brewery "Can")	L19 (fuel alcohol plant), L70 (corn silage; ?), L74 (brewery "C"; Japan), L76 (brewer "A"; USA), L79 (brewer "A"; USA), L80 (brewer "A"; USA), L82 (brewer "A"; USA), P62 (brewer "A"; USA),	Sequence conserved in all 27 isolates tested
Sequence anomalies	Original *horC* sequence described in [a] *Pediococcus* isolates all share: C545T, G869C, T988C, G1018A and G1088C	27-bp gap (results in HorC with a deletion of KLLQAKFKS at position 132-140)	27-bp gap; 3-bp gap (results in HorC-gap also minus cysteine at position 297); HorC point mutations: T51I, K180Q, A184T, V231I, N232G, T234A, V289I, V294A, S313F, L330F, T333F, F334V, S346A, T355A	None
[b] Secondary substrate capabilities	D1 = Retinol; Long chain fatty acids	D1 = LCFA; D2 = Ca^{2+}, N-acetyl glucosamine	D1 = Mg^{2+}, Glycerol; D2 = D-Alanine,	dCMP and dGMP (DNA)

*L07 (brewery "B"; UK) and P04 (brewery "E"; USA) contained a paralog of *horC*-full, while L68 (wine must; Japan), and P21 (brewery "A"; USA) contained *hor*C-gap and *horC*-CE297 paralogs, respectively.

[a] Described in (**36 – 37**).

[b] Predicted using RaptorX-Binding (**22**). D1, Domain 1; D2, Domain 2.

challenges or selective pressures, while *horB* concurrently has been genetically conserved to maintain *horC* activation and function writ-large.

Additional screening of the isolates via the BSR MTPX (using primers directed at a different locations in the *horB* and *horC* genes) provided further confirmation of the *horC* MTPX PCR data that *horB* and *horC* were present, and facilitated correlation analysis to determine if the presence of specific *horC* orthologs was linked to the presence of *hitA* and *horA* (Table 3.1). There is no statistical correlation between the presence/absence of *hitA* or *horA* with the presence of any *horC* ortholog, nor with the putative paralog in this data set, as analyzed by SPSS statistical software for Windows (ISBM, v. 19.0). This result is not unexpected, given that there have been no reports of these three genes consistently being acquired together, nor described as being located on the same plasmid structure.

Examining the isolate distribution of the *horC* orthologs (Fig. 3.2) reveals that the presence of *horC*-gap is exclusive to the *Lactobacillus* species analyzed here, as all *Pediococcus* isolates with this nucleotide gap, also have the 3-bp CE297 deletion and other associated point-mutations (Table 3.5). This *horC* ortholog distribution cannot be linked to the bacterial isolation source (Table 3.1 and 3.5), and instead likely is a function of the number and species distribution of the isolates analyzed. It is important to note, however, that both *Lactobacillus* and *Pediococcus* isolates putatively contained a *horC* paralog, which resulted in inconclusive *horC* sequencing results for these isolates (Table 3.1). This indicates that the *horC* primers described previously in the literature can falsely indicate the presence of *horC* in isolates from LAB in both genera (**13**). Further, this finding indicates the possibility that these paralogs are produced as a result of low-fidelity horizontal gene transfer (i.e., sequence loss and/or mutation) and raises questions as to the functional role that these paralogs may have for the cell.

3.4.2 HorC secondary structure prediction

Upon translation of the *horC* nucleotide sequences, it was found that almost directly downstream of the location of the 27-bp (nine amino acid) gap, is a six amino acid repeat of the middle section of the “gap” sequence; namely, LLQAKF (Fig. 3.1). This sequence is not repeated anywhere else in the gene. NCBI BLASTX indicates that this sequence is generally found in

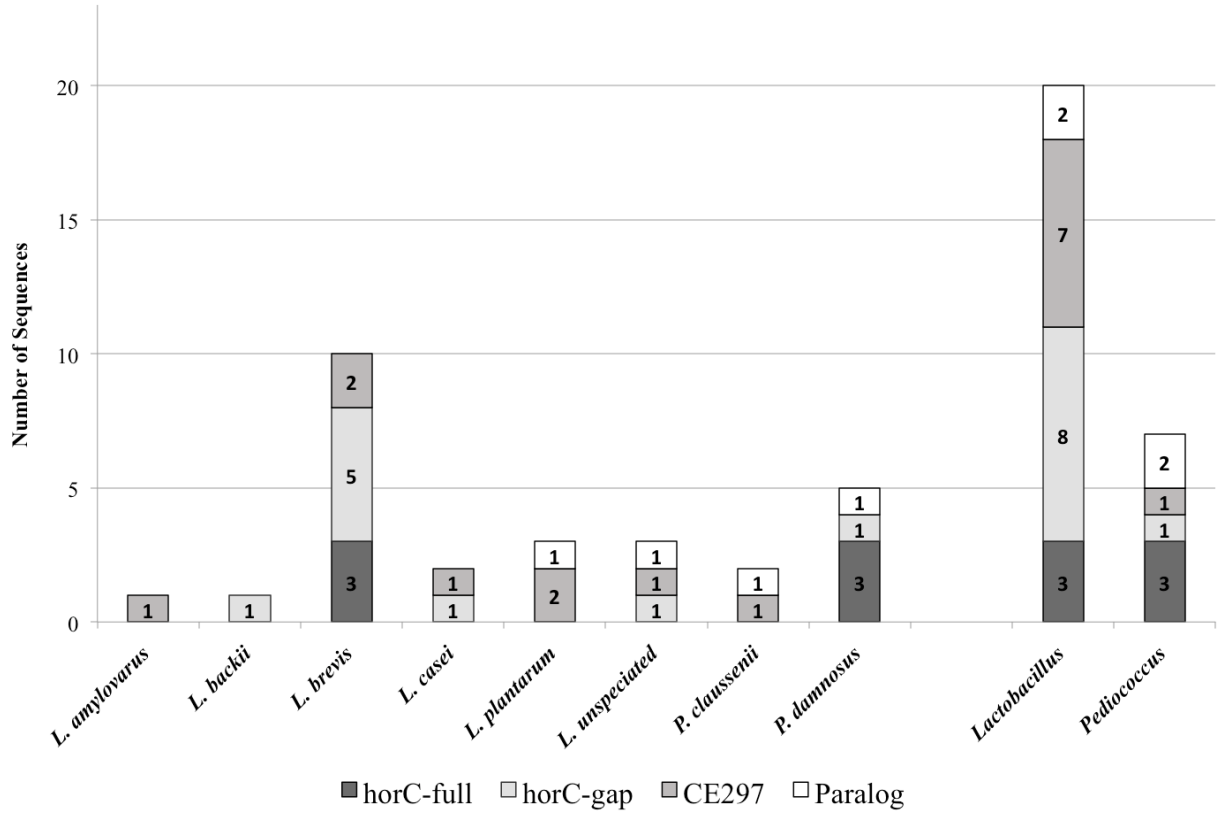

Fig. 3.2. Prevalence of *horC* orthologs and putative paralogs among the genera and species of the isolates analyzed.

Initial MTPX PCR testing of 27 *horC*+ isolates revealed the *horC*-gap to be prevalent among LAB BSOs, as 19 isolates contained this truncation. Upon full-length sequencing of *horC*-gap genes, it was revealed that eight of the *horC*-gap isolates had additional conserved sequence anomalies, and were subsequently grouped as *horC*-CE297. Four putative horC paralogs were found, including two, one, and one in isolates designated as *horC-full*, horC-gap, and *horC*-CE297, respectively.

membrane and lipoproteins, suggesting that the sequence of the gap and the repeated sequence following it are likely involved with anchoring the protein in the membrane and contribute to the physical structure of HorC rather than participating in protein interactions with potential substrates (**1**). Given that alterations in protein structure can alter substrate-binding efficiency, it may be possible that the loss of this sequence in the *horC*-gap isolates (i.e., the repeated LLQAKF motif) could alter HorC function.

To determine the functional effect of the discovered mutations in *horC,* it was important to first characterize the structure and function of the HorC-full protein; i.e., the originally described sequence by Suzuki et al., 2005 (**38**). Translated nucleic acid sequences were input into the Protter Secondary structure predictor (**25**), which confirmed the HorC protein resembled an RND transporter. This is interesting given that it has been found that proteins of the RND superfamily are underrepresented in gram-positive bacteria, whereas other multidrug efflux pumps such as Major Facilitator Superfamily (MFS) type pumps are much more prevalent (**6, 25**). However, both the HorC-full and HorC-gap 2D structures closely resemble exactly half of a characteristic RND transporter, i.e., there are six predicted transmembrane (TM) regions with a large extra-cellular region between TM's 1-2 (Fig. 3.3). This is an indication that HorC may function as a dimer to match the structure of the RND monomer, which would fit with a previous study showing that the fusion of two HorC proteins did not result in a loss of hop-tolerance and/or protein function (**19**).

3.4.3 Binding affinity for α-acids

SwissDock software was used to predict differences in binding affinity (**Δ**G; kcal/mol) to hop bitter acids that the different protein HorC orthologs may have (**11**). Each *horC* ortholog, and *horB,* were tested against three α-acids: humulone, colupulone and trans-isohumulone. These computational predictions suggest that HorC-full, HorC-gap, and HorC-CE297 have fairly similar α-acids binding affinity (ranging between -7.3 to -8.64 **Δ**G), which is unexpected considering the predicted changes to protein structure for the HorC-gap variants relative to HorC-full. Though *in silico* predictions of binding affinity require experimental validation, these values indicate HorC-gap has the greatest affinity measure for each substrate (-7.85 to -8.64 **Δ**G), and thus suggests that HorC-gap is the original protein rather than HorC-full; i.e., rather than

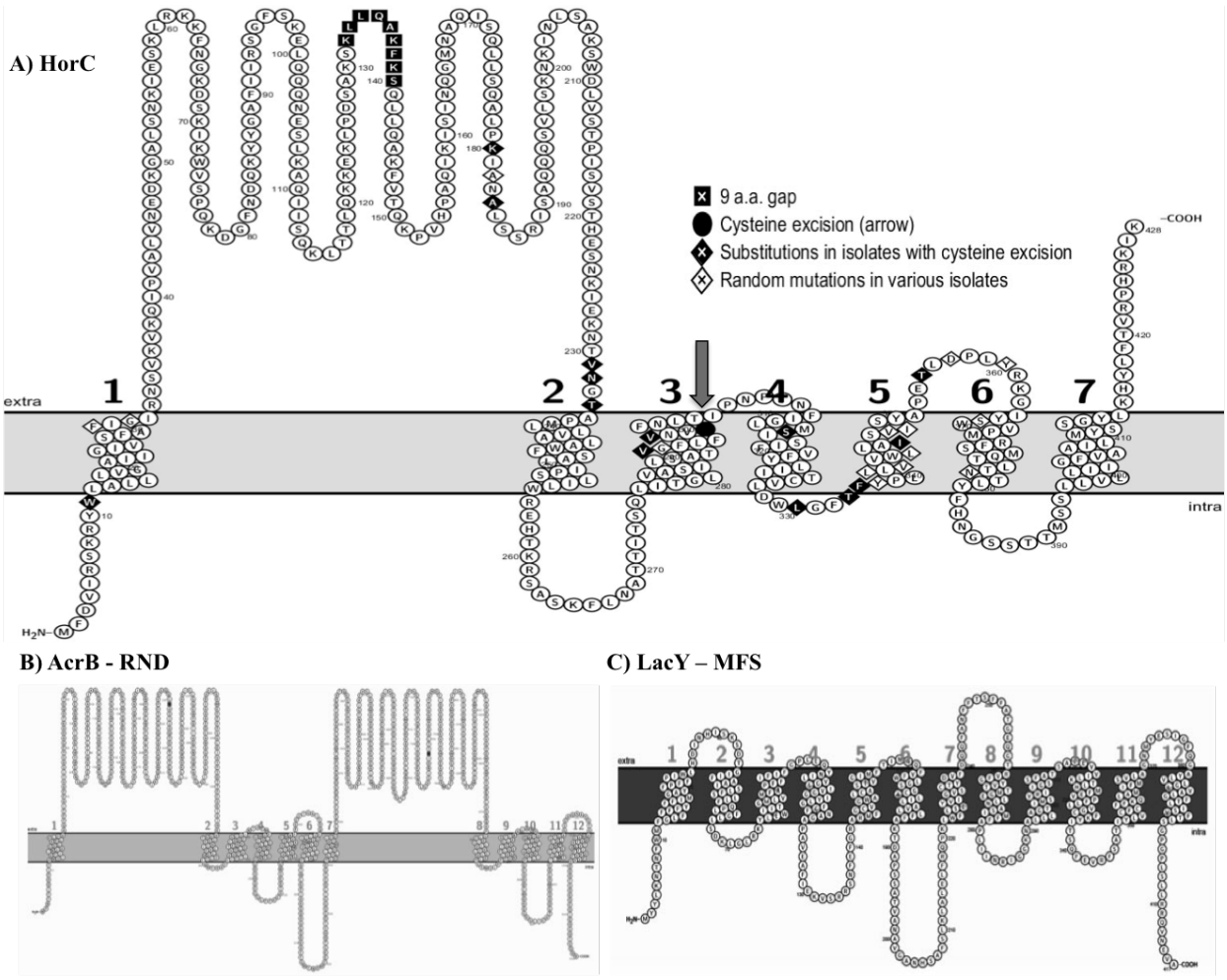

Fig. 3.3. Predicted 2D structure of HorC and comparison to example RND and MFS proteins.

A) shows the predicted structure of HorC-full, with the positions of sequence anomalies in HorC-gap (black squares) and HorC-CE297 (black circle and black diamonds) indicated. Loss of the nine amino acid sequence (black squares) from HorC-full does not significantly alter the predicted structure, only shortening the middle external protein loop in HorC-gap. The other mutations indicated (white diamonds) are at positions F28, G30, A182, G290, Y335, L338, V340, L343, V348, I349, D357, Y360, S366 and N379. Due to single base mutations within the codons for these amino acids, there are variable residues in HorC for some of the analyzed isolates. Note that the five base changes in *Pediococcus* isolates (see Table 3.5) are not indicated. B) and C) depict the 2D structure of RND and MFS proteins, respectively, showing that HorC more closely aligns with the RND structure. Numbers above the cellular membrane indicate trans-membrane regions for all three proteins.

a loss of sequence from HorC-full to produce HorC-gap, it is instead that HorC-full resulted from an insertion mutation, while HorC-CE297 experienced multiple point mutations from HorC-gap, causing two branching ortholog groups.

HorB was also analyzed by SwissDock to help characterize its function and interestingly multiple predicted binding sites were predicted for the different α-acids. The binding affinity measure of HorB is less (-6.72 to -7.4 **Δ**G) than compared to HorC orthologs, however if the hop α-acids were potentially to be passed from HorB to HorC, then HorC would necessarily have a greater binding affinity to overcome the activation energy of the transfer between the two proteins. Another theory, which incorporates the fact that HorB also has demonstrated putative RND transcription factor homology, is that HorB is a repressor of HorC that is activated upon binding of alpha acids to delocalize from DNA allowing *horC* transcription. This theory is based on three pieces of evidence: 1) HorB is predicted to have an affinity to hop bitter acids, 2) HorB has predicted homology via BLASTX to two RND transcriptional repressors: AcrR family transcriptional regulator (ref|WP_026883908.1; E = $3e^{-15}$), and TetR (dbj|GAD38909.1; E = $2e^{-52}$), and 3) many transcription factors require some sort of conformational change to their structure which increases/decrease its affinity to the target DNA sequence, and also allows it to function dependent on environmental conditions (**29**).

3.4.4 Putative substrate binding capabilities

Putative substrates for HorC and HorB were modeled through use of the RaptorX-Binding program (http://raptorx.uchicago.edu/BindingSite/) (Table 3.5). The predicted binding pockets are assessed by a measure of pocket multiplicity (PM) – a value that represents the frequency with which a given binding pocket is found in template ligand-binding protein structures within RaptorX-binding (**22**). A PM value above 40 is suggested by RaptorX to be an indication that the predicted binding site is true (i.e., this binding pocket is frequently found in a list of similar "template" protein structures). PM values below 40 indicate similar binding pockets are found at lower frequency in the template test set, and thus require experimental confirmation, nonetheless remaining as interesting prospective binding pockets.

Results indicate that Domain 1 of the HorC-full protein contains a valid binding site for the hops-like molecule, retinol, and that this binding occurs directly in the middle of its 3D structure (PM = 55). This may be evidence that HorC-full protein binds its substrate as an MFS rather than an RND transporter, given that a MFS transporter acts by binding a substrate in its center, then changing conformation to release it on the outer side of the membrane (**30**). Following release, an influx of protons into the cell allows conformational reversal of the protein before binding again. Other predicted substrates for the HorC-full Domain 1 were long chain fatty acids (PM = 13), which is interesting given that unsaturated long chain fatty acids have been shown to be up regulated in the membrane of *L. brevis* in response to ethanol and hops (**35**). This membrane alteration is hypothesized to decrease membrane fluidity and permeability in the face of the stressful and damaging beer environment.

Interestingly, the RaptorX program predicted the strongest binding for D-Alanine to the pocket in Domain 2 for HorC-CE297 (PM = 112). This amino acid is required for the creation and repair of the peptidoglycan cell wall (**21**). Given that the presence of alcohol has been shown to decrease hydrophobic interactions (and thus the binding strength) of membrane-associated enzymes involved in peptidoglycan cross-linking, D-alanine could be a potentially important substrate to uptake (**20**). Domain 1 is predicted to have affinity for Mg^{2+} (PM = 29) and glycerol (PM = 7), which both can effect ATPase function (**9**). Further, divalent cations such as Mg^{2+} have are known to specifically decrease the effect of hop α-acids on PMF (**34**), and one hop resistance marker, *hitA*, is a Mn^{2+} efflux pump (**16**). Strangely, the Domain 1 of HorC-gap only has weak affinity for long chain fatty acids in Domain 1 (PM = 6) and in Domain 2 has predicted capabilities for Ca^{2+} (PM = 29) and N-acetyl glucosamine (PM = 21).

These protein-modelling results together suggest that HorC is a transporter capable of moving multiple different molecules and thus is uniquely suited to defend against the acute stresses that hop bitter acids and ethanol present to the LAB cell. This protein modelling also indicates that alterations to the protein structure between orthologous HorC proteins can have dramatic impact on their binding capabilities and raises questions as to whether the evolution of these orthologs is necessarily advantageous? Nonetheless, long chain fatty acids and divalent cations, both previously implicated as being important in the LAB cellular response to beer, were predicted to

bind to multiple variants of HorC, thus lending credence to the hypothesis that HorC interacts with more than just hop α-acids when LAB grow in beer.

3.4.5 Phylogeny

Prior to performing a multiple *horC* nucleotide sequence alignment, the *horC* sequences were *cleaned*, such that each sequence began and ended at gene base pair positions 128 bp and 1,159 bp, respectively. This was done because the primers used for full-length sequencing could not ensure that the full CDS of the gene was sequenced and low quality base-reads at the beginning and end required trimming. Consequently, performing phylogentic analysis on translated, amino acid sequences was not performed.

Using the RND homolog of *L. brevis* ATCC 367 as the outlier, the resultant cladogram shows the three *horC* orthologs discretely separated (Fig. 3.4). There is shorter observed distance, as assessed by substitution per site value, between the *horC* sequences and the RND outgroup (0.04) than the MFS outgroup (3.0) (not shown), supporting the hypothesis that *horC* is an ortholog to RND, likely as an intermediate secondary transporter type that has yet to be well characterized. Interestingly, isolates VTT-E16, L86 and L101 all group together (Fig. 3.4), as even though these three isolates are designated as being *horC*-gap, these isolates share point mutation that place them closer to the *horC*-CE297 group than the *horC*-gap group, and at other point locations, match those found in the *horC*-gap isolates, and not the CE297 group. What is further interesting is that these three isolates were recovered from different geographical locations, and isolate L19 was not recovered from a brewery or beer (Table 3.1 and 3.5). There is stronger initial branching of *horC*-CE297 from the RND outgroup (Fig. 3.4) and the three grouped isolates VTT-E16, L86 and L101 appear as intermediaries or evolutionary links between *horC*-CE297 isolates and the *horC*-full and *horC*-gap isolates. Of note, *Pediococcus* isolates group together in each cladogram as a result of an additional common five SNPs shared within this group.

The phylogenetic relationships presented in Fig. 3.4 fit with the interpretation that the *horC*-CE297 ortholog group emerges first from the RND outgroup, followed by point mutation events that culminate in the *horC*-gap ortholog group. Lastly, an insertion event occurred which resulted

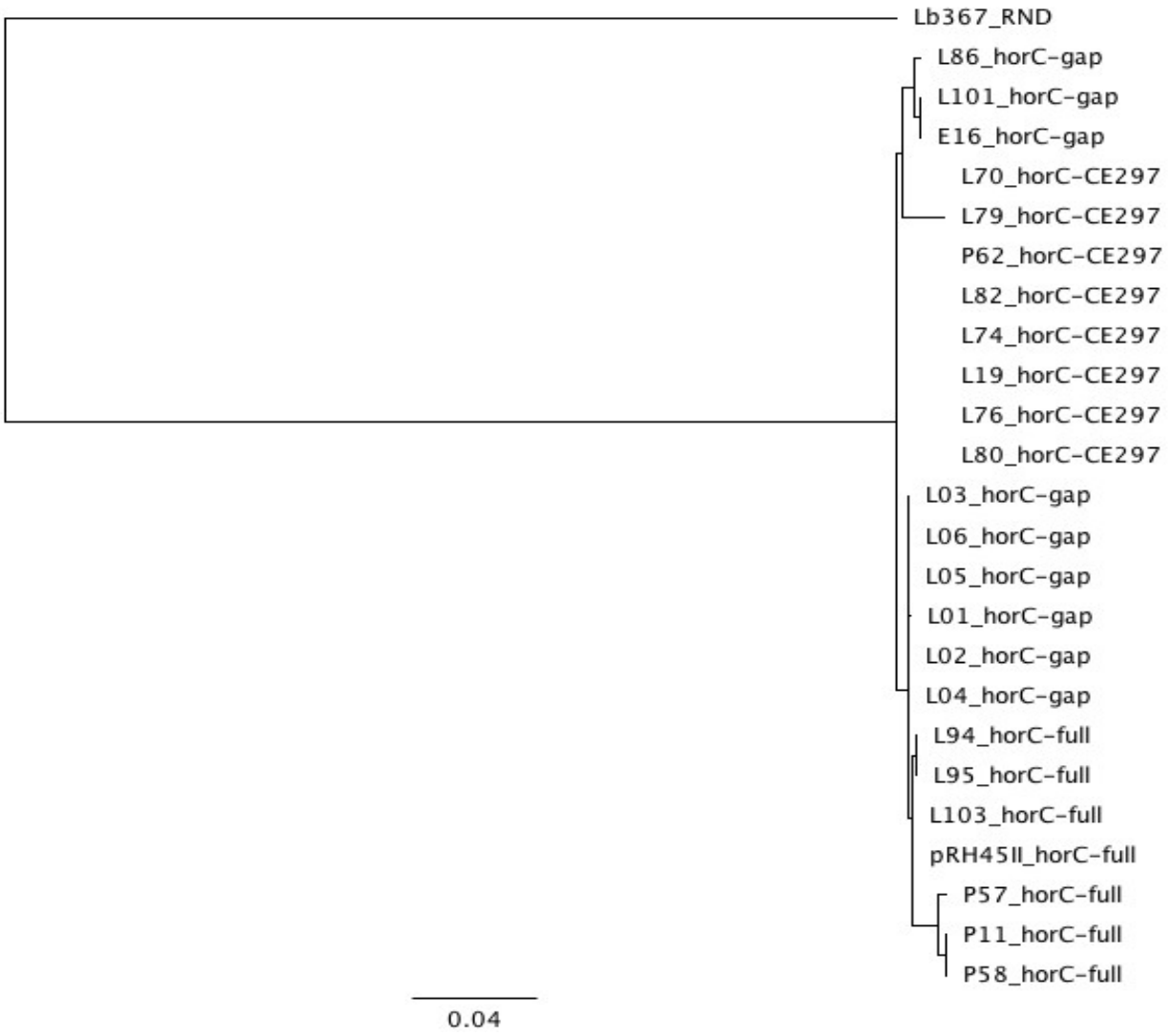

Fig. 3.4. Relatedness of *horC* to RND protein.

L. brevis ATCC 367 RND family protein, is used as the outgroup. *L. rhamnosus* GG MFS family protein was also used trialed an outgroup and revealed a substitution per site (branch length legend) of 3.0 relative to the score achieved above with RND (data not shown). This suggests *horC* evolved from the RND family. This cladogram suggests that *horC*-full was the most recent ortholog to emerge. Note that isolates L07, L68, P04 and P21 (containing putative *horC* paralogs) are not included in the analysis, though the original described *horC* sequence on pRH45II is included. The indicated branch length is sequence substitutions per site (the number of nucleotide changes divided by the length of the sequence).

in the *horC*-full ortholog group. This scenario suggests that development of the *horC* ortholog groups was not due to discrete events, but rather due to iterative events involving horizontal transfer of *horC* on plasmids, with the replication of the plasmids then not error free, despite apparent selective pressure from the environment the plasmid was acquired in. Overall, even though *horC*-full is not envisaged to be the first iteration of the *horC* protein, it must be kept in mind that the three-horC orthologous groups are not separated by great evolutionary distance.

3.4.6 Transcriptional activity

The isolates selected for *horB* and *horC* transcriptional analysis via ddPCR belong to different *horC* orthologs, and have similar hop-tolerance gene profiles (except L101; Table 3.1) and established hop-tolerance levels (**5**). The data firstly reveal that when the four selected isolates were grown under varying growth-limiting concentrations of hops. *horC* is expressed in response to hops regardless of ortholog group. Secondly, although increased hop levels resulted in increased *horC* expression within an isolate, different concentrations of hops were found to elicit comparable expression of *horC* in different isolates, thus indicating that *horC* is not uniformly efficacious in all isolates. Finally, the expression of *horB* is repressed in response to hops, potentially confirming that the role of *horB is* as a negative transcriptional regulator of *horC.*

ddPCR performs absolute quantitation of a given cDNA or DNA target, and it was found that the *horB* and *horC* DNA targets in both control and experimental samples existed at approximately a 1:1 ratio (Fig. 3.5), confirming previous studies (**7, 17**). Quantitation of cDNA levels for each target revealed that in control (mMRS) samples, there is higher expression of *horB* relative to *horC* indicating active transcription of *horB* in the absence of hops. This confirms that *horB* is hop-specific, thus its high sequence conservation, regardless of mutations that have occurred in *horC*. For all horC orthologs, *horC* expression relative to *horB* is increased dramatically in the presence of hops. Interestingly, this speaks more to the hop-specificity of *horB* than to the hop-specificity of the *horC* orthologs, as *horC* expression is induced to similar extents in isolate across a range of hop concentrations; i.e., an increase in hop levels does not uniformly increase the expression levels of *horC* (Fig. 3.5). This suggests that there are either subtle differences in the specificity of the *horC* orthologs towards hops, or that there exist redundant hop-tolerant

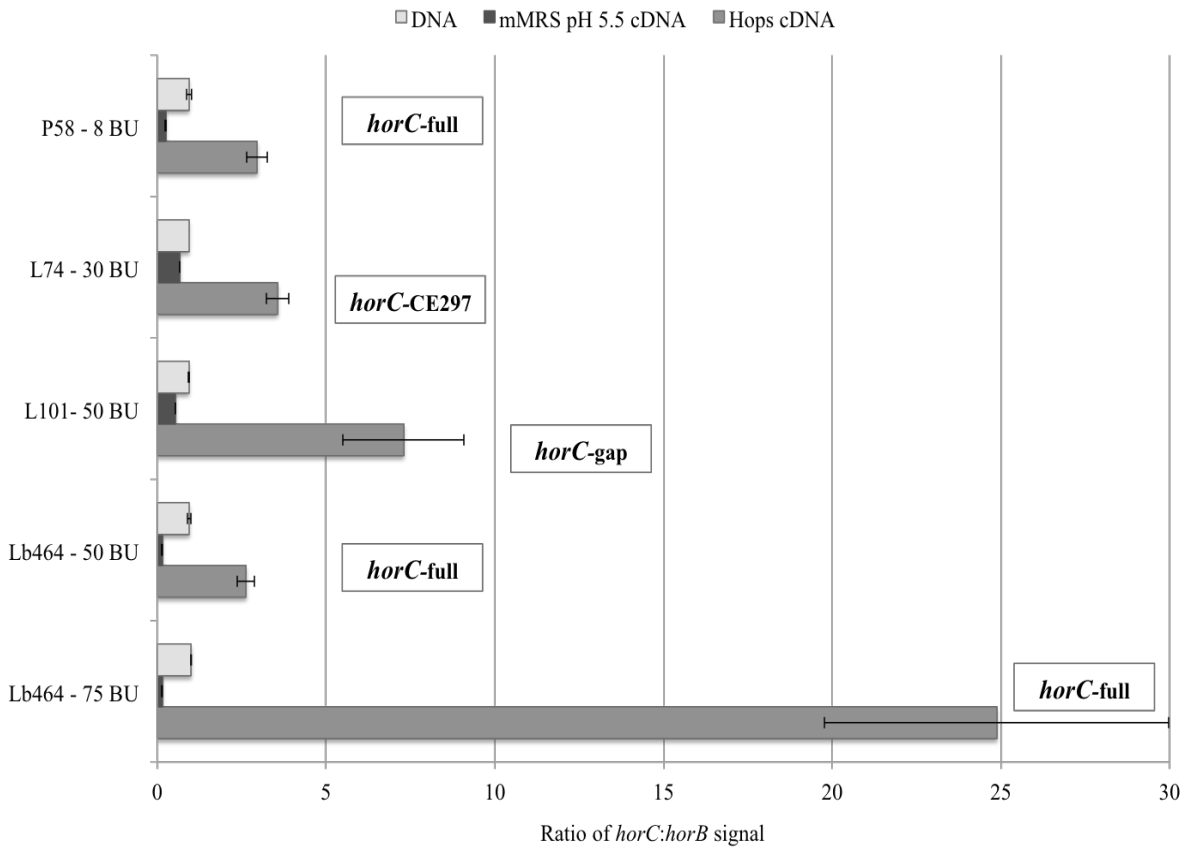

Fig. 3.5. Ratio of *horC*:*horB* gene and transcript abundance determined via ddPCR.

Error bars indicate standard deviation for four values. The *horC* and *horB* signal ratio for bacterial DNA was 1:1 for bacteria grown in both control (mMRS pH 5.5) and experimental (hops) conditions. The expression ratio of *horC* relative to *horB* is < 1 for all isolates in control conditions, reflecting increased *horB* expression in the absence of hops. Growth in the presence of hops results in a dramatically increased *horC*:*horB* transcript ratio.

mechanisms within isolates which would belie the existence of a single critical concentration of hops for use when analyze LAB hop-tolerance.

Further evidence to support the existence of redundant hop-tolerant mechanisms comes from the fact that despite previous characterization of each of these isolates as being highly-hop tolerant (**5**), different concentrations of hops were required to comparably limit the growth kinetics for different isolates (i.e., 8 bitterness units (BU) of hops slowed P58 growth, however, 50 BU of hops was required to see the same relative delay in the growth of L101; Table 3.4). Secondly, although isolate VTT-E16 has a supposedly functional *horC*-CE297 and *horB*, just as L74 does, VTT-E16 has no discernable hop-tolerance and cannot grow in modern beer. Though the transcriptional analysis presented in Fig. 3.5 does not indicate that sequence anomalies of *horC* strongly influence the gene's activity in the presence of hops, it does highlight that the presence of *horC* does not guarantee uniform hop-tolerance and highlights the variable and isolate-specific nature of LAB hop-tolerance. These observations support the idea that hop-tolerance is mediated by more than one gene and/or process.

Although the effect of *horC* sequence anomalies on beer-spoilage phenotype were not investigated here, a recent study which included the isolates analyzed in Fig. 5 found that hop-tolerance genes could not be used to adequately overall predict beer-spoilage phenotype (**7**). Consequently, like hop-tolerance, ability of a LAB to grow in and cause beer spoilage is genetically multi-factorial. This means solely using hop-tolerance genes to screen LAB for beer-spoilage potential writ large is folly. Certainly, the sum total of the data presented here confirms that presence of the *horC* hop-tolerance gene in a LAB does not guarantee a specific level of hop-tolerance. Moreover, the described sequence anomalies within *horC* greatly complicate the interpretation of both positive and negative results in screening assays where *horC* is the target gene for indicating hop-tolerance which, in turn, is assumed erroneously to indicate LAB beer-spoilage potential.

3.5 CONCLUSIONS

The results of this study show that the nucleotide sequence of the *horC* gene is not highly conserved and that there exist at least three *horC* orthologs: *hor*C-full, *hor*C-gap, and *horC*-CE297, as well as possible *horC* paralogs. In contrast, *horB* was found to be ultra-conserved, despite always being found together with *horC*. This likely reflects the fact that *horC* function (i.e., membrane transport) is redundant within LAB cells, whereas *horB* appears to

have a very specific regulatory action in response to hops. *In silico* characterization of the *horC*-orthologs predicted different *HorC* binding for specific substrates, although further testing is required to confirm that HorC can bind other substrates besides hop α-acids.

The data presented strongly support the following concepts. First, the adaptable nature of BSR LAB means that only one or even a few genes are not adequate to describe how all LAB grow in beer. Second, LAB found in the brewing environment, where adaptive genes are presumed to be required and transferred, likely harbour many yet un-described beer-specific genes. Third, using rapid-screening methods that rely on one or a few select genes for indicating LAB beer-spoilage potential is problematical, because sequence conservation of these genes and the linkage of these genes to overall beer-spoilage phenotype have not been ensured. This highlights the need to perform similar genetic variability analyses for the other traditional beer-spoilage markers *hitA* and *horA*, and the need to undertake detailed analysis of both genetic transfer within the brewery setting and the mobile elements (plasmids) expected to be involved in this transfer. Deploying MTPX PCR assays to identify present and future putative LAB beer-spoilage markers should be done with caution until sequence conservation of the assay target gene(s) is confirmed, so that both false-positive and false-negative assay results can be eliminated.

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Chapter 4: Role of plasmids in *Lactobacillus brevis* BSO 464 hop tolerance and beer spoilage

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4. INTERFACE

This chapter was previously published in *Applied Environmental Microbiology*, 81(4): 1234-1241, 2014, and is reprinted here with permission. This data demonstrates that the total plasmid profile of a beer-spoilage isolate is important in determining virulence and phenotype.

Jordyn Bergsveinson was involved with experimental design and validation, designed all primer sequences, performed HGA and HGAE plating and statistical analysis, and authored the manuscript.

Nina Baecker is credited with generating the plasmid variants used in this study and performing preliminary growth analysis. She also contributed to the editing of the manuscript prior to publication.

Vanessa Pittet provided insight into data analysis contributed to editing of the manuscript prior to publication.

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work.

4.1. ABSTRACT

Specific lactic acid bacteria (LAB) isolates can grow in the harsh beer environment, thus posing a threat to brew quality and economic success of breweries worldwide. Plasmid-localized genes, such as *horA*, *horC*, and *hitA* have been suggested to confer hop tolerance, a trait required for LAB survival in beer. Presence and expression of these genes among LABs, however, do not universally correlate with ability to grow in beer. Genome sequencing of the virulent beer-spoilage organism *Lactobacillus brevis* BSO 464 (Lb464) revealed the presence of eight plasmids, with plasmids 1, 2 and 3, containing *horA*, *horC*, and *hitA*, respectively. To investigate the role that these and the other five plasmids play in Lb464 growth in beer, plasmid curing with novobiocin was used to derive ten plasmid variants. Multiplex polymerase chain reactions were utilized to determine presence or absence of each plasmid, and how plasmid loss affected hop tolerance and growth in degassed (non-carbonated) beer was assessed. Loss of three of the eight plasmids was found to affect hop tolerance and growth in beer. Loss of plasmid 2 (*horC* and 28 other genes) had the most dramatic effect, with loss of plasmid 4 (120 genes) and plasmid 8 (47 genes) having significant, but smaller impact. These results support the contention that genes on mobile genetic elements are essential for bacterial growth in beer, and that beer-spoilage ability is not dependent solely on the three previously described hop-tolerance genes, nor on the chromosome of a beer-spoilage LAB isolate.

4.2. INTRODUCTION

Beer is a very harsh environment for bacterial growth due to unique physiochemical attributes. Specifically, the presence of antimicrobial hop compounds (15-55 ppm iso-α-acids) and ethanol (0.5-14% *v/v*) (25), coupled with the low availability of nutrients, microaerophilic environment, low pH and, finally, pressure in packaged product due to CO_2 content all together mean that contaminating microorganisms must employ compensatory mechanisms to survive in this milieu. When successful microbial growth in beer does occur, the result is a spoiled product with unappealing off-flavours and an altered sensory profile, ultimately leading to issues with brand-confidence and economic loss for brewers globally (2).

Isolated beer-spoiling organisms (BSOs) frequently belong to the *Lactobacillus* or *Pediococcus* genera, both of which are classified as lactic acid bacteria (LAB) (**19, 21**). The ability to spoil beer, however, is not intrinsic to all members of a given species in each genus,

and is instead *isolate-specific*. This indicates that BSOs have undergone a degree of genetic specialization relative to non-BSO LAB isolates of the same genus and species. LAB are a diverse group of organisms and given species can be isolated from a number of environments. For example, *Lactobacillus plantarum* isolates can be found not only in spoiled beer, but also in spoiled meats and the human intestinal microbiome (**14**). Due to the varied nature of these environments and frequency with which specific bacterial species are found within them, opportunity arises for genetic material from different species or isolates to be shared with neighbouring cells. With specific reference to BSOs, transfer of genetic material likely occurs with some frequency in breweries where microbial biofilms containing LAB occur (**12, 13, 30**). The close physical proximity of cells within biofilms is highly conducive to plasmid transfer events between cells, supporting the hypothesis that beer-spoilage ability is largely mediated by plasmid-encoded genes.

Only a limited number of genes thus far have been proposed to confer the ability to tolerate hops, and thereby are considered relevant to bacterial beer-spoilage. These include *horA* (**23**), *horC* (**28**), and *hitA* (**9**), all of which were initially found on plasmids and are purported to participate in counteracting the antimicrobial effects of hops. Through the action of bitter-acid compounds such as isomerized α-acids which function as protonophores, hops are able to dissipate the bacterial cell's trans membrane pH gradient, thus depleting the proton motive force (PMF) and killing the organism (**24, 26**). The hop-resistance genes are proposed to counteract the action of hops by removing iso-α-acids from the cell through ATP-binding cassette-type multidrug resistance transporters such as HorA (**23**), or through PMF-dependent multidrug transporters such as HorC (**10**) and its transcriptional regulator HorB (**11**). Involvement of HitA in hop resistance is believed to be through divalent cation transport (**9**).

Despite several reports on the role of *hitA*, *horA*, and *horC* in hop tolerance and beer-spoilage ability in specific BSOs (**6, 11**), these plasmid-based genes are not found in every BSO, nor are they in a consistent combination when present (**7, 27**). This indicates that hop tolerance and, by extension, the overall ability to grow in beer are not mediated solely by the products of these three hop-tolerance genes. Rather, it seems likely that there are other, as of yet uncharacterized, beer-spoilage-related (bsr) gene products that facilitate LAB growth in beer compared to isolates of the same LAB species unable to grow in beer. These novel gene products may well function in conjunction with plasmid-coded HorA, HorC, and/or HitA, and are hypothesized to similarly be derived from genes found on plasmids.

To test this hypothesis and investigate the role plasmids (i.e., the genes encoded on specific plasmids) have in the beer-spoiling phenotype, we employed a reverse-genetics approach to analyze the effects of plasmid loss on *Lactobacillus brevis* BSO 464 (Lb464), the most rapidly growing (i.e., most virulent) BSO within our culture collection of over 200 LAB isolates (**3, 25**). This bacterium is capable of spoiling beer very quickly, as it establishes growth in degassed beer within 24 h. We have sequenced the genome of Lb464 (unpublished data) and determined that it contains the *horA, horC,* and *hitA* bsr genes located across three of its eight plasmids (Table 4.1.). We intentionally cured Lb464 of its endogenous plasmids in various combinations and assessed the resultant Lb464 plasmid variants for altered hop tolerance and growth phenotype in beer.

4.3. MATERIALS AND METHODS

4.3.1. Plasmid variants

Full plasmid sequences of the original strain of *L. brevis* BSO 464 ($Lb464^{OG}$) were obtained following genome sequencing via the Roche 454 Genome Sequence FLX platform. Paired and unpaired reads were obtained during two separate runs, resulting in a final coverage of ~30X. Genome assembly was done using Newbler GS *De Novo* assembler (v. 2.5.3), and contigs were joined using via sequencing of polymerase chain reaction (PCR) amplicons. Plasmids were fully circularized using various bioinformatic approaches to identify plasmid sequences (Table 4.1), with confirmation done by PCR (Fig.4S1[2] and Table 4S2). Plasmid variants of the original strain of Lb464 ($Lb464^{OG}$) were derived by incubating in De Man-Rogosa-Sharpe broth (**20**) (MRSB; pH 5.5; Lactobacilli MRS Broth, Difco, BD Diagnostic Systems, Franklin Lakes NJ, USA) containing a sub-lethal concentration of the DNA-gyrase inhibitor and plasmid-curing agent novobiocin (sodium salt ≥ 90% pure, Sigma-Aldrich St. Louis, MO, USA) (**5**). $Lb464^{OG}$ was first incubated in MRSB at 30°C for 48 h and 10 µl of this culture was transferred into 12 mL of MRSB containing novobiocin at levels ranging from 1 to 20 µg/ml. These cultures were then incubated for 48 h at 30°C. Cultures with visible growth at higher concentrations of novobiocin were streaked on MRS agar plates and incubated for 4 days at 30°C. Individual colonies were resuspended in 40 µl of autoclaved reverse-osmosed deionized H_2O and vortexed at high speed for 20 s. These cell preparations served as template for downstream multiplex PCRs as described below to assess the plasmid

[2] Fig. 4S1 is presented in Chapter 6 as part of Fig. 6.1.

Table 4.1. Lb464 Plasmids

Plasmid	Size (bp)	Accession Number	Genes of Interest	CDS [a]	Hypothetical proteins [b]
pLb464-1	15,324	CP005978	*horA*	17	4
pLb464-2	28,459	CP005979	*horB*, *horC*	39	13
pLb464-3	21,365	CP005980	*hitA*	25	4
pLb464-4	84,941	CP005981	Phage-related proteins, ABC transporters	120	50
pLb464-5	10,867	CP005982	Toxin/antitoxin system	10	3
pLb464-6	5,018	CP005983	Cadmium transporter	8	1
pLb464-7	2,353	CP005984	Cryptic plasmid [c]	1	0
pLb464-8	49,838	CP005985	Xyloside transporter, ferrodoxin reductase	47	9

[a] Number of coding sequences as annotated by (**16**).

[b] Number of hypothetical proteins as annotated by (**16**).

[c] Cryptic plasmid with only one protein encoded for its own replication.

Table 4S1. Lb464 Plasmid Multiplex PCR primers

Plasmid	Primer Name	Sequence (5'→ 3')	Amplicon (bp)	5' Binding Location (bp) [a]
Multiplex A				
pLb646-2	P2-F	GTTACACAGAAACCCGTCAG	291	17537
	P2-R	CCACGCCAATGCCACTAG		17827
pLb464-5	P5-F	GCTAACCGCTACCAAATCAAC	526	3195
	P5-R	GGATAACACGAACCGAGAAGA		3720
pLb464-6	P6-F	GAGCTATTAAGCCGACCATCCG	432	3573
	P6-R	TCTCTTCTCCGTCACTATCACC		4004
pLb464-7	P7-F	TTCACCCAAACCTGACAAGC	179	175
	P7-R	CGTAGATAGCCTTATTGAGCCG		353
pLb464-8	P8-F	CTAAGGATTGTTGCGGTCTTGG	643	29279
	P8-R	CAACCAAATTCGCCATAGCC		29921
Multiplex B				
pLb464-1	P1-F	TTCAAATCCGTTCCAGTCAG	372	3914
	P1-R	CGAAGTCCGTCTAGTCAAATCG		4283
pLb464-3	P3-F	CAGAATAACGGCAACCAGTGTC	562	2712
	P3-R	GAGCGTAGCAGAAGAACCTAAG		3273
pLb464-4	P4-F	ATCAGTCTTTGGAGGACAGC	668	18012
	P4-R	CCTTGCTTGCTTCGTCAGTA		17345
16S rRNA gene	386F [b]	CTGATGGAGCAACGCCGCGT	148	Chromosomal
	534R [c]	ATTACCGCGGCTGCTGG		

[a] Primers were designed to plasmid specific regions such that they could be distinguished from other plasmids and chromosomal elements.

[b] Described in (**7**).

[c] Described in (**15**).

Table 4S2. PCR primers used for validation of full plasmid sequences

Plasmid; Size (bp)	Primer Name	Primer Sequence (5' → 3') [a]	Amplicon (bp)	5' Binding Location
pLb464-1 15,324	LbC132-1F	TCCAGACGACATTGCTGG	1,601	7713
	LbC133-1R	AGTTCTGAGTTCTGAGTGTTGG		9313
	LbC133-3F	ACCTTGGCTTACCATTGAAGTTC	2,570	13521
	LbC132-3R	CTTGTGAGAGTAAGACTAAAATGGG		666
pLb464-2 28,459	LbC134-4R	AATCGCATATTCTAACGAACCTTC	1,751	1519
	LbC135-1R	ACATGGAATTTCCCCATCTCG		3269
	LbC135-1F	GAAGGCAGCACATATTCAGC	432	7957
	LbC136-1R	AGCCAATGCCATATTCTCTGG		8388
	LbC136-2F	TTGGTATCGCAATCGCTGC	1,649	10131
	LbC137-1R	GCACCCAAAGCATACTCCC		11779
	LbC137-2F	GCCAATCTCGAATCATTCATCG	1,203	15046
	LbC138-1R	TCAATCGAAGAGAGGGATTCC		16248
	LbC138-2F	TCCTGTAGCACTTGTCAACG	354	17215
	LbC139-1R	ATCTGTGCAGGGTGAACG		17568
	LbC139-2F	GTGGCGACCTTCCTTATCATC	1,374	24337
	LbC140-3R	AACCTCAAGACGAGACCC		25710
	LbC140-2F	GCAAGACTATTCGACAACTTTCAG	1,975	26976
	LbC134-3F	AATACTAACCAGTGCTAGTACGATC		491
pLb464-3 21,365	LbC141-1F	GACACGATAAAGAGTCTAGCTTAC	1,437	9744
	LbC142-1R	CGAGATTACCGTTATGGAGCG		11180
	LbC142-2F	ACCTGTATTTCGGGACACATC	713	12647
	LbC143-1R	ATTGTCGCCCTGTAACCG		13359
	LbC143-2F	CCGCTTGTCTGTGATATGTC	1,309	14221
	LbC144-1R	GCACTTTACGGCACACAATC		15529
	LbC144-2F	GTTCATCTTCGCTTAGTTCAACTG	1,804	19785
	LbC141-2R	TGACACCTTGGTTGTGGC		223
pLb464-4 84,941	LbC121-1F	TCCCTGGAATCAGCTATTTGG	569	68517
	LbC122-1R	GTGTACTGTGTACGGTGTACTG		69085
	LbC122-1F	TGATCTCTGGCAGTACACAG	1,415	76931

	LbC123-1R	GTCGGCCGATAACGATAACAG		78345
	LbC123-2F	CTGCTTGTATCAAGGGATTGGG	1,902	78865
	LbC124-2R	GCCATCACTACCAAATGTACG		80766
	LbC124-3F	GTGTGCTGCTTTGACTGGTTG	1,146	84446
	LbC149-3F	TCTACTTAACAACATTGGCTGGC		650
	LbC149-1R	TGATACCAGCCGCAATCG	1,204	2151
	LbC148-2F	CGACTGTGCTCCTTACCG		3354
	LbC148-1R	CTCTTTGGAACTCACACGAGC	260	5577
	LbC147-1F	GCCTGCTATGCAATACTAGACC		5836
	LbC146-1R	CCTCGTTTCACTCGGTCTTC	1,686	15301
	LbC145-1F	GACTCCCTGCGAATACAGG		16986
	LbC145-3R	GAAGATATTCACTAACGACTTCTGC	893	18240
	LbC120-1R	CAACCATGATTAGGACAAGTTCAG		19132
	LbC150-1F	GCGATTCTATGGTTATCAGCAG	738	289
	LbC151-1R	GGAACTAACAGCTTATGCAACAAG		1027
pLb464-5	LbC151-2F	ACCCGCCAACATGAGTTG	1,318	7404
10,867	LbC152-1R	CTGCTCGAAGAATACAAGGC		8721
	LbC152-3F	CTGTATATCTTGGGAGAGTAAGGC	815	10173
	LbC150-3R	GTCTGCTGATAACCATAGAATCGC		312
pLb464-6	LbC153-1F	TACAAGCGGACTAGCACAAATG	382	4899
5,018	LbC153-1R	CAATTCATGGGGTTTGGCAAG		262
pLb464-7	LbC155-1F	GAACAAGTTCCCAATCCAAATC	614	2093
2,353	LbC155-1R	CGTAGATAGCCTTATTGAGCCG		353
	LbC125-1F	GCTGATGGAACAAGGCTATG	1155	2160
	LbC126-1R	CAACGGCAGTTATGAGCG		3070
	LbC126-1F	TGTAATGAGGTCTGGCAGG	1,764	11216
	LbC127-1R	TCACACAAGCAATCATGGC		12979
pLb464-8	LbC127-1F	ATTTATGCCTGCCCTCCTG	1,878	16183
49,838	LbC128-1R	TGGATGAGATGCTTGAACAC		18060
	LbC128-2F	GGACGAAGCCTTAATGACCA	2,248	25427
	LbC129-1R	GCTCACTTGCTGGATCTCAC		27674
	LbC129-2F	CTCCTAACTTACGCTGTTCC	1,483	32069
	LbC130-1R	ATGGCATCTGGCTCAACAC		33552

LbC130-1F	TCATTTTGTACCACAGTATGCA	2,340	42090
LbC131-1R	TCACTCATCTAATCGCCTCC		44429
LbC131-3F	GCACCGTATCTACTTCCCAATGACC	1,909	47623
LbC125-3R	CAAGTGATACTAGCCCACTAAACTC		49531

[a] Primers were designed previously during genome assembly of Lb464 to amplify sequence regions between contigs and circularize plasmids. Based on primer binding locations and size of plasmids, plasmid sequence is verified every ~7 Kb for pLb464-1; ~4 Kb for pLb464-2; ~5.3 Kb for pLb464-3; ~10 Kb for pLb464-4; ~3.6 Kb for pLb464-5; ~5 Kb for pLb464-6; ~2 Kb for pLb464-7; and ~7 Kb for pLb464-8.

profile of cells comprising individual colonies. If incubation with novobiocin was successful at curing the cell of plasmids in a novel combination, 10 µl of the resuspended colony was inoculated into 8 mL of MRSB and grown overnight at 30°C. From this culture, 25% (v/v) glycerol stocks were made and stored at -80°C.

4.3.2. Multiplex PCR

The presence or absence of each of the eight Lb464 plasmids was determined through use of two multiplex PCRs (Table 4S1). PCR Multiplex B contained primers for the 16S rRNA geneto verify the presence of bacterial DNA (**7, 15)**. All primers were designed and ordered using the Oligoanalyzer program provided by Integrated DNA technology (IDT, website: http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/). Absence of alternate bindingsites in the Lb464 genome was confirmed via local BLAST (**1**). Each multiplex PCR was prepared as a 15 µl reaction consisting of Bioline MyTaq Reaction Buffer, 0.3 U of MyTaq HS DNA Polymerase (Bioline, Taunton, MA, USA), 0.2 µM of each plasmid primer, or 0.1 µM of the 16S rRNA primers (in Multiplex B), and 1.2 µl of a resuspended Lb464 colony as template. Negative reactions were prepared that did not contain Lb464 cells to ensure the multiple PCRs were contamination-free. All multiplex reactions were run in a 2720 Thermal Cycler (Applied Biosystems, Life Technologies, Carlsbad CA, USA). The PCR program consisted of 95°C for 1 min, followed by 35 cycles of 95°C for 15 sec, 57°C for 15 sec, and 72°C for 1.5 min, and a final elongation step for 2 min at 72°C. Multiplex PCR amplicons were visualized on 1.5% agarose gel by electrophoresing 12 µl of each PCR sample for 40 min at 130 volts. Gels were stained in 0.5 mg/ml ethidium bromide and visualized using a BioRad Gel Doc XR UV camera.

To verify that plasmid variant profiles were correct, primers previously designed for the PCR gap-closing steps of plasmid assembly were used to verify the presence of complete plasmid sequences and to verify the absence of those plasmids detected as missing via the multiplex screen (Table 4S2). Verification PCR's were performed and visualized using the same reagents and equipment described above, except for a PCR annealing temperature of 55°C for 1 min with a 2.5 min elongation step at 72°C being used. To confirm that hop-tolerance-related genes *hitA*, *horA*, and *horC* did not remain within plasmid variants that had lost the plasmids, carrying these genes, a previously described hop-tolerance multiplex-PCR was utilized (**7**).

4.3.3. Hop-gradient agar plates

Hop tolerance was evaluated using hop-gradient agar (HGA) plates, described previously in (**8**), except that the hop gradient ranged from 0 to 135 bitterness units (BU). Hop and ethanol gradient agar (HGAE) plates were prepared in the same manner as HGA plates, but with 5% (*v/v*) ethanol added to both agar layers. Control plates were prepared that contained no hops or ethanol in either agar layer. All isolates were grown in modified MRSB (mMRSB; pH 5.5, no Tween 20 present) for 24 h at 30°C and then stamped onto gradient agar plates via the edge of a sterile glass microscope slide. A stamp of sterile H_20 was included on each plate as a negative control for growth. Stamped cultures were allowed to dry for 30 min on the agar surface under sterile conditions, and the plates were then taped closed on two sides and incubated upside down for 48 h at 30°C in a candle jar. Hop tolerance was measured as growth distance (assessed in 0.5 cm increments) across the hop gradient having a maximum allowable growth distance of 6 cm.

4.3.4. Ethanol Tolerance

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol for each plasmid variant was assessed in three separate trials, according to the method described in (**4**). In short, variants were incubated in MRS broth containing varying concentrations of ethanol at 30°C and after one week assessed for the lowest concentration that inhibited visible growth (the MIC) and the highest concentration from which viable cells could be recovered (the MBC).

4.3.5. Growth in degassed beer

Assessment of growth kinetics of $Lb464^{OG}$ and its plasmid variants was performed in a degassed (i.e., non-carbonated) lager beer (pasteurized 5% (*v/v*) ethanol beer, pH 4.2, and 11 bitterness units). Degassed beer was prepared on the day of use by pouring fresh 4°C beer (commercially bottled beer) successively between beakers 30 times (**4**). The poured beer was partially covered and incubated under sterile conditions for 2 h at room temperature, and then subjected to another 30 successive pours. Bacteria taken from -80°C stocks were first grown for 48 h in MRSB at 30°C, and 20 µl were used to inoculate 12 mL of 85/15 medium (85% (*v/v*) beer and 15% (*v/v*) double-strength mMRSB). Following incubation at 30°C for 48 h, 100 µl of this culture were placed into 16 mL of degassed beer and incubated at 30°C. This process normalized starting bacterial populations to the same inoculation level (between 5.0

and 5.5 log CFU/ml), to allow for comparable growth analysis. To assess bacterial growth over time, 50 µl of degassed beer culture were sampled every 12-24 h up to a maximum of 14 days and colony forming units (CFU) determined by plating on MRS agar using the drop-plate method (**10**). CFU were enumerated after incubation at 30°C for 4 days. Individual colonies were selected at various time-points along the growth curve and screened via the two multiplex PCRs to verify that the plasmid profile of the variant under analysis was maintained. Growth of each plasmid variant in degassed beer was assessed on two separate occasions, each done in duplicate with the exception of $Lb464^{45}$ and $Lb464^{5}$ growth, which were each performed once in duplicate.

To assess growth kinetics, the lag phase and mean generation time were calculated and averaged across trials for each plasmid variant. Lag phase was determined by extrapolating back from two points along the exponential phase of the growth curve to the CFU level of the starting inoculum and determining the corresponding time value. Mean generation time (time required for CFU numbers to double during exponential growth) was calculated by dividing the time interval of the exponential phase, by the value of $g = (log_{10}N_t - log_{10}N_0) / log_{10}2$, where N_o and N_t are initial and final CFUs of the exponential growth phase, respectively.

4.3.6. Statistical analysis

Levene's tests for homogeneity of variance was first performed, followed by one-way ANOVA, and Tukey HSD post-hoc tests (SPSS for Windows, Version 19.0, IBM SPSS Statistics) to assess differences in hop tolerance and in mean generation times between all variants at an $\alpha = 0.05$ significance level. A paired-T test ($\alpha = 0.05$) was employed to assess the difference between the mean growth distance of plasmid variants on HGA and HGAE plates.

4.3.7. Plasmid copy number

The plasmid copy number (PCN) of Lb464 plasmid variants when grown in degassed beer was determined via quantitative real-time PCR (qPCR). Cells in mid-exponential growth in degassed beer were harvested by centrifugation. DNA was then extracted using the MoBio UltraClean Mirobial DNA Isolation Kit according to manufacturer's specifications, however, with an optional heating step for 10 min at 70°C to increase cell lysis efficiency before bead-beating. To linearize DNA template for use in qPCR reactions, 300 ng of DNA was digested

with 20 U of restriction enzyme PmlI (New England Biolabs) for 16 h at 37°C followed by a heat inactivation step at 65°C for 20 min. To ensure full digestion of template, ten-fold serial dilutions of digest material were made and then assessed via PCR using primers that spanned the Pml1 cut sites (**18**). Three biological replicates were analyzed using three technical replicates in two different trials, with 6 ng of digested DNA used in each qPCR reaction. A technical replicate was excluded from analysis if the Cq value contributed to a standard deviation greater than 0.02 for that triplicate set. Previously validated and described primers for two single-copy chromosomal reference genes *proC* and *pcrA* (**3**) were utilized, and qPCR primers specific for each plasmid are listed in Table 4S3**.** The absolute number of each plasmid was determined from standard curves of each plasmid amplicon and this value then was divided by the average of the absolute copy number of two chromosomal reference genes, as outlined in (**18**).

4.4. RESULTS AND DISCUSSION

4.4.1. Derivation of plasmid variants

Treatment with sub-lethal concentrations of novobiocin was an effective means of curing Lb464 plasmids from Lb464 cells. A total of 160 individual colonies were screened with the multiplex PCRs to reveal ten unique plasmid variants (Table 4.2). While larger plasmids are suspected to be more easily lost due to being at presumed lower copy number, it should be noted that pLb464-4 and -8, the two largest Lb464 plasmids, actually were not the ones most easily lost. Instead, pLb464-1 and pLb464-3, which harbour *horA* and *hitA*, respectively, were the most frequently undetected plasmids, being easily driven from the cell at novobiocin at concentrations below 15 µg/ml.

PCRs that targeted plasmid sequence every several thousand base pairs, and a hop-tolerance gene multiplex were used to ensure that individual plasmid variants were not false positives (i.e., remaining plasmids were incomplete), nor false negatives (fragments of “missing” plasmids remained within a plasmid variant). Although this PCR-validation can not rule out the presence of single base changes, small truncations in gene sequence or small sequence rearrangements within genes, it shows to a high degree of confidence that the basic sequences of all plasmids were not altered during plasmid-curing and that this process was efficient at removing entire plasmids.

Table 4S3. Primers used for qPCR analysis of PCN

Plasmid	Primer Name	Primers (5'→ 3') [a]	Amplicon (bp)
pLb464-1	qP1-F	GTTCCAGTCAGACGATCTTCTTC	112
	qP1-R	GTCCATTTGCGGTTCTCGG	
pLb464-2	qP2-F	CACAGAAACCCGTTCACCC	144
	qP2-R	GAACACTTTGTTGCTGTGCG	
pLb464-3	qP3-F	AGGCTGGAGATTAAGATAACCG	137
	qP3-R	CGAACATTATTTGCCTACACC	
pLb464-4	qP4-F	TGAGGTTCCGAACAGGG	102
	qP4-R	CCGCAACTTCGCCTGC	
pLb464-5	qP5-F	CGTCTCCAGAATTAAGTCCAC	149
	qP5-R	CGAATACGGGGATTCCAACC	
pLb464-6	qP6-F	GGTTCCGAGGCTCAACG	167
	qP6-R	CTAATAAGTCTAATGCTGTCGC	
pLb464-7	qP7-F	CAAACCTGACAAGCTGAACC	127
	qP7-R	GAGGCTTTAAGGAGTTGATAGAC	
pLb464-8	qP8-F	CATTCAACTGCTGGTTCTG	133
	qP8-R	CAAGACTTTGATTTAGGCACG	

[a] qPCR primers were designed to amplify a smaller region (100 – 250 bp) within the amplicon produced in the Multiplex PCR (Table 4.1.) to be suitable for qPCR analysis.

Table 4.2. Growth kinetics of Lb464 and Lb464 plasmid-variants in degassed beer

Plasmid Variant [a]	**Plasmids Absent**	**Hop-tolerance Genes Present**	**Lag Phase** [b] **(h) ± SD***	**Mean Generation Time (h) ± SD**
$Lb464^{OG}$	None	*hitA, horA, horC*	≤ 8	5.1 ± 1.0
$Lb464^{1245678}$	1	*horA, horC*	≤ 8	5.3 ± 1.3
$Lb464^{245678}$	1, 3	*horC*	≤ 8	5.2 ± 1.1
$Lb464^{24578}$	1, 3, 6	*horC*	≤ 8	6.4 ± 1.1
$Lb464^{2458}$	1, 3, 6, 7	*horC*	≤ 8	7.2 ± 1.8
$Lb464^{245}$	1, 3, 6, 7, 8	*horC*	≤ 8	6.5 ± 1.4
$Lb464^{258}$	1, 3, 4, 6, 7	*horC*	20 ± 2.0	10.3 ± 2.5
$Lb464^{458}$	1, 2, 3, 6, 7	None	89 ± 5.0	14.4 ± 4.5
$Lb464^{58}$	1, 2, 3, 4, 6, 7	None	74 ± 6.0	12.4 ± 2.0
$Lb464^{45}$	1, 2, 3, 6, 7, 8	None	105 ± 5.0**	19.1 ± 3.0**
$Lb464^{5}$	1, 2, 3, 4, 6, 7, 8	None	108 ± 4.0**	15.1 ± 2.5**

[a] Superscripted numbers indicate the plasmids present in each variant.

[b] Lag phase was calculated by extrapolating the slope of the exponential growth to the corresponding X-axis value (time in h) at the level of the starting inoculum.

* SD, standard deviation

** N = 2 for these variants. N = 4 for all other variants analyzed.

Despite efforts, pLb464-5 could never be cured from cells. This indicated pLb464-5 likely carries genes necessary for survival. Genetic annotation via RAST (**16**) revealed that in addition to a Type I restriction-modification system, pLb464-5 also contains three hypothetical proteins. Two of these hypothetical proteins show homology to the Xin-Antitoxin System, Toxin component RelE family of *L. brevis* KB290 (NCBI Reference Sequence Accession: YP_007655365) and to the Antitoxin component RelB/DinJ family of *L. brevis* subsp. *gravesensis* ATCC 27305 (GenBank Accession: EEI71732). As both toxin and antitoxin are encoded on this genetic structure, pLb464-5 would be critical to keep within cells in order to provide the antitoxin when in the presence of other Lb464 cells producing the toxin.

The derivation of a plasmid variant harbouring only plasmids 2 and 5 (i.e., Lb464^{25}) was desired for comparison against Lb464^{245}, Lb464^{258}, Lb464^{458}, Lb464^{58}, Lb464^{45}, and Lb464^{5} to more clearly elucidate the potential roles of pLb464-4 and pLb464-8. However, such an isolate was not detected in screening of 30 colonies following routine novobiocin treatment of Lb464^{245} and Lb464^{258} (with hopes that pLb464-4 or-8 were cured from the cells); nor was it obtained in subsequent trials wherein an additional 20 colonies were screened following incubation of Lb464^{245} or Lb464^{258} in 85/15 medium or 1st degassed beer with 25 µg/ml novobiocin.

4.4.2. Hop tolerance of plasmid variants

Ability to deal with the antimicrobial effects of hop compounds is believed to be a major physiological and therefore genetic requirement of BSOs. Hop tolerance was determined using HGA plates containing a hop concentration of 135 BU at the top of the gradient. Fig. 4.1. shows the growth distances on HGA plates of the ten Lb464 plasmid variants relative to the parent strain across five trials. As growth is read to the nearest 0.5 cm increment on the plate grid, all isolate data sets were assessed by calculating Cook's Distance for potential data outliers, which led to one data point for isolates Lb464^{245678}, Lb464^{24578}, and Lb464^{258} being replaced with the average growth distance for the remaining four trials in each case. A Levene's test for homogeneity of variance within the mean growth distances for all isolates across trials demonstrated homoscedasticity (P = 0.99). A one-way ANOVA test was therefore conducted, and a significant difference was found among the mean growths of the plated isolates (P < 0.05). A post-hoc Tukey HSD test was performed to discover which

plasmid variants exhibited significantly different growth from others. The results show that the growth means of isolates Lb464^{458}, Lb464^{58}, Lb464^{45} and Lb464^{5} did not differ from each other, but each were significantly lower than the growth means of Lb464^{OG} and the other plasmid variants ($P < 0.05$). While the loss of pLb464-3 (containing *hitA*), and added loss of pLb464-1 (containing *horA*) did decrease the hop-tolerance level, this change is not statistically significant compared to Lb464^{OG}, indicating that *horA* and *hitA* are not the primary hop-tolerance genes within Lb464. Though this contrasts with previous studies wherein the loss of *horA* was shown to dramatically decrease hop-tolerance of beer-spoilage isolate *L. brevis* ABBC45 (**22**), its does align with further studies that found the combined loss of *horA* and *horC* from *L. brevis* ABBC45 resulted in a non-hop tolerant (i.e., non-beer-spoilage) variant (**29**).

Our present results clearly demonstrate that the loss of pLb464-2 (containing *horC*) has the most profound effect on Lb464 hop tolerance (Fig. 4.1), with those plasmid variants containing pLb464-2 having significantly higher hop tolerance than those variants lacking it ($P < 0.01$). This fits with our previous transcriptional analyses that demonstrated that among *horA, horC,* and *hitA, horC* was the only putative bsr gene utilized by Lb464 during mid-exponential growth in beer (**3**). These two separate studies collectively indicate that there exists a hierarchy for which hop-resistance mechanism is most efficacious among present bsr genes, with *horC* essentially being solely relied upon when all three genes are present. This observation raises the questions as to why slightly enhanced beer-spoilage ability is observed when more than just one bsr gene is present within a BSO (**3**, **26, 27**) and why Lb464 retains all three bsr genes when they are harboured on different plasmids and clearly not equally contributing to hop resistance? One possible explanation is that the mechanism of action of *hitA* and *horA* is only to contribute low-level or basal support to the anti-hop response if *horC* is present, i.e., in helping maintain the PMF. Alternatively, these two genes may have a role in the uptake of compounds in beer that facilitate the overall bacterial anti-hop response.

In a similar vein, the role other plasmids play in hop-tolerance is also highlighted. When pL464-6 and -7 are lost in addition to plasmids pL464-1 and -3, no further decrease in hop tolerance is seen (e.g., Fig. 4.1; Lb464^{2458} compared to Lb464^{245678}). As plasmids pLb464-6 and -7 are small with limited coding capacity (with pLb464-7 being a cryptic plasmid; Table 4.1), this is not surprising. In contrast, the subsequent loss of either pL464-4 or -8 from Lb464^{2458} to Lb464^{245} or Lb464^{258} leads to a further significant reduction in hop tolerance

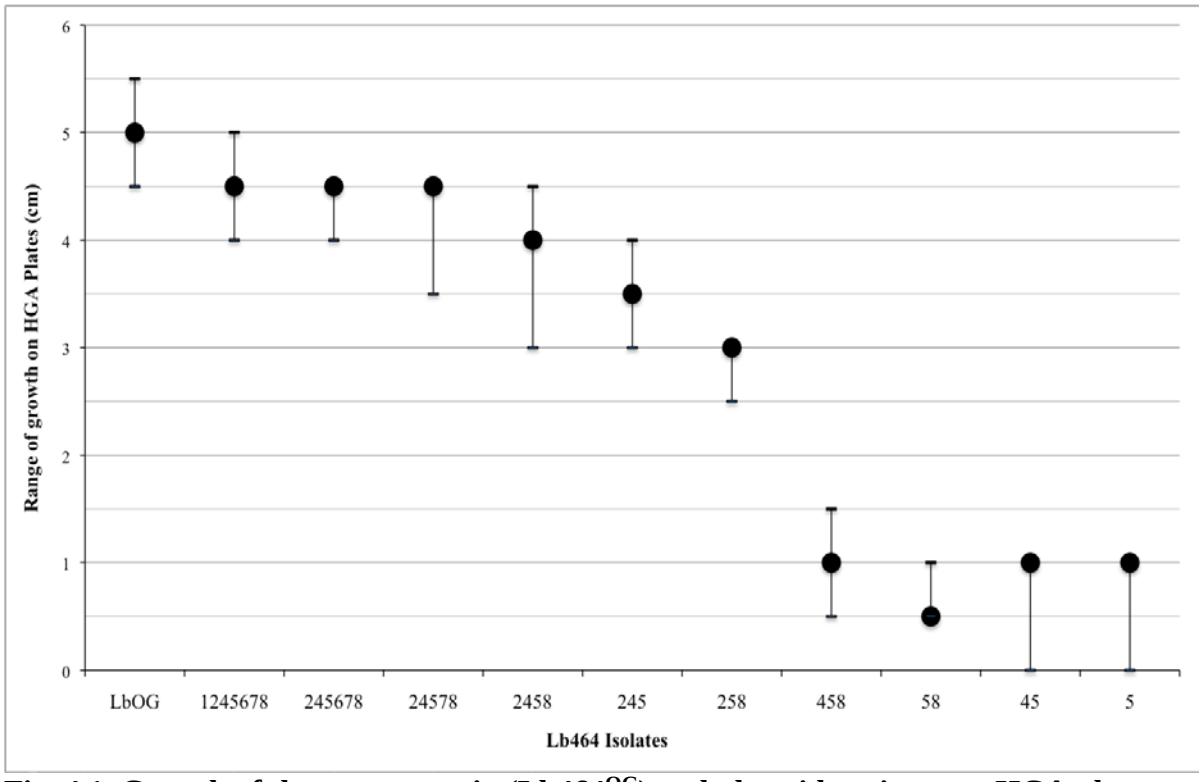

Fig. 4.1. Growth of the parent strain (Lb464^{OG}) and plasmid variants on HGA plates.

The hop gradient ranged from 0 to 135 BU. Higher hop tolerance is reflected by a greater growth distance across the gradient of hops. Control plates without hops showed growth across the entire 6 cm length of the plate. N = 5 for all strains; median and range of values are shown.

relative to the parent strain ($P < 0.05$). These two plasmids are the largest plasmids in Lb464, each encoding a large number of hypothetical proteins (particularly so for pL464-4; Table 4.1). As such, it is not surprising to find that loss of either plasmid has a negative effect on hop tolerance. However, we do not see the importance of pL464-4 or -8 highlighted when we compare growth of Lb464^{458} and Lb464^{58}, Lb464^{45} or Lb464^{5} on HGA plates (Fig. 4.1). This is likely due to the lower level of hop tolerance exhibited by Lb464 now lacking pL464-2, regardless of which other plasmids remain. Finally, the residual and essentially equivalent low level of hop tolerance exhibited by Lb464^{458} and Lb464^{58}, Lb464^{45} or Lb464^{5} indicates two additional facts; that pL464-5 plays no role and that the Lb464 core genome plays at best a minimal role in the high hop tolerance exhibited by Lb464^{OG}.

It is clear overall that having more plasmids correlates with better hop tolerance for Lb464 (Fig. 4.1). While the roles played in Lb464 hop tolerance by pL464-4 and -8, and certainly pL464-1 and -3, are much less dramatic than that seen for pL464-2, these four plasmids appear to contain genetic coding capacity that contributes to the *robustness* of the Lb464 anti-hop response. These plasmids may enhance the hop-tolerance capability of Lb464 by containing novel hop-tolerance-related genes (potentially efflux proteins, or membrane transport or modification proteins). Alternatively, these Lb464 plasmids likely contain genes with yet-uncharacterized mechanisms for dealing with hops, or for mechanisms that act in synergy with the hop-tolerance mechanisms coded on pLb464-2 (including *horC*). An intriguing possibility, though not confirmed via HGA plate analysis, is that genes on Lb464 plasmids 1, 3, 4, and 8 may play a more central role in handling the other stressors found in beer such as acid pH, carbohydrate starvation and dissolved CO_2 (in gassed beer).

Finally, since we have previously shown some BSO isolates demonstrate enhanced hop tolerance if ethanol is present (**8**), the hop tolerance of Lb464^{OG} and the ten plasmid variants was also assessed with hop gradient plates containing 5% (v/v) ethanol. Results (not shown) indicate that the presence of this level of ethanol in addition to the challenge of hops does not significantly affect the parent isolate's nor any plasmid variant's ability to tolerate hops. Considering that the ethanol tolerance of Lb464 is quite high (MIC ~ 20% *v/v*) this result is not surprising given the lower ethanol level used in testing for enhancement of hop tolerance.

4.4.3. Ethanol tolerance of plasmid variants

The assessment of ethanol MIC and MBC for all variants revealed that these levels do not differ significantly from the full plasmid strain (data not shown). Though variants Lb464^{245}, Lb46^{258}, Lb464^{458}, Lb464^{58}, Lb464^{45}, and Lb464^{5} exhibited slightly lower MIC and MBC levels when assessed after 7 d and 9 d respectively (15-18% compared to 20%), all variants except Lb464^{45} and Lb464^{5} did eventually exhibit ethanol tolerance levels equivalent to Lb464^{OG}; i.e., after additional incubation time (~ 4 d). These observations indicate that the initial lower MIC and MBC of some variants, and correlation with their prolonged lag phase in degassed beer, is likely due to general decreased cell fitness as a result of carrying fewer plasmids. Ultimately, Lb464 ethanol tolerance cannot be attributed to the presence of a specific plasmid, but rather it is intrinsic to the cell and largely mediated through chromosomal genes.

4.4.4. Growth rate analysis of plasmid variants

To delineate the role that individual plasmids play in the beer-spoilage capability of Lb464, the ten-plasmid variants were assessed for growth in degassed beer (Table 4.2, Fig. 4.2). Degassed beer was the chosen growth media because it can be prepared in a standardized fashion, limiting the effects of potential fluctuation in carbon dioxide level that can occur when using gassed beer, while retaining normal levels of ethanol (**4**). Paralleling the hop data, it was found that loss of certain plasmids correlated with both delayed and slowed Lb464 growth in degassed beer. Specifically, the loss of pLb464-1 (*horA*) and pLb464-3 (*hitA*) did not notably alter the growth kinetics of Lb464 relative to the parent strain (Table 4.2, Fig. 4.2). Moreover, no significant effect on Lb464 replication was observed if pLb464-6 and pLb464-7 are additionally lost, clearly demonstrating that plasmids pLb464-1, -3, -6, and -7 are not critical for the establishment of rapid Lb464 growth in degassed beer. As was the case for hop-tolerance, it was found that loss of pLb464-2 (*horC*) most greatly inhibits the ability of Lb464 to immediately establish growth in beer. Despite losing pLb464-1 and pLb464-3, variants containing pLb464-2 replicate during the exponential phase markedly faster than isolates missing pLb464-2 (i.e., variants Lb464^{458,} Lb464^{58}, Lb464^{45}, and Lb464^{5}), and do not have a prolonged lag phase (Table 4.2). This data provides further support for the significance of the *horC* gene, as well as providing impetus to further analyze the contribution to growth in beer of other genes located on pLb464-2. RAST annotation of pLb464-2 indicates the presence of an anaerobic respiratory reductase, glycosylation-related

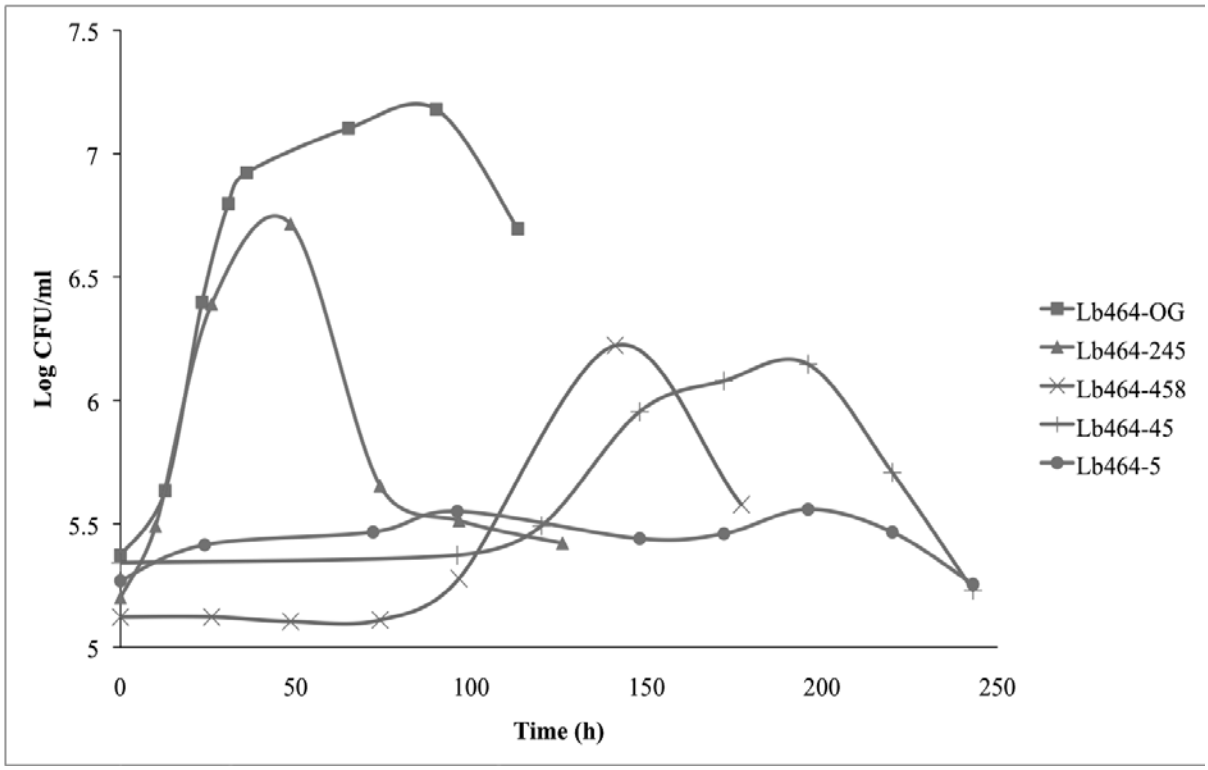

Fig. 4.2. Growth of Lb464^{OG} (parent strain), Lb464^{245}, Lb464^{458}, Lb464^{45} and Lb464^{5} in degassed beer.

Despite the loss of pLb464-1,-3,-6,-7, and -8 from the parent strain in Lb464^{245} there is maintenance of a short lag phase, albeit with a decrease in the robustness (height) of the growth curve and the length of the stationary phase (i.e., cell death occurs more rapidly). With the loss of pLb464-2, there is a dramatic increase in the lag phase (see Lb464^{458}, Lb464^{45} and Lb464^{5}). The presence of pLb464-8 results in a shortened lag-phase and more robust exponential growth for Lb464^{458} compared to Lb464^{45} (Lb464^{45} only exhibits a half-log fold increase in growth). Lb464^{5} is not capable of establishing growth in degassed beer, but does remain bacteriostatic in beer.

proteins, DNA-damage inducible genes, and 17 hypothetical proteins. These genes may directly contribute to the beer-spoilage ability of Lb464 through protein products mediating damage to the cell incurred as a consequence of specific stresses in the harsh beer environment. Alternatively, the action of these protein products may contribute to a larger synergistic network of protection mechanisms, which also involves the expressed protein products of genes located on other plasmids. Thus, the specific combination of plasmids present in conjunction with pLb464-2 may increase the overall fitness of the cell and allow for greater initial growth success.

This hypothesis is supported by the finding that the combined presence of pL4646-2 and pL4646-4 (in absence of various other plasmids) results in more successful growth in beer relative to a variant containing pLb464-2 and pLb464-8 (i.e., compare $Lb464^{245}$ to $Lb464^{258}$; Table 4.2). With pLb464-4 lost and pLb464-8 retained, there is a marked increase in both lag phase and mean generation time. One possible reason for this is that genes on pLb464-4 are better able to compensate for the absence of genes on pLb464-8, as it is the only other plasmid (apart from pLb464-2 and pLb464-8) coding for proteins involved in redox regulation and anaerobic respiration. In addition, pLb464-4 harbors genes for various ABC-transporter systems, integral membrane proteins, and a quite large number of uncharacterized hypothetical proteins that may participate in the beer-growth phenotype to a greater extent than those proteins resulting from genes localized on pLb464-8. Because pLb464-4 is the largest Lb464 plasmid (~85 Kb), it is expected to be found in low copy number in order to reduce energy burden on the cell, thus allowing $Lb464^{245}$ to grow efficiently within beer, despite the loss of other plasmids, and the genes encoded thereon.

In the absence of pLb464-2, the role of pLb464-4 and pLb464-8 is more confounding as we see those isolates containing Lb464-8 exhibit a shorter lag phase than the two isolates without it ($Lb464^{458}$ or $Lb464^{58}$ ~80 h lag phase; $Lb464^{45}$ or $Lb464^{5}$ ~ 100 h lag phase). Additionally, without pLb464-2 or Lb464-8, plasmid variants $Lb464^{45}$ or $Lb464^{5}$ do not establish robust growth in beer, showing less than a log-fold increase in CFU (Fig. 4.2). This data demonstrates that the role of pLb464-4 in the absence of pLb464-2 is negligible. In fact, it suggests pLb464-8, compared to pLb464-4, plays a larger role in establishing any growth in beer in the absence of pLb464-2 (decreased lag phase and mean generation time for plasmid variant $Lb464^{58}$ compare to $Lb464^{45}$; Table 4.2). Though pLb464-8 does not contain any putative beer-spoiling genes, it does contain nine hypothetical proteins, in addition to proteins

involved in diverse activities such as a Type 1 Restriction modification system, ferredoxin reductase (involved in redox regulation and anaerobic respiration), xyloside transport, and efflux pump systems which may provide some minimal defense for the cell against the general physical attributes of beer. Thus, in the absence of pLb464-2, the presence and/or activity of one or more of these pLb464-8 proteins appears to be more greatly relied upon than are those proteins coded for by pLb464-4 to begin cell growth in beer.

Though the growth kinetics data reveal pLb464-4 and pLb464-8 as interesting players in Lb464 beer-spoilage ability depending on the presence of other plasmids, it is of interest to note that their significance was not clearly indicated by our HGA plate data. This strongly suggests that pLb464-4 and pLb464-8 do not contribute directly to hop-tolerance, but aid the cell in circumventing other growth stresses present in beer. This suggests that for a bacterium to be able to grow in and thus spoil beer, possessing hop-tolerance mechanisms is in itself not sufficient. Consequently, further investigation into the role and transcriptional activity of plasmid-encoded genes in direct response to each of the non-hop-related growth selection pressures in beer is warranted, since this research should reveal which specific genes from each plasmid, and thus which metabolic pathways, are relied upon *in toto* to circumvent the harsh growth environment posed by beer.

4.4.5. Plasmid copy number (PCN) in plasmid variants

Assessment of the PCN in plasmid variants exhibiting phenotypic differences from the original strain during growth in degassed beer was carried out via qPCR (Table 4.3.). Considered within the context of both hop and beer growth data, we see that during mid-exponential growth in beer pLb464-2 increases in plasmid variants Lb464^{245} and Lb464^{258} compared to Lb464^{OG}, while pLb464-4 and pLb464-8 increase in copy number in plasmidvariants with most other plasmids removed (Lb464^{458}, Lb464^{45} and Lb464^{58}). The increase in pLb464-8 copy number, however, does not correlate with increased growth success in beer, relative to variants with fewer copies of pLb464-8 (Lb464^{458} *versus* Lb464^{2458}). Instead, it underscores the hypothesis that pLb464-8 is important for decreasing lag phase in variants lacking pLb464-2. This data also may explain why the role of pLb464-4 in variants with few plasmids is confounding relative to its apparent importance in Lb464^{245} compared to Lb464^{258}, given that its copy number does not increase and its role in growth in beer remains stable regardless of what other plasmids are present.

Table 4.3. Plasmid copy number during mid-exponential growth in degassed beer

Plasmid Variant	Plasmid Copy Number [a,b]		
	pLb464-2	**pLb464-4**	**pLb464-8**
$Lb464^{OG}$	2.3 ± 0.1	0.9 ± 0.1	0.6 ± 0.3
$Lb464^{1245678}$	2.7 ± 0.1	0.9 ± 0.2	0.6 ± 0.3
$Lb464^{2458}$	3.2 ± 0.2	1.1 ± 0.4	1.2 ± 0.1
$Lb464^{245}$	5.7 ± 0.2	0.9 ± 0.3	--
$Lb464^{258}$	3.8 ± 0.6	--	1.3 ± 0.2
$Lb464^{458}$	--[e]	0.9 ± 0.1	1.7 ± 0.2
$Lb464^{58}$	--	--	2.3 ± 0.4
$Lb464^{45}$	--	1.2 ± 0.3	--

[a] Copy number for pLb464-1, -3, -5, -6, and -7 in $Lb464^{OG}$ were found to be 1.9 ± 0.2; 1.5 ± 0.2; 2.1 ± 0.3; 3.6 ± 0.5 and 6.2 ± 1.0, respectively. The PCN for these five plasmids did not vary significantly for any plasmid variant in which they are found (data not shown).

[b] Standard deviation calculated from the average of N = 12 to 16 reactions.

[e] Plasmid not present

Though this data highlights once more the importance of pLb464-2, -4, and -8 during growth in beer, it must be noted that an increase in copy number does not necessarily correlate to an increase in transcriptional activity for all genes on these plasmids. Further transcriptional analysis is necessary to determine which genes exhibit increased expression in conditions where PCN is increased. The results of this study show that the loss of specific plasmids can dramatically affect both the hop-tolerance and growth in beer (i.e., beer-spoilage) ability of Lb464 (Table 4.4). pLb464-2, which bears *horC* (and the gene for its transcriptional regulator HorB), was found to play the most significant role in both hop tolerance and growth in beer. In contrast, loss of pLb464-1 and -3 (which harbour *horA* and *hitA*, respectively) and -6 and -7 had a minimal effect, which indicates *horC* is utilized preferentially when all three bsr genes are present. Most importantly however, is the compelling evidence that successful LAB growth in beer is a multifactorial process requiring complex genetics beyond that contained within the bacterial chromosome.

This conclusion is firstly based on finding that Lb464, when lacking specific plasmids, loses the ability to grow in beer or even tolerate hop compounds. Secondly, the three previously described bsr (i.e., hop-tolerance) genes are not the only genes important for beer-spoilage by LAB, as the presence of plasmids pLb464-4 and pLb464-8, which do not harbour any annotated bsr genes, correlate with increased growth success of Lb464 in degassed beer. These plasmids encode protein products involved with anaerobic respiration and membrane transport, as well as other unknown proteins that may function synergistically with the products of other plasmids (most likely pLb464-2) to decrease the length of time required to achieve growth in the beer environment. What is now needed is a detailed look at transcription levels for individual genes on plasmids pLb464-2, -4, and -8 in the original Lb464 strain and various plasmid variants when grown in the stressful environment of beer. This research will further delineate the genetics utilized by LAB to be successful as BSOs. Inturn, this will indicate potential new markers for beer-spoilage capacity that can be used to improve microbial quality control within breweries.

4.6. ACKNOWLEDGEMENTS

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Table 4.4. Role of Lb464 plasmids in hop-tolerance and degassed beer-growth phenotype [a]

Lb464 plasmid	Role in hop - tolerance	Role in growth in beer
pLb464-1	No	No
pLb464-2	Yes	Yes
pLb464-3	No	No
pLb464-4	No	Auxiliary role[b]
pLb464-5	No	No
pLb464-6	No	No
pLb464-7	No	No
pLb464-8	No	Auxiliary role

[a] Role in hop-tolerance, see Fig. 4.1; role in growth in beer ; see Table 4.2.

[b] Auxiliary roles are proposed for these two plasmids as their presence and/or absence influences the beer growth kinetics of Lb464 plasmid variants. The role that these two respective plasmids have is not directed at mediating hop- or ethanol- tolerance. Instead, their gene products likely mediate other growth stresses found in beer.

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Chapter 5: Dissolved carbon dioxide selects for lactic acid bacteria able to grow in and spoil packaged beer

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5. INTERFACE

This work has been previously published in *Journal of American Society of Brewing Chemists*, 73(4): 331-338, 2015, and is reprinted here with permission. This work is the first of its kind to demonstrate that the presence of headspace pressure and thus dissolved CO_2 in a standard North American beer bottle selects for beer-spoilage virulence of known beer-spoiling LAB.

Jordyn Bergsveinson carried out the experimental design, as well as the development of the degassing method and Vernier pressure reading procedures and validation. In addition, she performed all statistical analysis, HGA plating, bioinformatic analysis, and wrote the manuscript.

Anna Redekop is credited with performing growth analysis of isolates in degassed and gassed beer at 30°C and screening of isolates for hop-tolerance genes. She also contributed to the editing of the manuscript prior to publication.

Sheree Zoerb is credited with performing validation steps of the Vernier apparatus and conducting growth analysis of isolates in degassed and gassed beer at 20°C and 30°C. She also contributed to the editing of the manuscript prior to publication.

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work.

5.1. ABSTRACT

Lactic acid bacteria (LAB) are frequently found as beer-spoilage organisms (BSOs). Correctly identifying a LAB as a BSO is problematic, given there are few known genetic markers that distinguish beer-spoiling from non-beer-spoiling LAB. Currently, genes purported to participate in hop-tolerance mechanisms are heavily relied upon to indicate LAB isolates with the potential to spoil beer, even though these genes do not consistently correlate with beer-spoilage. Though the presence of hops certainly is a significant physiological stress for bacteria in beer, we demonstrate here that the presence of CO_2 dissolved in beer is a strong selective pressure for *true* LAB beer-spoilage ability, i.e., the ability to grow in and spoil a finished and packaged beer. We screened twenty LAB for their capability to survive and grow in gassed beer at 22 and at 30°C, and discuss the results in relation to ethanol- and hop-tolerance. Functional gene comparisons of nine dissolved CO_2-tolerant and non-tolerant genome-sequenced isolates reveal potential metabolic pathways of interest for further study, specifically those that deal with cell dormancy and stress responses. These results further our understanding of LAB BSOs and have implications for how best to analyze these bacteria in laboratory settings and to test for these bacteria in the brewery.

5.2. INTRODUCTION

Bacterial beer-spoilage results in significant revenue losses for the global beer industry each year as a result of the loss of saleable product, and reduced consumer confidence and brand preference. Understanding mechanisms by which bacterial isolates spoil beer, and the ability to effectively screen for and detect these virulent isolates in a brewery setting, is critical for quality control and product consistency. Our limited understanding and appreciation of the genetic variability and complex physiology of these beer spoilage organisms (BSOs), however, limits the accuracy with which detection is performed.

Although isolates belonging to the group of Gram-positive lactic acid bacteria (LAB) are common beer-spoilers, not all isolates of a given LAB-species are capable of growth in beer (**27, 28**). This strongly indicates genetic specialization in LAB BSOs, enabling growth in the niche environment of the brewery and beer. Nonetheless, specific and universal genetic markers for beer-spoilage by LAB remain elusive. Genes involved with mediating the cell-damaging effects of hops (i.e., hop-tolerance) have long been proposed to be the main determinants of spoilage (**15, 17, 28**). However, studies have shown that there are instances

where the presence or absence of these hop-tolerance genes does not correlate with the ability or inability to grow in and spoil beer, respectively (**4, 5**). This provides cause to expand the scope of analysis beyond the stress of hop compounds and mechanisms of hop-tolerance to consider bacterial genetics related to mediating other selective stresses present in beer.

One of the last growth-adverse aspects of beer that has yet to be adequately explored is that of dissolved CO_2 (dCO_2) and the role of headspace pressure in finished, packaged beer. Although it has been reported previously that high-pressure treatment can supress the beer-spoilage capacity of *Lactobacillus plantarum* in beer (**12**), standard levels of bottle-headspace pressure and dCO_2 have yet to be broadly analyzed as determinants of beer-spoilage virulence, alongside hop-tolerance, for LAB BSOs of different genera and species. Further, it has been shown that the presence of dCO_2 limits or alters the growth kinetics of bacteria (**10, 20**), and affects transcriptional activities and physiology of food-related microorganism (**31**), however, these effects have yet to be detailed for LAB BSOs, which is surprising given that the presence of dCO_2 (and headspace pressure) contributes to the harsh nature of the beer niche environment. We hypothesized that only isolates capable of growing in a pressurized/gassed finished beer product are "true" beer-spoilers and that such isolates should be characterized for potential unique genetic elements that could be used for diagnostics in the brewery. Here we demonstrate that the presence of headspace pressure and dCO_2 are, indeed, major contributing factors to the ability of LAB to spoil packaged beer, and that dCO_2-tolerance genetics should be explored and potentially screened for in addition to hop-tolerance genes.

5.3. EXPERIMENTAL

5.3.1. Bacteria

In all, 18 LAB organisms comprised of 12 lactobacilli and 6 pediococci isolates, 17 of which were brewery- or beer-isolated, were analyzed for survival in degassed and gassed lager beer (Table 5.1). The same commercial lager beer was used in all experiments (pasteurized 5% [v/v] ethanol beer, pH 4.2, 11 Bitterness Units [BU]). Two isolates were each available as two colony morphology variants when plated on agar; namely, *Lactobacillus brevis* MC9-4g (glossy colony) and *L. brevis* MC9-4p (puffy colony), and *Pediococcus damnosus* MC9-6s (small colony) and *P. damnosus* MC9-6b (big colony). Isolates stored as -80°C culture stocks were grown overnight in MRS medium (de Man, Rogosa, Sharpe medium) at 30°C (**9**). Strains were then adapted to the lager beer environment by transferring 20 µl of an overnight

Table 5.1. Isolate dCO_2, Ethanol, and Hop Tolerance Profile.

Gassed beer survival, Isolate [a]	Presence of hitA/horA/horC[b]	Ethanol MIC [c]	Hop-tolerance[d]
No survival			
L. harbinensis MC9-2[e]	-/+/-	12	1.5×
L. helveticus CCC B1186	-/+/-	10	2×
L. plantarum CCC B1301	-/+/-	12	3. 5×
L. rhamnosus ATCC 8530[e]	-/-/-	12	1×
L. sakei ATCC 15578[e]	+/+/+	18	3.5×
P. acidilactici ATCC 8042	-/+/-	20	1×
P. claussenii ATCC BAA-344NR[f]	-/+/-	12	2×
P. damnosus MC9-3	+/+/+	12	4×
P. damnosus MC9-6s[d]	+/+/+	10	2.5×
P. parvulus ATCC 43013	-/-/-	15	0.5×
Static survival			
L. brevis BSO 310[e]	+/+/-	12	3×
L. brevis CCC 96S2AL	-/+/-	12	4.5×
L. casei CCC B1205[e]	+/+/+	18	2.5×
L. casei MC9-8	-/-/-	20	2.5×
P. claussenii CCC B1098R[f]	-/-/+	15	3.5×
Growth			
L. backii L101[e,g]	-/-/+	10	3.5×
L. brevis BSO 464[e]	+/+/+	15	5×
L. brevis MC9-4g[e] and -4p[f]	+/+/+	12	4×
P. damnosus MC9-6b[e,f]	+/+/+	15	3×

[a] Survival groups at 7 days after inoculation: no survival, no recoverable CFU; static survival, 75 to 150% viable cells present; growth, 300 to 1600% increase in viable cells present at 22 and 30°C. ATCC = American Type Culture Collection, Manassas, VA; BSO = beer spoilage organism, Brewing Research Foundation, Oxoid, UK; CCC = Coors Culture Collection and MC = MillerCoors, MillerCoors, Golden CO.

[b] Hop-tolerance gene profile; + and - indicate gene is present or absent, respectively.

[c] Minimum inhibitory concentration (MIC) of ethanol (% v/v) that inhibits growth after 1 week.

[d] Hop-tolerance reported as concentration range, indicating isolate's established growth; for example, 1.5× means growth the full distance on a 1× hop-gradient plate of 0 to 27 BU and growth part way on a 2× hop-gradient plate of 27 to 54 BU; while 2× means growth the full distance on a 2× hop-gradient plate of 27 to 54 BU and no growth on a 3× hop gradient plate of 54 to 81 BU).

[e] Genome sequence available.

[f] Colony morphology variants: NR = non-ropy as compared to R = ropy; b = big colony as compared to s = small colony; g = glossy colony as compared to p = puffy colony.

[g] *L. backii* L101 was isolated from commercial packaged product produced by a craft brewery in Saskatoon SK, Canada.

MRS culture into 12 ml of 85% (v/v) lager beer with 15% (v/v*)* double-strength mMRS (MRS without Tween 80) (85/15 medium), with incubation at both 22 and 30°C. This adaptation ensures that cells are acclimatized to the lower pH of beer such that when they are introduced into full-strength beer they are able to establish growth in a reasonable time period, provided they possess beer-spoilage ability.

5.3.2. dCO_2 Determination

Gas pressure within the beer bottle was determined using a Gas Pressure Sensor from Vernier Software and Technology (Beaverton, OR). This system involves a silicone rubber stopper that fits a standard North American beer bottle, fitted with a connection to a gas pressure sensor that, in turn, is linked to a detection manifold which tracks the change in pressure (kPa) over time (Fig. 5.1). The manifold is capable of recording readings from three separate beer bottles simultaneously, and from these pressure readings, the amount of dCO_2 was calculated according to VitalSensors, LLC Carbonation Calculation (**23**) as described in the ASBC Method Beer-13B, *Pressure Method for Beer in Bottles and Cans* (**1**). The calculation is as follows:

$$\text{True } CO_2 \text{ (v/v)} = \frac{5.16 \times (GP + BP)}{(T + 12.4) \times SG \times (1 + E/0.789)}$$

where *GP* is Gauge Pressure in pounds per square inch (converted from kPa), *BP* is the Barometric Pressure (14.65 pounds per square inch absolute), *T* is temperature in °F, *SG* is specific gravity of the beer (1.01), *E* is ethanol in w/w, and values of 5.16 and 0.789 correspond to the Henry's Law Water Constant and the density of ethanol, respectively.

5.3.3. Evaluation of Methodology

To assess the effectiveness of the Vernier Sensor apparatus and the process of recapping, (developed to perform bacterial growth analysis in gassed beer), direct comparison of final pressure levels were made after varying lengths of time, between unopened bottles and bottles that had been recapped at 4, 22, and 30°C (data not shown). Recapping is performed by uncapping a 4°C glass beer bottle, then immediately wrapping the lip of the bottle in 2.5 cm wide strips of parafilm wax and recapping using a bench capper from Enotria (London, UK). This entire process was consistently performed within 15 sec, and the process verified to ensure no significant loss of pressure or dCO_2.

Fig. 5.1. Vernier gas pressure sensor setup.

Three bottles are fitted with stoppers that sit flush to the lip of the bottle and then sealed in place using foil tape. These stoppers are fitted with connectors that lead to gas pressure sensors (small black boxes) that are then connected to the system manifold (pictured in inset). Change in pressure is recorded over time from all three bottles simultaneously. During use, the bottles and sensors are shifted to the appropriate temperature incubator.

Trials were also performed to ensure effectiveness of an adapted pouring method for degassing beer (**7, 30**). Full degassing involved pouring 4°C beer continuously for 30 times in a sterile environment, then leaving the beer covered for 2 hr, followed by another 30 pours for near complete removal of CO_2. The 2 hr rest period allows for foam and bubbles formed by the initial 30 pours to reduce and to recover a reasonable volume of beer necessary for experimentation. To assess the effect of pouring on dCO_2 levels, bottles of 4°C beer were emptied and poured 7, 15 or 60 times with no rest period and added back to bottles which were then fitted ("recapped") with the Vernier Apparatus sensor and changes in pressure monitored (Fig. 5.2).

5.3.4. Growth in Degassed Beer

Degassed beer was prepared on the day of experimentation and 100 µl of an 85/15 culture grown for 40 hr was inoculated into 16 ml of degassed beer. Samples were incubated at 22 or 30°C and aliquots were taken at 12 – 24 hr intervals for 7 days, diluted, and plated on MRS agar plates via the drop plate method (**16**). Plates were sealed with parafilm and incubated at 22 or 30°C for 2 to 4 days, and resulting colony forming units (CFUs) were enumerated.

5.3.5. Growth in Gassed Beer

In a sterile environment, 2.1 ml of 85/15 culture grown for 40 hr was inoculated approximately 2 cm below the surface of beer in a freshly uncapped, 4°C beer bottle (341 ml). The bottles were then recapped as described above and incubated at 22 or 30°C. Individual bottles of beer were used for each data point, with sampling done in 12- to 24-hr intervals for 7 days. To sample, beers were uncapped in a sterile environment and half the volume poured off. The remaining liquid was then swirled ten times to dislodge any cells from the bottom of the bottle and combined with the initial volume poured off. After mixing to get a homogeneous cell suspension, aliquots were then taken and diluted, plated, and enumerated in identical fashion as in the degassed trials, however, plates were incubated in a candle jar to facilitate colony formation at both 22 or 30°C within 2 – 4 days. For growth in both degassed and gassed beer, analyses for each isolate was performed in duplicate in two separate trials. If isolates demonstrated either apparent death (no recoverable CFU at 7 d) or static growth (CFU recovered at 7 d was between 75 and 150% of the original viable bacteria in the inoculum) in the first trial, adjustments were made to the sampling schedule to ensure that clear evidence was found of death (more frequent sampling soon after inoculation) or static

maintenance of cell numbers (more frequent sampling later in the 7 day period). For the five isolates designated as growing in gassed beer at both 22 or 30°C, CFU numbers peaked between 300 and 1,600% of the CFU levels provided by the inoculum.

5.3.6. Assessment of Culture Viability

For isolates that had no recoverable CFU's from gassed beer after 48 hr, 800 µl of culture was taken and inoculated into 8 ml of MRS and incubated for 1 week at 22 or 30°C. If there was visible cell growth in MRS after one week, the 16S rRNA sequences of the culture was verified via PCR using primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') (**11**) and 534R (5'-ATTACCGCGGCTGCTGG-3') (**22**) to rule out contamination with any other microbes that may have been present in a given bottle of beer and carried over into the MRS broth medium.

5.3.7. Hop-Tolerance Genes

All twenty isolates were screened for the putative hop-tolerance genes *horA, horC,* and *hitA* (Table 5.1) through use of a previously described multiplex PCR (**13**). Screening for the maintained absence or presence of these genes was performed for each growth trial using as PCR template cells from an initial 85/15 culture as well as cells from individual colonies grown on agar plates from samples taken at various time points during growth in both degassed and gassed beer.

5.3.8. Hop and Ethanol Tolerance

Hop-tolerance for each isolate was assessed on three separate occasions (Table 5.1), using the established method of growth on hop-gradient agar (HGA) plates (**14**). In short, overnight MRS culture of each isolate is stamped along an agar plate, containing a concentration gradient of hop iso-α-acids (Isohop® isomerized hop extract; 28 to 32% [w/w] iso-α-acids in an aqueous solution of potassium salts; John I. Haas Inc., Washington, DC). The relative length of growth up the gradient indicates the level of a bacterium's hop-tolerance. The ethanol MIC (% v/v) level for each isolate was assessed in two separate trials (Table 5.1), according to the methodology described in (**26**). The mean ethanol- and hop-tolerance levels for each survival group of bacteria (i.e., no survival, static, and growth) (Table 5.1) were compared to one another via an independent *t*-test using SPSS for Windows, Version 19.0 (ISBM SPSS Statistics).

5.3.9. Analysis of Genetic Sequences

Genome sequences were available from NCBI for isolates *L. brevis* BSO 464 (**5**), *L. rhamnosus* ATCC 8530 (**25**), and *P. claussenii* ATCC BAA-344NR (**24**). In-house total genome sequencing of isolates *L. backii* L101, *L. brevis* BSO 310, *L. casei* CCC B1205, *L. harbinensis* MC9-2, *L. sakei* ATCC 15578, and *P. damnosus* MC9-6b was performed via the Illumina MiSeq platform (250 bp, paired-end reads) at the National Research Counsel Plant Biotechnology Institute in Saskatoon, Saskatchewan. Raw reads were processed and assembled into scaffolds using the Genius Software (version 8.0.5; http://www.geneious.com) (**19**). Draft genomes of these organisms were uploaded into the Rapid Annotation using Subsystem Technology (RAST) server (**3**), and functional-assignments of genes analyzed and compared between isolates of varying dCO_2 tolerance.

5.4. RESULTS AND DISCUSSION

5.4.1. Effectiveness of dCO_2 Determination Method

The Vernier Gas Pressure Sensor provided effective and reproducible monitoring of the internal gas pressure within standard North American beer bottles (Fig. 5.1). To convert the temperature-pressure data to relevant data concerning the level of dCO_2 present in beer, the Vital Sensors, LLC Carbonation Calculator was used, as it has been developed to account for dynamic variables of specific gravity and alcohol, which can affect the solubility of CO_2 (**23**). Both of these tools verified that recapping the bottles modeled the environment of a pressurized, unopened beer bottle as evident by the equivalent final pressure measurements of beer bottles that were unopened until time of pressure reading and those bottles that were opened and recapped within 15 sec (data not shown). It is acknowledged that the uncapping of a beer bottle unavoidably results in the loss of some minimal amount of CO_2, however, this loss at 4°C when CO_2 is exceptionally soluble, followed by immediate recapping, did not affect the outcomes of the results presented here.

Trials performed to ensure that the sensor detected small fluctuations in headspace pressure, and resulting dCO_2, by degassing beer in the bottles to different extents were successful. From the data in Fig. 5.2, we see that it takes approximately 3-4 hr for beer initially at 4 to 6°C to warm to 30°C as indicated by stabilization of the headspace pressure and thus dCO_2.

According to Henry's Law, at a constant temperature, the amount of dCO_2 is directly proportional to the partial pressure of the un-dissolved CO_2 above the solution. As CO_2 will move from a region of higher partial pressure to one of lower pressure, CO_2 therefore dissolves according to its partial pressure, with temperature influencing the process. This explains why it is possible to over-carbonate beer - beer will continue to absorb CO_2 if the partial pressure of un-dissolved CO_2 above the liquid is greater than the partial pressure of dCO_2.

For a beer that is recapped with no pours, we see the starting headspace pressure is greater than in that of bottles with poured beer (Fig. 5.2), indicating that bottles with poured beer have an initial loss of headspace pressure and dCO_2. The no-pour beer then demonstrates a cycling pattern of headspace pressure and dCO_2, due to dCO_2 escaping from solution as a result of decreased solubility at the higher temperature, thereby increasing headspace pressure: which, in turn, forces some CO_2 back into solution. Over the 24-hr observation time, the headspace pressure and dCO_2 for the no-pour beer slowly continues to increase, as equilibrium between the partial pressures of dCO_2 and headspace CO_2 has not yet been reached.

Seven pours is insufficient for CO_2 removal (Fig. 5.2). Though the initial pressure reading indicates the loss of some CO_2, the headspace pressure and dCO_2 levels are slightly above that of a no-pour, recapped beer when the bottle system reaches 30°C. This is because the agitation of seven pours increases the rate at which dCO_2 (in the form of micro-bubbles) escapes from solution, forcing a highly pressurized headspace which in turn forces CO_2 back into solution, at a rate influenced by the temperature and the interfacial area between the beer and headspace. These factors establish the observed equilibrium between the rates at which CO_2 is dissolving (dCO_2 [v/v]) and escaping from solution (headspace pressure) over the 24-h assessment time for beer poured 7 times and explain the observed limited cycling or fluctuation in the headspace pressure and dCO_2 levels. Ultimately, since the no-pour beer and the beer poured seven times have close to the same headspace pressure and dCO_2, readings at 24 h, it is clear that the seven-pour procedure is not sufficient for CO_2 removal. In contrast, after 15 pours, there is a clear reduction in both headspace pressure and resultant dCO_2 level. Here, the considerable loss of CO_2 from solution (lower partial pressure of dCO_2) does not sufficiently pressurize the headspace to force evident CO_2 cycling. Nonetheless, with the shift in temperature from 4 to 6°C to 30°C, the slow increase in both headspace pressure and

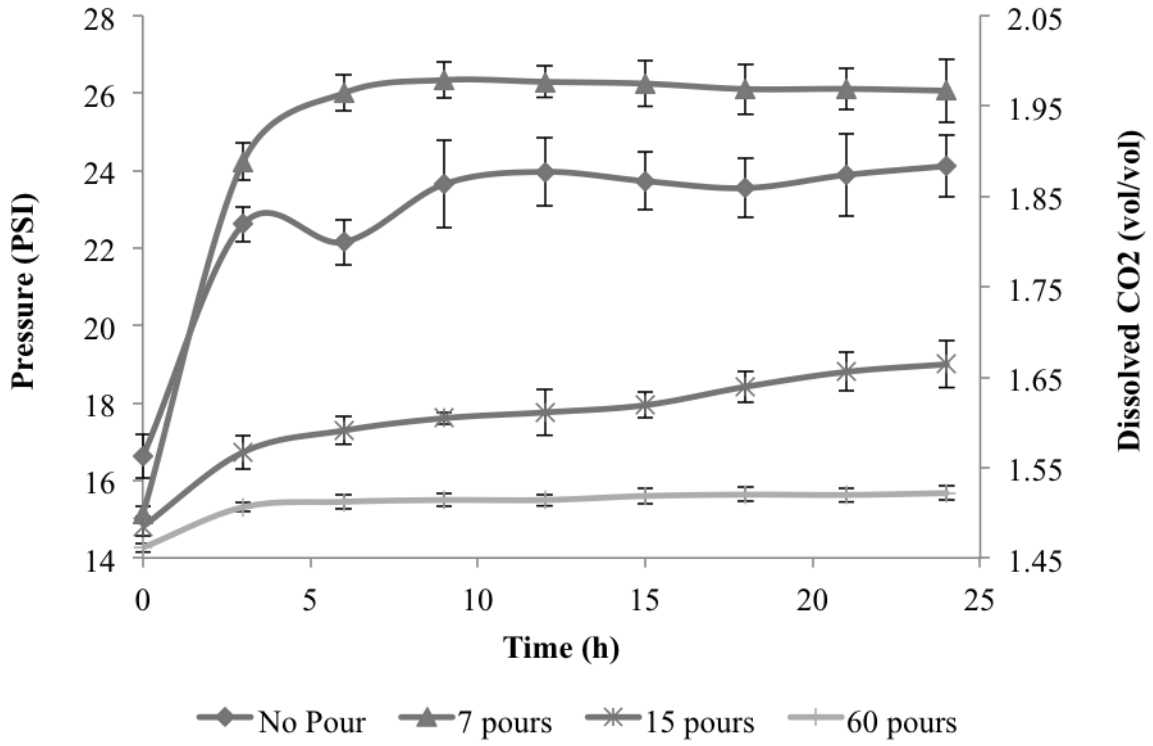

Fig. 5.2. Effect of degassing method (number of pours) on headspace pressure (PSI) and dCO_2 (vol/vol) levels at 30°C.

At 0 hr, the temperature of the beer is 4 to 6°C; following assembly of the sensor, the bottle is shifted to 30°C. After 3 to 4 hr, the bottle and the beer reach equilibrium with the temperature of its surroundings. The same trend is observed when performed at 22°C (data not shown). The shown data are averaged from duplicate readings across two separate trials (n = 4; error bars indicate standard deviation).

dCO_2 over the 24-h period indicates that when starting with much lower dCO_2, it takes more time for the partial pressures of residual dissolved and non-dissolved CO_2 to reach equilibrium. Pouring the beer 60 times lowers the amount of dCO_2 to near negligible levels (i.e., beyond that provided by normal atmospheric pressure), as evident by the decrease in headspace pressure. As such, 60 pours provides beer for assessing bacterial growth in the absence of any dCO_2 remaining from the CO_2 added during packaging. Likewise, our modified full degassing method of 60 pours, with a 2-hr rest at 30 pours utilized to enable preparation of large volumes of experimental degassed beer, provides equally effective removal of dCO_2 (data not shown).

5.4.2. Effect of Temperature and Bacterial Fermentation on dCO_2 Cycling

The concept that CO_2 is very soluble in beer but less so at higher temperatures is corroborated by Figure 5.3 (ASBC CO_2 Chart: Solubility of Carbon Dioxide in Beer Pressure-Temperature Relationships) (**1**). As expected from Figure 5.2, as dCO_2 escapes from solution, there is a resultant increase in the headspace pressure, as evident by the dramatic increase in pressure readings from when a bottle is initially shifted from 4°C (0 hr) to 30°C and the bottle slowly warms (reaching 30°C in approximately 3-4 h). Also as expected from Figure 5.2, bottles that are immediately recapped with no bacterial inoculum experience a continuous cycling of both headspace-pressure and dCO_2. In contrast, this fluctuation pattern is less evident at 22°C (Fig. 5.3.A), since following an initial spike in pressure, CO_2 returns to solution as a result of both lower dCO_2 partial pressure and its increased solubility at 22 relative to at 30°C (Fig. 5.3.B).

When *L. brevis* BSO 464 is present and growing in the pressurized gassed beer, it produces CO_2 as a result of normal fermentation and metabolism, and an increase in pressure is observed at both growth temperatures, as additional CO_2 is cycling in and out of solution, resulting in distinctly higher maximum pressure levels relative to sterile bottles kept at the same temperature (Fig. 5.3.A and 5.3.B). Comparatively, we see that the initial pressure spike at 30°C is greater than the one occurring at 22°C as a result of more rapid release of dCO_2 from solution at the higher temperature. Further, the notable delay in pressure increase observed when *L. brevis* BSO 464 is grown in pressurized beer at 22°C relative to the same conditions at 30°C is explained by the longer growth lag-phase observed at 22°C due to LAB preference for growth at 30°C (**29**).

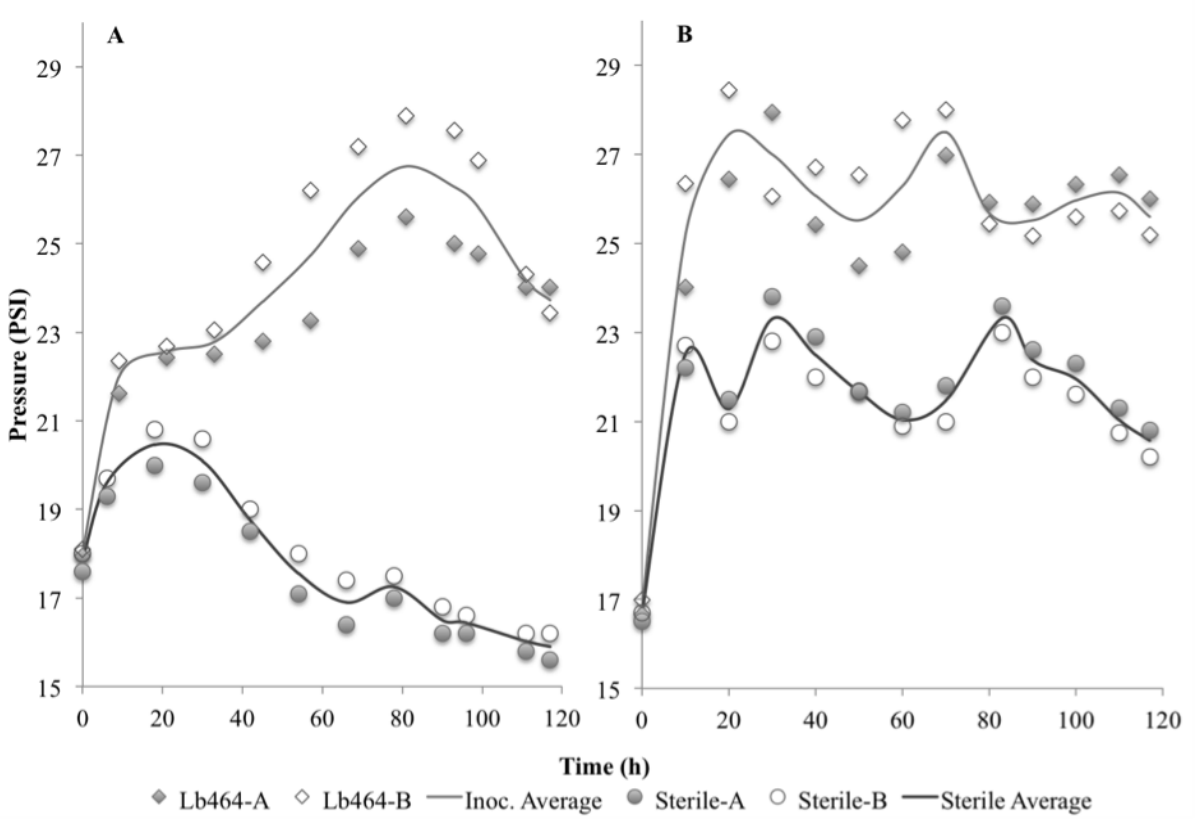

Fig. 5.3. Change in headspace pressure (PSI) during growth of *Lactobacillus brevis* BSO 464 in pressurized gassed beer versus sterile beer at A, 22°C and B, 30°C.

A 4°C bottle of beer was either inoculated with *L. brevis* BSO 464 and recapped, or not inoculated and recapped, and shifted to either 22 or 30°C. The resultant change in dCO_2 (vol/vol) levels at both temperatures directly follows the pattern of headspace pressure cycling (see Fig. 5.2); therefore, this axis is not shown. Data shown are from two experiments at each temperature done with the same batch of commercial lager beer; an inoculated beer and a control sterile beer were run concurrently in each (the curves represent the average of the duplicate data).

5.4.3. Effect of dCO_2 on Ability of LAB Isolates to Grow in Beer

Of the 20 isolates assayed, 19 were able to establish sigmoidal-growth curves with a 10-fold or greater increase in CFU in degassed beer within 7 days. The exception was *P. parvulus* ATCC 43013 which grew slowly, with only a threefold increase in viable cells. Of the 19 isolates able to grow in degassed beer (and thus, originally considered to be BSOs), only five grew (>300% increase) in the pressurized gassed beer environment at both 22 and 30°C; namely, *L. backii* L101, *L. brevis* BSO 464, both colony morphology-variants of *L. brevis* MC9-4 (g and p), and *P. damnosus* MC9-6b (Table 5.1). The colony morphology-variant *P. damnosus* MC9-6s was not stable as it produced *P. damnosus* MC9-6b colonies following culture in both degassed and pressurized gassed beer. Small colonies were not recovered from the pressurized gassed environment when a pure inoculum of the small colony-variant was used; therefore, only *P. damnosus* MC9-6b is considered to grow in gassed beer.

Colony variants (i.e., phase variants) of the same isolate are the result of differences in genetic expression behaviour and not the underlying genetics or genome (**8, 36**), and can be the product of either a random event or a response to external stress factors (**2, 35**). This is an interesting phenomenon in the context of brewing microbiology, though one that is not well investigated, for it is possible that the beer environment induces the expression of phase-genes causing a change in colony phenotype, thereby selecting for beer-fit subpopulations of a given isolate. Indeed, the results observed here suggest that culturing in gassed beer selects for the specific big colony morphology of *P. damnosus* MC9-6b. Further investigation into the underlying genetic mechanisms of beer-induced colony or phase variants, and physiological adaptations of these isolates to gassed beer could give insight into the overall stress responses and genetic activity necessary for LAB survival in beer.

In all, 5 isolates that had survival between 75 and 150% of the original inoculum over 7 days were designated as having static survival in gassed beer, and 10 isolates that had no recoverable CFUs over 7 days were labeled as no-survival (Table 5.1.). Though it should be noted that in brewery settings, incubation times to allow for colony formation often extend up to 7 days and beyond, all isolates analyzed here were capable of establishing CFUs from degassed beer did so within a 2 to 4 days. Those capable of growth or “static” cell numbers in gassed beer also

produced countable CFUs within 2 to 4 days. Thus, if isolates when grown in gassed beer could not establish growth in this window, they were termed “no growth” and occasional continued monitoring of these plates up to 7 days did not reveal any colony formation. Further, all 10 no-survival isolates were able to establish visual growth after 24 to 48 hr following passage back from pressurized gassed beer cultures into MRS medium. This culture was verified by 16S rRNA gene sequencing to be the same as the original inoculum isolate (i.e., free of potential bacterial contamination acquired from the beer bottle). This indicates that some of the cells of each of the 10 isolates can enter a viable, but non-culturable (VBNC) state while in the pressurized-gassed beer environment (**32**). However, it should be noted that although *L. harbinensis* MC9-2, *L. plantarum* CCC B1301, and *L. sakei* ATCC 15578 were designated as no-survival in gassed beer due to no recoverable CFU at 7 days, these bacteria did show recovery of very low levels of CFU’s when plating from beer incubated for a longer time (i.e., 10 to 14 days instead of 7). This indicates that at least for some LAB, a greatly lengthened adaptation period to the gassed beer environment at a given temperature is required, relative to comparable growth in degassed beer.

That the majority of isolates studied were not able to grow in the pressurized gassed beer environment and that these bacteria appear to be able to enter a VBNC phase fits with the previous finding by Molina-Guiterrez et al (**21**) of two distinctive effects of pressure treatment (albeit high pressure) on beer-related microorganisms. The primary effect observed in their study was an increased permeability of the cytoplasmic membrane resulting in decreased cell fitness, while complete cell death remains a separate event that does not depend on the length or strength of pressure treatment, but rather on the total growth environment conditions during treatment (**21**). This indicates that pressure, which necessarily maintains the presence of dCO_2, serves to exacerbate the effects of other physiologically adverse factors in beer such as ethanol, hop compounds, and low nutrient availability, even though organisms may have a demonstrable tolerance or adaptation to one or more of those singular factors. Indeed, Ulmer et al (**34**) found that pressure treatments of 200 MPa affect the viability of *L. plantarum* cells slightly by increasing the permeability of the cytoplasmic membrane and inactivating hop-resistance mechanism HorA, and Gaunzle et al (**12**) showed that treatment of highly pressurized *L. plantarum* TMW 1.460 cells with 5 to 10% ethanol enhanced physiological stress and death during storage (**12**). These results indicate that the pressure treatment that occurs in packaged

beer is not sufficient for cell death but does negatively impact the majority of LAB isolates and can induce a nonculturable state.

Recent work investigating the presence and effect of intracellular gas bubbles, or nano-bubbles, in fermenting yeast also suggests another potential mechanism by which the pressurized gassed beer contributes to inducing a VNBC state. Swart et al (**33**) demonstrated that CO_2 produced intracellularly by yeast (a function which heterofermentative LAB also perform) can actually deform organelles in yeast cells before the CO_2 is released to the environment. Though prokaryotic LAB cells do not possess organelles, these nano-bubbles are also suspected of contributing to high intracellular pressure and affecting cellular osmosis (**33**). Thus, if CO_2 nano-bubbles are also produced in LAB, then the increased intracellular pressure combined with increased headspace pressure of the beer bottle and dCO_2 present in the surrounding solution could also greatly alter normal cell function and/or induce a VNBC state. Thus, published information, together with the results presented here strongly suggest that mild pressure (such as that created by the CO_2 used when packaging beer, together with the resultant dCO_2) can limit the growth of contaminating LAB by negatively impacting innate physiological defences against the different individual stresses found in packaged beer. As such, we believe headspace pressure and dCO_2 to be the final major selection factor that determines whether a given LAB is, indeed, able to grow in and spoil packaged beer.

5.4.4. Ethanol- and Hop-Tolerance

With respect to ethanol tolerance, all isolates demonstrated a wide range of tolerance across gassed beer-growth phenotype and all exhibited tolerance to alcohol levels higher than found in most domestic beer, as expected of most LAB isolates (**18, 24**). Comparison of the three groups of bacteria shown in Table 5.1 found no significant difference with regard to ethanol tolerance ($P > 0.25$ for all three comparisons). As such, ethanol-tolerance is not in any way predictive of LAB ability to grow in pressurized gassed beer (or even in degassed beer as 19 of the 20 isolates grew well in degassed beer).

The plasmid-encoded genes *hitA*, *horA* and *horC* have been characterized for their hop-tolerance mechanisms and are often used as indicators of not only hop-tolerance, but also beer-spoilage

ability (**13, 28**). However, we found no uniform hop-tolerance gene profile amongst isolates capable of growing in gassed beer (Table 5.1), because *L. backii* L101 only possesses *horC*, while the other four isolates able to grow in gassed beer had all three hop-tolerance genes. Moreover, there are four isolates that have all three hop-tolerance genes that are not capable of establishing growth in gassed beer (Table 5.1). It should be noted that the hop-tolerance gene profile of isolates as determined via multiplex-PCR, have been shown to change following cultivation in different growth media as a result of potential changes in plasmid copy number in response to growth environment (**13**). To assess whether this played a role in the experiments reported here, the hop-tolerance gene profile was first assessed for each isolate when grown in 85/15 media (which contains beer) and then throughout its growth in fresh beer. Because the continuous beer environment provides a selective pressure for these genes, the plasmid/gene profile was found to be essentially stable (data not shown).

The lack of a clear relationship between beer-spoilage ability in pressurized gassed beer and the presence of hop-tolerance genes raises concern as to the predictive capabilities of these hop-tolerance genes. In considering this, several facts are relevant. First, it must be remembered that any multiplex-PCR for these genes does not ensure that the detected genes are actually functional. Second, it has been proposed before that the hop-tolerance gene profile may change as a result of changes in plasmid copy number, which can be influenced by the growth environment of the isolate (**13**). Lastly, given that these known hop-tolerance genes are either efflux pumps or ATP-binding cassettes (ABC transporters) that counteract the damaging effects of hops, it seems highly likely that these three specific genes are not alone in conferring hop-tolerance in beer-spoilage LAB isolates. Thus, we propose that these three hop-tolerance genes cannot be used with reasonable probability to predict the ability of LAB to grow in packaged beer.

This suggestion is supported by comparison of the hop-tolerance for isolates in each of the three groups of bacteria in Table 5.1. In contrast to the ethanol-tolerance for the same three groups of bacteria, hop-tolerance for the isolates that die in gassed beer show a trend to lower hop-tolerance or do have significantly lower hop-tolerance compared to isolates that are static or can grow in pressurized beer ($P = 0.106$ and $P < 0.025$, respectively). In contrast, hop-tolerance was not significantly different for isolates showing static survival compared to isolates with growth in

gassed beer ($P > 0.25$). Although these findings support the expectation that hop-tolerance is needed by LAB to survive and grow in pressurized gassed beer, the presence or absence data (Table 5.1.) for the putative hop-tolerance genes *hitA*, *horA*, and *horC* shows that these three genes even collectively are not sufficient on their own for predicting which LAB can grow in packaged beer. It should be noted, however, that this does not negate potential utility of these three genes for assessing the likelihood of LAB growth in beer during production stages prior to packaging with high levels of dCO_2 (**13**).

5.4.5. Potential Adaptations of dCO_2-Tolerant LAB

LAB are a diverse group of organisms, with variability in genome size, coding capacity, and niche adaptations between and within species (**6**). Indeed, the existence of BSOs suggest that genetic adaptations have taken place in specific isolates of the same species to allow for growth in the niche environment of beer. This variability, paradoxically and unfortunately, has made it difficult to ascertain a small, universal subset of genes that allow for the adaptation to growth in beer at all stages of production. Further, the apparent range of ethanol- and hop-tolerance, and overall virulence in the beer environment among LAB (Table 5.1) suggest that genetic adaptation to all the simultaneous stresses presented by beer is likely incremental. By this, we mean that a LAB isolate may have intrinsic, developed or acquired tolerance to one or even multiple stresses, yet as a net result of their genetic capacity are less capable, or incapable, of overcoming the total beer environment, particularly in the final pressurized gassed packaged product. Therefore, we believe the isolates that are capable of either static survival or growth in the pressurized-gassed beer environment are the model isolates to be focused on for more in-depth study as to the genetic and physiological requirements allowing LAB to grow in beer at all stages of production, including, most importantly, in the final pressurized packaged product.

Given that the nine LAB genomes analyzed here existed in different stages of assembly completeness, direct sequence comparisons were difficult; therefore, the RAST annotation program was used to perform function-based comparisons between dCO_2-tolerant and intolerant organisms. This analysis revealed interesting insights into important metabolic networks that are worthy of further characterization (Fig. 5.4). Comparing the nine genomes to one another, revealed considerable differences in basic metabolic functions such as amino acid, carbohydrate

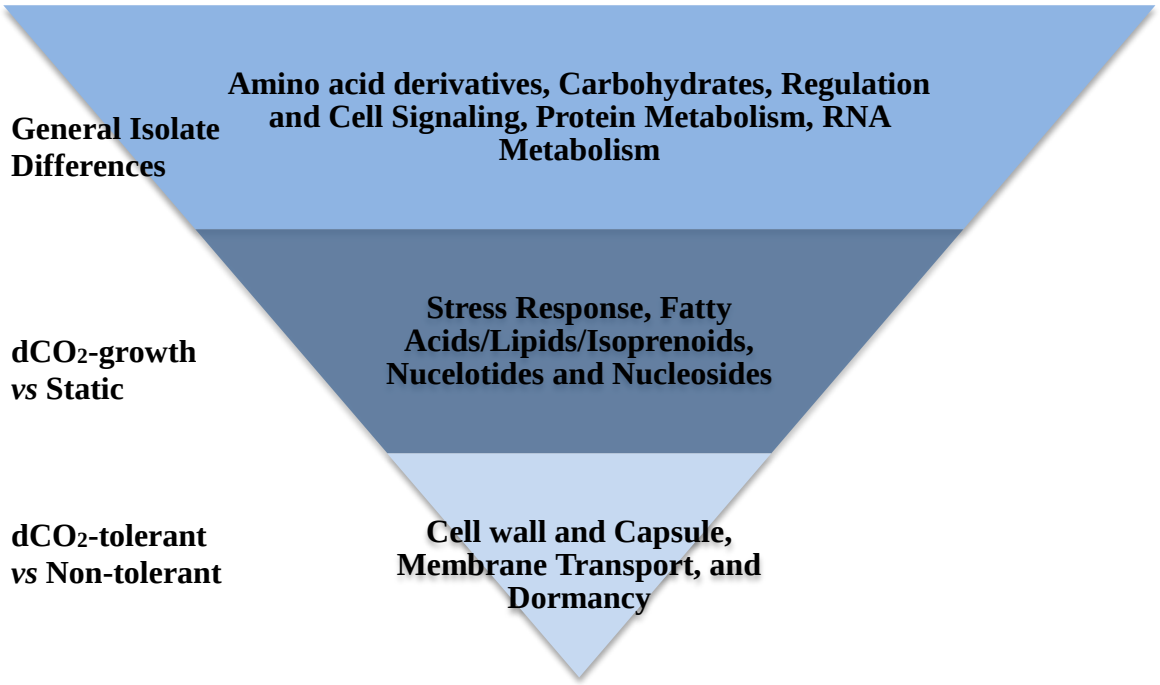

Fig. 5.4. Schematic of differences in RAST metabolic subcategories for the nine genome-sequenced isolates under study.

In general, individual isolates differ most notably in the categories of amino acid and carbohydrate metabolism. When comparing isolates that were capable of growth in gassed beer to isolates to those that were statically able to survive in gassed beer, the next tier of most notable differences included the categories of stress responses, fatty acid metabolism, and nucleotide processing. dCO_2-tolerant (those that can remain static or grow in pressurized gassed beer) and non-tolerant organisms differed most notably in the categories of cell wall and capsule, membrane transport, and dormancy.

and protein metabolism, cell regulation and cell signaling. When isolates were grouped according to their dCO_2 tolerance, it was noted that genes belonging to stress response networks and fatty acid processing broadly delineated the groups of static survival and dCO_2-growth isolates. In addition to these two tiers of differentiation in metabolic functionality, functions of cell wall, membrane transport, and dormancy processes notably differed between dCO_2-tolerant (both static survival and growth) and non-tolerant isolates. These findings support the idea that dCO_2 (as a result of headspace pressure) damages cell membrane processes generally, and membrane-located hop-defense mechanisms specifically.

As suggested from RAST analysis, if dCO_2-non-tolerant isolates have fewer, or lack important membrane transport, lipid metabolism, or cell dormancy genes, it stands to reason they will be more susceptible to the damaging effects of dCO_2 and therefore are unable to grow in and spoil packaged beer. Although the growth data suggest that dCO_2-non-tolerant isolates may enter into a dormant state in pressurized gassed beer, it could be that the regulation or absence of key genes prohibits their successful exit from this state compared to dCO_2-tolerant isolates. Further gene annotation analysis and investigation of metabolic pathways is necessary to determine why a given LAB isolate can or cannot enter a true dormancy state in gassed beer.

Finally, while these functional gene groupings of LAB help our understanding of the general physiology of virulent BSOs, and lend support and direction for further analysis in this regard, they most importantly also suggest that distinction between dCO_2-tolerant and nontolerant isolates may be feasible by way of genetic characterization of only a select few gene pathways. As more LAB genome sequences become available, in-depth within- and between-species comparisons will allow for the elucidation of specific genes belonging to membrane transport, stress responses and cell wall pathways that could be targeted for use in distinguishing between dCO_2-tolerant or beer-spoilage LAB and non-spoilage LAB.

5.5. CONCLUSIONS

The results of this study indicate that headspace pressure and resultant dCO_2 levels present in packaged beer are selective for the growth of beer-spoilage LAB, irrespective of their ethanol- and hop-tolerance. Though LAB isolates generally were able to recover culturable cells over

extended incubation at a consistent temperature in gassed beer, the difference in adaptation time among isolates indicates that there is a scale of genetic specialization in relation to the growth stresses in gassed beer. This tolerance is likely not conferred by one (or even just a few) specific genetic element(s), but is probably the result of the accumulation of various genetic adaptations. Genomic comparisons of dCO_2-tolerant and intolerant LAB isolates show that genes involved in pathways for membrane transport, cell wall and capsule processes, stress responses, and dormancy are potentially suitable for defining virulent, beer-spoiling LAB. These data also strongly suggests that beer-spoilage LAB recovered from brewery settings or packaged beer should be maintained or passaged routinely in conditions of pressurized-gassed beer during laboratory analysis in order to maintain selective pressure for the genomic and transcriptional elements required for LAB-beer spoilage.

5.6. ACKNOWLEDGEMENTS

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Chapter 6: Genome sequencing of beer-spoiling organism *Lactobacillus brevis* BSO 464 and transcriptomic analysis for growth in degassed and gassed beer

Jordyn Bergsveinson, Emily Ewen, Vanessa Pittet, and Barry Ziola

6. INTERFACE

The complete genome assembly of *Lactobacillus brevis* BSO 464 (Lb464) is described here as well as its complete transcriptome when grown in basic nutritive conditions (mMRS pH 5.5) and in conditions of degassed and gassed beer. The sequencing data obtained were used to ascertain transcripts specifically expressed in response to beer and dissolved CO_2. It is important to note that these samples were prepared and sequenced simultaneously with samples of Lb464 and *Pediococcus claussenii* ATCC BAA-344^T (Pc344) grown in growth-limiting concentrations of hops (and Pc344 grown in mMRS pH 5.5) which are discussed in up-coming Chapter 7.

Jordyn Bergsveinson lead PCR gap-closing efforts for Lb464 genome assembly and prepared all RNA samples for sequencing. She performed all subsequent bioinformatics processing and analysis of RNA sequencing reads, including differential expression analysis and genome validation. She authored the manuscript.

Emily Ewen assisted in PCR gap-closing efforts for genome assembly efforts.

Vanessa Pittet is attributed with preparing Lb464 DNA for genome sequencing and performing initial *in silico* genome assembly steps and providing insight into RNA sequencing experimental design and during the processing of RNA sequencing reads.

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work.

6.1. ABSTRACT

Lactobacillus brevis BSO 464 (Lb464) is a beer-spoilage isolate of great interest given its unique physiological attributes; specifically, its high hop tolerance and ability to grow in pressurized/gassed beer (Chapters 2, 4, 5). The initial genome assembly for Lb464 involved *in silico* and PCR amplicon sequencing methods, and revealed a 2.7 MB genome together with eight plasmids ranging from 2 Kb to 85 Kb (discussed in Chapter 4). GC-skew analysis of the genome revealed a potential mis-assembly of the genome, however, sequencing and subsequent mapping of RNA transcripts to the genome did not indicate assembly errors. Analysis of differentially expressed transcripts in both degassed and gassed beer by two separate statistical analysis packages reveal a set of transcripts that are critical for growth in beer regardless of dissolved CO_2 (dCO_2) content. Agmatine metabolism, putrescine transport, energy production and redox homeostasis, and cell membrane and wall fortification are all important processes. Most notably, genes involved in peptide and amino acid transport and nitrogen metabolism, and histidine kinases are strongly implicated as being critical for Lb464 growth in beer, which creates new areas of investigation. Gene ontology (GO) enrichment analysis reveals that in general, DNA metabolism and nitrogen metabolism are most notably enriched in both degassed and gassed beer relative to basic nutritive media. The presence of dCO_2 shifts transcriptional activity towards transcripts involved in cell wall and membrane fortification and osmoregulation, indicating that headspace pressure and dCO_2 enhances the cell-damaging action of beer stressors. The differential expression of various families of transcriptional regulators during growth in beer suggests that the regulatory network is likely critical for effective survival during growth in beer, as well as avoiding a viable, but non-culturable state. GO enrichment reveals that DNA recombination, integration, metabolism and subsequent nitrogen metabolism are the processes most strongly induced by the additional stress of dCO_2 and thus the Lb464 genome may have a degree of instability that allows for adaptation and rapid growth in this environment. pLb464-2 is the most transcriptionally active and important plasmid for growth in beer with the hop-tolerance genes *horC* being the most significantly differentially expressed transcript. This plasmid is also implicated as being important in ferrous iron control, along with a chromosomal ferrous iron regulator protein, suggesting that ferrous iron metabolism is a critical oxidative stress defense in face of the harsh gassed beer environment. pLb464-4 and pLb464-8 both appear to contribute to the physiological response to beer through transcript products involved in DNA

repair and recombination, as well as several hypothetical proteins. The functional role of plasmid and chromosomal hypothetical proteins highly expressed during growth in beer is unknown, though it is highly likely that they are contributing to the regulatory control within the Lb464 cell under stressful conditions.

6.2. INTRODUCTION

Lactobacillus brevis isolates have long been recognized as among the most common and major beer-spoilage contaminants (**5, 19**). Despite the recognized impact these isolates have on the brewing industry, the only complete *L. brevis* genomes currently available from NCBI are non-beer-spoiling strains, namely, the type strain *L. brevis* ATCC 367^T (Lb367) (**31**) and *L. brevis* KB290 (KB290), which was isolated from suguki, a Japanese fermented vegetable (**20)**. Adaption of lactic acid bacteria (LAB) to niche environments is known to be accompanied by or the result of either genomic reduction, rearrangements, mutations or horizontal gene transfer events, and given that beer-spoilage ability is not a conversed trait of a particular LAB species, the use of Lb367 and KB290, or other non-beer-spoiling *L. brevis* genomes, for understanding beer-spoilage genetics is not particularly useful. Consequently, the full genome assembly of *Lactobacillus brevis* BSO 464 *(*Lb464) was undertaken and general analysis of genome features are presented here.

Though resolving the full genome sequence and architecture provides for great insight into ancestral or shared gene sets, key gene acquisition, and potential evolutionary relationships between niche adapted LAB isolates, the use of RNA sequencing (RNAseq) or transcriptome sequencing is the among the most useful modern tools for assessing which genes (i.e., transcripts) are critical for niche-behavior or growth attributes (**48**). Previous RNAseq analysis of beer-spoilage isolate *Pediococcus claussenii* ATCC BAA-344^T-NR (Pc344) when grown in beer and basic nutritive conditions revealed several important insights into the genetic strategy this bacterium employed to grow in beer (**37**). Specifically, the fatty acid biosynthesis *fab* operon and genes involved in mannitol, trehalose and the agmatine deiminase operons were shown to be up-regulated to provide means for efficient energy production and moderation of oxidative stress. Additionally, plasmid-based transcripts involved in various stress mechanisms and non-coding RNAs (ncRNAs) were highlighted as being important for growth in beer (**37**). It should be noted

that the beer growth medium used to analyzed gene expression by Pc344 was not defined as degassed or pressurized/gassed beer, as has been recently defined or prepared in (**11**) and instead was a growth environment more intermediate in terms of dCO_2 content. Thus, the transcriptional sequencing of Lb464 was performed in both degassed and pressurized/gassed beer (hereafter referred to as gassed beer) in order to not only determine the necessary transcripts for Lb464 tolerance to beer, but also to dCO_2 content specifically, and to allow for comparison to the transcriptional response of Pc344 when grown in beer.

6.3. MATERIALS and METHODS

6.3.1. Isolate and DNA extraction

Lb464 was obtained from the Brewing Research Foundation (Oxoid, UK). To isolate genomic data, the isolate was first taken from -80°C stock and grown overnight in MRS broth culture at 30°C. DNA was then extracted through use of the UltraClean Microbial DNA Isolation Kit (Mo-Bio Laboratories, Carlsbad CA) according to the manufacturer's instructions, with an additional heating step for 10 min at 70°C prior to bead-beating to optimize cell lysis.

6.3.2. DNA sequencing and assembly of reads

Sequencing was performed using the Roche 454 Genome Sequence FLX platform at the National Research Council Plant Biotechnology Institute (NRC PBI) in Saskatoon, SK. Paired and unpaired reads were obtained during two separate runs resulting in a final coverage of ~30X. There were a total of 567,735 paired reads which were assembled using Newbler GS *De Novo* assembler v. 2.5.3, producing 236 contigs arranged across 17 scaffolds. The scaffolds were then visualized using Hawkeye (**41**) to identify gaps that could be closed using subsequent sequencing of polymerase chain reaction (PCR) amplicons. Sequencing PCR amplicons via the ABI 3700*xl* platform at NRC PBI closed gaps between contigs. The assembled Lb464 genome and plasmids are depicted in Fig. 6.1.

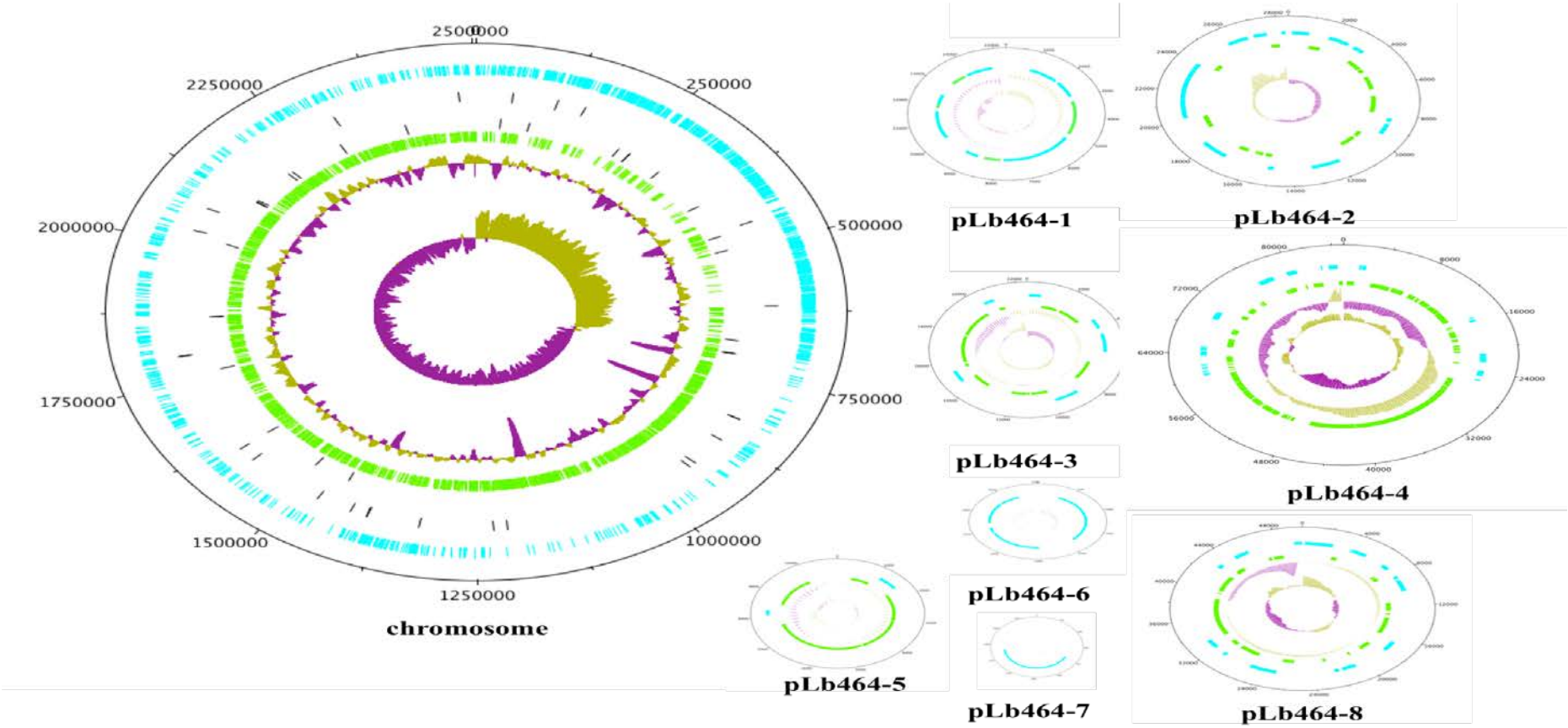

Fig. 6.1. Assembled Lb464 genome and plasmids visualized via DNA Plotter[3].

The outer blue and inner green rings indicate CDS on the positive and negative strands, respectively. The black dashes indicate location of transposons (outer and inner rings are for the positive and negative strands, respectively). The second and inner most rings display the GC plot and the GC skew, respectively.

[3] Plasmid plots originally presented in (**12**; Chapter 4) as Fig. S1 and S2.

6.3.3. Genomic Annotation

Initial genome annotation of Lb464 was done via two separate pipelines; the Prokaryotic Genome Automated Annotation Pipeline (PGAAP) via NCBI (**44**) and RAST (Rapid Annotation using Subsystems Technologies) (**4**) (Table 6.1). The PGAAP annotation of the Lb464 genome was used for downstream analysis of transcriptome data, with known hop-tolerance genes *hitA, horA, horB* and *horC* added to the annotation.

6.3.4. Growth in degassed and gassed beer

Lb464 was taken from -80°C stock and grown overnight in MRS broth at 30°C. Twenty µl was then passaged into 85/15 media (85% lager beer, 15% double-strength modified MRS broth [without Tween 80]) and grown for 2 d. From this 85/15 culture, 100 µl was taken and inoculated into 16 ml of freshly degassed beer (1st degassed beer) and 2.1 ml inoculated into a 4°C bottle of beer and recapped (1st gassed beer) (degassing and recapping methods described in **11 and 12**; Chapters 4 and 5). These 1st beer cultures were incubated at 30°C for 30 h and 55 h (late-log) for degassed and gassed beer, respectively. From the 1st beer degassed culture, 6.25 ml was used to inoculate 1 L of freshly prepared degassed beer, in duplicate. For gassed beer, fresh 4°C bottles were opened, 8 ml of beer removed, and then 10.3 ml of 1st gassed beer was immediately added, followed by bottle recapping. For one replicate, three separate beer bottles were inoculated and recapped. All degassed and gassed beer cultures were grown at 30°C until mid-exponential growth was established, taking, 22 h for degassed beer and 48 h for gassed beer (Fig. 6.2).

From Lb464 grown in MRS medium, 1 ml was also taken and used to inoculate 100 ml of mMRS broth pH 5.5 in duplicate (L-mM). Cells were harvested during mid-exponential growth (14 h; OD_{600} nm ~ 0.3). L-mM samples are used to assess Lb464 growth in basic, nutritive conditions for comparison against growth in degassed and gassed beer, and in the presence of hops (Chapter 7).

Table 6.1. Annotation pipeline outcomes for Lb464

	Size (bp)	GC (%)	CDS	rRNA (5S, 16S, 23S)	tRNAs	Hypothetical/ Unknown Function	Conserved Hypo. Proteins	Transposase	Prohapage Proteins	Unique Pipeline Attributes
Xsome	2,503,991	45.7	2,363 [a]; 2,425 [b]	18; 18	47 ; 47	851; 370	N/A[d]; 84	51 ; 36	6 ; 8	**PGAAP**:
pLb464-1	15,324	41.0	17 ; 17	-[c] ; -	- ; -	7 ; 7	N/A; -	2 ; -	- ; -	36 psuedogenes;
pLb464-2	28,459	42.4	35 ; 39	- ; -	- ; -	10 ; 11	N/A; 6	6 ; 2	- ; -	19 frameshifted
pLb464-3	22,411	40.7	20 ; 25	- ; -	- ; -	8 ; 7	N/A; 3	5 ; -	- ; -	genes; 1 CRISPR
pLb464-4	89,941	39.5	114 ; 121	- ; -	1 ; 1	80 ; 57	N/A; 7	5 ; 2	6 ; 15	region: (29 bp, 3 spacers from
pLb464-5	10,867	39.1	9 ; 10	- ; -	- ; -	2 ; 3	N/A; -	- ; -	- ; -	2,448,910 – 2,449,121 bp)
pLb464-6	5,018	39.1	6 ; 8	- ; -	- ; -	2 ; 2	N/A; -	- ; -	- ; -	**RAST:**
pLb464-7	2,353	38.2	1 ; 1	- ; -	- ; -	- ; -	N/A; -	- ; -	- ; -	functional
pLb464-8	49,835	39.6	41 ; 47	- ; -	- ; -	16 ; 6	N/A; 3	6 ; 12	3 ; 16	assignment of genes

[a,b] Numbers from PGAAP [a]; RAST [b] annotation, respectively

[c] -; not present

[d] N/A indicates specific annotation not provided by the indicated pipeline.

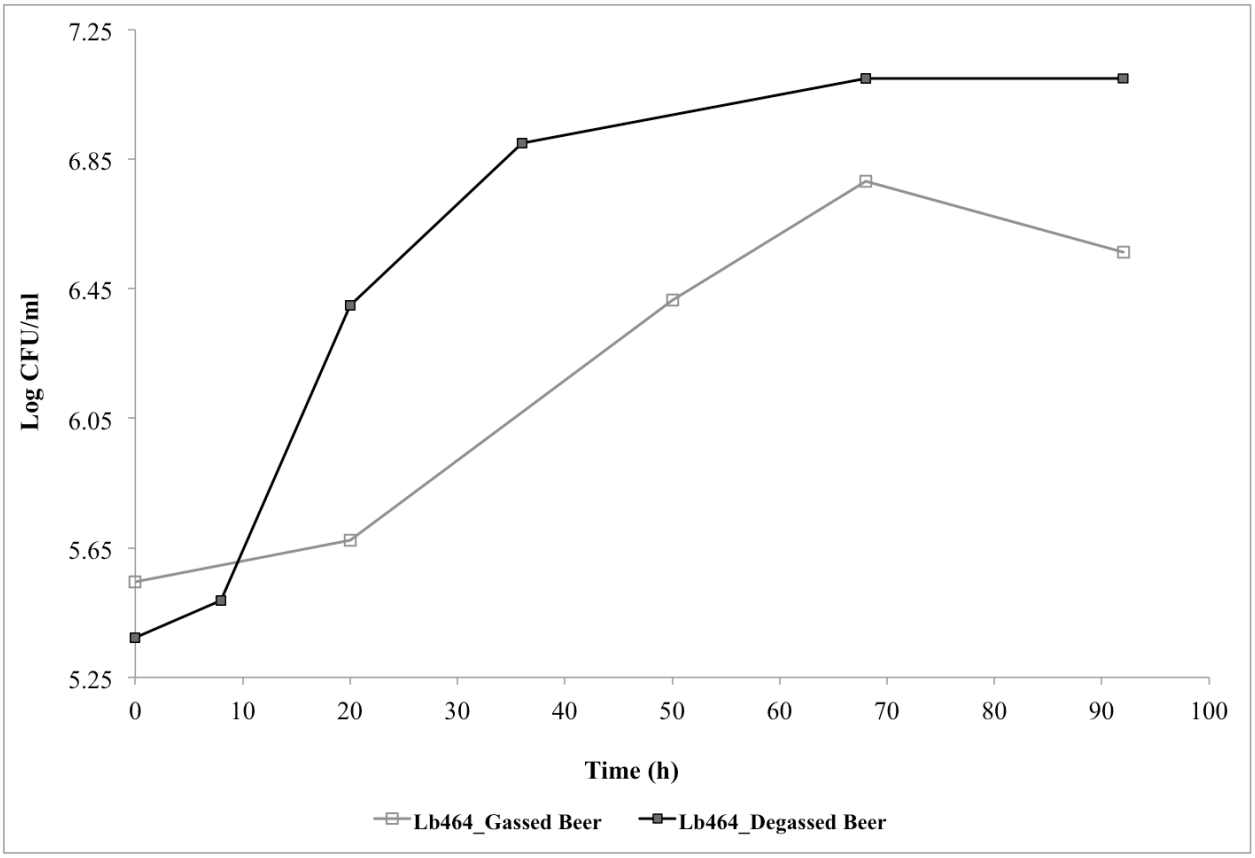

Fig. 6.2. Growth of Lb464 in degassed and gassed beer at 30°C.

When grown in gassed beer, there is a markedly increased lag time before Lb464 establishes exponential growth, relative to growth in degassed beer. Further, in degassed beer Lb464 is able to establish a greater level of CFU's than in gassed beer. Lb464 cells were harvested for RNA extraction from each growth condition when mid-exponential growth was reached (degassed beer, 22 h; gassed beer, 48 h).

6.3.5. RNA isolation and mRNA processing

The 1 L volumes of each beer culture replicate (degassed beer and pooled bottles of beer for gassed beer) were centrifuged at 4,000 × g for 10 min at room temperature (done in duplicate for each medium). Resultant pellets were re-suspended in 35 ml of degassed or gassed beer medium and centrifuged once more at 10,000 × g for 3 min. Pellets were flash-frozen with liquid N_2 and stored overnight at -80°C. For mMRS control samples, 35 ml aliquots were centrifuged at 10,000 × g for 3 min. These pellets were also flash-frozen and stored overnight, and the pellets for one replicate pooled during RNA extraction. Total RNA isolation for all samples was done with the PowerMicrobiome™ RNA Isolation Kit (MOBIO) according to the manufacturer's instructions, except that 70% ethanol was used in place of solution PM4 to prevent small RNA species (5S, tRNA and degraded RNA) from co-precipitating with mRNA and ribosomal RNA (rRNA). A 15 min on-column DNase digest was included in this protocol (DNase I, MOBIO). To the 100 µl elutate of total RNA, 1 µl of SUPERNase-In™ RNase Inhibitor was added (Ambion). A further DNase treatment was then performed on the elutate using 6 U of TurboDNase (Ambion), according to the manufacturer's specifications. To ensure that DNA removal was complete, cDNA was prepared using the SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen) and was assessed via qPCR, along with a no-reverse transcription (noRT controls), using primers for genes *proC* and *rpoB* as previously described (**10**; Chapter 2).

rRNA was removed from each sample with the Ribo-Zero™ Magnetic Kit for Gram-positive bacteria according to manufacturer's instructions (Epicentre). Samples were then concentrated using RNeasy® MiniElute® Cleanup kit to a volume of 12 µl, according to the manufacturer's specification (Qiagen). Samples were assessed for rRNA removal efficiency and overall quality both pre- and post- rRNA removal with use of Experion™ RNA StdSens Assay (BioRad) and samples were quantified via a Qubit® 2.0 Fluorometer (Invitrogen).

6.3.5.1. mRNA sequencing, processing of reads and validation of Lb464 genome assembly

Purified mRNA was prepared with the Illumina TruSeq RNA Sample Preparation Kit and sequenced via the Illumina HiSeq platform at NRC PBI, Saskatoon, SK. Samples were indexed

and multiplexed on one lane along with four other samples (Chapter 7) to achieve paired-end, 100 bp reads.

Illumina reads were visualized via FastQC version 0.9.3 for quality and Trim Galore version 0.3.3 was used to remove Illumina adaptors from read ends and reads of poor quality such that a Phred quality score of ≥ 30 across the entire library was achieved (Barbraham Bioinformatics; http://www.bioinformatics.babraham.ac.uk/). Any resulting reads that were less than twenty nucleotides (nt) long were also discarded. Reads were then aligned to the Lb464 genome available from NCBI (BioProject Accession No. PRJNA203088) via Bowtie 2 version 2.2.3 (ran in –M mode, --very-sensitive for end-to-end alignments and –X 400 for maximum fragment length) (**27**).

Bowtie 2 output files in SAM format were converted via SAMtools to sorted, indexed BAM files (**28**). These BAM files, along with the Lb464 genome and PGAAP annotation files available from NCBI, were loaded into Artemis v. 14.0.0 (**13**) to examine read coverage across the chromosome and each plasmid.

6.3.5.2. Differential expression analysis

Sorted, indexed BAM files and the Lb464 annotation file were processed using HTSeq-count version 06.1 (**2**) in –m union mode to produce count tables of gene features. These feature count tables were generated using the PGAAP annotation file with all annotations for rRNA and tRNA sequences removed, and hop-tolerance genes re-annotated. DESeq 2 (version 1.8.1) was implemented in RStudio to perform differential expression (DE) analysis on these read counts, with a false discovery rate (FDR) of 0.1 (**30**). As this intent of this study is to elucidate the general processes and physiological mechanisms induced in Lb464 by the beer environment, transcripts with *P*-adjusted (FDR; Table S6.1a,-b,-c) values less than 0.1 were taken as significant and examined further. For discussion of biologically relevant transcripts in each sample comparisons, transcripts that are expressed at or above 2 Log_2 fold change (i.e., expression fold change of $\geq$ 4 between conditions) are discussed. Fold-change values are log transformed for reporting for reason that this transformation minimizes skew in the data set by reducing variance in gene expression levels.

6.3.6. Gene ontology (GO) annotation and enrichment analysis

Lb464 proteins were annotated for gene ontology (GO) terms using Blast2GO v.3.0 by using a BLASTx search Expect value of 1.0^{-3}, and default settings for GO annotations (**1**, **16**). Proteins that were significantly expressed (FDR < 0.1) in DESeq 2 comparisons were taken and used to perform enrichment analysis against the complete genome GO annotation via Fisher's Exact Test in Blast2GO (using FDR < 0.1).

6.4. RESULTS and DISSCUSSION

6.4.1. Genome sequence and assembly of *Lactobacillus brevis* BSO 464

Efforts to completely assemble the genome of Lb464 through *in silico* and PCR gap-closing methods resulted in one circular chromosome (2,503,991 bp); and eight plasmids (pLb464-1 (15,324 bp), pLb464-2 (28,459 bp), pLb464-3 (22,411 bp), pLb464-4 (84,941 bp), pLb464-5 (10,867 bp), pLb464-6 (5,018 bp), Lb464-7 (2,353 bp), and pLb464-8 (49,835 bp)) (Fig 6.1, Table 6.1). A total of 147 PCR amplicons were used to join contigs and scaffolds, or confirm contig joining suggested by Hawkeye. Due to the presence of repetitive (putative transposon) regions, 8,461 bp cumulatively in the chromosome and a 1,000 bp gap in pLb464-8 remain to which accurate sequence could not be obtained by PCR amplicon sequencing. The sequences for *Lactobacillus brevis* BSO 464 were deposited in GenBank under accession numbers CP005977, CP005978, CP005979, CP005980, CP005981, CP005982, CP005983, CP005984, and CP005985 for the chromosome and pLb464-1 to -8, respectively.

De novo assembly of the Lb464 chromosome and plasmids revealed the overall G+C content of the genome to be 45.7%, with plasmids ranging from 39.1% to 42.4%. Interestingly, when the GC-skew of the Lb464 chromosome was mapped via DNA Plotter (**14**), an atypical GC-skew was observed (Fig. 6.1), in that it does not display a symmetric bias in nucleotide composition of leading and lagging DNA strands relative to the origin of replication (**15**). The AT-rich lagging strand encroaches into the normally G-C rich leading strand by approximately ~500,000 bp. This would appear to indicate that there is a potential inversion or mis-assembly of a roughly 500 Kb section of the Lb464 genome, although it was revealed that Lb367 had a similar atypical GC-skew when mapped via DNA plotter (see Chapter 8).

Though it is possible that the joining of eight large sequence scaffolds at the end of Lb464 genome assembly efforts resulted in a misassembly, it is difficult to ascertain whether other small inversions or changes in genome architecture are "false" given that genome rearrangements, gene loss, and mutations can be influenced by multiple genetic events, including the adaptation to different niche environments (**33**, **42)**. For example, previous whole genome comparisons of a milk-adapted *Lactobacillus bulgaricus* isolate against related organisms revealed a roughly 300 kbp area with perturbation in genome synteny, as well as a 47.5-kbp inverted repeat in the replication termination region (**47**). Further, there have been reports of atypical AT-skews in *Firmicutes* (**15**), meaning whether this predicted abnormal skew is valid is difficult to determine. Therefore, RNA sequencing reads were subsequently used to further investigate the assembly of the Lb464 genome (see section 6.4.4).

6.4.2. Lb464 genome annotation

Annotations of Lb464 were performed through use of PGAAP via NCBI, as well as the RAST annotation server (Table 6.1). Both annotation pipelines were used in order to balance the strengths and weaknesses, and to exploit the different analysis tools that are offered through each method. For example, the RAST server allows for comparative functional or sequence analysis between multiple organisms and in-house metabolic modeling analysis (**4, 25)**. In contrast, the functional annotation of genes by RAST is not as stringent, given that it solely utilizes FIGfams, their internal protein families' database to make annotation calls (**4**). Conversely, the NCBI PGAAP pipeline incorporates traditional predictive measures for finding protein-coding sequences (Glimmer3) (**18**), with statistical evidence of coding potential from closely related organisms (**44**). This pipeline functions under the principle that closely related organisms maintain conserved proteins and thus total annotation of new genomes allow for comparisons of "core" and "pan" genomes of populations of related organisms (**44**). Further, implementation of a two-pass method for improvement of the original gene predictions allows for the detection of frameshift events, and small ncRNAs, and "fast evolving genes" such as phage and CRISPR (clustered regularly interspaced short palindromic repeat) are screened for. Thus, the overall accuracy and efficiency of the gene calling process is improved utilizing the NCBI PGAAP pipeline.

Comparing results of these annotation methods shows there is a general trend for RAST to provide a greater number of predicted coding sequences (CDS) than does PGAAP (Table 6.1). Further, the PGAAP annotation categorizes a higher proportion of CDS as hypothetical proteins (37% of the genome) than does RAST (22% of the genome). In the end, the PGAAP annotation was used for all subsequent downstream RNAseq analysis because it is publically available from the NCBI GenBank FTP site and is expected to be more stringent than RAST (note that missing or un-annotated CDSs can be readily cross-referenced to RAST predictions).

6.4.2.1. General Features

Lb464 contains six rRNA operons (16S-23S-5S) and only has 47 chromosomal tRNA genes, which is different from both KB290 and Lb367 which contain five rRNA operons and ~ 60 tRNAs specific for all 20 amino acids (**20, 31**). Lb464 does not appear to have tRNAs specific for cysteine, histidine, and tryptophan according to both RAST and PGAAP. Though it is possible that these tRNAs are present within a gap in the chromosomal sequence, it is likely that Lb464 employs the use of base wobble in the third position of tRNAs that are present to allow for utilization of these amino acids. Interestingly, it was found in a survey of genomes from three different phyla that amino acids for cytosine, histidine and tryptophan are used infrequently, perhaps explaining why tRNAs specific for these amino acid codons are lost from the Lb464 genome to allow acquisition of other beer-niche advantageous genes (**46**). This notable difference in genome characteristics aside, Lb464 and KB290 both contain a high number of plasmid elements, with Lb464 containing eight plasmids and KB290 containing nine, the highest reported number in finished *Lactobacillus* genomes (**20**).

In general, the RAST server is useful for readily visualizing general functional features of the Lb464 genome, which reveals that carbohydrate utilization and/or related functions comprise a large proportion of chromosomal functional capacity (~19%), which is likely a reflection of adaptation to the nutrient deplete and variable niche of beer (**33**). Genes related to groups of "fatty acids, lipids and isoprenoids", "membrane transport", "cell wall", "stress response" and "virulence, disease, and defense" then each comprise between 3.5 to 5.9% of the chromosomal functional systems. For plasmids, DNA metabolism-related functions comprise roughly 43% of

the functional coding capacity, phage-related elements encompass 24%, and functions belonging to categories of “stress response” and “respiration” total 8 to 10% of functional assignments.

NCBI PGAAP annotation of Lb464 indicates that approximately 13% of the genome (both chromosome and plasmids) codes for membrane transport systems, which is in line with previous accounts of LAB genomes having a larger proportion of transporters than found in many other bacteria, most likely as a result of adaptation to nutrient variable environments (**42)**. As well, Lb464 contains more than four copies of each of the LysR, GntR, MarR, MerR, TetR and Xre family of transcriptional regulators which is expected according to previous analysis of LAB genomes by (**39**), as well as more than four copies of LytR and Rrf2 transcriptional family regulators. There is also the presence of 79 copies of transposase genes across the Lb464 genome and plasmids, dominated by the ISL3 and IS30 family, which is more than in either Lb367 or KB290 (**20**).

There are 36 annotated (disrupted) pseudogenes in the Lb464 genome, with 26 being chromosomally located and the remaining ten dispersed on pLb464-1, -2, -3, -4 and -8 (Table 6.1). The plasmid-based pseudogenes are hypothetical proteins or transposase-related products, with the exception of a pyrimidine dimer DNA glyosylase on pLb464-4 and a nickase on pLb464-8. On the chromosome, there are several membrane transport protein pseudogenes (major facilitator superfamily (MFS), manganese transporter, ABC transporter permease, acetoin ABC transporter ATP-binding protein,) as well as transcriptional regulators (RNA polymerase sigma factor RpoD) and enzymes (aldo/keto reductase, glycosyl transferase, NAPH:quinone reductase). The majority of the genes have intact paralogs present in the gene, or have likely paralogs based on presumed function (i.e., “MFS transporter”). The only genes that do not have redundant paralogs are a multicopper oxidase (L747_09245), nicotinamide mononucleotide transporter (L747_13715), aggregation-promoting factor surface protein (L747_10710), hydroxyethylthiazole kinase (L747_12230), and γ-glutamylcysteine synthetase (L747_13650), indicating that these functions are lost from the cell.

RAST was used to compare the carbohydrate pathways of Lb464 to Lb367 and KB290, which revealed Lb464 to uniquely contain proteins involved in citrate metabolism, transport and

regulation (notably citrate lyase), mannose metabolism, and a fructuronate transporter (and D-Galacturonate and D-glucuronate utilization). Citrate uptake, and citrate lyase specifically, which are involved in fatty acid biosynthesis were implicated by RNAseq analysis as being important for growth of Pc344 in beer (**37**). Looking specifically at the RAST functional category of fatty acid, lipids and isoprenoids, Lb464 contains several unique transcripts relative to KB290 dealing with isoprenoids for quinones, and unique transcripts relative to both to KB290 and Lb367 dealing with glycerolipid and glycerophospholipid metabolism.

Type 1 restriction-modification proteins (subunit M protein and enzyme R protein) were found on pLb464-5, and a Type 1 restriction endonuclease was found on pLb464-8, however, none are functionally annotated to exist within the chromosomal sequence. There is, however, a CRISPR region within Lb464 detected by PGAAP (211 bp length total; Table 6.1), which is expected to provide a type of bacterial immunity against invading DNA, such as from bacteriophages. KB290 also has one CRISPR region, whereas Lb367 has two regions (**20, 31**). Both annotations predict the presence of eight phage-related proteins dispersed throughout the Lb464 genome and a phage island (~23 kb) is contained on pLb464-4 along with a tRNA gene coding for methionine. The presence of this tRNA is proposed to increase the fitness of phage with different coding capacity from their host and to facilitate phage integration (**6**). In order to connect the interesting genetic features of Lb464, with its ability to grow in the pressurized beer environment, full transcriptome analysis (RNAseq) was used to investigate how specific genes are utilized.

6.4.3. RNA isolation, sequencing and read processing

The isolation of Lb464 mRNA from experimental samples was deemed successful by various assessments (i.e., Experion, Qubit quantification and qPCR assessment). Further, the library preparation and Illumina paired-end sequencing of mRNA performed at NRC PBI was also successful, given that a total of 170,794,473 reads were obtained from one lane with a total of 12 samples multiplexed, when generally 150 million reads are expected (**22**). Upon quality processing of these reads to discard reads below 20 nt and/or with a Phred score < 30, and subsequent mapping of these reads to the Lb464 genome via Bowtie 2, there was a high percentage (91 to 99%) of total reads which mapped to the genome (Table 6.2). Of the aligned,

Table 6.2. Bowtie 2 alignment of RNA sequencing reads for Lb464

Sample [a]	Total Paired Reads		% Aligned Reads [d]		% rRNA Reads [e]		% Annotated CDS [f]		# Single Reads mapping to CDS [g]
	Unfiltered [b]	QC [c]	Unfiltered	QC	Unfiltered	QC	Unfiltered	QC	QC
L-deg-I	14,536,859	14,418,996	98.2	98.7	15.8	15.9	74.9	75.4	21,748,136
L-deg-II	13,954,174	13,954,174	99.1	99.1	18.0	18.2	74.5	75.1	20,985,400
L-gas-I	13,775,070	13,658,680	98.4	99.0	0.3	0.3	88.8	89.4	24,425,970
L-gas-II	14,354,212	14,240,958	97.9	98.5	0.8	0.8	85.9	90.1	24,620,116
L-mM-I	14,482,096	13,652,921	89.4	94.9	76.4	81.1	11.5	12.2	3,324,704
L-mM-II	14,465,266	14,027,270	88.9	91.6	75.0	77.3	12.2	12.6	3,537,528

[a] Samples denoted as "L-" (Lb464), "deg" and "gas" are degassed beer and gassed beer, respectively, mM is mMRS pH 5.5, and "I" and "II" denote replicates.

[b] Values obtained from raw sequencing files with no prior quality processing or trimming.

[c] Values obtained following processing of raw sequencing reads to remove low quality reads via FastQC.

[d] Percentage of all reads aligned to Lb464 genome using Bowtie-2 alignment.

[e] Percentage of aligned paired-end reads corresponding to rRNA genes.

[f] Percentage of non-rRNA aligned paired-end reads corresponding to annotated CDS regions.

[g] Total number of high-quality, single read fragments aligning to CDS regions.

quality-controlled (QC) reads, between 0.3 to 81% of these reads mapped to rRNA regions across samples. These levels of rRNA reads, even after physical rRNA removal is not unexpected, although the wide range in values across experimental samples is surprising given that all samples were processed identically (**21, 22**). This notably large disparity in rRNA removal efficiency of samples is likely a function of the fact that more rapidly growing cells, like Lb464 cells in L-mM controls *versus* in beer require more ribosomes to cope with demand for increased protein synthesis. Further, efficieny of rRNA removal may be affected by the *total* RNA extraction efficiency of these samples. For instance, the extraction of quality RNA from "L-gas" samples (Table 6.2) was extremely difficult given the physiological adaptation of the Lb464 cells to the harsh beer environment, with fewer cells grown in this medium (**10**; Chapter 2). In fact, following rRNA removal, the absolute minimum of mRNA allowable for library preparation remained for "L-gas" samples. In contrast, RNA extraction from Lb464 cells grown in the control MRS medium was comparatively easy and yielded more total RNA. Such higher amounts of total RNA for some samples means there is a higher number of rRNA molecules present and input into the rRNA removal procedure, which results in a decrease in the efficiency with which the rRNA can be removed (Table 6.2).

rRNA accounts for 80 to 95% of total bacterial RNA, thus representing a large percentage of available reads, even with rRNA-removal steps performed during the mRNA preparation (**21, 22**). Any rRNA reads that remain can skew analysis away from recognizing significantly expressed small or rare RNA transcripts. Therefore, rRNA and tRNA genes were removed from the Lb464 annotation files prior to counting the number of reads that mapped to CDS features and undertaking downstream differential expression analysis (**36**).

The disparity and relatively low mapping rate of remaining non-rRNA reads to annotated CDS regions across samples initially appears to be concerning. For each sample, there is a large proportion of reads that align to the genome, but in "no feature" regions – i.e., indicating they map to intergenic regions (see section 6.4.4). This indicates potential genomic DNA (gDNA) contamination of these rRNA samples, which is highly surprising given that assessment of samples prior to sequencing by both Qubit spectrometry, and qPCR assessment indicated negligible levels of DNA present. BLAST analysis of these reads revealed that they do belong to

Lb464 and given the high level of initial alignment to the genome confirms that this contamination is not from an outside source, but that either DNase treatment was not as efficient as quantitative readings indicated or more likely that the great sequencing depth of theses samples included detection of very low level genomic DNA (**1, 21**). Once more, the affect of high total RNA yield is observed in relation to increased rRNA and potential gDNA contamination levels, with mMRS samples having higher proportions of gDNA-mapping reads and thus a lower proportion of read belonging to CDS regions.

When "no feature" reads are added to the number of reads mapping to CDS regions, there is still a proportion of QC reads that do not align to the genome (between 1 and 4% of reads). This small proportion of non-aligning reads are similar to previous studies, where roughly 5% of reads did not align to the genome (**37**), and it is likely that this proportion of reads are artificial sequencing chimeras (**29**).

Regardless of inefficient rRNA removal and presence of gDNA, the number of quality, non-rRNA read pairs that map to Lb464 CDS loci for all samples is still sufficient for detection, as previous studies have found that between 5 and 10 million non-rRNA fragments allow detection of all but a few of the most low expressed genes in diverse bacteria growing under a variety of conditions (**21**). This same study also found that the use of biological replicates provides for differential expression analysis of genes with high statistical significance, even when the number of reads per sample is reduced to 2 to 3 million, as was the case for the mMRS samples ("L-mM; Table 6.2) (**21**).

6.4.4. Verification of Lb464 genome using RNA sequencing reads

Despite the potential inversion and mis-assembly of the Lb464 genome, the entire Lb464 genome had RNA sequencing coverage as visualized by Artemis and Hawkeye (data not shown). This indicates that the vast majority of CDS regions in the Lb464 genome were encoded for in the eight assembled scaffolds prior to scaffold joining and that scaffolds were only dis-jointed as a result of repetitive, non-coding regions (i.e., transposases, rRNA or tRNAs). Though the orientation of these scaffolds (and thus CDS) may be inverted, the accuracy with which RNA sequencing reads map to these regions is not affected as the sequencing was non-strand specific

and paired-end. Genomic regions with read-coverage, yet no existing annotation were investigated via Artemis to reveal "new" or un-annotated genes. This did not prove fruitful, as potential open reading frames (ORFs) >100 bp did not reveal functional coding sequences. Though with increased effort it may be likely that there are smaller ORFs that may encode some small RNA species, specifically ncRNAs, as was found previously in (**37**) – these regulatory RNA species require much more analysis before they can be useful in screening for beer-spoilage ability given their elusive and presumed complex role in regulating general cellular physiology in growth environments. Additionally, reads that did not align to the assembled genome were analyzed via BLAST to determine if any reads corresponded to cysteine, histidine and tryptophan tRNA exons and no transcripts related to these tRNA molecules were found.

Interestingly, there is a large stretch of pLb464-4 that corresponds to a phage island which had minimal read coverage – i.e., had very low-level transcriptional activity in mMRS and gassed beer samples, but greater expression in degassed beer. Given that the rest of the plasmid sequence has notable transcriptional activity in all three-growth media (mMRS, degassed and gassed samples), it is most likely that only the degassed beer environment (mild-stress) triggers some minimal transcriptional activity of this phage region. DESeq 2 retains these features in its differential expression analysis (DE) (i.e., regions which have no reads in one condition, yet have coverage/reads in another), and though these differences between conditions may be considered to be statistically significant, the Log_2 FC in expression between the two conditions is too low to be meaningfully calculated. Thus, these specific transcripts are not included in description of significant DE (SDE) genes in one condition over another, yet are listed as part of the complete statistical output of DESeq 2 in Supplementary Table S6.1a,-b,-c.

6.4.5. Differential gene expression analysis

Transcriptomic analysis of spoilage organisms is done with intent of revealing a set of genes and/or genetic pathways important for the spoilage phenotype and that could be used with reasonable confidence to identify other potential spoiling isolates. The goal of the present study is to elucidate candidate "beer-spoilage indicator" genes or pathways that should be focused upon in future transcriptomic studies with other BSR LAB. Thus, exact quantification of

transcript expression change is not critical, but rather what is important is the qualitative, "meta" understanding of how Lb464 survives in the beer environment.

DESeq 2 was selected for differential expression analysis given its well-studied efficacy and widespread use. This software package is adept at analyzing experiments with small numbers of replicates, and implements a scaling factor normalization procedure to account for varying sequencing depths of different samples, applying the Benjamini–Hochberg procedure to control the false discovery rate (FDR = 0.1) (**3, 30**). DESeq 2 uses a negative binomial model for analysis that operates on the hypothesis that most genes are not DE and tests whether for a given gene the change in expression strength between the two conditions is larger than the variation observed within each replicate group. Normalization is achieved by applying a scaling factor for comparisons between samples with different read library sizes. However, to confirm that the disparity in the number of quality mapped reads across experimental samples did not affect the outcome of differential analysis (Table S6.1a,-b,-c), the SAMtools package was used to subsample aligned reads from the "L-gas" BAM files at levels of 50% and 25% of reads. The subsamples "L-gas" reads were used to perform differential analysis with DESeq 2 package against the full "L-mM" reads. Using different subsampling levels did not change the overall statistical output and characteristic SDE genes and Log_2 fold change (FC) values. The only observed difference was that subsampling fewer number of reads results in an increase in the number of genes that did not exhibit coverage in either condition, thus forcing DESeq 2 to not perform testing of these genes. Therefore, read files were not subsampled prior to any DE analysis as DESeq 2 was found to competently handle differences in sample library sizes.

Comparison of experimental samples, using a *P*-adjusted (FDR) of < 0.1 as a cut off for SDE predicted greatest DE and SDE transcripts when comparing mMRS to gassed beer (relative to mMRS compared to degassed beer, and degassed beer compared to gassed beer) (Table 6.3). For all subsequent comparative analysis between experimental conditions, transcripts that are SDE at or greater than 2 Log_2 FC (i.e., 4-FC) are considered for discussion of beer-specific physiological responses. It must be noted that some of these transcripts may belong to operons that have components showing SDE at levels lower than a 2 Log_2 FC, and are thus not explicitly listed or discussed (Table S6.1a,-b,-c).

Table 6.3. DESeq 2 differentially expressed Lb464 genes during growth in different media

Sample Comparisons	# DE Genes (%)[a]	# increased SDE genes [b]	# genes Log_2 Fold change > 2 [c]	pLb464-1[d]	pLb464-2	pLb464-3	pLb464-4	pLb464-5	pLb464-8
L-deg-I L-deg-II	1765 (66.8%)	836	240	1	19	2	75	0	10
L-mM-I L-mM-II		929	134	5	8	13	11	8	12
L-gas-I L-gas-II	1796 (68.0%)	905	205	7	20	4	16	1	24
L-mM-I L-mM-II		891	207	7	7	9	11	5	7
L-gas-I L-gas-II	1641 (62.1%)	846	16	8	23	10	12	6	32
L-deg-I L-deg-II		794	73	1	6	5	88	0	3

[a] Total number of significant differentially expressed (SDE) transcripts based on FDR < 0.1.

[b] Number of significant differentially expressed (SDE) transcripts during growth in one medium, compared to the other.

[c] Number of genes that are SDE, that are expressed at over Log_2 FC of 2 in during growth in one medium compared to the other.

[d] Number of SDE (FDR < 0.1) plasmid-localized genes during growth in one medium compared to the other. pLb464-6 and pLb464-7 are not listed as they are cryptic; i.e., are small and have limited coding capacity.

6.4.6. Lb464 transcripts DE in degassed and gassed beer relative to mMRS medium

Several Lb464 transcripts are SDE in both degassed and gassed beer relative to mMRS medium, that were previously implicated as important for growth in beer in a transcriptomic study of *Pediococcus claussenii* ATCC BAA-344 (Pc344) (**37)** (Table S6.1a,-b). Specifically, agmatine deiminase and putrescine carbamoyltransferase (L747_12850 and L747_12860; energy production and pH regulation), ATPase (L747_06760 and L747_07730; maintenance of proton motive force), manganese transport protein (L747_13605; hop-tolerance and oxidative stress), methionine sulfoxide reductases MsrA (L747_05475; oxidative stress), glutathione reductase (L747_11980; oxidative stress), as well as other metal transport (L747_09900) and energy homeostasis proteins (L747_06040, L747_10025, L747_05335) all are among the most highly Lb464 SDE genes in both degassed and gassed beer, as they were for Pc344 in (**37**). Agmantine and putrescine are among the most prevlant biogenic amines found in beer (**8, 24**), with the levels being affected by raw materials, brewing techniques and microbial contamination during brewing (**8, 24**). The formation of agmatine specifically is seen in mashing and wort boiling, and other biogenic amines are produced likely as a result of potential enzyme activity in the malt and the main fermentation (**24**). Both fermenting yeast and potential contamination by LAB have also been shown to procude specific biogenic amines, specifically putrescine by *L. brevis* (**52**). The metabolism of agmatine to produce ATP, CO_2, putrescine and ammonia therefore appears critical for the efficient production of energy when organisms are growing in and spoiling beer.

Several transcripts up-regulated in Lb464 likely function as structural components of the cellular membrane ("membrane proteins"), in addition to multiple general membrane transport mechanisms (ATP-binding cassette type (ABC), major facilitator superfamily (MFS) transporters, multidrug transporters, efflux (ion) pumps, and permeases), similar to what was found for Pc344 (**37**). Additionally, the MFS transporter *horC* involved in hop-tolerance (L747_00215) is up-regulated in both beer conditions as are several other transcripts found on pLb464-2. This the only Lb464 plasmid which has SDE genes above 2 Log_2 FC in both beer conditions, apart from a hypothetical protein originating from pLb464-4 (L747_00880). This finding corroborates previous analyses that demonstrated pLb464-2 strongly contributed to both the hop-tolerance and beer-spoilage ability of Lb464 (**12**). The apparent similarities between the present Lb464 data sets and that for Pc344 (**37**) is important given that the study of Pc344 involved the use of

different control medium (MRS pH 6.5 with added Tween 80, not mMRS pH 5.5), and that the dCO_2 content of the beer used in the Pc344 study was not well defined (**37**). Thus, these commonalities lend credence to these transcripts being critical for BSR LAB survival (i.e., non-species specific) in beer.

Despite these similarities, there are several notable differences or unique processes up-regulated in Lb464 relative to Pc344 when grown in beer. For instance, the Lb464 data set reveals that there is a signal peptidase I (L747_09825) involved with processing and maturation of secretory and membrane proteins that is up regulated, whereas a similar peptidase was down-regulated in the case of Pc344. Given that several transcripts encoding Lb464 membrane proteins are shown to be SDE, it would make sense that processes involved in membrane protein maturation and functioning also are SDE. Further, the universal stress protein UspA (L747_10150) is up-regulated in Lb464 in both degassed (2.6 Log_2 FC) and gassed beer (2.3 Log_2 FC), whereas no specific stress response proteins were strongly expressed by Pc344 when growing in beer suggesting that Lb464 mounts a strong general stress response to the beer environment, in addition to deploying more beer-specific mechanisms.

There are also notable differences in the carbohydrate utilization and metabolism genes up-regulated in beer between the Lb464 and Pc344. For example, Lb464 expresses at greater than 2 Log_2 FC key genes of operons involved with maltose metabolism (L747_07715 and L747_09905; maltose O-acetyltransferase), histidine metabolism (L747_01385; imidazolonepropionase), arabinose metabolism (L747_07720) and butanoate metabolism (L747_08660; alpha-acetolactate decarboxylase). These processes were not implicated in the transcriptional study of Pc344 during growth in beer (**37**).

The up-regulation of several histidine metabolism-related genes suggests that Lb464 scavenges trace amounts of this amino acid from the environment, as it does not have direct tRNA synthesis capacity for it. Histidine can be used as a source of carbon, energy and nitrogen, and histidine metabolisms shares biosynthetic pathways with purine metabolism (i.e., L747_08270) and alanine (i.e., L747_05595), aspartate (i.e., L747_07260) and glutamate metabolism (i.e., L747_10940, L747_01690) (**9**), which are all processes that have transport and metabolism

genes up-regulated in both beer media (Table S6.1-c). A recent study of beer-spoilage *L. brevis* isolates suggested that a specific signal transduction histidine kinase was enriched in beer-spoilage related strains, and the presence/absence of this gene was used as the first node in a dichotomic decision tree for determining beer-spoilage ability of an unknown *L. brevis* isolate (**7**). Lb464 contains one signal transduction kinase (L747_02770), which was not the specific transcripts discovered in (**7**), but nonetheless was SDE at 1 and 0.5 Log_2 FC in degassed and gassed beer media, respectively. In previous transcriptomic data of Pc344, no significant expression of histidine kinases in beer was observed (**37**). Signal transduction kinases are involved in signal transduction across cellular membranes (**32**), and thus transcriptional data suggests some importance for a signal transduction histidine kinase during growth in unpressurized and pressurized (packaged beer) for *L. brevis*, however the usefulness of the specific gene suggested in (**7**) as a chromosomal (i.e., stable) genetic marker for *L. brevis* beer-spoilage ability is still unclear.

Lb464 genes involved in arabinose metabolism are up-regulated as well and given that trace amounts of arabinose have been found in beer (**37**), provides further testament to the nutrient scavenging ability of this bacterium. Alpha-acetolactate decarboxylase (L747_08660) is an enzyme involved with butanoate metabolism using pyruvate as a precursor, leading to the production of diacetyl and acetoin, both of which produce a buttery off-flavour in spoiled beer (**50**). Acetoin is also an external energy store for some fermentative bacteria, and during exponential growth can prevent the over-acidification of the cytoplasm and surrounding growth medium as a result of acidic metabolism product accumulation (i.e., acetic acid). Upon entering into the stationary phase, acetoin can be used to maintain the culture density (**50**). None of the carbohydrate utilization genes predicted as unique to Lb464 by RAST (Section 6.4.2.1) are shown to be SDE in beer or are up-regulated to a small extent in mMRS (L747_09170; citrate lyase and L747_10635; fructonate transporter). This is interesting given that citrate lyase metabolism is important for the biosynthesis of fatty acid during Pc344 growth in beer. Nonetheless, Lb464 still shows SDE of pyruvate metabolism transcripts and genes involved in fatty acid production in Lb464 (i.e., L747_06040-06060, L747_11960) (Table S6.1a,-b,-c). This indicates that citrate was present in low quantities for scavenging during mid-exponential Lb464 growth and that Lb464 made use of other carbon sources to produce pyruvate and feed fatty acid

metabolism. Again, this difference is noted with the caveat that the study of Pc344 used the same beer however with slightly different experimental conditions, however, it also indicates that the response of a given BSR LAB is likely to be individualistic based on the background genome and transcriptional regulation abilities. This finding also enforces the need for transcriptional studies to be done in parallel with comparative genomics, with both analyses done on a variety of BSR LAB with beer-spoilage ability.

One of the most distinct differences in the transcriptional pattern of Lb464 *versus* Pc344 when growing in beer involves the greater number of peptide and amino acid transport transcripts found to be up-regulated in Lb464 (Table S6.1a,-b,-c; Fig. 6.4). Peptide and amino acid transport has been proven advantageous to bacterial cells in defense against osmotic stress conditions (1**7, 51)**, specifically proline, which can function as water-sequestering compounds as well as chaperones for protein folding, preventing aggregation (**51**). Proline cannot be used by primary fermenting yeasts and has been shown to be present in finished beer, often contributing to haze-production (**38**). In addition, peptide and amino acid compounds and their uptake are important for nitrogen metabolism, a process known to be critical for malolactic fermentation by wine-fermenting LAB. Studies of assimilation of free available nitrogen in beer are largely concerned with the ability of fermenting yeast to utilize these compounds; in contrast, the importance of nitrogen uptake and cycling for BSR LAB is not well understood. However, the present transcriptomic data strongly indicates that nitrogen uptake and utilization is an efficient way for Lb464 to obtain energy in face of carbohydrate starvation, facilitating rapid growth (i.e., cell metabolism and replication) in beer. In addition to the increased number of peptide transport transcripts, Lb464 has several genes involved in nutrient cycling (L747_08270; xanthine permease/purine transport, L747_04210; glutamine synthetase/nitrogen metabolism, L747_07260; asparagine synthase, L747_07255; ammonia permease/transport; L747_01930l, and aminopeptidase C/amino acid processing, L747-01500 serine protease/peptide bond cleavage). There are no apparent extracellular peptidases annotated or up-regulated in beer to suggest Lb464 actively breaks down extracellular peptides or uses amino acids for scavenging. However, there is a phosphohydrolase (L747_10665; periplasmic or membrane bound), which may facilitate breakdown of nitrogen sources in the beer environment prior to uptake into the cell. Pc344 contains coding capacity for amino acid and peptide transport and metabolism, however

its limited dependence on these transcripts relative to Lb464 could likely be a function of differences in genome size and coding capacity and suggests that efficient or increased nitrogen metabolism is advantageous for growth in beer.

6.4.7. Transcriptional response in gassed beer relative to degassed beer

The defining difference between degassed and gassed beer is that the gassed beer is maintained with roughly 150 KPa headspace pressure at 30°C (levels found in a standard North American beer bottle with a fermenting organism growing in it). This means approximately 2 vol of CO_2 are forced into solution (**11**; Chapter 5). Therefore, pressure is a necessary condition for dCO_2 to be present, however, neither condition is present at levels that would constitute "high pressure" or lethal concentrations of CO_2. Nonetheless, the presence of both has been shown to greatly influence the beer-spoilage ability of BSR LAB (**11**; Chapter 5).

DESeq 2 predicts a set of genes that were SDE in degassed beer over 2 Log_2 FC that were not expressed at that level in gassed beer, and vice versa (Table S6.1-c). This is not to say that genes in each condition-specific list are not SDE in both conditions, but rather that the specific condition enhances a given gene's transcriptional activity to over 2 Log_2 FC relative to the othe condition. The top "degassed beer-specific" transcripts are several stress proteins (L747_00955 (pLb464-4) and L747_05870 (chromosomal); universal stress protein UspA, and L747_11695; alkaline shock protein) and two manganese transporters (L747_9040 and L747_09570). Further, there are several other SDE transcripts involved in processes already discussed such as amino acid (i.e., histidine) transport and metabolism, and oxidative stress management. Thus, growth in degassed beer is not distinct relative to the general Lb464 response to beer, but is clearly specifically geared towards eliciting stress responses, which can help protect the cell membrane and osmoregulation of the cell. Here it is important to note once more that these genes and other related genes are significantly transcribed in gassed beer as well, however, the gassed beer environment selects for stronger transcription from other loci and thus these "degassed-specific" transcripts appear down regulated in the gassed beer dataset. Alternatively, it could be that because the gassed beer environment elicits a stronger response related to membrane defense mechanisms, the total gassed beer environment in effect becomes less stressful for the cell compared to the degassed beer environment, which must elicit a stronger general stress response.

Regardless, the fact that they are "degassed-specific" highlights the importance of these physiological responses in beer without increased dCO_2 presence.

"Gassed-specific" transcripts, i.e., genes predicted to be SDE > 2 Log_2 FC in gassed beer and not degassed beer, contain fewer hypothetical proteins and are specifically targeted at cell wall and membrane strengthening, transport, and rRNA metabolism and transcriptional activity (Table S6.1-c). Interestingly, there are more plasmid-based transcripts that are highly SDE in the gassed beer environment relative to the degassed environment, which is dominated more by strong expression from chromosomally located loci (Table S61-c). This interestingly suggests that the presence of pressure in the beer environment (i.e., headspace pressure) and the resultant increase in dCO_2 in gassed beer drives a specific response that is to an important extent reliant on extra-chromosomal coding capacity. The overall nature of these transcripts appears to be primarily involved in cell wall fortification (L747_02040; bactoprenol glucosyl transferase, L747_07965; cell surface protein), and energy and transcriptional regulation (L747_11320; cold-shock protein/transcriptional regulation, L747_04040 50S ribosomal protein L32, L747_08550; DEAD/DEAH box helicase, L747_10825; ribonulceotide reductase). There are also two very specific transporter proteins up-regulated, L747_10285; Na^+:H^+ antiporter and L747_04825; ammonia permease, and L747_11245; glycine/betaine ABC transporter, which enable energy maintenance and osmoregulation.

The shift towards cellular wall defense and osmoregulation cannot explicitly be credited as either a pressure-response or as a dCO_2–specific response. Extracellular pressure undoubtedly creates osmotic stress for the bacterial cell, as well as affecting cell morphology and cytoskeleton integrity and disrupting cell division (**17, 23**). dCO_2 can increase the cell membrane permeability and thus cause osmotic shock and disruption of cellular processes and transcription (**35**). It has also been proposed that small CO_2 bubbles can be formed intracelluarly in some bacterial cells and create high intracellular pressure (**43**). The direct damage to the cell wall integrity by these two stress elements enhances the membrane and PMF-damaging effect of other beer stresses such as ethanol and low pH. Thus, it is difficult to ascertain what gassed-beer transcripts are specifically responding to apart from strong osmotic shock induced by the combined presence of headspace pressure and CO_2.

Despite these targeted modifications and stress responses, it is most interesting that Lb464 does not enter a viable, but non culturable state (VNBC), as is common for BSR LAB when grown in gassed beer and for LAB in general in response to (usually high) pressure treatments (**11, 53**; Chapter 5). In fact, a recent transcriptome study of *Vibrio parahaemolyticus* in a VNBC state indicated that many of the general processes up-regulated in its non-culturable state are very similar to those that are up-regulated during Lb464 growth in gassed beer, namely, amino-acid fermentation, specific transport and binding proteins and cold-shock transcriptional regulators (**34**). Lb464 therefore appears to be able to perform extreme-stress survival mechanisms that VNBC cells perform, yet still maintains cellular division and growth. Pivotal to understanding how Lb464 is able to avoid the VBNC state when growing in beer is the transcriptional regulation occurring during this growth condition.

Determination of the transcriptional regulatory network of Lb464 in response to gassed beer would likely require the use of time-course transcriptomic samples, especially in light of the fact that the transcriptional induction of the VNBC state in LAB is not well understood and because it is likely largely dependent on specific environmental characteristics. However, previous work has identified a LysR-type transcriptional regulator, OxyR, which regulates oxidative stress-related genes that when mutated result in the induction of VNBC state in gram-negative *Vibrio vulnificus* (**26**). Lb464 has four LysR transcriptional regulators, one of which is SDE expressed in gassed beer relative to mMRS (L747_12175; 1.5 Log_2 FC). Lb464, however, has a number of different transcriptional regulator families and a large portion of hypothetical proteins that are likely non-coding RNAs (ncRNAs), which are hypothesized to perform some regulatory role. DE testing between the expression data from degassed beer and gassed beer datasets reveals a Fur (ferric uptake regulator) transcriptional regulator (L747_08475) is up-regulated ~ 3 Log_2 FC in gassed beer relative to degassed beer. This family of regulators is important in protecting against reactive oxygen species, controls the uptake of other metals such as manganese, and are important in virulence of bacteria (**45**). Indirect activation of Fur can also be via small RNAs, which involve some of the hypothetical proteins that are up-regulated simultaneously (**45**). This highlights the potential complexity of the transcriptional regulatory network of Lb464 while

indicating potential important regulators for survival in gassed beer and avoidance of the VNBC state.

Pending corroboration, SDE genes above 2 Log_2 FC in gassed beer relative to degassed beer have potential to be suitable as markers or predictive of "gassed-beer" (Table 6.4). These include genes encoding proteins involved in metal transport and homeostasis, as well as amino acid uptake. Once more, non-descriptive membrane and hypothetical proteins are indicated as being important in this set of circumstances. BLASTx analysis of these hypothetical proteins indicates that some are found in other *Lactobacillus* species; however, the beer-spoilage status of these organisms has not been investigated (**1**). Overall, the analysis of transcriptional response in gassed beer compared to degassed beer enforces the notion the presence pressure and dCO_2 shifts transcriptional energy and effort towards osmoregulation through cell wall enforcement and transport activities, and may explain how Lb464 is adept at avoiding entry into a VNBC state.

6.4.8. Transcriptional response of Lb464 plasmids and hop tolerance genes

The importance of plasmids in the beer-spoilage ability and hop tolerance of Lb464 in degassed beer has been previously demonstrated (**12**; Chapter 4). pLb464-2 was found to most greatly contribute to the beer-spoilage capacity, with pLb464-4 and pLb464-8 contributing in an ancillary way. These findings are reflected in the transcriptional data analysis via DESeq 2 (Table S6.1a,-b,-c).

Interestingly, there is greater Log_2 FC of some plasmid transcripts in degassed beer relative to mMRS medium as compared to in gassed beer relative to mMRS media, though plasmid-based transcripts were more important overall for the specific response elicited in gassed beer (Table 6.3 and Table S6.1-a,-b,-c). The relative differences in Log_2 FC are likely a result of changes in the plasmid copy number (PCN) for each growth condition. It was previously demonstrated that the overall plasmid profile of Pc344 was highly variable, with PCN increased during growth in beer (**37**). They also showed that plasmid-based transcriptional changes (i.e., relative differences in expression) were influenced by this change in PCN. The Lb464 plasmid profile, however, is apparently much more stable than that of Pc344, in that Lb464 plasmids have not been shown to

Table 6.4. Lb464 transcripts with Log_2 Fold Change ~ 2 in gassed beer *vs.* degassed beer

Locus Tag	Protein	RAST annotation and/or Function	Log_2 FC	Location
L747_10285	Na^+:H^+ antiporter	PMF maintenance	3.5	chromosome
L747_09230	Inner membrane protein	Structural support or membrane transport	3.5	chromosome
L747_08465	Membrane protein	Structural support or membrane transport	3.3	chromosome
L747_12895	Hypothetical protein	Unknown	3.0	chromosome
L747_08475	Fur family transcriptional regulator	Ferrous iron metabolite control	3.0	chromosome
L747_10935	Peroxiredoxin	Defense against toxic oxygen species	2.9	chromosome
L747_12900	Hypothetical protein	Unknown	2.8	chromosome
L747_08470	Membrane protein	Structural support or membrane transport	2.6	chromosome
L747_09365	D-alanyl-D-alanine carboxypeptidase	Cross linking peptidoglycan in cell wall	2.3	chromosome
L747_02045	Hypothetical protein	Unknown	2.2	chromosome
L747_02040	Bactoprenol glucosyl transferase	Cell membrane integrity	2.2	chromosome
L747_01900	Metal ABC transporter substrate-binding protein	Zinc-transporter binding protein; ZnuA	2.1	chromosome
L747_03425	Alanine glycine permease	Amino acid transport	2.1	chromosome
L747_01235	Hypothetical protein	Unknown	2.1	pLb464-8
L747_11200	MFS transporter	Multidrug transport	2.1	chromosome
L747_12445	Hydrolase	Peptidoglycan lytic protein P45; cell wall-associated or secreted signal peptidase	2.0	chromosome
L747_04675	Amino acid ABC transporter permease	Amino acid transport	2.0	chromosome
L747_08070	Hypothetical protein	Unknown	1.9	chromosome
L747_08500	Hypothetical protein	Unknown	1.9	chromosome
L747_05850	Hypothetical protein	Unknown	1.8	chromosome
L747_07230	Tyrosine protein phosphatase	Cellular regulation	1.8	chromosome
L747_08120	Hydrolase	Peptidoglycan lytic protein P45; cell wall-associated or secreted signal peptidase	1.8	chromosome
L747_13025	MFS transporter	Benzoate transporter; BenK	1.7	chromosome

[a] Protein annotation according to RAST server and predicted cellular function or role.

be spuriously lost during extraction steps, and the copy number of Lb464 plasmids has already been demonstrated to be increased by degassed beer (**12;** Chapter 4). The presence of pressure and dCO_2, however, could stress the cell such that PCN is reduced in order to conserve energy. Regardless of changes in PCN and/or the contribution of PCN to transcriptional changes, the conclusion remains that specific plasmids, and thus specific plasmid transcripts, are important for facilitating growth of Lb464 in beer.

pLb464-5, which was unable to be cured from the Lb464 cell (**12**; Chapter 4) did not demonstrate any SDE genes in either beer environment, but instead had several transcripts more strongly induced in the mMRS growth environment. Small plasmid pLb464-6 and cryptic plasmid pLb464-7 were also both up-regulated during Lb464 growth in mMRS medium. Transcripts from all three plasmids had sequencing coverage in both degassed and gassed beer, indicating that pLb464-5, -6 and -7 transcripts are not relied upon for growth in beer and/or that they are more beneficial for basic plasmid maintenance (i.e., through RepA proteins) or transcriptional regulation or DNA repair when growing in nutritive conditions (Table S6.1a,-b,-c).

For pLb464-1, the hop-tolerance gene *horA* was SDE in both degassed beer (0.7 Log_2 FC) and gassed beer (1.2 Log_2 FC relative) to mMRS medium. Interestingly, the cassette of surrounding genes that *horA* is always associated with did not show comparable transcriptional activity as also observed during Pc344 growth in beer **(37**). In degassed beer, the only other SDE pLb464-1 genes are transposases and in gassed beer an additional hypothetical protein and glycosyl transferase show increased transcription. On pLb464-3, the putative hop-tolerance gene, manganese transporter *hitA*, is minimally SDE transcribed in degassed beer (0.4 Log_2 FC) and not SDE at all in gassed beer. The only pLb464-3 transcript that is SDE in both conditions is L747_00305, a transcriptional regulator (1.2 FC in degassed beer and 0.7 in gassed beer).

pLb464-2 has the highest proportion of its transcripts SDE in the beer environment, lending credence to the idea that it is a niche-adapted genetic element. Indeed, it is the only plasmid that has plasmid-replication genes (i.e., L747_00265; RepB) up-regulated in both beer environments relative to mMRS medium during mid-exponential growth. All other plasmids do not have SDE

plasmid replication proteins in the beer environment (conditions (Table S6.1a,-b,-c). Most interestingly, the hop-tolerance gene, putative MFS transporter *horC* is the most highly transcribed pLb464-2 transcript (4.6 FC in degassed beer vs. 5.4 FC in gassed beer), however, its putative transcriptional regulator *horB* is not SDE in either beer environment relative to mMRS. Transcription of *horB* is in fact significantly increased during Lb464 growth in mMRS medium. Thus this transcriptional data confirms the results of Chapter 3, which suggested *horB* acts as a repressor of *horC* activity under nutritive conditions and transcription of *horB* is ceased in response to the presence of hops. There are also seven hypothetical proteins and two membrane proteins induced to comparable levels in both beer growth conditions. Interestingly, there is a ferritin protein responsible for storing and controlling the release of intracellular iron encoded on pLb464-2 (L747_00270) transcribed in both degassed (2.1 FC) and gassed beer (1.7 FC) relative to mMRS medium. This, in conjunction with the finding that a chromosomal Fur regulator is an important transcript for growth in gassed beer relative to degassed beer, suggests that pLb464-2 provides a very direct and specific advantage to Lb464 for utilizing ferrous iron likely as a means to mediate oxidative stress. Though understanding of iron levels in beer is rather limited, it has been shown that iron is present in differing levels across light and dark beers, and that beer consumption is correlated with increased ferritin intake (**40**, **49**). The control of environmental ferrous iron could be important in oxidative stress regulation, and thus important for rapid growth of LAB in pressurized/gassed beer. An enolase enzyme (L747_00180) and a glycosyltransferase (L747_00230) also are both SDE in degassed beer and to a greater extent in gassed beer, further suggesting that pLb464-2 is able to contribute to the gassed beer-response.

pLb464-4 and pLb464-8 have been previously hypothesized as providing important functions for growth in beer that are either redundant for chromosomal processes and/or synergistic with the coding capacity of pLb464-2 (**12**; Chapter 4). These plasmids were previously found to maintain in fairly low PCN in degassed beer, however, the transcriptional data suggests that several genes located on both are still induced during Lb464 growth in both degassed and gassed beer. The fact that the phage island and other transcripts experienced such low level of sequencing coverage, but had the highest relative coverage in degassed beer supports the contention that pLb464-4 transcripts, and the plasmid in general provides a supportive, rather than critical role for growth in beer. Its PCN is slightly increased in the presence of degassed beer, indicating it may have

transcripts specifically induced by the stress of beer, but the introduction of pressure/dCO_2 in the gassed beer results in a potential decrease in PCN to reduce the energy burden on the cell and thus lower levels of transcription. Thus, pLb464-4 transcripts appear to be a large portion of SDE plasmid genes. Largely, these genes are hypothetical proteins or phage-related, however, there are several membrane proteins and transport proteins (L747_00425; multidrug ABC transporter permease, L747_00980; MFS transporter and L747_00905; metal ABC transporter substrate-binding protein) that are also SDE. Uniquely, there are several Lb464-4 transcripts involved in either DNA excision or DNA repair that are SDE in degassed beer (L747_00985; pyrimidine dimer DNA glycosylase, L747_00680; HNH endonuclease, L747_00785; recombinase, L747_00900; DNA integrase). These transcripts, which range from 3.4 to 6.7 FC in degassed beer, however, are not SDE in gassed beer. In the presence of gassed beer, six hypothetical proteins, an ABC transporter binding-protein and transposase-related transcripts are the only SDE pLb464-4 transcripts (Table S6.1a,-b,-c).

pLb464-8 transcripts appear to have the opposite relationship with gassed beer in that there are several more transcripts SDE during Lb464 growth in this environment relative to degassed beer. Transcripts that are transcribed in both beer environments include transposase proteins, two hypothetical proteins (L747_01235 and L747_01295) and L747_01140; NAD(FAD)-dependent dehydrogenase and L747_ 01290; FAD-dependent pyridine nucleotide-disulfide oxidoreductase, both of which participate in oxidative stress production. In gassed beer, there is increased transcription of DNA damage-repair protein urvX (L747_01230) and a DeoR family transcriptional regulator (L747_01115), a global regulator. Thus, it is clear that the two largest Lb464 plasmids (-4, and -8) do contribute useful gene functions during growth in both degassed and gassed beer. Since these two plasmids are relatively large (Table 6.1) they concurrently poses a large energy burden to the stressed Lb464 cell. Overall, these two plasmids provide transcripts that are largely involved in DNA repair and excision and rearrangement (transposases, phage and integrase products), with various membrane proteins and hypothetical proteins also being transcribed.

6.4.9. GO enrichment

Investigation of what general molecular functions and biological processes (GO terms) are enriched during growth in both degassed and gassed beer relative to mMRS medium reveals that DNA integration (recombination) and metabolism, and nitrogen metabolism are of major importance (Fig. 6.3). This is somewhat surprising given the number of Lb464 SDE transcripts annotated as “membrane” proteins and cell wall maintenance (Table S6.1a,-b), suggesting their importance for growth in the beer environment regardless of dCO_2 content. However, some of these non-specific membrane proteins, along with hypothetical proteins are unable to be assigned GO terms, which limits their representation in enrichment analysis. Further, it is apparent that transcripts belonging to DNA and overall cellular metabolism regulation are proportionally greater amongst the SDE beer-important transcripts relative to basic nutritive medium (Table S6.1a,-b).

The GO enrichment map of transcripts SDE in degassed beer relative to gassed beer depicts processes important for growth in beer in the absence of dCO_2 (Fig. 6.3). Interestingly, fatty acid metabolism, cellular lipid metabolic processes and structural cellular components are all enriched for, which confirms previous analysis of Pc344, wherein these were proposed to be important adaptations for LAB to deal with the stress of ethanol and oxidative stress (**37**). Biosynthetic processes and primary cell metabolism processes are expectedly enriched given that cells were harvested foranalysis during mid-exponential growth in beer. In the presence of dCO_2, these activities are assuredly still important, however, there is a notable enrichment (up-regulation) of transcripts involved in processes associated with DNA metabolism (both recombination and integration) and subsequent nitrogen metabolism (Fig. 6.3). DNA metabolism is critical for repairing cellular damage and during cell growth, thus suggesting that the dCO_2 environment elicits more damage to the cell writ-large. Further, the enrichment of recombination, integration and transposition activity suggests that the Lb464 genome is increasingly unstable under conditions of pressurized/gassed beer and therefore amenable to genomic rearrangement and gene loss. While analysis of individual SDE transcripts (Table S6.1a,-b,-c) indicates hypothetical proteins and membrane-associated proteins as being critical for growth in gassed beer (Table 6.4), and thus possible candidacy as selective markers for pressurized/gassed-beer survival, these

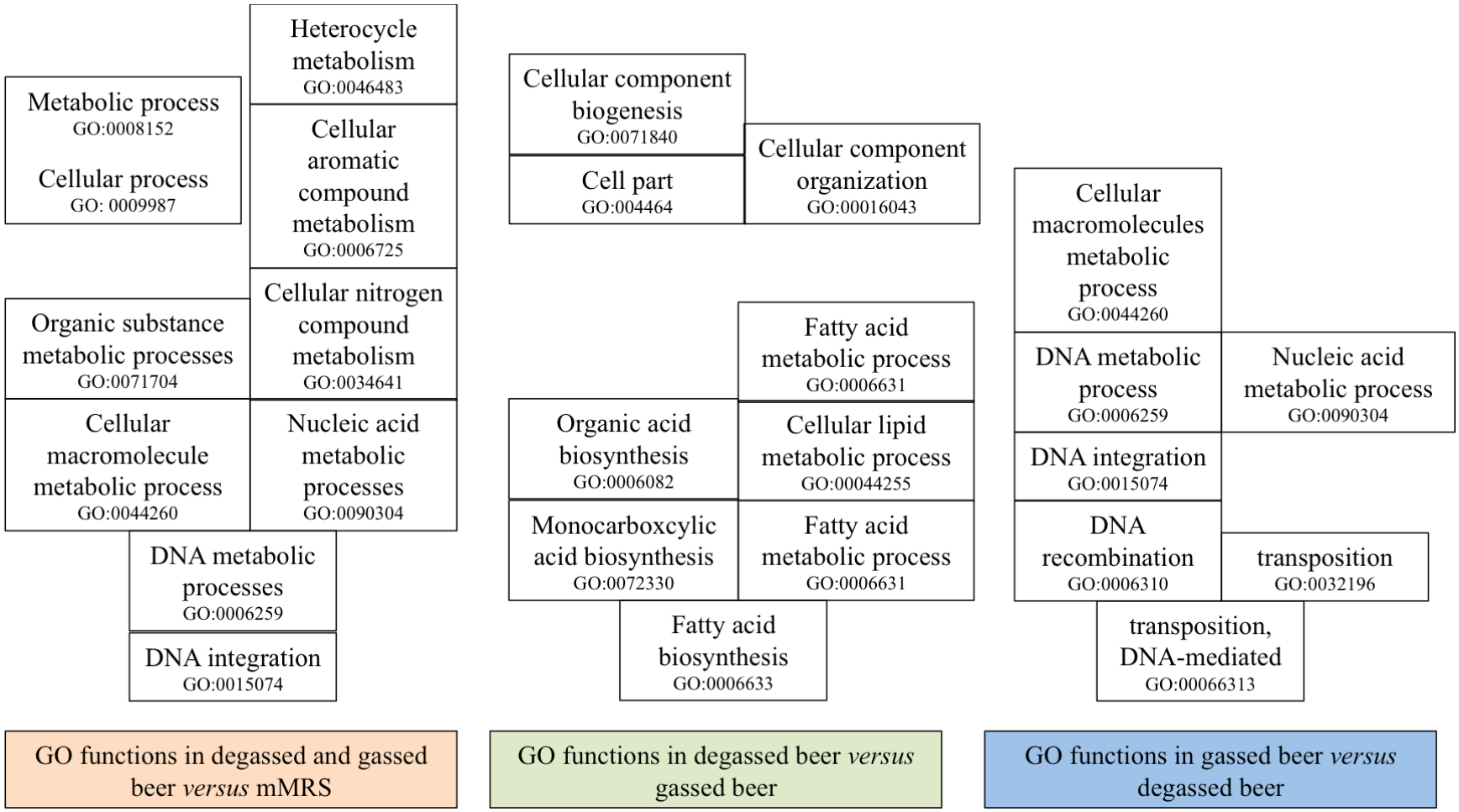

Fig. 6.3. Enriched common GO functions for Lb464 in different growth conditions.

Specific GO terms were determined to be enriched at a FDR level of < 0.1. Position and grouping of boxes indicates close association of GO terms and/or processes.

transcripts are either not amenable to GO categorization and/or comprise proportionally less of the DE transcripts.

6.5. CONCLUSIONS

The *de novo* genome assembly of rapid beer-spoiling organism Lb464 produced an atypical GC skew that did not influence the accuracy with which RNA sequencing reads were aligned to the genome. Lb464 is unique in that it contains eight plasmids, and lacks tRNA codons for cysteine, histidine and tryptophan. The absence of these genes, the presence of repetitive regions and apparent large rearrangement suggests that the Lb464 genome is adaptable and amenable to recombination and gene reduction. Despite these sequence anomalies, the transcriptional analysis of Lb464 when grown in degassed and gassed beer confirmed coverage of the genome and thus lends high confidence to conclusions of transcriptional activity during growth in beer. Transcriptional behavior in both the degassed and gassed beer environments is largely similar, which is unsurprising given that these two environments differ only in the presence of headspace pressure and dCO_2. Together with previous transcriptomic data of Pc344 (**37**), the Lb464 transcriptional data indicate that the most important beer niche-specific genetic elements for LAB areas are related to (a) metabolism of agmatine, (b) membrane modification and membrane transport, and (c) efficient transcriptional regulation. Most interestingly, however, is the importance and efficiency of amino acid metabolism and nitrogen cycling for Lb464 during growth in beer, which was not observed during the comparable analysis of Pc344. This finding, coupled with the notable difference that Lb464 induces significant DE of multiple general stress proteins during growth in beer, whereas Pc344 does not, may explain Lb464's greatly increased virulence/growth success in beer and tolerance to dCO_2 relative to the more slowly growing Pc344 in beer.

The presence of headspace pressure in bottled beer, and thus dCO_2, drives Lb464 transcription to increase cell wall and membrane modification, transport and overall osmoregulation. GO enrichment analysis reveals the importance of lipid metabolism and fatty acid synthesis, confirming the long-held suspicion that these processes are important for adaptation to growth in beer. Additionally, increased activity of hypothetical proteins, hop-tolerance gene *horC*, and DNA repair and recombination from pLb464-2, -4, and -8 are observed in this stressful

environment. The coding capacity of pLb464-2 appears to be most advantageous for growth in both degassed and gassed beer and thus is suitable to use for comparative analysis against other plasmids recovered from putative BSR LAB. The control of ferrous iron via both chromosomal and plasmid-based transcripts provides the first indication that control of this metal is critical during exponential growth in gassed beer. Overall, the upregulation and role of transcriptional regulators is implicated as being critical for avoidance of the VNBC state by Lb464 in face of the harsh environmental posed by beer, and is likely the key to defining "true" (i.e., pressure/dCO_2-tolerant) BSR LAB.

The analyses presented here indicate a complex and multifactorial transcriptional response occurs during LAB growth in beer and should move the research paradigm of BSR LAB away from the notion that there are "absolute" genetic markers specific to the beer environment. Rather, it is likely that BSR LAB will share a broad set of stress response mechanisms with non BSR LAB, while having a greater proportion of their coding capacity dedicated to membrane modification and transport and/or transcription factors. Thus, it will be critical to perform further transcriptional studies of more BSR LAB, under various conditions and time points, in conjunction with BSR LAB whole-genome analyses. This is needed to confirm not only the prevalence of transcripts in beer-specific responses, but to also grasp the extent of variability of BSR LAB responses to beer and whether a given response is genus-, isolate- or isolation-environment- (i.e., brewery) specific. Further, increased investigation into profiling "conserved" hypothetical proteins, and orthologs or paralogs among BSR LAB will greatly enlarge the pool of available data that may serve as potential markers during screening procedures for BSR LAB.

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Chapter 7: Transcriptional response of *Lactobacillus brevis* BSO 464 and *Pediococcus claussenii* ATCC BAA-344^T during growth in the presence of hops

Jordyn Bergsveinson, Emily Ewen, Vanessa Pittet and Barry Ziola

7. INTERFACE

Previous RNA sequencing of both *Lactobacillus brevis* BSO 464 (Lb464) and *Pediococcus claussenii* ATCC BAA-344^T (Pc344) during growth in beer has indicated several transcripts that may mediate specific stresses induced by the presence of hops in beer. In order to confirm this, and to better understand the total transcriptional response to hops, RNA sequencing of both isolates in growth limiting concentrations of hops was performed.

Jordyn Bergsveinson prepared all RNA samples for sequencing and performed all subsequent bioinformatics processing and analysis of RNA sequencing reads; including differential expression analysis and genome validation. She authored the manuscript.

Emily Ewen is attributed with isolating all Pc344 plasmid variants and performing growth analysis in beer.

Vanessa Pittet provided insight into RNA sequencing experimental design and during the processing of RNA sequencing reads and data.

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work.

7.1 ABSTRACT

Whole-transcriptome analysis of beer-spoilage organisms *Lactobacillus brevis* BSO 464 (Lb464) and *Pediococcus claussenii* ATCC BAA-344^{T} (Pc344) when grown in growth-limiting concentrations of hops was performed, in order to analyze the total cellular response to hops and to help delineate hops-specific responses from the overall physiological response to the total beer environment. Firstly, the genome of highly hop-tolerant isolate Lb464 demonstrated less differential expression in response to a stronger challenge of hops compared to Pc344, which highlights the variable nature of hop-tolerance in beer-spoilage related lactic acid bacteria (BSR LAB). However, the most basic physiological response to hops in Lb464 centers around pathways involved in acid tolerance and intracellular pH homeostasis, which are processes noted to be important for mediating hop-stress. Interestingly there is significant expression of an ATP binding cassette (ABC) transporter for taurine, which may have some cross specificity with hop-iso-α-acids and therefore provide redundancy and increased efficiency in exporting hops from the cell. However, the plasmid-located hop-tolerance gene *horC* is the most significantly differentially expressed gene providing final confirmation of its importance for Lb464 hop-tolerance. In Pc344, there is more differential genetic expression in response to hops, with many transcripts previously shown to be important for Pc344 growth in beer also being expressed in hops. Specifically, transcripts for putrescine carbamoyltransferase, monooxygenase, agmatine/putrescine antiporter, peptide methionine sulfoxide reductase, and metal ion transport, all of which are proposed to help mediate oxidative stress and maintain homeostasis and which were also significantly expressed in response to beer by Lb464. This data raises interesting questions as to what differs between the two isolates that necessitates Pc344 being so strongly responsive to oxidative stress, where Lb464 is able to withstand increased levels without eliciting the same type of response? Lastly, it was noted that both Lb464 and Pc344 both harbor at least one plasmid structure that appears critical for maintaining overall beer-spoilage virulence. Profiling of these plasmids reveals that these plasmid structures harbor *horC* and *horA,* respectively, as well as a toxin/antitoxin system and DNA-damage inducible or repair proteins. Overall, the response to hop-tolerance is shown to be generally directed at maintaining cellular pH homeostasis, however is individual to each isolate.

7.2. INTRODUCTION

Bitter acid compounds derived from hops have long been viewed as the defining physiological stress for microorganisms growing in the niche environment of beer. Accordingly, hop-tolerance is viewed as the attribute that separates beer-spoiling lactic acid bacteria (LAB) from benign, or non-spoiling isolates (**30**). Hop bitter acid compounds have demonstrated antimicrobial activity as they act as proton-ionophores, dissipating the pH gradient (proton motive force; PMF) across the cell membrane and reducing PMF-dependent activities such as nutrient uptake (**23**). Eventually, this loss of function and decrease in cell permeability inhibits cell growth and/or results in cell death (**26**). Further, as an exchange of proton and divalent cations (Mn^{2+}) is involved, intracellular depletion of Mn^{2+} levels results in both oxidative stress and a loss of some enzymatic function (**3, 4**). Efforts to characterize the genetic elements that mediate this hops challenge to the PMF and onset of oxidative damage has lead to the identification of three principal genes, namely *hitA* (**14**), *horA* (**24**), and *horC* (**31**), which are all plasmid-located, and code for membrane-associated transporters that serve to maintain the integrity of the cell in the presence of hops.

Hop-tolerance genes are frequently utilized as predictive "markers" for not only hop-tolerance, but also the overall beer-spoilage ability of a LAB isolate. Unfortunately, these genes fail to perfectly correlate with beer-spoilage ability and are insufficient to confer hop-tolerance to all LAB beer-spoilage organisms (BSOs) (**5 – 7**, **18**; Chapter 2, 5). The fact that the presence of only one or even several hop-associated genes fails to sufficiently ensure tolerance is not surprising as it is apparent that multiple cellular defenses are likely employed to mediate hop damage (**3 – 4, 30**). Further, given the diversity and heterogeneity of LAB in general, and the number of membrane transporters and transport systems that they contain, it is not surprising that that the three genes fail to consistently confer a specific-stress tolerance across all beer-spoilage LAB (**18, 19**).

In order to better understand the important genetic elements and specific transcriptional response to the presence of hops, whole-transcriptome analysis (RNA sequencing) was used to assess beer-spoilage isolates *Lactobacillus brevis* BSO 464 (Lb464) and *Pediococcus claussenii* ATCC BAA-344^T (Pc344; also available as DSM 14800^T and VTT E-032355^T) during growth in basic

nutritive media and the same media with a growth-slowing concentration of hops. These isolates are ideal for such targeted hop-transcription analysis, as a wealth of physiological and genomic data is known and available for both (**6, 8, 11, 21 – 22**; Chapter 2, 4, 6). Further, both isolates previously have had completed whole-transcriptome analysis of their growth in the beer environment (**22;** Chapter 6). Thus these previous transcriptional data sets provide an interesting and detailed framework within which to consider whole-transcriptome sequencing of Lb464 and Pc344 growing under hop-induced duress.

Importantly, the Pc344 strain used in this present study has a "non-ropy" phenotype in that it did not contain pPECL-7, similarly to the strain used in previous beer-specific transcriptional analysis (**22**). pPECL-7 encodes the glucosyltransferase gene, *gtf* that is responsible for producing exopolysaccharide or "rope" that interferes with RNA extraction. It should be noted that the non-ropy phenotype does not differ from the type strain phenotype in tolerance to ethanol, hops, or beer (**13, 21 – 22**). All other seven plasmids remained in this Pc344 variant, however, as pPECL-1 and pPECL-2 are small and cryptic, the Pc344 variant analyzed in the present study is denoted as $Pc344^{34568}$. Further, it is critical to consider that the previous transcriptome analysis of Pc344 during growth in beer was done with a Pc344 isolate that also did not contain pPECL-4 and -6 as these plasmids were found to be lost during RNA preparation; this isolate is then similarly denoted as $Pc344^{358}$ (**22**).

7.3. MATERIALS and METHODS

7.3.1. Isolate and Growth Conditions

$Lb464^{OG}$ (original, full plasmid profile) (**8**; Chapter 4) and $Pc344^{34568}$ were taken from -80°C stock and cultivated in MRS broth at 30°C overnight. From the overnight cultures, 1 ml was inoculated, in duplicate, into 100 ml of modified MRS, pH 5.5 (mMRS; medium lacks Tween 80) and also into 100 ml of mMRS, pH 5.5, containing different bitterness units (BU) of hops. These media are referred to as mM and mM+H, respectively. $Lb464^{OG}$ and $Pc344^{34568}$ were grown in mMRS broth, containing 50 and 30 BU, respectively. The preparation of the mM control for Lb464 (L-mM) was discussed previously in Chapter 6. For mM+H, hops was added to mMRS broth (hops: (hops: Isohop® isomerized hop extract; 28–32% w/w iso-α-acids in an

aqueous solution of potassium salts; John I. Haas Inc., Washington, DC, diluted 1:4 of hop: 95% (*v/v*) ethanol). Cultures were then incubated at 30°C until mid-logarithmic growth was established ($Lb464^{OG}$ = 14 h for L-mM controls, OD_{600}nm ~ 0.3; and 28 h for L-mM+H samples, OD_{600}nm ~ 0.1; $Pc344^{34568}$ = 5 h for P-mM controls, OD_{600}nm ~ 0.25; and 35 h for P-mM+H samples, OD_{600}nm ~ 0.1), at which point cells were harvested for RNA extraction (Fig.7.1).

7.3.2. Total RNA Isolation and RNA sequencing

Bacterial control and experimental cultures were portioned into 35 ml aliquots and harvested by centrifugation at 10,000 × g for 3 min. Resultant pellets were flash-frozen in liquid N_2, stored overnight at -80°C and pooled during extraction. Total RNA isolation, mRNA purification, rRNA removal, and cDNA preparation was performed as in Chapter 6. cDNA was assessed for quality using previously described primers for genes *proC* and *rpoB* for $Lb464^{OG}$ and *ldh* and *gyrA* for $Pc344^{34568}$ (**6**; Chapter 2).

Samples were then sequenced simultaneously along with the samples described in Chapter 6, with the data processed as described in Sections 6.3.5 – 6.3.5.1. Unfiltered data reads were used for downstream analysis as the same validation was performed for these samples as was done in Table 6.2. Respective reads of for each isolate were then aligned to the Lb464 genome available from NCBI (BioProject Accession No. PRJNA203088) and the Pc344 genome available from NCBI (BioProject Accession No. PRJNA81103), using Bowtie 2 version 2.2.3. Count tables of reads aligning to specific features were performed with the use of the same annotation feature file for Lb464 described in Chapter 6 and for a feature file available from NCBI for Pc344. As in Chapter 6 for Lb464, the annotation file for Pc344 also had all rRNA and tRNA sequences removed, and had several transcripts added in that had been identified in a previous transcriptome study of Pc344 (**22**), but had not yet been added to the NCBI Pc344 annotation file (Table 7.1). Differential expression (DE) analysis was performed exactly as in Chapter 6, section 6.3.5.2.

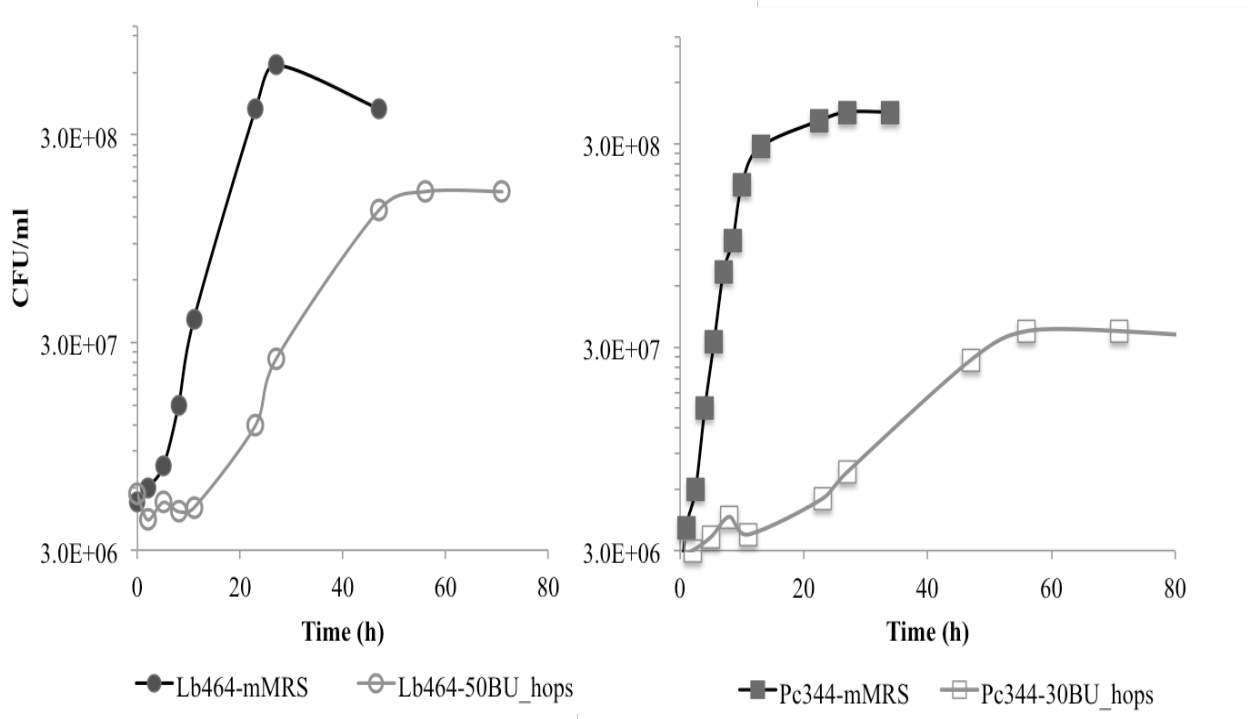

Fig. 7.1. Effect of hops in mMRS pH 5.5 on Lb464^{OG} and Pc344^{34568} growth at 30°C.

Cells of each isolate were harvested for RNA extraction during mid-exponential growth in mMRS, and mMRS and hops.

Table 7.1. List of transcripts added into the Pc344 annotation file before transcriptome reads were aligned.

Locus_tag	Structure	Location	Description
PECL_2059	Chromosome	175194-175817	putative ncRNA between PECL_171 and PECL_172 (pncRNA-1)
PECL_2057	Chromosome	499951-500037	bacterial small signal recognition particle RNA
PECL_2056	Chromosome	590129-590500	transfer messenger RNA; *ssrA*
PECL_2046	Chromosome	603906-604151	hypothetical protein
PECL_2060	Chromosome	698797-698985	putative ncRNA between PECL_686 and PECL_687 (pncRNA-2)
PECL_2047	Chromosome	799027-799155	hypothetical protein
PECL_2048	Chromosome	863743-863970	hypothetical protein
PECL_2049	Chromosome	879505-879915	Prophage Lp1 protein 7, nonsense mutations
PECL_2050	Chromosome	966418-966747	hypothetical protein
PECL_2058	Chromosome	1016600-1016967	Ribonuclease P (RNase P) class B
PECL_2051	Chromosome	1099087-1099236	50S ribosomal protein L33
PECL_2052	Chromosome	1222725-1223096	vanZ like family protein
PECL_2061	Chromosome	1410556-1410791	putative ncRNA between PECL_1459 and PECL_1460 (pncRNA-3)
PECL_2062	Chromosome	1435477-1435632	putative ncRNA between PECL_1482 and PECL_1483 (pncRNA-4)
PECL_2053	Chromosome	1527892-1528242	hypothetical protein
PECL_2054	Chromosome	1540183-1540503	hypothetical protein
PECL_2055	Chromosome	1584901-1585119	hypothetical protein
PECL_2063	pPECL_4	36037-36328	putative ncRNA between PECL_1907 and PECL_1867 (pcnRNA-7)
PECL_2064	pPECL-8	4-375	putative ncRNA between PECL_2033 and PECL_2044 (pcnRNA-5)
PECL_2065	pPECL-8	2152-2430	putative ncRNA between PECL_2033 and PECL_2034

***** These transcripts were discovered and annotated in (**22**), however, are not updated in the annotation file available from NCBI.

7.3.3. Differential analysis and GO term enrichment

As in Chapter 6, DESeq 2 version 1.8.1 was implemented in RStudio to perform DE analysis on Lb464 and Pc344 read counts, with a false discovery rate (FDR) of 0.1 (17). Transcripts with *P*-adjusted (FDR; Table S7.1 and S7.2) values less than 0.1 were taken as significant and examined further. Transcripts that are DE at or above 2 Log_2 fold change (i.e., expression fold change of 4 between conditions) are considered biologically relevant and therefore are discussed. Fold change values are log transformed for reporting as this transformation minimizes skew in the data set by reducing variance in gene expression levels. Lb464 and Pc344 proteins were annotated for gene ontology (GO) terms using Blast2GO v.3.0 and a BLASTx search Expect Value of 1.0^{-3}, and default settings for GO annotations (10). Proteins that were significantly expressed (FDR < 0.1) in DESeq 2 comparisons were taken and used to perform enrichment analysis against the complete genome GO annotation via Fisher's Exact Test in Blast2GO (using FDR < 0.1).

7.3.4. Generation and analysis of plasmid variants

Plasmid variants of Pc344 were generated according to procedure described in (**8**; Chapter 4) by using plasmid-curing agent novobiocin, and screened for using a Pc344-plasmid specific multiplex assay (Table 7.2). The growth kinetics of Pc344 variants in degassed beer was performed in triplicate, as described in (**8;** Chapter 4). The plasmid profile of each Pc344 variant was confirmed through the use of the Pc344-plasmid multiplex PCR throughout its growth in degassed beer.

7.4. RESULTS and DISCUSSION

7.4.1. RNA sequencing and mapping

$Lb464^{OG}$ and $Pc344^{34568}$ cells were both isolated in duplicate during mid-exponential growth in a basic nutritive medium (i.e., mM) and basic nutritive medium containing growth-limiting concentrations of hops in mMRS (i.e., mM+H, Fig.1). RNA extraction, mRNA purification and sequencing steps for both isolates were successful by a number of verification methods and by

Table 7.2. Multiplex PCR primers for detection of Pc344 plasmid variants*

Multiplex	Plasmid Target	Primer Name	Sequence (5' → 3')	Primer Binding Location	Amplicon Size (bp)
Mix A	pPECL-3	PECL_25-1F	CACTCGCCAAGACTGGTGTTC	12785-12805	275
		PECL_25-2R	CGTGGCATGACCATGAATGATCG	13059 – 13037	
	pPECL-5	p5_1F	CAGATCAACGCCAAGCTCAAGTG	1257-1279	515
		p5_2R	GCCTCGACCGTCTGTTATGATACC	1770 – 1747	
	pPECL-8	horA-RT-F	GGATCATCAACTCAATCGGTC	8380 – 8359	155
		horA-RT-R	CCAAAGTGTTGTTCGCAGC	8534 - 8553	
	pPECL-4	p4_MFS-1F	CCGCAGCTGGCACTAAGGAC	18690 – 18671	335
		p4_MFS-2R	ACTGGACTGGGTCTCCTTCC	18356 – 18375	
Mix B	pPECL-6	p6_4F	CACGTTCTTCAAAGACCAAGGTTGC	12017 – 12041	612
		p6_5R	ATTTAAGCCAGAATCAAGGGACGAC	12629 – 12605	
	16S rRNA	386F	CTGATGGAGCAACGCCGCGT	16S rRNA	148
		534R	ATTACCGCGGCTGCTGG		

***** For all resulting Pc344 plasmid variants, the plasmids that remain within a variant are listed as superscripted numbers.

the alignment results produced by Bowtie 2 (Table 7.3). The removal of rRNA from extracted mRNA using RiboZero™ Magnetic Kit was successful as reads mapping to these genes comprised a small percentage of mapped reads relative to previous similar studies (Table 7.3; **22**).

7.4.2. Differentially expressed transcripts in response to hop stress

As shown in Table 7.3, roughly 64% of the Pc344^{34568} genome was significantly DE (SDE; FDR < 0.1) during growth in 30 BU hops, whereas only 23% of the Lb464^{OG} genome was SDE when growing in 50 BU hops. The difference in total SDE transcripts between the two organisms was unexpected given that the presence of hops was noticeably affecting the growth rate of each organism (Fig. 7.1). It is surprising the sub-lethal level of 50 BU hops is still not enough to elicit a hop-stress response comparable to the strength of the response observed in Pc344 by 30 BU hops, though this enforces the assertion that Lb464 is a highly hop-tolerant organisms (**6 – 8**; see Chapter 2 – 5). As such, there are considerably fewer genes SDE at levels above 2 Log_2 fold change in the Lb464 ^{OG} data set compared to Pc344^{34568}, however, the genes that are expected to indicate the basic physiological response of Lb464 ^{OG} to the presence of hops.

7.4.3. Lb464 response to hops

The most highly expressed transcript in Lb464 ^{OG} in response to hops is that of membrane-transport protein *horC* found on pLb464-2 (Table S7.1-a). This finding supports the long held contention that *horC* is an important hop-tolerance gene (**30**; Chapter 3, 6) and is especially so for Lb464 ^{OG} (**6**; see Chapter 2). Interestingly, the putative transcriptional regulator of this hop-gene, *horB,* is not differentially expressed in hops, in fact it appears to have increased expression levels in mMRS medium (Table S7.1-a). This finding confirms previous droplet digital PCR (ddPCR) analysis of these two genes in several different beer-spoilage-related lactic acid bacteria (BSR LAB) that suggested *horB* is a repressor of *horC* activity (Chapter 3). Other putative hop-tolerance genes, *hitA* and *horA* are induced within Lb464 when growing in 50 BU hops (relative to mMRS medium), however, when grown in degassed and gassed beers have apparently divergent importance (Fig. 7.2). The manganese transporter *hitA* is not SDE expressed in either

Table 7.3. Growth in hops transcriptome sequencing data for Lb464^{OG} and Pc344^{34568}

	Bowtie2 Alignment of QC reads					DESeq 2		
Sample [a]	**Paired Reads**	**% Aligned Reads [c]**	**% rRNA Reads [d]**	**% CDS [e]**	**# Single reads mapping to CDS [f]**	**# DE Genes (%) [g]**	**# increased DE genes [h]**	**# genes > 2 Log_2 Fold change [i]**
L-mM+H-I [b]	15,215,309	99.84	90.4	23.3	3,535,765	629 (23.8%)	352	14
L-mM+H-II	12,414,348	99.57	90.0	24.9	3,094,728			
L-mM-I	13,652,921	94.93	76.4	11.5	3,324,704		277	5
L-mM-II	14,027,270	91.68	75.0	12.2	3,537,528			
P-mM+H-I [b]	13,158,012	97.18	6.4	80.1	21,097,813	1230 (64.4%)	607	95
P-mM+H-II	14,059,550	97.60	4.8	82.8	23,300,698			
P-mM-I	14,122,554	86.41	67.4	17.2	4,872,276		623	10
P-mM-II	15,256,954	89.85	63.6	23.9	7,294,295			

[a] Samples coded as; L- (Lb464^{OG}) and P- (Pc344^{34568}); "mM" (modified MRS broth, pH 5.5) and "mM+H" (mMRS broth + hops). "I" and "II" denote replicates.

[b] Lb464^{OG} cells were grown in the presence of 50 BU of hops; Pc344^{34568} cells were grown in presence of 30 BU hops.

[c] Percentage of quality-controlled reads aligned to Lb464 according to Bowtie-2 alignment.

[d] Percentage of aligned paired-end reads corresponding to rRNA genes.

[e] Percentage of non-rRNA aligned paired-end reads corresponding to annotated CDS regions.

[f] Total number of high-quality, single read fragments aligning to CDS regions.

[g] Total number of significant DE transcripts based on FDR < 0.1.

[h] Number of significant DE transcripts in given medium.

[i] Number of genes that are DE; i.e., expressed at over Log_2 fold change of 2 in on medium compared to the other.

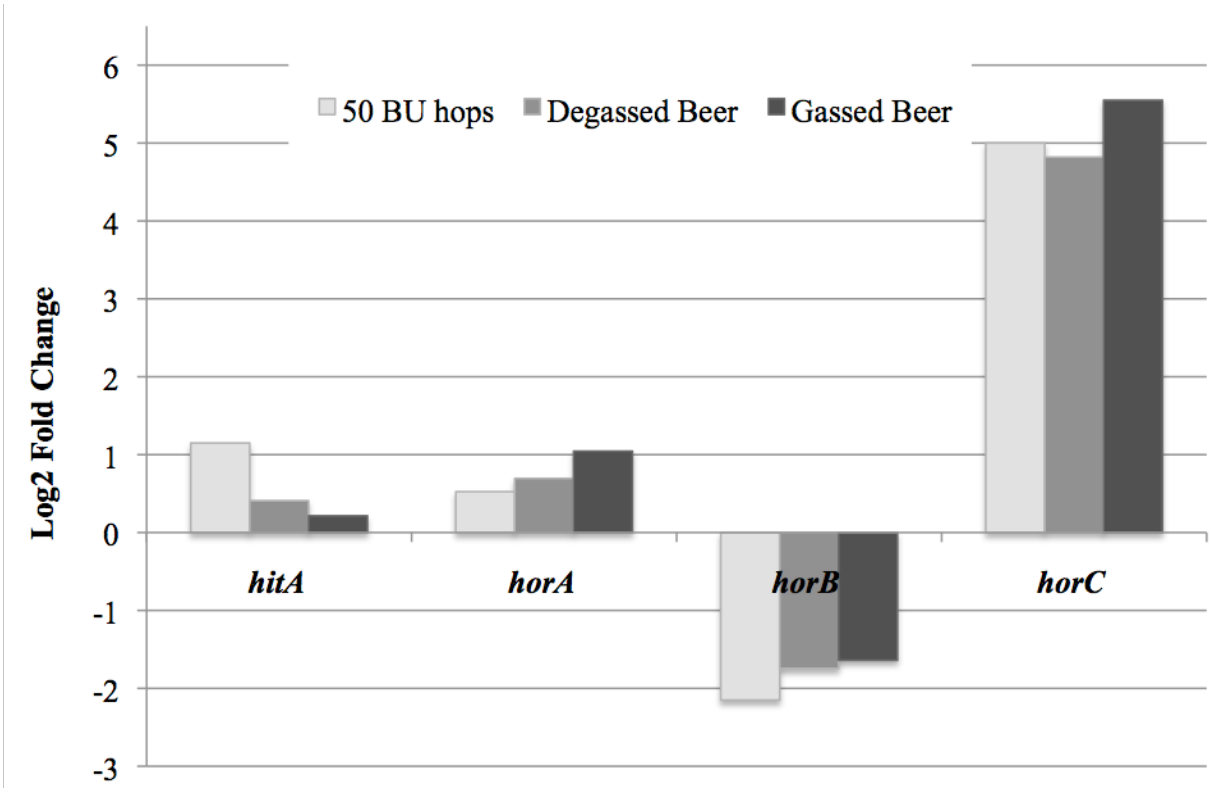

Fig. 7.2. Log_2 fold change of $Lb464^{OG}$ hop-tolerance genes.

The relative expression strength of putative hop-tolerance genes is shown in experimental conditions relative to mMRS pH 5.5 control medium.

beer conditions, whereas transcription of ABC-transporter *horA* is slightly increased in beer (relative to its expression in 50 BU hops), though to a considerably less extent than *horC.* That a putative pseudogene of a chromosomal manganese transporter is more highly expressed in 50 BU hops than *hitA* (Table S7.1-a) suggests that manganese transport is an ancillary or secondary response to the oxidative stress induced by hops presence while *horA* and *horC* have specificity to hop compounds. As these three genes are located on separate plasmids, it is likely that plasmid copy number (PCN) could affect these expression values, with specific plasmids increasing in number in response to specific environmental factors (i.e., increasing stress). In either event, the data show these specific plasmids (pLb464-1, *horA*; pLb464-2; *horC*) and/or plasmid-specific transcripts are inducible by the beer environment.

Other notable SDE Lb464 transcripts in response to hops are involved in glutamate metabolism: glutamate:γ-aminobutyrate antiporter (L747_01685), glutamate decarboxylase (L747_01690) and glutamyl-tRNA synthetase (L747_01695). These genes have been implicated as important in acid tolerance and intracellular pH homeostasis by consuming protons in a decarboxylation reaction that produces γ-aminobutyrate (GABA) from glutamate. The antiporter system couples the uptake of glutamate to the efflux of GABA (**12**). Glutamate also is a key metabolite in linking nitrogen and carbon metabolism, which Lb464 ^{OG} apparently does efficiently in stressful environments such as degassed and gassed beer (**12;** Chapter 6). Other highly SDE genes are largely involved in the general stress response, including the GNAT family acetyltransferase (L747_07675 and 10185) which plays a role in transcriptional regulation of the stress response (**33**), a universal stress protein UspA (L747_10150), transcriptional regulators belonging to the LytR family (Lb464_12540 which regulate putative membrane signal transducers (**20**), and a gene in the MerR family (L747_11460) which is involved in oxidative stress and metalloregulation (**9**). Further, there are several SDE transcripts involved in general pH/proton motive force (PMF) maintenance as in L747_06760, an ATPase and L747_09105, a gluconate:H^+ transporter. Several genes involved in regular cell metabolism are also SDE, such as L747_12555, the cell division protein FtsW; L747_11740, a tRNA synthetase β-subunit, and L747_11680, an oxidoreductase.

Of particular interest is the apparent SDE of L747_12545, which is a taurine ABC transporter ATP-binding protein. This protein typically functions as part of an ATP-binding cassette transporter (ABC transporter), which transports the amino sulphonic acid, taurine. However, no other components of the taurine ABC transporter are found in the Lb464 genome, thus indicating that this is a newly acquired gene, yet incomplete operon in Lb464 ^{OG}. As taurine has also not been reported to be a natural component of most beers, the presence and increased transcription of this specific transcript indicates potential cross specificity with another organic acid.

Overall, Lb464 ^{OG} transcriptional activities in the presence of growth-limiting concentrations of hops shifts towards expressing genes involved in PMF maintenance and transcriptional regulation of stress genes. The only notable hop-specific transcripts are that of *horC*, and glutamate and GABA metabolism.

7.4.4. Comparison of Lb464 ^{OG} DE transcripts in hops, degassed beer and gassed beer

Differential analysis was performed comparing RNA sequencing reads for Lb464 ^{OG} grown in the presence of 50 BU hops to growth in degassed and gassed beer in order to differentiate what transcripts are specific to hops and what transcripts which are critical for growth in both beer environments (Table S7.1-b,-c). Analysis reveals transcripts involved in glutamate metabolism and carbohydrate metabolism (i.e., L747_12590, fructokinase; L747_06910, ribose pyranse; L747_12625, phospholglycerate kinase) are up-regulated during growth in 50 BU hops. This is intuitive given that the background growth medium of the 50 BU hops is mMRS which has many more available nutrients than does beer, thus alternative energy pathways are not utilized or transcribed (i.e., amino acid metabolism; Chapter 6). Further, this suggests that glutamate metabolism which is an effective means for mediating hop stress directly by moderating proton movement across the membrane. This response, however, is eclipsed during growth in beer due to the stronger stress responses in beer – such as transcriptional regulation of cellular metabolism, cellular wall and membrane modification, and increased metabolism of alternative energy sources such as nitrogen (Chapter 6).

Interestingly, the citric acid metabolism operon of Lb464 ^{OG} (involved in citrate metabolism and fatty acid production) is up-regulated in hops relative to mMRS medium, yet not in degassed or

gassed beer relative to mMRS medium. (Chapter 6; Table S7.1-a,-b,-c). This is surprising given that this operon was shown to be SDE in Pc344^{358} growth in beer, as changes or elongation in the fatty acid composition of BSR LAB's membrane has long been thought to be an adaptation for beer (**22**). However, during Lb464^{OG} growth in beer, there is a significant shift in transcriptional activity towards cell-wall associated catabolism and pyruvate metabolism, which is not readily observed in the Pc344^{34568} dataset, thus suggesting low levels of citrate are available to Lb464^{OG} in beer media (Table S7.1-a,-b,-c). These results indicate that BSR LAB, and specifically Lb464^{OG}, are adaptable to limitations in their environment, yet also highlights the need to perform further transcriptional analysis of additional BSR LAB isolates in different (unique) beers (i.e., beer having diverse nutrient and metabolite compositions).

Gene ontology (GO) term enrichment analysis of Lb464^{OG} transcripts during growth in 50 BU hops that are SDE relative to transcripts expressed in both beer media unsurprisingly indicates categories of plasma membrane ATP regulation and activities, and carbohydrate metabolism processes as being increased proportionally in conditions of hops (Fig. 7.3). As indicated, the background medium of mMRS contains much more available nutrients for the bacteria than does beer, and known hop-tolerance mechanisms are centrally involved membrane transport and regulation of proton and solute, thus maintaining the PMF of the cell (**16**, **22**).

7.4.5. Pc344^{34568} response to hops

The most highly expressed Pc344^{34568} transcripts in response to growth-limiting concentrations of hops relative to nutritive conditions is an ABC transporter protein, PECL_1630, together with the third most highly DE transcript, PECL_1629, a TetR family transcriptional regulator that likely regulates PECL_1630 activity (Table S7.2). This is surprising giving that Pc344^{34568} has the hop-specific ABC transporter *horA* encoded on pPECL-8 that is SDE to roughly half the extent that PECL_1630 is (2.7 Log_2 fold change *vs.* 5.4 Log_2 fold change, respectively). In previous RNAseq analysis of Pc344^{358}, it was found that *horA* was expressed to a greater extent than PECL_1630 when growing in beer (though both genes were SDE), together with a demonstrable increase in pPECL-8 PCN. The difference in expression levels observed here could be the result of a difference in PCN observed in the response to hops compared to the beer used in (**22**); nonetheless, redundant resistance mechanisms to hops encoded for on the

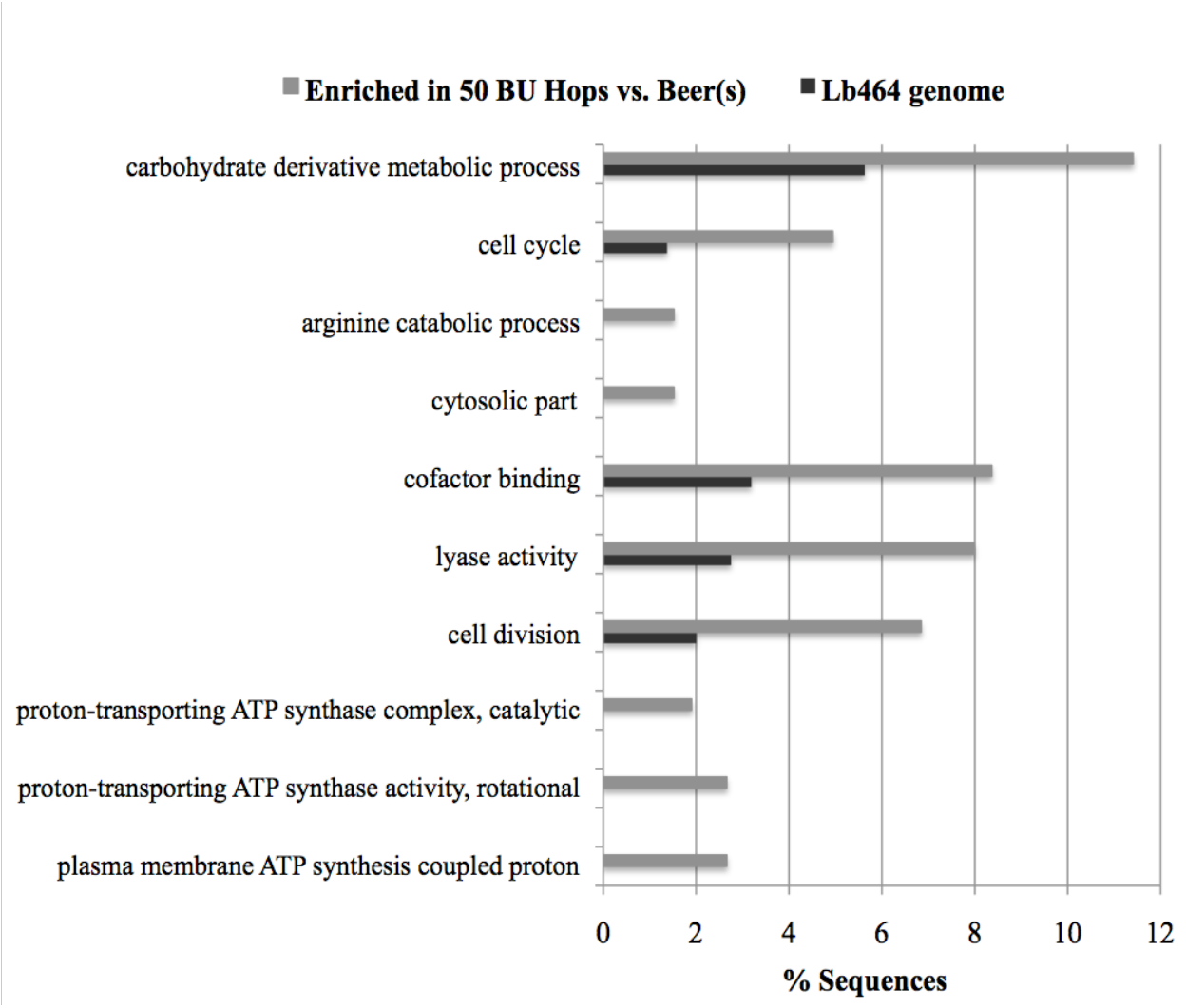

Fig. 7.3. Enriched GO terms when Lb464^{OG} is grown in 50 BU hops relative to degassed and gassed beer.

Transcripts that are SDE in 50 BU hops compared to degassed and gassed beer were taken and compared against all annotated GO terms for the Lb464 genome (FDR < 0.1). The % of sequences annotated as a particular GO terms in the Lb464 genome are provided, as are the % of sequences SDE in 50 BU hops of those same GO terms.

Pc344 chromosome are still indicated (**22**). PECL_1630 is a 447 amino acid ABC-2 type transport system protein, which is notably smaller than HorA, at 583 amino acids. A BLASTx search of PECL_1630 using default settings does recover some significant (E value < 0) alignments in other available LAB sequences (**2**). Most similar sequences are annotated as ABC transporter permease, however, either at or just below 68% identity, suggesting a *P. claussenii* species- or isolate-specific gene capable of responding to hops.

The comparison of hop-specific transcripts from Pc344^{34568} and beer-specific transcripts from Pc344^{358} analyzed in (**22**) are made with the noted caveat that the background plasmid profile of the Pc344 strains are different. Secondly, the basic nutritive media used for comparison against hops and against beer differed slightly, given that the different expression test of beer took place against MRS, pH 6.5 that contained Tween 80 (**22**). Tween 80 has been shown to interfere with the antimicrobial action of hops (**25**), thus, in this present study MRS with Tween removed was used to assess the affect of hops. Thirdly, slightly different RNA extraction methods and processing, and subsequent data treatment steps were used compared to those described in (**22**). Nonetheless, general statements concerning the functional roles and characteristics of expressed transcripts in each data set are made to highlight notable similarities and differences.

Of the top twenty-five most highly DE Pc344^{34568} genes during growth in the presence of hops, 11 of these are hypothetical proteins, with seven chromosomally located and four found on pPECL-8 (Table S7.2). Other highly expressed transcripts are involved with managing oxidative stress and homeostasis and/or providing for an energetically favorable means of metabolism and energy production, such as a VIT family protein, putrescine carbamoyltransferase, monooxygenase, agmatine/putrescine antiporter, peptide methionine sulfoxide reductase, and proteins involved in metal ion transport. Putrescine carbamoyltransferase, and agmatine/putrescine antiporter were also among the top twenty most highly expressed transcripts in the beer-specific Pc344^{358} RNAseq analysis, suggesting that these genes respond to the oxidative stress (and subsequent energy stress) elicited specifically by the presence of hops (**22**).

Putative non-coding RNA (ncRNA) species were also demonstrated to be important for growth of Pc344^{358} in beer (**22**), and are hypothesized to be present in Lb464^{OG} when grown in beer as

well (i.e., unannotated hypothetical proteins) (Chapter 6). Comparatively, there are considerably fewer ncRNA transcripts SDE in Pc344^{34568} when grown in hops, namely, chromosomal PECL_2059 and PECL_2060. Although these two transcripts show no similarity to previously described RNA families, they were confirmed in **(22)** to be significantly transcribed in beer via real-time quantitative PCR (RT-qPCR). Given that these two transcripts are still SDE in hops suggests that their activity is not specific to beer, but involved in the response to general stress and/or functioning and regulation of the cell. Overall, the total beer environment appears to elicit transcription of ncRNA and small regulatory RNAs to a greater extent than does the stress of hops.

Previous analysis with Pc344^{358} suggested that malolactic (PECL_1506) and citric acid fermentation genes (PECL_253 – 258), which were SDE in beer, generated buffering capacity and PMF, and thus were involved in counteracting the action of hops and low pH (**22**). Indeed, for Lb464, malolactic operon components are expressed at ~2.3 Log_2 fold change and the citrate operon between 1.6 – 1.8 Log_2 fold change in hops (Table S7.2), and these genes were among the top twenty-five most highly SDE genes in the prior Pc344 beer-transcriptome data set (**22**). This confirms that these genes are expressed in response to oxidative or PMF stress, but not necessarily specifically in response to hops. The chromosomal *fab* operon, which is responsible for fatty acid biosynthesis, was expressed at 2- to 3- fold higher in the earlier beer data set, which was thought to be important for dealing with the simultaneous presence of both membrane-damaging hops and ethanol in beer (**22**). This contention is supported in the present research by the finding that the *fab* operon is not SDE in response to direct challenge of growth-limiting concentrations of hops (Table S7.2). Therefore, observed LAB membrane adaptation through the synthesis or alteration of fatty acid composition is in response to the total stress environment of beer (i.e., compounding damage of multiple stresses) and not necessarily as an immediate adaptation to hops as suggested by (**3**). Finally, a number of genes were transcribed in Pc344^{358} grown in beer that are also SDE in Pc344^{34568} when grown in hops. Included here are two metal ion transporters specific for manganese transport (PECL_313, _638), methionine sulfoxide reductastes, *mrsA* and *mrsB* (PECL_936, _935) as was well as two other metal transport and homeostasis proteins (PECL_793, _1579), suggesting these genes are primarily involved in

mediating oxidative stress and PMF maintenance, and can be specifically induced by the presence of hops (**22, 27**).

The summation of transcriptional data of both Lb464^{OG} and Pc344^{34568} when growing in the presence of hops separately from beer, depicted in Fig. 7.4, allows for compartmentalization of processes specifically responding to hop-stress, and those induced when the other stresses of beer are also present. We see that there are more individual (i.e., isolate-specific) responses during growth in beer than there are during growth in hops, even though both isolates have one specific plasmid structure that harbors distinct hop-tolerance genes important for growth in both hops and beer. In general, PMF regulation and fatty acid metabolism are the only responses that are up-regulated in both hops and beer, with the addition of beer eliciting transcription of processes such as cell wall modification, specific nutrient scavenging transporters and metabolism and overall transcriptional regulation. The up-regulation of the agamatine deiminase operon and putrescine processing for energy generation are the major shared transcripts induced in both isolates during their growth in beer, which is an apparent beer-niche adaptation given the ubiquitous presence and availabilty of agmatine and putrescine found in beer styles from around the world (**33**). This overview indicates that there is potential for identifying at least species-specific markers for growth in beer, keeping in mind that there is going to be large variation in how given BSR LAB isolates are able to efficiently respond to and mediate challenges such as nutrient starvation, cell wall modification and overall energy regulation.

7.4.6. Role of plasmids in response to hops

7.4.6.1 Lb464^{OG} plasmids

Lb464 $^{\mathbf{OG}}$ plasmids have been shown to be important for growth in beer (**8;** Chapters 6); indeed, the most highly SDE Lb464$^{\mathbf{OG}}$ transcript is hop-tolerance gene *horC* encoded on pLb464-2 (Table S7.1-a). This confirms previous data that suggested the loss of the plasmid carrying *horC* results in a reduction in hop tolerance and provides further support that *horC* is a highly active hop-tolerance gene (**6, 8, 15**; Chapter 2, 4). Of the eight Lb464$^{\mathbf{OG}}$ plasmids, pLb464-2 has the greatest number of SDE transcripts in response to hops, which is interesting given there is considerably less overall plasmid-based transcriptional activity for Lb464$^{\mathbf{OG}}$ when growing in hops relative to when growing in beer medium (Table S7.1-a,-b,-c). pLb464-8 demonstrates the

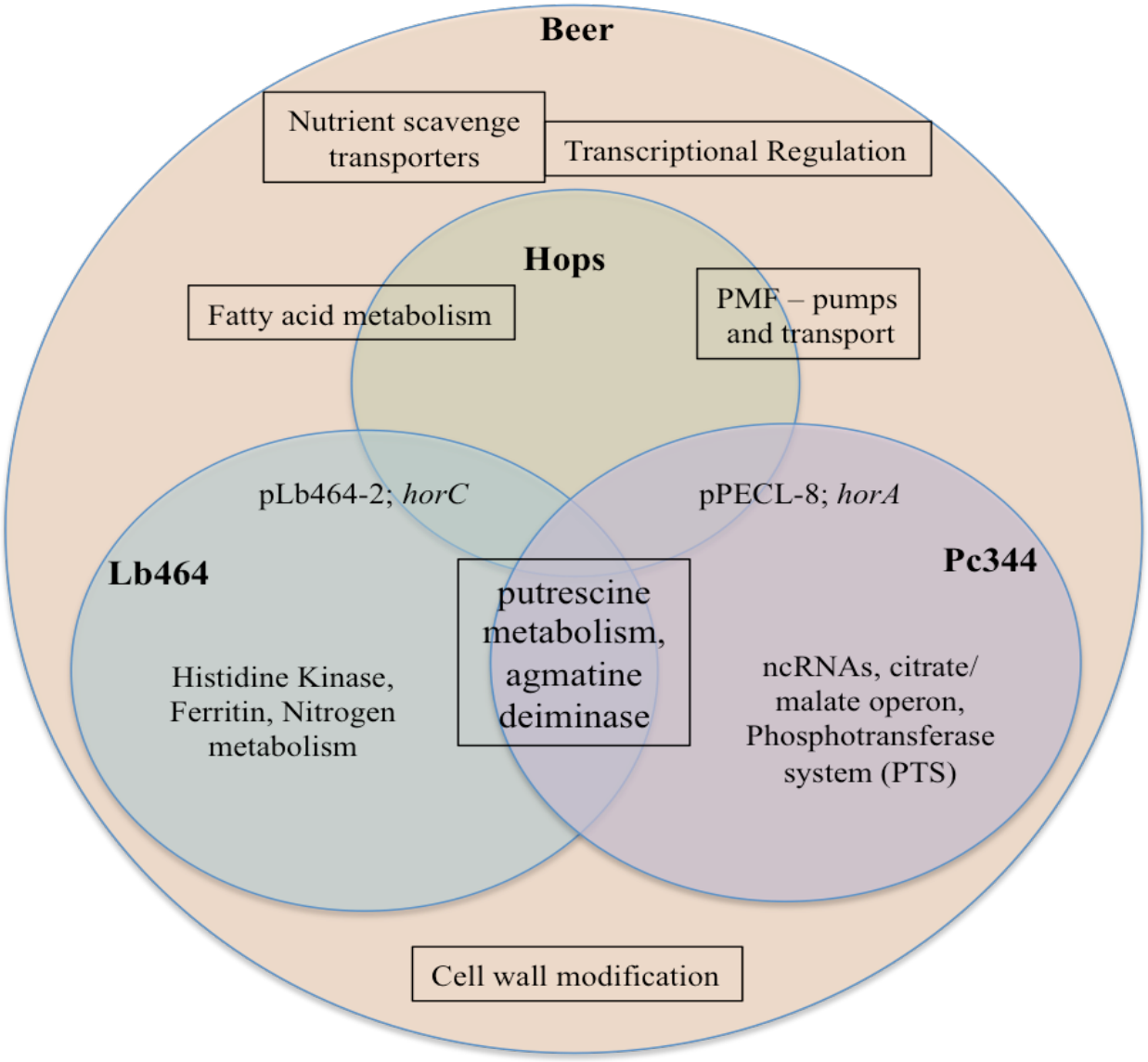

Fig. 7.4. Summary of transcriptional processes of both Lb464^{OG} and Pc344^{34568} in beer and hops.

Processes that are important for both isolates are bordered in black, and those processes that are unique to, or more critical for a specific isolate, are grouped with the isolate's respective circle. Hop stress is represented as a small portion of the total beer environment. The most specific processes that are shared by both Lb464^{OG} and Pc344^{34568} during growth in beer are the activation of putresecine carbamoyltransferase and agmatine deiminase (there is slight over lap with the "hop" circle, as there is some activity of these genes during Pc344^{34568} growth in hops).

greatest amount of transcriptional activity in response to hops after pLb464-2, with only *hitA* and *horA* from pLb464-1 and -3, respectively, being minimally SDE in response to hops (1.2 and 0.5 Log_2 fold change, respectively). Finally, the increased SDE (1.4 Log_2 fold change) of a TetR transcriptional regulator on pLb464-3 located approximately 300 bp upstream of *hitA* on the opposite DNA strand, is the first suggestion of a regulator for this transporter.

Overall, there appears to be considerable redundancy in tolerance mechanisms encoded for on the $Lb464^{OG}$ genome (manganese transporters, efflux pumps, membrane modification proteins) that are capable of mediating hop-stress. This chromosomal coding capacity could also explain the increased beer-spoilage virulence of Lb464, considering the theory that hop-tolerance (and thus beer-spoilage) is largely accepted as being inherited via plasmids. Given the apparent propensity of LAB to undergo DNA recombination, insertion and transposition events in stressful environments such as gassed beer (Chapter 6), it is conceivable that advantageous plasmid-based genes or genes from other organisms encountered in the brewery have been incorporated into the $Lb464^{OG}$ genome.

7.4.6.2. $Pc344^{34568}$ plasmid response to hops

The original genome sequence of Pc344 revealed that it harbors eight plasmids, ranging in size from 1.8 to 36 Kb (**21**), with its defining hop tolerance gene *horA* found on pPECL-8. The plasmids pPECL-1 and pPECL-2 are small and cryptic, and do not show notable activity in any growth medium. In the previous transcriptional analysis of $Pc344^{358}$, it was found that pPECL-4 and pPECL-6 were lost during the preparation of samples for RNA sequencing (**22**). In the present study however, both of these plasmids were present and specific transcripts shown to have increased expression during growth in hops. Two transcripts on pPECL-4 (a transposase and a plasmid-mobilization protein) were significantly expressed during growth in modified MRS medium and were the only plasmid-localized transcripts to be significantly DE in this growth condition.

All SDE transcripts from pPECL-4 and pPECL-6 are expressed between 0.5 and 2.5 Log_2 fold change greater in hops, with the highest DE transcripts being hypothetical proteins (Table S7.2). pPECL-6 harbors several ABC transporters, (PECL_1939, 1940) which are both SDE at only

~0.5 Log_2 fold change in hops. The most highly expressed transcripts on both pPECL-4 and pPECL-6 apart from hypothetical proteins are an antitoxin of a toxin-antitoxin stability system of the RelB family (PECL_1912 and PECL_1929 on each plasmid, respectively). Similarity between the pPECL-4 and -6 SDE transcripts indicates that they have similar or redundant functions encoded.

Despite the additional presence of pPECL-4 and -6 in this study, it was found that pPECL-3, -5 and -8 demonstrated the most significant transcriptional response to hops, just as they did in response to beer (Table S7.2) (**22**). The most highly expressed transcript from pPECL-3 is that of *dps* that provides DNA protection during starvation at 2.8 Log2 fold, while on pPECL-5 and pPECL-8 several hypothetical proteins are expressed at greater than Log_2 fold change in response to hops. Thus, it appears pPECL-3, -5 and -8 transcripts remain the most important plasmid elements for Pc344 in response to mediating the stress of either hops or beer, indicating that the response to growth in either is not mediated by solely one gene, i.e., *horA* (pPECL-8).

pPECL-8 shows the greatest number of SDE plasmid-based transcripts, as the majority are over 2 Log_2 fold change during growth in hops (Table S7.2). Though many pPECL-8 genes encode for hypothetical proteins, there is the notable presence of the hop-tolerance gene *horA* (2.7 Log_2 fold change), and genes involved in cellular maintenance, such as a Type 1 restriction-modification system (2.5 Log_2 fold change), a transcriptional regulator belonging to the Xre family (3 Log_2 fold change) and a DNA-repair protein (2.8 Log_2 fold change) (Table S7.2). Interestingly, copies of similar genes are also located on the chromosome; however, they do not have as high of a Log_2 fold change as the pPECL-8 transcripts. This indicates that pPECL-8 may have increased in PCN in response to hops, and certainly suggests that transcriptional activity off this plasmid is of importance to the cell for basic cellular maintenance and repair.

Also found on pPECL-8 is a cassette of five genes usually found surrounding *horA*. Four of these genes encode for products involved with cell wall or phospholipid biosynthesis (**22, 28)**. During $Pc344^{358}$ growth in beer, each of the five genes had greater than 3 fold change in transcription levels, and were among the most highly expressed transcripts on pPECL-8 (**21 – 22**). In hops, these transcripts are still differentially expressed, but to a lesser fold change than in response to

beer. This strengthens the theory that PCN is most greatly increased in response to beer, but that *horA* and its surrounding gene cassette are transcribed in response to hop-damage.

7.4.6.3. The role of pPECL-8 and analysis of plasmid variants

To confirm the importance of specific plasmids for growth in beer, plasmid variants of Pc344 were generated and tested for their overall hop-tolerance and growth kinetics in beer, as has been previously done for Lb464 (Fig. 7.5) (**8**). Despite the apparent role of pPECL-3, -5, and -8 transcripts in response to hops, when these plasmids are lost from the cell there is no statistically significant difference in hop tolerance levels as compared to the Pc344 parent strain which contains all eight plasmids (as assessed via hop gradient agar plates (**13**); data not shown). This corroborates the suggestion that there are redundant, chromosomally located proteins capable of either exporting hops, or dealing with the hop-induced damage to the PMF (i.e., ABC transporter, PECL_1630).

In terms of overall beer-spoilage, the relative importance of pPECL-8 for Pc344 beer-spoilage capacity is confirmed, as is overall this plasmid's involvement in beer-spoilage kinetics (Fig. 7.5). Most interestingly, there is no notable difference in the beer-spoilage phenotype or growth kinetics of $Pc344^{34568}$ and $Pc344^{358}$, as previously analyzed in (**22**). However, the subsequent loss of either pPECL-6 and pPECL-8 (yielding $Pc344^{345}$) or pPECL-4 and pPECL-8 (yielding $Pc344^{356}$) alters the "normal" beer-growth pattern. Though $Pc344^{345}$ grows well initially, it experiences a dramatic death phase compared to that of $Pc344^{34568}$ and $Pc344^{358}$, only to regain growth again after ~6 d. $Pc344^{356}$ experiences both a prolonged lag phase and small death phase before being able to establish successful exponential growth, with CFU/ml increasing 4-log fold in a period of 5 d. Though it its noted that the starting inoculum of both $Pc344^{345}$ and $Pc344^{356}$ was not as high as the two other variants, a higher inoculum for these two strains was never reached using the standardized growth-assessment protocol (**8**; Chapter 4). Thus the loss of pPECL-8 appears to result in an inability of Pc344 to maintain a basic static survival in beer as evident by the two distinct death phases experienced by both variants lacking this plasmid ($Pc344^{345}$ and $Pc344^{356}$). This data highlights the complexity of plasmid-transcription and apparent synergism between plasmid coding capacities that plays into beer-spoilage ability. For example, the combined loss of pPECL-4 and pPECL-8 is noted to be more

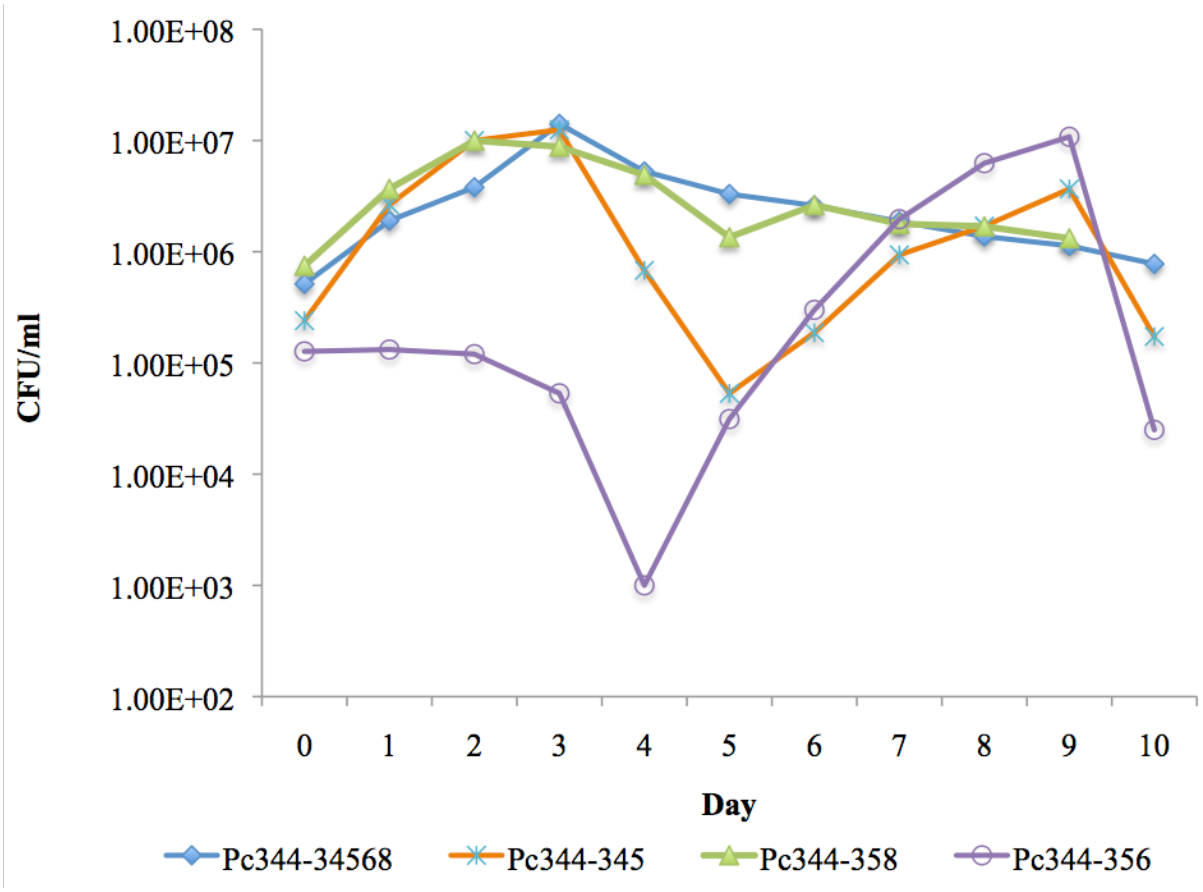

Fig. 7.5. Growth of Pc344 plasmid variants in degassed beer.

The growth kinetics of Pc344^{34568} (blue) and Pc344^{358} (green; the isolate analyzed in **22**), are compared. Variants Pc344^{345} (orange) and Pc344^{356} (purple) both have lost pPECL-8.

detrimental for the ability to establish growth in beer in a timely manner ($Pc344^{356}$), relative to the loss of pPECL-6 and pPECL-8 ($Pc344^{345}$) which is capable of establishing rapid initial growth in beer.

7.4.7. "*Beer-niche*" plasmids

Ultimately, it appears that pPECL-8 is the most important Pc344 plasmid for determining beer-spoilage virulence, just as pLb464-2 is most central for the beer-spoilage phenotype of Lb464 (**8, 22**; Chapter 4). Incidentally, both of these plasmids harbor hop-tolerance genes, pLb464-2 (*horC*) and pPECL-8 (*horA*). Transcriptional data has confirmed that both of these genes are expressed in response to hops, however, it is also evident that other transcripts, largely hypothetical proteins and transposase, on these two plasmids are highly advantageous to the cell for growth in beer (Table S7.1-a,-b,-c and Table S7.2). These two plasmids may be exemplary "beer-niche" plasmids that have acquired and grouped advantageous genes for growth in beer on the same plasmid structure. However, upon analysis of protein similarity between the two plasmids and the originally described beer-spoilage-related plasmid that harbors *horC*, pRH45II (**29, 31**), it is clear that there is limited similarity in coding capacities between pPECL-8 and pLb464-2 (Fig. 7.6).

This finding is not wholly surprising given that the LAB containing these plasmids were acquired in two separate breweries and are from two different genera. Nonetheless, the two plasmids, both contain genes encoding proteins advantageous to bacterial survival under stressful growth conditions. pPECL-8 contains a plasmid partition protein (PECL_2034), a Type 1 Restriction/Modification system and DNA repair protein UmuC (PECL_1961), and two glycosyl transferase proteins as part of the *horA* gene cassette (PECL_1951 and 1954). Similarly, pLb464-2 also contains a plasmid partition protein (L747_00155), a DNA-damage-inducible protein (L747_00140), a glycosyl transferase (L747_00230), and an antitoxin and toxin/antitoxin system (L747_00095 and _00100). Both beer-important plasmids thus appear to be stable, given they code their own plasmid partitioning and replication genes, carry DNA repair and modification systems, and are very amenable to carrying critical beer-adaptation genes. Such genes include not only their respective hop-tolerance genes, but also phospholipid biosynthesis enzymes for pPECL-8, and for Lb464, several membrane proteins that may help with membrane transport or

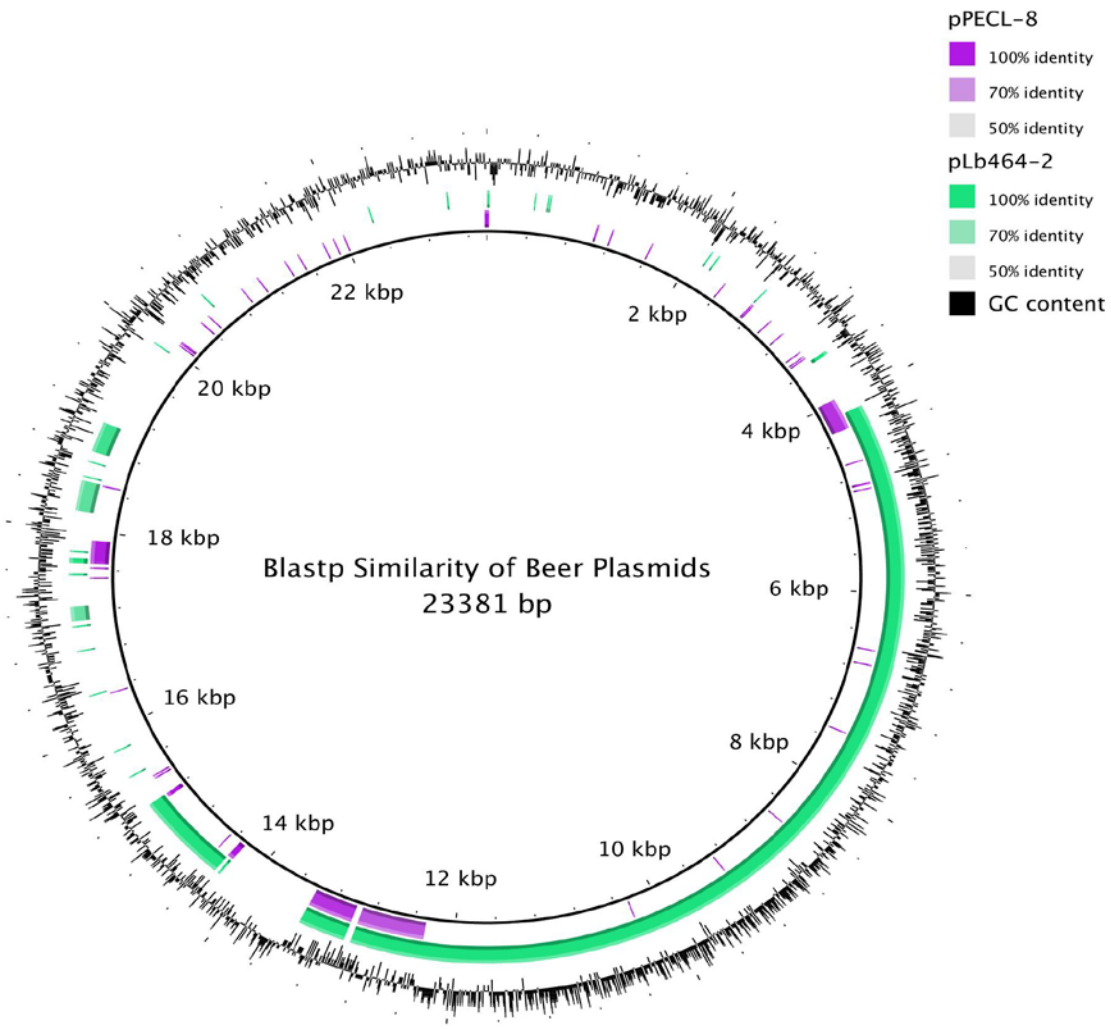

Fig. 7.6. BLASTp comparison of pPECL-8 and pLb464-2 relative to *L. brevis* pRH45II.

pRH45II (23,381 bp) was among the first plasmids to be isolated from beer-spoilage *L. brevis* isolates (**31**). Using this plasmid as a reference, the pPECL-8 and pLb464-2 sequences were analyzed for homologous sequences via Blast-p analysis and drawn using BRIG v0.95 (**1**).

fortification and a ferritin enzyme that is implicated as important for Lb464 growth in gassed beer (Chapter 6).

7.5. CONCLUSIONS

The whole-transcriptome sequencing of Lb464^{OG} and Pc344^{34568} during growth in sub-lethal concentrations of hops highlights the unique nature of these two isolates in their ability to tolerate hops. Lb464^{OG} is highly hop-tolerant, and even with a challenge of 50 BU of hops did not elicit a transcriptional response as strong as Pc344^{34568} did in response to only 30 BU (i.e., less of the Lb464^{OG} genome was DE transcribed in response to the hops stress). This inherent difference could be explained by difference in underlying coding capacity of the two isolates, with Lb464^{OG} containing a high proportion of transporters that could adeptly maintain the PMF under any set of stress conditions and thus not need an array of genes showing tight transcription in relation to hops compounds. Nonetheless, there are shared or common transcripts expressed by both isolates in response to hops, with these largely concerned with mediating oxidative stress (i.e., *mrsA* and *mrsB).* The expression of putrescine and agamatine deiminase metabolism products in both hops and beer by Pc344^{34568}, and in degassed and gassed beer by Lb464^{OG} indicate that these are highly suitable candidates for further investigation in other BSR LAB as indicators of beer-spoilage ability. Analysis of the how the levels of these two substrates change over the course of BSR LAB fermentation in beer and hops would be useful to gain insight into what extent they are scavenged for and if this behavior is uniform in all BSR LAB.

Though this data helps to parse the bacterial transcriptional response to the stress of hops from the stress of beer, indicating that hop-tolerance is but one component of beer-spoilage ability, it still underscores the inherent isolate variability in hop-tolerance and thus the genetic and physiological responses of LAB to beer. For example, although the hop-tolerance genes *horA* and *horC,* were demonstrated to be induced by both hops alone and the beer environment, Lb464OG and Pc3443456 still variably express these two genes. In response to hops, *horA* in Pc344 was not the most SDE ABC transporter, and in Lb464, *horA* activity decreases when growing in beer in apparent favor of *horC.* These isolates clearly make different use of the same hop-tolerance genes relative to each other and in different environments. Thus, these hop-

tolerance genes may be found widely since they can be recovered from BSR LAB of different species and genera, however, they are not universally important to each BSR LAB isolate's ability to grow in beer. The prediction of beer-spoilage ability for LAB isolates could be much improved with the inclusion of detection of beer-specific transcripts and investigations into BSR LAB genetics that challenge the isolates with the total beer environment and not just hops.

7.6. ACKNOWLEDGMENTS

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Chapter 8: Comparison of *Lactobacillus* and *Pediococcus* genomes in terms of beer-spoilage ability

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8. INTERFACE

Previous chapters indicate the potential for isolate- and species-specific adaptations to the beer environment. This chapter provides a genomic comparison of LAB isolates along non-beer-spoiling and beer-spoiling characteristics. All publically available *Lactobacillus brevis* genomes are first analyzed, followed by analysis of multi-species LAB.

Jordyn Bergsveinson prepared all DNA samples for genome sequencing, and performed all bioinformatics analysis and comparisons of non-beer-spoiling and beer-spoiling lactic acid bacteria. She authored the manuscript.

Ilkka Kajala is credited with performing initial *in silico* genome assembly efforts of four lactic acid bacteria isolates sequenced in-house.

Barry Ziola provided guidance and assisted with experimental design, provided editing of the manuscript, and held the research grant that supported this work.

8.1. ABSTRACT

Comparative genomics is a powerful tool to determine genetic elements common to a group of isolates occupying a specific niche, such as a beer. In the context of beer-spoiling-related lactic acid bacteria (BSR LAB), it is still unclear as to what level of comparisons are required to enable "beer-specific" genes to be elucidated, given that LAB isolates of different species and genera can exhibit beer-spoilage ability and that horizontal gene transfer is known to occur within the brewing environment where multiple BSR LAB may be present. Thus, the extent to which genus-, species- or environment- (i.e., brewery-) level variability influences beer-spoilage phenotype is unknown. By performing intra-species comparison of all publically available *Lactobacillus brevis* genomes, inter-species comparisons of BSR and non-BSR *Lactobacillus*, and inter-genus comparisons of BSR and non-BSR *Lactobacillus* and *Pediococcus* genomes, it is evident that there is considerable difference between BSR LAB of different species, and that the isolate source (brewery niche) further influences the genetic profile of a BSR LAB. However, analysis at the *L. brevis*-species level has lead to the conclusion that transcripts related to carbohydrate nutrient scavenging of sorbose, and enzymes related to the breakdown of plant materials provide, an important adaptation of *L. brevis* and lactobacilli to the beer environment, and suggests that BSR LAB recovered from a brewery have likely at one point been plant-niche adapted. The coding capacity of plasmids is shown to contribute to niche-specific attributes, and in the case of BSR LAB, plasmids are likely to be highly-brewery specific, encoding important, yet different, virulence factors and adaptations to individual BSR LAB. Together, this data supports the theory that beer-spoilage phenotype is a result of a "Swiss-army knife"-like approach, wherein multiple and variable genes can be acquired and added to the total genetic arsenal. The sum total of this arsenal, dictates beer-spoilage spoilage virulence under different environmental circumstances. Further research into how genetic elements are transferred and harbored by the brewery environment, and the total composition of BSR LAB microflora of breweries is critically important information for further comparative genomics studies of BSR LAB.

8.2. INTRODUCTION

The discrimination of beer- or food-spoiling lactic acid bacteria (LAB) has focused on finding a niche-specific "barcode" of genes that distinguishes niche-adapted isolates from non-adapted

counter parts (**5, 25**). However, in addition to variability among beer-spoilage-related (BSR) LAB at the genus and species level, it is known that the actual brewery niche can influence the variability of an organism as a result of horizontal gene transfer (HGT) events, and presumably by the total microflora present and the nature of the brewery itself (i.e., raw substrates used, design, process). How these factors work together to influence variability and similarities between BSR LAB is simply not understood.

There have been recent studies attempting to both characterize and quantify the extent of genetic differences at the level of group (i.e., beer-spoiling *vs.* non-beer-spoiling LAB) (**26**), at the level of genus (i.e., beer-spoiling *Pediococcus* species *vs.* non-beer-spoiling *Pediococcus* species) (**32**) and at the level of species (i.e., beer-spoiling *Lactobacillus brevis vs.* non-beer-spoiling *L. brevis*) (**5**). Work done previously **(26)** concluded that adaptation to the brewery environment of BSR LAB writ-large could not be attributed to the inheritance of brewing-specific chromosomal genes (i.e., hop-tolerance genes), but instead was largely dependent on how genes for nutrient acquisition and secondary metabolite-energy genetic mechanisms were regulated. Redundancy and variability in both plasmid and chromosomal nutrient acquisition and energy metabolism was found, however, and thus it was concluded that no specific genes/proteins were predictive of beer-spoilage ability across the *Lactobacillus* and *Pediococcus* genera (**26**).

In. **(32)**, the comparison of three *Pediococcus* isolates, *P. damnosus* LMG 28219 (beer-origin), with *Pediococcus claussenii* ATCC BAA-344^T (beer origin) and *Pediococcus pentosaceus* ATCC 25745 (plant origin), revealed that genes involved in *de novo* folate biosynthesis were unique to the strains isolated from beer, in addition to, unsurprisingly, plasmid-localized hop-tolerance mechanisms. However, genes related to stress responses such as osmotic shock were common among the compared strains, a finding that undermines the concept of BSR LAB being more highly stress-adapted than non-BSR LAB. Interestingly, transcriptional regulators were enriched in the genomes of *Pediococcus* capable of growth in beer, and this confirms recent transcriptional analysis of *P. claussenii* ATCC BAA-344^T (Pc344) and *L. brevis* BSO 464 (Lb464), wherein transcriptional regulators of multiple families were strongly expressed during the growth of both isolates in beer (**28;** Chapter 6). This buttresses the argument that overall

cellular regulation is an important adaptation for beer-spoilage, and not necessarily overall genetic content of the cell (**26, 32**).

Finally, in **(5),** several specific genes involved in nutrient transport, transcriptional regulation, and catalytic activities, that were not distinguished as chromosomally- or plasmid-localized, were purported to correlate with *L. brevis* beer-spoilage ability. Thus, it would appear that at species-level resolution, it is possible to distinguish niche-adapted genes. However, there has not been adequate testing of whether putative "beer niche" genes identified at the species level, holds true for all available BSR *L. brevis* isolates, nor if the idea applies to other closely related *Lactobacillus* species and thus truly demarcating a beer-spoilage phenotype. These are important questions to answer, as the answers would provide insight into the depth of genomic data and future investigation required to probe questions of universal genetic markers or "barcodes" for LAB beer-spoilage ability.

Therefore, the present study attempts to make use of available genomic data for BSR LAB and related non-BSR LAB in order to answer the questions: (1) are beer-niche genes most strongly influenced by environment- (specific recovery location), species-, or genus-level variation; (2) what are genes of interest (non-hop-tolerance-related) that can serve as candidates for future targeted analysis in BSR LAB and non-BSR LAB; and (3) what influence do non-hop- tolerance plasmid-encoded genes have on the beer-spoilage phenotype?

8.3. MATERIALS and METHODS

8.3.1. Acquisition of publically available genomes

To facilitate genome comparison of *L. brevis* isolates of both beer-spoiling and non-beer-spoiling nature, all available *L. brevis* genome assemblies were retrieved July 4th, 2015, from GenBank (http://www.ncbi.nlm.nih.gov) (see Table 8.1). At the same time, the genomes for beer-spoilage *Pediococcus claussenii* ATCC BAA-344^{T} (Pc344) (**27**), and three non-beer-spoilage related genomes *Lactobacillus casei* ATCC 334 (Lc334) (**22**), *Lactobacillus sakei* subsp. *sakei* 23K (Ls23K) (**8**), and *P. pentosaceus* ATCC 25745 (Pp25745) (**22**) were retrieved in order to facilitate comparisons of similarities and differences between LAB isolates with the capability to

Table 8.1. LAB genomes acquired from NCBI.

Isolate and Reference [a, b, c]	Assembly Level	GenBank Assembly Accession	Isolation Source/Identifier	Size (MB); # of plasmids [d]	GC%	rRNA [e]	tRNA	Genes [f]
ATCC 367 (**22**)	Complete	GCA_000014465.1	Starter culture for sourdough and silage	2.3 ; 2	46.2	5	63	2174
KB290 (**16**)	Complete	GCA_000359625.1	Suguki (fermented vegetable)	2.5 ; 9	46.1	5	63	2458
BSO 464 (**6,7**)	Chromosome with gaps	GCA_000807975.1	Spoiled beer	2.7 ; 8	45.7	6	48	2606
subsp. *gravesensis* ATCC 27305	Scaffold	GCA_000159175.1	Gastrointestinal tract	3.1 ; N/A	45.9	1	57	2721
DSM 20054	Scaffold	GCA_000875905.1	Gastrointestinal tract	2.4 ; N/A	46.4	1	51	2291
15f (**39**)	Scaffold	GCA_000875905.1	Fecal matter of healthy adult	3.1 ; N/A	44.1	3	57	2256
EW (**20**)	Contig	GCA_000474675.1	*Drosophila* intestine	2.8 ; N/A	44.2	3	63	2806
AG48	Contig	GCA_000526755.1	Sheep ruemen	2.5 ; N/A	42.0	5	69	2424
WK12	Contig	GCA_000784455.1	Kimchi	2.6 ; N/A	42.8	1	63	2486
DmCS_003	Contig	GCA_000814725.1	*Drosophila melanogaster* gut	2.8 ; N/A	43.1	2	69	2758
TMW 1.465 (**5**)	Contig	GCA_000833395.1	Soft-drink/Brewery; Beer-spoiling	2.5 ; N/A	44.9	2	60	2104
TMW 1.313 (**5**) [g]	Contig	GCA_000833405.1	Beer; Beer-spoiling	2.7 ; N/A	41.7	3	63	2312
TMW 1.6 (**5**)	Contig	GCA_000833415.1	Feces; Non beer-spoiling	2.5 ; N/A	43.5	1	59	2435
47f	Contig	GCA_001010995.1	Fecal matter of healthy adult	2.6 ; NA	43.1	1	66	2449
P. claussenii ATCC 344^T (**27**)	Complete	GCA_000014465.1	Brewery; beer-spoiling	2.3; 8	37.0	4	57	1,928
P. damnosus 9-6b (**6**)	Scaffolds	Not deposited (**6**)	Brewery; beer-spoiling	2.2; 6	39.1	5	61	2,166
P. damnosus LMG 28219 (**32**)	Contig	GCA_000962875.1	Beer; beer-spoiling	2.2; N/A	38.2	3	56	2,178
L. casei ATCC 334 (**22**)	Complete	GCA_000014525.1	Emmental cheese	2.9; 1	44.3	4	59	2,902
L. sakei subsp. sakei 23 K (**8**)	Complete	GCA_000026065.1	French sausage	1.8; 1	41.2	6	63	1,889

P. pentosaceus ATCC 25745 (**22**)	Complete	GCA_000014505.1	Wild-type strain	1.8; 1	37.4	4	55	1,795

a Isolates specifically associated with spoiled beer are highlighted in grey. Isolates above the second bold line are *L. brevis* isolates.

b "*L.*" denotes *Lactobacillus* and "*P.*" denotes *Pediococcus* isolates.

c *P. claussenii* ATCC 344^T = Pc344; *P. damnosus* 9-6b = Pd58; *P. damnosus* LMG 28219 = Pd28219; *L. casei* ATCC 334 = Lc334; *L. sakei* subsp. sakei 23 K = Ls23K; *P. pentosaceus* ATCC 25745 = Pp25745.

d For complete genomes, the total genome size includes genome and plasmid sequence.

e rRNA operons; 5S-16S-23S rRNA.

f Total gene count includes chromosomal and confirmed plasmid genes.

g Genome contains many frame-shifted proteins.

spoil beer and those that cannot (see Table 8.1). The genome for *P. damnosus* 96-b was previously sequenced and analyzed in-house, as described in (**6**; Chapter 5).

8.3.2. In-house genome sequencing of four beer-spoiling LAB isolates

8.3.2.1. DNA preparation and sequencing

Lactobacillus brevis BSO 310 (Lb310), *L. casei* CCC B1025 (Lc03) and *Lactobacillus spp.* ATCC 15578 were obtained from their respective culture collections (see footnotes in Table 8.2). *Lactobacillus backii* (L101) was isolated from a local lager beer in-house [40 bitterness units (BU), 5.2% alcohol by volume (ABV)]. These cultures were taken from -80°C stocks and 10 µl transferred into 8 ml of de Man, Rogosa and Sharpe (MRS) medium and grown overnight at 30°C. Overnight culture was adapted for transfer into the beer environment by first culturing for 48 hr in 85/15 medium [85% lager beer, pH 5.2, 5% (*v/v*) alcohol, 11 bitterness unit (BU); 15% double strength modified MRS (MRS without Tween 80)]. Next, 100 µl of this culture was taken and transferred into 16 ml of degassed lager beer and incubated for 5 d. From this beer-adapted culture, 20 µl was taken and added once more into 85/15 medium and grown for ~35 hr to facilitate DNA extraction.

Cells were harvested by centrifugation and DNA extracted from the resultant pellet via the MoBio UltraClean Microbial DNA extraction kit according to the manufacturers protocol, with an additional heating step at 70°C for 10 min to help lyse cells efficiently prior to bead-beating. DNA was quantified and analyzed for quality via a QuBit Fluorometer (LifeTechnologies).

All samples were sequenced at National Research Council Plant Biotechnology Institute (NRC PBI) in Saskatoon, SK on an Illumina MiSeq platform using paired-end and 250 bp inserts library preparations. Lb310 and L101 were sequenced together, multiplexed in one lane along with three other samples. Lc03 and Ls74 were sequenced in a separate run, again multiplexed in one lane along with an additional three other samples.

Table 8.2. Genome assembly statistics of four beer-spoilage *Lactobacillus* isolates.

Isolate [a]	**Label [b]**	**Locus Tag [c]**	**# Reads [d]**	**# Scaffold [e]**	**Size (MB)**	**Plasmids Complete; Putative [f]**	**GC %**	**rRNA [g]**	**tRNA [g]**	**Genes [g]**	**Psuedo genes[g]**	**Frame-shifted Genes [g]**
Lactobacillus backii L101	L101	ACX53	2,765,936	116	2.6	1 ; 3	40.1	4	58	2,533	130	32
Lactobacillus brevis BSO 310	Lb310	ACX49	2,729,650	136	2.6	4 ; 1	43.5	4	59	2,653	122	29
Lactobacillus casei CCC B1025	Lc03	ACX51	1,780,406	84	3.2	- ; 8	44.5	5	57	3,221	120	53
Lactobacillus spp. ATCC 15578	Ls74	ACZ99	2,143,540	28	3.4	- ; 6	42.0	6	68	3,299	88	39

[a] L101 = isolated in-house from local beer; BSO = Institute for Biotechnology, Oxoid, UK; CCC = Coors Culture Collection, Golden Colorado, USA; ATCC = American Type Culture Collection, Manassas, Virginia, USA.

[b] Internal isolate label; used throughout the manuscript .

[c] NCBI locus_tag; data made publically available online January 1st, 2016.

[d] All reads obtained using Illumina MiSeq with 250 bp inserts, paired end. Lb101 and Lb310 were run together in one lane, and Lc03 and Ls74 were run together in one lane in a separate run.

[e] Scaffolds assembled using A5 pipeline.

[f] Plasmids determined via Geneious 7.8 program.

[g] Values obtained from PGAAP annotation of genomes.

8.3.2.2. DNA sequence analysis, assembly and annotation

Raw sequences were input into the A5 assembly program, and were subsequently analyzed in the Geneious 7.8. software (**10, 19**). Whole genome sequence (WGS) assemblies of all four isolates were submitted to NCBI under accession numbers: Lb310 (SAMN03813855), L101 (SAMN03813857), Lc03 (SAMN03813856), Ls74 (SAMN03813858). Sequences were annotated through the NCBI Prokaryotic Genome Automatic Annotation Pipeline (*PGAAP*) (**33**).

8.3.3. Beer-spoilage ability assessment

Isolates tested for beer-spoilage ability are indicated in Tables 8.1. All those tested in-house were assessed for growth in degassed and gassed beer and previously reported in (**6**; Chapter 5). In short, an isolate was adapted to the beer-environment by passage through 85/15 medium and then incubated in degassed or pressurized lager beer (pH 5.2, 5% (*v/v*) alcohol, 11 BU) at 30ºC for up to one week. Changes in colony forming units (CFU) were monitored to confirm whether the cell population experienced death, growth, or remained static.

The beer-spoilage ability of strains TMW 1.313, 1.465 and 1.6 (Tables 8.1) was reported in (**5**) and was assessed according to a procedure of resazurin dye reduction described in (**29**). In short, cell cultures are incubated with the indicator dye, resazurin, which changes color upon metabolic activity over the duration of incubation.

8.3.4. Genome conservation, gene interrogation, and orthologous protein analysis

To analyze gene conservation amongst all *L. brevis* isolates, the BLAST ring image generator (BRIG) v. 0.9.5. was used (Fig. 8.1 and 8.2) (**2**). Protein annotation files from Genbank for *L. brevis* isolates were assigned clustered ortholog group (COG) identities via OrthoMCL software (http://orthomcl.org/) (**9**). Isolates were then grouped according to their known-beer-spoilage ability (Fig. 8.3), and parsed for overlapping and unique COG terms. COG terms common within each group were then compared to the other three groups and unique COGs inspected for candidate “beer-niche” specific genes, based on corroboration with previous transcriptional data of Lb464 and Pc344 (Chapter 6 and Chapter 7; Table 8.3). The genomes of

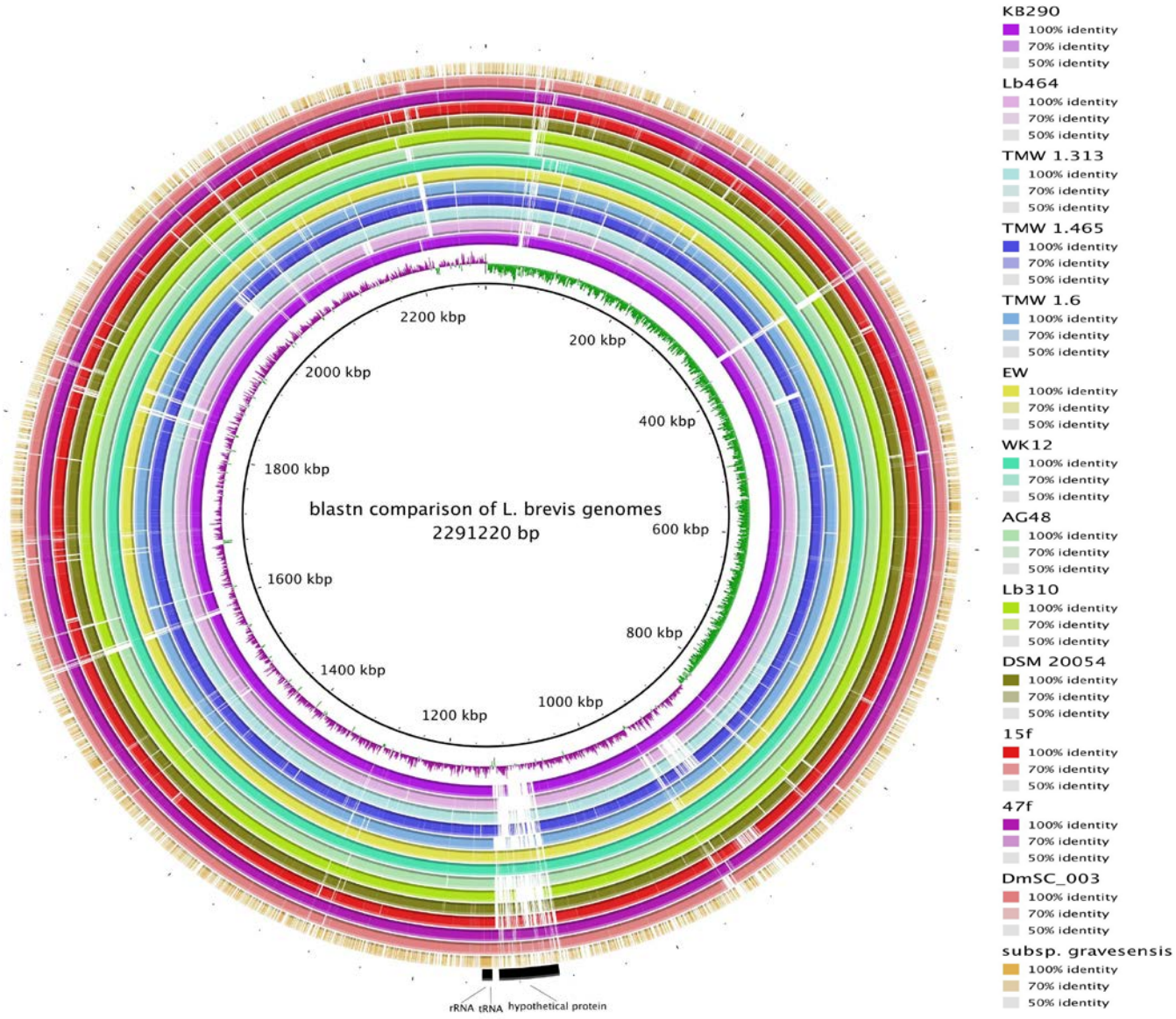


Fig. 8.1. Whole genome comparison of the 14 available *L. brevis* genomes, using Lb367 (type strain) as a reference.

There is a notable atypical GC-skew of Lb367 that was also observed in Lb464 (Chapter 6).

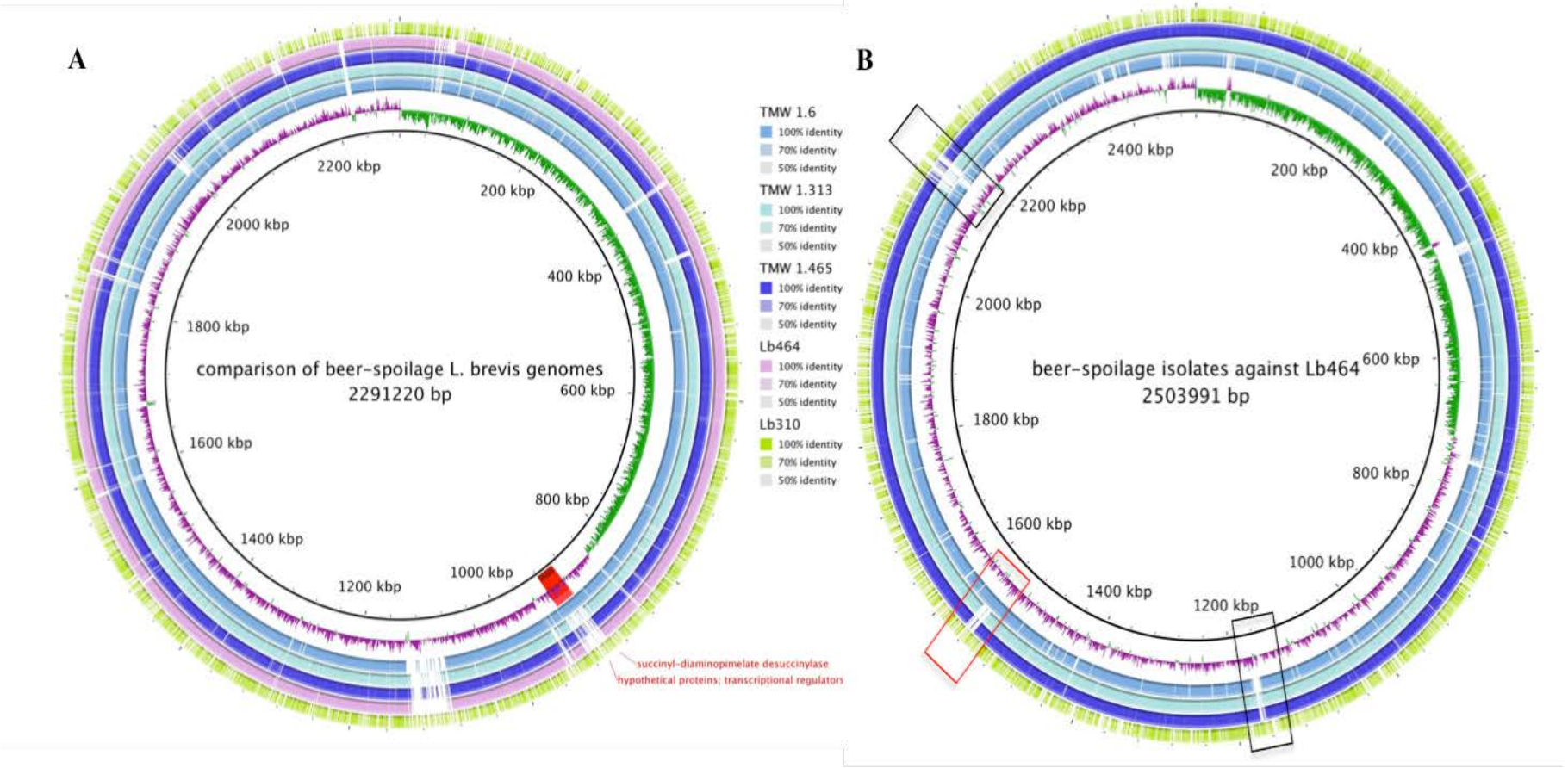


Fig. 8.2. BLASTN comparison of beer-spoilage related *L. brevis.*

(A) is the Lb367 genome and (B) is the Lb464 genome. The genome of TMW 1.6 is included as a non-beer-spoilage control. In B), black boxes denote example areas of low identity against the Lb464 genome (i.e., suggesting areas unique to isolate Lb464) and the red box denotes an example of regions unique to beer-spoilage isolates TMW 1.313 and 1.465 (i.e., niche/location-specific identifiers).

Table 8.3. Presence/absence of putative beer-niche specific genes in LAB genomes

Phenotype	Isolate Label	signal transduction histidine kinase [c, d]	arsR/ cinA [d]	Poly-galacturonase [d]	Arabinose ABC permease [e]	Rrf2/ HxIR/ LytR	Fur family transcriptional regulator	coniferyl aldehyde dehydrogenase	PTS, sorbose-specific IIC subunit
Known *L. brevis* Beer-Spoilers	Lb464 [a]	+	-/ -	+	+	4/2/4	2	+	+
	Lb310 [a]	-	-/ - (+)	+	+	5/2/4	2	+	+
	TMW 1.313 [b]	+	+/ +	+	+	3/4/5	2	+	+
	TMW 1.465 [b]	+	+/ +	+	+	3/3/4	2	-	+
Known *L. brevis* Non-Beer-Spoilers [a]	Lb367 [a]	-	-/ -	-	efflux	5/-/3	-	-	-
	KB290 [a]	-	-/ -	-	-	1/-/2	-	-	-
	TMW 1.6 [b]	-	-/ +	-	-	2/4/4	2	+	-
Untested for beer-spoilage *L. brevis* (but non-beer-related)	EW	+	-/ - (+)	endo-polygalacturonase	-	2/3/4	1	+	-
	subsp. *gravesensis*	-	-/ - (+)	-	-	1/4/0	2	-	+
	DSM 20054	-	-/ - (+)	-	-	2/6/1	2	putative	-
	15f	-	-/ - (+)	-	+	3/2/2	-	-	-
	47f	-	-/ - (+)	-	-	3/2/4	-	-	-
	AG48	-	-/ -	-	-	4/2/5	2	-	-
	WK12	-	-/ -	-	-	2/2/5	2	-	-
	DMsc_003	-	-/ - (+)	endo-polygalacturonase	-	3/4/4	1	+	-
Known	L101 [a]	-	7/ +	-	-	4/4/4	2	-	sorbitol-

***Lactobacillus* beer-spoilers** [a]	Lc03 [a]	-	5/ +	-	-	6/1/3	1	-	specific +
	Ls74 [a]	-	5/ +	-	-	4/3/4	2	+	+
Known *Pediococcus* Beer-Spoilers [b]	Pc344 [a]	+	5/ +	-	-	-/1/-	-	-	+
	Pd58 [a]	-	- /+	-	-	1/2/-	1	-	+
	Pd28219 [b]	-	3/ +	-	efflux	2/1/2	2	-	-
Non-beer-related Type Strain Isolates	Lc334	+	4/ +	-	+	-/-/-	-	-	-
	Ls23K	-	2/ -	-	-	-/-/-	2	-	-
	Pp25745	-	2/ -	-	efflux	-/1/1	1	-	-

[a] Isolates tested in-house for beer-spoilage ability.

[b] The beer spoilage ability of TMW 1.313, TMW 1.465 and TMW 1.6 were tested as in (**5**), and the beer-spoilage ability of Pd28219 was tested as in (**32**).

[c] The presence/absence of a gene is indicated as "+" or "-" if single copy; numbers indicate how many copies of a given gene are present.

[d] Parameter suggested in (**5**). Values for the parameters provided above the second bold black line were investigated using specific *L. brevis* primer sequences to detect presence/absence; values below this line for non-*L. brevis* isolates denote presence/absence of this type of gene and/or enrichment. Results given as (+) indicate that the specific primer sequence suggested by **(5)** could not be found, however, the genome has an annotated *cinA* gene.

[e] Parameter suggested in (**32**).

every isolate were searched for genes of interest, by both inspection of existing individual genome annotations available from Genbank, and by performing local BLASTn and BLASTx searches of their genome sequences, using annotated Lb464 sequences as a query (3). The RAST server (rapid annotations using subsystems technology) was used to parse the functionality of plasmid protein sequences of Lb464, Pc344 and KB290 downloaded from Genbank (4).

8.4. RESULTS and DISCUSSION

8.4.1. Beer-spoilage *vs.* non-beer-spoilage *L. brevis*

All available *L. brevis* genomes and other representative LAB genomes publically available from NCBI were downloaded for comparative analysis (Table 8.1). Of these 14 isolates, Lb367, Lb464, KB290, Pc344 and Pd58 had been tested for beer-spoilage ability in-house according to methods described in (**6**; Chapter 5). Isolates TMW 1.313, TMW 1.465 and TMW 1.6 have been tested for beer-spoilage ability in (**5**) using a previously described resazurin dye reduction assay (**29**). Although the remaining isolates have not been tested for growth in beer, they are taken as representing an interesting non-beer-niche-related cache of isolates due to being recovered from non-beer (brewery) sources such as the human gastrointestinal and feces, or the *Drosphila melangaster* gastrointestinal tract. Further, many of these genome sequences are only assembled to contig or scaffold level, making in-depth sequence analysis and comparison difficult given that sequences may be missing (such as transposase or repetitive regions, 16S rRNA or tRNA sequences); nonetheless, all 14 *L. brevis* genomes appear to have similar GC content (%) (Table 8.1). Interestingly, Lb464 has the fewest number of tRNA species, yet greatest coding capacity among the *L. brevis* isolates, although both Lb464 and Lb310 (Table 8.3) have fewer tRNA coding sequences than non-beer-spoiling Lb367 and KB290. It is also notable that Lb310, Lb464, Lb367 and KB290 have the greatest number of rRNA operons (5S-16S-23S) compared to the other *L. brevis* isolates (with the exception of AG48). The beer-spoilage strains TMW 1.313 and 1.465 have fewer numbers of rRNA operons as well as relatively low coding capacity compared to Lb464 and Lb310. Though the genome assembly stage for some isolates may affect the number of observed rRNA operons, it is notable that isolates not recovered from digestive environments or feces, i.e., strains that are beer-adapted, food-related, or fermentative (Lb367; sourdough and KB290; suguki), have higher rRNA operon copy number. As suggested in (**21**),

the greater number of rRNA operons may correlate with an isolate's ability to respond to harsh or changing environmental conditions (i.e., beer), by optimizing protein synthesis capacity in face of variable nutrient availability.

8.4.2. Genome sequencing and assembly of BSR *Lactobacillus*

The genomes of four beer-spoilage *Lactobacillus* isolates were sequenced and annotated in-house (Table 8.2). These isolates were all recovered from either spoiled beer or a brewery, and like the beer-related *L. brevis* isolates, the non-*L. brevis* BSR isolates have between four and six rRNA operons. These four BSR isolates all have higher coding capacities than the majority of *L. brevis* isolates, and are similar to that of Lb464, within the range of 2,533 to 3,299 genes. Further, all beer-related isolates (Table 8.1 and 8.2) have apparent higher coding capacity than non-beer-spoiling counter parts (i.e., type-strain isolates in Table 8.1) with the exception of TMW 1.313 and TMW 1.465, which do not possess increased coding capacity. This suggests that these two strains may have undergone a reduction in genome size or lack significant plasmid coding capacity. Further, as the beer-spoilage ability of these isolates was assessed through use of a resazurin-dye reduction assay and not a beer-culture test (5, 6**, 29**; Chapter 5), it is possible that these strains have a low beer-virulence compared to Lb464, Lb310 and Lb101, Lc03, and Ls74. The high number of putative pseudogenes found in the four non-*L. brevis* beer-spoilage isolates in Table 8.3 is also interesting, suggesting that numerous lateral gene transfer, gene duplication or DNA recombination events had occurred in these isolates (**11**, **17**).

8.4.3. *L. brevis* genome conservation

BLASTn comparison of all *L. brevis* isolates against the Lb367 reference genome in Fig. 8.1 reveals a high level of conservation among all isolates. The most interesting observation is that Lb367 also exhibits an atypical GC-skew similar to the one displayed by Lb464 such that the skew is not symmetrical around the replication origin and terminus (Chapter 6). The replication terminus also corresponds to the most notable section of variable sequence identity, as this region of the Lb367 genome is annotated largely with hypothetical proteins (Fig. 8.1). When the same comparison is performed using just beer-spoilage related organisms against the Lb367 reference genome (Fig. 8.2A), and against the Lb464 genome (Fig. 8.2B), using TMW 1.6 as a

non-beer-spoilage control, visible areas of non-identity are increased. These areas often correspond to areas of hypothetical proteins in either reference genome and/or regions dense with transcriptional regulators. This type of analysis could be useful for determining location or niche-specific genetic markers (i.e., which have been acquired by isolates in a specific brewery, or acquired by isolates recovered from different locations). Overall, there are no regions of genomic dissimilarity that are particularly remarkable between beer-spoilage and non-beer-spoilage *L. brevis* genomes. This strengthens the notion that the genome coding capacity of BRS LAB and non-BSR LAB will be largely similar, with main differences lying in the overall cellular regulation of the two different groups of LAB and/or extra-chromosomal (i.e., plasmid) coding capacity.

8.4.4. *L. brevis* ortholog protein enrichment

COG identities were assigned to each *L. brevis* genome and then isolates were grouped according to three phenotypes: known-beer spoilers (Lb310, Lb464, TMW 1.313 and TMW 1.465); known-non-beer-spoilers (Lb367, KB290, TMW 1.6); and isolates with unknown beer-spoilage ability (all other isolates). The latter isolates were grouped together given they were recovered from similar environments (i.e., digestion-related) and were termed “environmental”. COGs that were common within the three groups (i.e., common to BSR isolates, common to non-beer-spoilers, and common to environmental isolates) were taken so as to eliminate isolate-specific “hypothetical proteins” or phage/transposons that may inflate values. When these three groups of COG’s were compared, it was revealed that there is a large core of similar protein COGs shared between all *L. brevis* isolates (Fig. 8.3), with a smaller subset of genes then specific to each isolate “group” that would presumably assist isolates growing in the beer-environment. Genes specific to the BSR group include several short chain dehydrogenases, which have roles in lipid, amino acid, and carbohydrate metabolism (**18**), as well as permease type transporters. Non-beer-spoilers appear enriched for a phosphotransferase transport system (PTS) specific for galactictol subunit IIC, Type III restriction modification systems, and ion transporters; and digestive-related or “environmental” isolates are enriched for amino-acid specific-acetyltransferases and glycosyl hydrolases, as well as several ABC transporters. The enrichment of different transporter types in niche-specific *L. brevis* groups highlights the

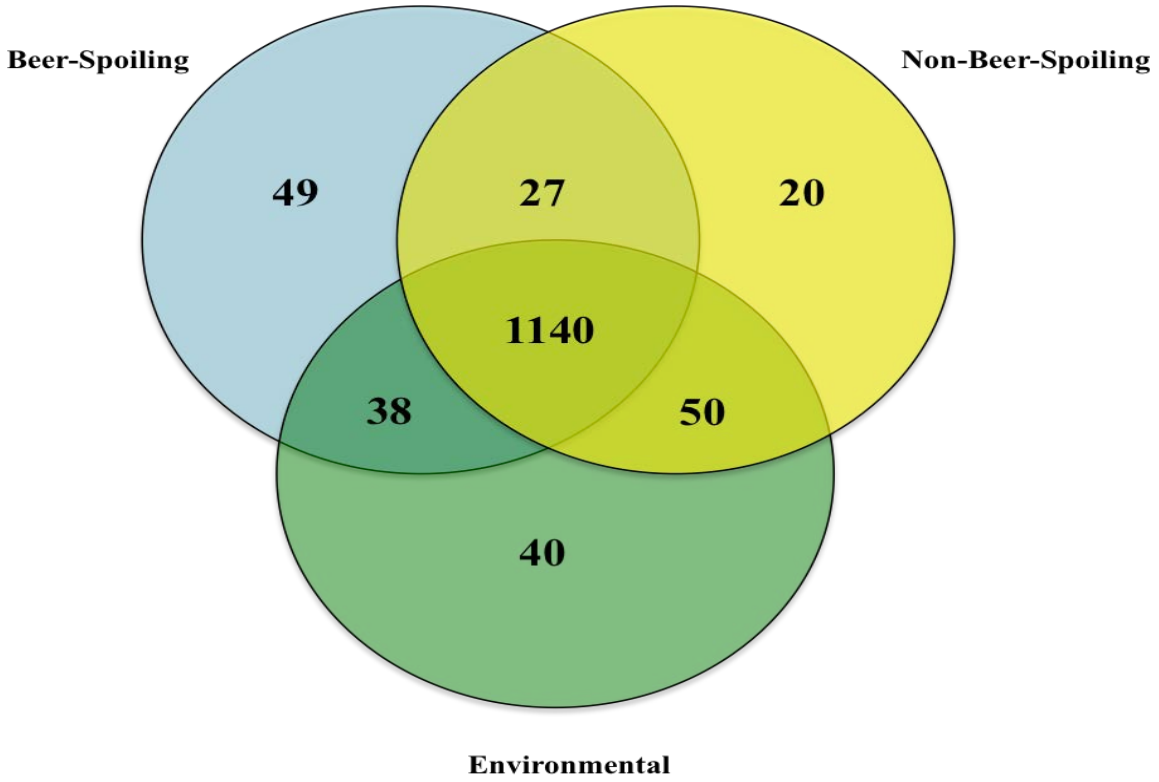


Fig. 8.3. Common clustered ortholog group (COG) terms enriched *L. brevis* isolates.

Beer-spoiling isolates include Lb464, Lb310, TMW 1.313 and TMW 1.465; non-beer-spoiling isolates include Lb367, TMW 1.6, and KB290; environmental samples include all other available *L. brevis* isolates (Table 8.1). *L. brevis* subsp. *gravesensis* was not included in any group as part of this analysis given that contains a large number of unique COGs and is largely dissimilar to the *L. brevis* genome (Fig. 8.1).

importance and critical adaptations of membrane interaction and transport with specific growth environments. Inter-genus comparison of COGs was not performed on the basis of previous studies yielding indeterminate results, or results not being useful for informing selection of a few genetic markers (**26**). As an example of this, the study of BSR and non-BSR *Pediococcus* revealed that *Pediococcus* BSR isolates had an enrichment of transcripts for *de novo* folate synthesis, however, these same transcripts could not be found in most *L. brevis* isolates, regardless of beer-spoilage ability (**32**). Table 8.3 serves as proof of concept that genetic differences elucidated within one genus, do not necessarily have application in describing or predicting beer-spoilage ability in another genus.

8.4.5. Beer-niche-specific gene screen of LAB

All LAB genomes were screened for the presence or absence (if a single copy gene) or enrichment (if a multi-copy gene) of a subset of putative beer-nice genes (Table 8.3). These genes were implicated in either previous studies (**5, 32**) and/or by transcriptional anlaysis of Lb464 and Pc344 during growth in beer, (Chapter 6) in conduction with the COG analysis of beer-adapted isolates (Fig. 8.3).

A recent study proposed on the basis of comparative genomic hybridization analysis that a specific signal transduction histidine kinases gene, a specific ArsR transcriptional regulator, and the competence-damage inducible gene *cinA*, were all suitable as markers of *L. brevis* beer-spoilage potential (**5**). Primers for these sequences, provided.in **(5)** were queried against all genomes using a BLASTn search [(Signal histidine transduction forward – ACACCGTACGGGGGATTGGCT and reverse – GGGCGCGTGATTTGTTCGGC); (ArsR: forward – TTTGTCCCAAGCTACTTCATCTGGC and reverse – TGGGCCATCCCCTGAGTCGT); (CinA forward – AGTGCAGCCGAAAGTTTAACTGGGG and reverse – ACAGCCACGAGCCATTGAGCG)].

Results in Table 8.3 indicate that querying the specific signal transduction kinase, ArsR transcriptional regulator and *cinA*, are not robust indicators of *L. brevis* (or LAB writ-large) beer-spoilage potential. The presence of these genes only correlates well with TMW 1.313, 1.465 and 1.6, all isolates used in the original study (**5**). Thus, the use of these primer sets only appears to resolve beer-spoilage ability of the original set of genomes analyzed by Behr et al. (**5**); i.e., they

do not appear to be suitable as universal *L. brevis* beer-spoilage markers, but rather markers suitable for use in the specific brewery they were recovered from. Thus, using a small subset of beer-related *L. brevis* genomes, from one specific isolation source, skews the identification of unique beer-related genes to that particular locale. We see this specifically with the use of primers for *cinA*, a gene that was functionally annotated in most *L. brevis* genome [indicated as (+)]. However, using these specific primers, we do not detect the *cinA* gene in any *L. brevis* isolate not recovered from the sources in (**5**). These *L. brevis*-specific primers were not used to query the genomes of non-*L. brevis* genomes; instead these genomes were probed for the absence/presence and/or enrichment of these types of genes (Table 8.3). The presence/absence of signal transduction histidine kinases and a *cinA* gene do not correlate with beer-spoilage ability of LAB writ-large, nor is there an apparent enrichment of ArsR transcriptional regulators in BSR LAB.

In contrast, querying each genome via BLASTx for a polygalacturonase gene (L747_13085) does reveal this gene to correlate well with *L. brevis* beer-spoilage ability (Table 8.3). This enzyme breaks down oligogalacturonides in the cell walls of plants, and thus may provide beer-spoilage LAB a niche-adaptation for breaking down barley or other grain to obtain nutrients and energy (**30**). This lends credence to the theory that beer-spoilage isolates are adept at scavenging carbon sources from their environment. Interestingly, the two *L. brevis* isolates recovered from *Drosophila* intestine/gut both possess an endo-polygalacturonase, which cleaves oligogalacturonides in a random fashion, however, none were found in human-GI tract associated isolates which may reflect differences in the two organism's digestive processes.

The remaining genes screened for in Table 8.3 were selected based on the findings of **(32)** on beer-adapted *Pediococcus* isolates, wherein COG terms for arabinose efflux permease was suggested to be enriched in isolates of beer-origin. In Chapter 6, transcriptional analysis of Lb464 during growth in beer revealed that an arabinose ATP binding cassette (ABC) transporter permease was significantly up-regulated in beer (Chapter 6) and, thus, in conjunction with COG term analysis of beer-specific isolates (Section 8.4.1.3.), the arabinose ABC permease (L747_12740) was selected as a potential marker for beer-spoilage ability (Table 8.3). Analysis of the transcriptional data of Lb464 and COG term enrichment of BSR *L. brevis* resulted in the

remaining putative “beer-niche” specific CDS listed in Table 8.3 (Chapter 6). Namely, transcripts related to Rrf2, HxlR and LytR and Fur (ferrous iron uptake regulator) transcriptional regulator families were analyzed for enrichment, and the presence/absence of coniferyl aldehyde dehydrogenase (ALDH) (L747_10870), and a PTS, sorbose-specific IIC subunit (L747_12995) were screened for.

Although the study in **(32)** found an arabinose efflux permease to be enriched in beer-associated LAB isolates, transcriptional data of Lb464 suggests an ABC-type transporter mediating arabinose transport is important for growth in beer (Chapter 6). The ABC characterization of this permease is an apparently important distinction given that the use of this transporter requires energy expenditure to uptake arabinose from the environment. Table 8.3 shows that the ABC arabinose permease is largely specific to beer-adapted *L. brevis* isolates, thus potentially making for inclusion into a set of suitable candidate markers for beer-spoilage ability when isolates are recovered from beer-related sources.

Transcriptional regulators belonging to Rrf2, HxlR, LytR, and Fur families are all up-regulated in the response of Lb464 to beer (Chapter 6), although there is no apparent enrichment of these regulators in BSR compared to non-BSR *L. brevis*. Interestingly, there is an enrichment of these transcriptional regulators in *Lactobacillus* species that are both beer and environmental-related (Table 8.3). There is limited presence of these genes in *Pediococcus* isolates and the type strains of both genera recovered from food sources, suggesting these transcriptional regulators are correlated with *Lactobacillus* species (Table 8.3). Thus, future focused analysis on transcriptional families and regulation of BSR LAB is worthwhile, as these types of genes are not currently capable of resolving beer-spoilage virulence at any level (i.e., genus, species, isolate), despite indication BSR LAB have altered or specialized transcriptional regulation.

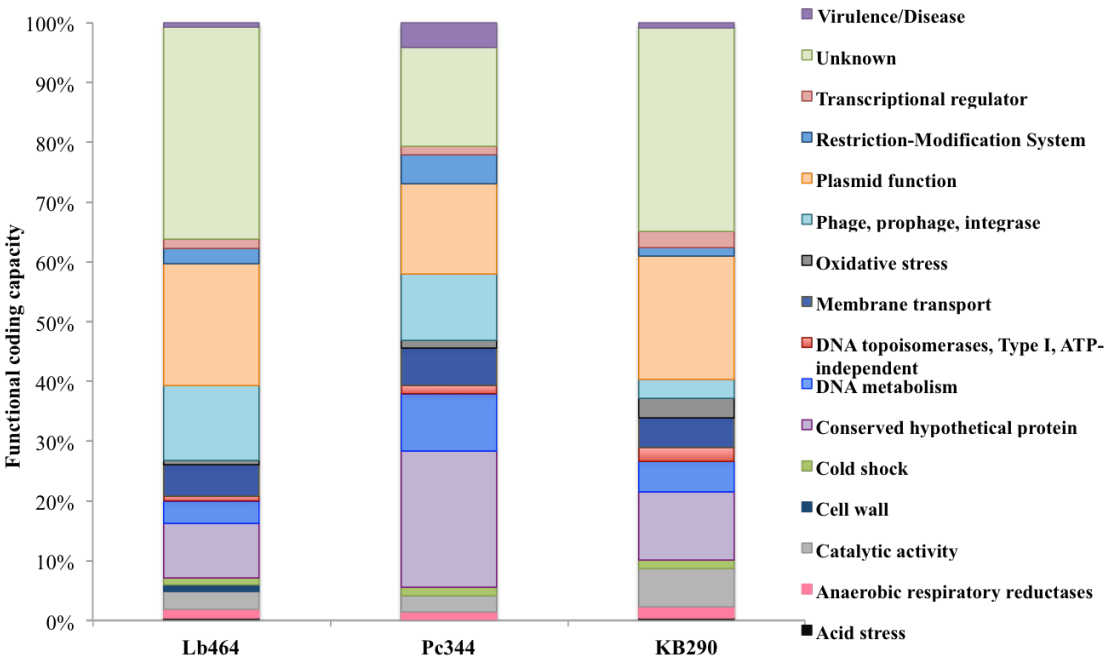
Lastly, genes for coniferyl aldehyde dehydrogenase (ALDH) and a sorbose-specific IIC subunit of the phosphotransferase (PTS) transporters appear, to strongly distinguish beer-niche adapted *L. brevis,* and beer-adapted *Lactobacillus* and *Pediococcus* from non-beer adapted counterparts, respectively (Table 8.3). This is strong evidence that nutrient scavenging processes are the most notable processes that define BSR LAB. Coniferyl ALDH is an oxidoreductase that reduces

ferulate, a component of plant cell walls (i.e., barley, grains), to coniferyl aldehyde by reducing nictotinamide adenine dinucleotide phosphate (NADPH) and 2 H^+ (**1**). This supports the theory that all BSR LAB were originally associated with plant material prior to their introduction into the brewery, i.e., carried into the brewery on raw materials (e.g., barley, wheat). It should be noted that sorbose is a naturally occurring monosaccharide that is not completely fermented, if at all, by brewing yeast, making it a viable nutrient source for beer-spoilage isolates to utilize (**38**). The fact that only the gene for PTS, a sorbose-specific IIC component, resolves beer-spoilage ability in LAB isolates belonging to both *Lactobacillus* and *Pediococcus* isolates, suggests that this carbon source may be commonly exploited by LAB in most beer styles, regardless of the brewing-yeast strain used.

What is most interesting is the finding that transcripts for the three candidate nutrient-scavenging-related genes polygalacturonase, coniferyl ALDH and PTS sorbose-specific component IIC are not significantly differentially expressed in response to beer in Lb464 (Chapter 6). This could likely be a result of the fact that transcriptional analysis was performed during exponential growth, and thus available oligogalacturonides, ferulate, or sorbose, had already been largely consumed in order to establish growth and/or as a function of the particular style of beer used for analysis. This finding once more supports the need for confirmatory proteomic or metabolic studies, as well as further transcriptional analysis of both Lb464 and other BSR *L. brevis* at other (importantly, earlier) time points during growth in beer.

8.4.6. Beer-spoilage plasmids

Of the published and publically available *Lactobacillus* and *Pediococcus* genomes, beer-spoilage isolates Lb464 and Pc344, and non-beer spoilage isolate KB290, contain the greatest number of plasmids (Table 8.1). While Lb464 and Pc344 each harbor eight plasmids and one and two cryptic plasmids, respectively, they also differ greatly in genome size (2.7 MB vs. 1.9 MB) and overall coding capacity. Lb464 can also grow in pressurized beer while Pc344 cannot (**6**; Chapter 5). KB290 has demonstrated ability to survive in stimulated intestinal and gastric juices, and harbors nine plasmids (Fig. 8.4) (**16**). Further, there is no cross-resistance of any isolate to these different niche environments, i.e., Lb464 and Pc344 are not able to survive in intestinal/gastric



# of plasmids	8	8	9
growth in:	pressurized beer	gassed beer	stimulated gastric and intestinal juices
cryptic plasmids	pLb464-7	pPECL-1, -2	none
size range (Kb)	2.3 – 84.9	1.6 – 3.6	5.8 – 42.4

Fig. 8.4. Functional coding capacity of plasmid genes for LAB with multiple plasmids.

Cryptic plasmid is defined as a plasmid structure with coding capacity solely for its own replication. Transcripts that belong in categories of Virulence/Disease, Transcriptional regulator(s), Oxidative stress, Membrane transport, Cell wall and Catalytic activity are further described in Table 8.5.

juices and KB290 has no ability to survive or grow in beer (data not shown). These findings, along with previous analyses demonstrating the loss of specific plasmids or combinations of plasmids from all three isolates can affect their observed, respective tolerance-phenotypes (**7, 16,** Chapter 7) suggest that plasmid content strongly influences niche adaption. In light of the fact that there is an energy burden upon the cell to carry plasmids (**14**), the maintenance of the total coding capacity of plasmids present must truly impart critical function to the cell. Thus, analysis of the functional coding capacities of each of the three isolates' respective plasmids was undertaken.

Annotated proteins sequences from all plasmids were assigned to functional categories via RAST and manually curated (Fig. 8.4). It was revealed that several functions and specific transcripts are common to plasmids of all three isolates. These mainly include proteins involved in plasmid function (replication, plasmid partition, copy number control proteins), and DNA metabolism products such as DNA damage inducible proteins, site-specific recombinases, and a nucleotidyltransferase transcript, which is involved in DNA repair. Further, Lb464 and KB290 plasmids both code for PemK-like and PemI-like proteins, which are involved in stable plasmid maintenance during autonomous replication, suggesting that the plasmid profile of these two genes is highly stable (**34**). The absence of these genes from Pc344 may explain why its plasmids have been previously noted to be easily lost from the cell during both storage and experimental manipulation (**28**). All three isolates have plasmids that bear Type 1 restriction-modification subunits R, S, and M, which can function as a defense system against foreign DNA, although all isolates also contain a number of plasmid-based integrases and transposases largely belonging mostly to the IS30 family. Lb464 and Pc344 contain more phage and transposon-related products than KB90, and Lb464 has a large prophage region on its largest plasmid, pLb464-4, which at 85 Kb is also the largest plasmid among the three isolates.

Other plasmid-coded shared functions among the three isolates are the LtrC-like protein (low temperature requirement C protein), which is a putative cold-shock chaperone protein, and multiple copies of electron transfer flavoprotein-ubiquinone oxidoreductase (EC 1.5.5.1), which is a gene that can assist in anaerobic respiration (**37**). Multiple copies of ATP-dependent protease ATP-binding subunit clpL were only found in Lb464 and KB290, a gene proposed to

help with both acid shock, and the general stress response, by degrading misfolded proteins and providing chaperone activity (**13, 36**). The presence of this plasmid gene in only these two isolates interestingly correlates with the fact that Lb464 and KB290 can grown in environments that have a high oxidative stress level (pressurized beer and gastric juices, respectively) compared to degassed beer, which is the beer medium that Pc344 can grow in and spoil (**6**; Chapter 5).

These three isolates also have similar plasmid-based coding capacity for antitoxin of toxin-antitoxin stability systems (Virulence/Disease functional category), and ferroxidase (EC 1.16.3.1), which is an enzyme likely to be involved in enhancing tolerance to oxidative stress (**31**). Lastly, all three isolates have a number of conserved hypothetical proteins, i.e., proteins that RAST detects closely related products for in other organisms, and a higher proportion of unknown hypothetical proteins, suggesting most of these products are specific to each respective isolate.

These three niche-adapted isolates all share transcripts related to DNA maintenance and metabolism, and general oxidative stress-enhancing mechanisms, however, the differences in plasmid coding capacity are also highly interesting (Table 8.4). In addition to Lb464 and Pc344 each containing hop-specific membrane transport systems, they also share plasmid-encoded metal resistance operons, a lipid A export ABC permease protein, MsbA (involved in glycerophospholipd export and biogenesis of the cellular membrane), and the catalytic enzymes glycosyl transferase and phospho-beta-glycosidase (Table 8.4). Relative to KB290, the plasmids of Lb464 and Pc344 also contain a higher number of plasmid-encoded ABC type transporters, thus potentially allowing for both greater nutrient scavenging or poison (i.e., hops) export ability. As expected, there are also notable differences that separate Lb464 from Pc344; specifically, Lb464 has more plasmid-encoded transcriptional regulators than does Pc344, in addition to harboring transporters for xylose. Xylose, like sorbose, is a carbohydrate not fermented completely, if at all, by brewing yeast thus leaving it behind as a nutrient source for BSR LAB (**38**). This suggests that Lb464 has a greater arsenal of genetic adaptations for growth in beer than does Pc344; i.e., it may be more capable of regulating plasmid-encoded transport activities and/or can scavenge for additional carbohydrate sources, thereby explaining the greatly increased

Table 8.4. Unique attributes of Lb464, Pc344 and KB290 plasmid coding capacities

Category [a]	Lb464 plasmids [b]	Pc344 plasmids [b]	KB290 plasmids [b]
Virulence/ Disease	-2: • Antitoxin of toxin-antitoxin stability system -6: • Cd^{2+} resistance protein; • Cd^{2+} efflux system -4: • Universal stress protein, UspA -8: • As^{3-} efflux pump protein	-3: • Antitoxin of toxin-antitoxin stability system -5: • As^{3-} resistance efflux pump; • As^{3-} resistance operon repressor -6: • Lantbiotic permease protein; • Lanthionine biosynthesis protein, LanM -7: • Antitoxin of toxin-antitoxin stability system • Cu^{2+} homeostasis transpoter; • Exopolysaccharide gene, *gtf*	-5: • Antitoxin of toxin-antitoxin stability system -7: • Lactococcin A immunity protein
Transcriptional regulator	-2: • Hop-tolerance, HorB (TetR family); • Two Xre family -3: • TetR family -4: • Diaglyercol kinase; • TetR family	-6: • RNA polymerase RpoB	-2: • Two AcrR family; • MarR family -5: • ArsR family; • TetR family
Oxidative stress	-2: • Ferroxidase (EC 1.16.3.1) -8: • Ferredoxin reductase; • NADH peroxidase, Npx (EC 1.11.1.1)	-3: • Ferroxidase (EC 1.16.3.1); • glutathione reductase -5: • NADH oxidase	-4: • Ferroxidase (EC 1.16.3.1) -5: • Thioredoxin reductase -6: • Ferroxidase (EC 1.16.3.1)
Membrane transport	-1: • Hop-tolerance, HorA; • Lipid A export ATP-binding/permease protein, MsbA -2: • Hop-tolerance, HorC; • CrcB protein (ion transport)	-4: • ABC transporter -6: • Two ABC transporters -8: • Hop-tolerance, HorA; • Lipid A export ATP-binding/permease protein, MsbA	-2: • Kup (K^+ uptake); • Mg^{2+}/Co^{2+} transporter; • Na^+:H^+ antiporter; • Voltage-gated Cl^- channel family protein -3: • Serine transporter;

Catalytic activity	-3: • Hop-tolerance, HitA; • Mg^{2+}/Co^{2+}, CorA -4: • Three ABC transporters -8: • Two Xyloside transporter, XynT -1: • Glycosyl transferase; • Phospho-beta-glycosidase -2: • Enolase (EC 4.2.1.11); • Glycosyltransferase -8: • SAM-dependent methyltransferase; • Pyridoxamine-phosphate oxidase-related, FMN-binding; • D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95)	-8: • Two cytosine deaminase (EC 3.5.4.1); • Glycosyl transferase; • Phospho-beta-glycosidase	• $Co^{2+}/Zn^{2+}/ Cd^{2+}$ resistance; • Two ABC transporters -5: • Amino acid ABC transporter -6: • Peptide ABC transporter ATP-binding protein, ComA -1: • Cellulose synthase catalytic subunit [UDP-forming] (EC 2.4.1.12); • Glycosyl transferase -2: • Histidinol-phosphatase (EC 3.1.3.15); • GCN5-related N-acetyltransferase -3: • Long-chain-fatty-acid, CoA ligase; • Phosphopantetheinyl transferase -5: • Aspartate racemase (EC 5.1.1.13); • Disulfide isomerase; • GCN5-related N-acetyltransferase; • Nicotinate phosphoribosyltransferase
Cell wall	-2: • Teichoic acid glycosylation protein -4: • Lyzozyme M1 (1,4-beta-N-acetylmuramidase); • N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28)	No related products	No related products

[a] Categories as presented in Fig. 8.4.

[b] Plasmid numbers for each isolate are denoted as -number: (e.g., pLb464-2 and pKB290-2 are both indicated as -2 in the respective columns of each isolate).

virulence of Lb464 in beer. Of additional interest is the fact that genes for xylose transporter, XynT, can be found on putative plasmids of isolate Lb310, which suggests Lb310 and Lb464may have been recovered from the same location. If this were the case, this transporter potentially could be an example of a brewery/niche-specific adaptation, and plasmid marker that is transmitted within that specific location. Alternatively, such a transporter could simply be an example of one type of an ancillary adaptation to growth in beer.

Most significantly, there is the presence of plasmid-encoded cell wall metabolism-related enzymes in Lb464 that are not found in either Pc344 or KB290. This provides very strong evidence that Lb464 has acquired specific coding capacity that allows for its adaptation to and survival in the pressurized gassed environment, which acts to weaken the cellular membrane and increase oxidative stress in the cell (**24, 35**). Modification of the cell wall can fortifiy the cell against increased cell permeability under pressure, and this was confirmed to be an important response by transcriptional anlaysis of Lb464 during growth in pressurized/gassed beer (Chapter 6). These cell wall associated proteins are encoded by plasmids pLb464-2 and -4, which were both previously implicated as contributing to the overal beer-spoilage ability of Lb464 in (**7**; Chapter 4). The fact that cell-wall modification proteins are found on Lb464 plasmids and not found on Pc344 plasmids further highlights divergent adaptation to the brewing environment. The disparate beer-spoiling strengths of these two isolates (**6**; Chapter 5) underscores the hypothesis that beer-spoilage ability can be acquired incrementally, with some genes or adaptations being more useful or contributing to spoilage-virulence to a greater extent.

Relative to the two beer-spoiling isolates, the plasmids of KB290 differ most notably in the type of membrane transporters encoded thereon. Most are involved in ion and proton transport, foreseeably allowing for efficient cellular proton motive force (PMF) regulation and homeostasis when growing in the presence of highly concentrated electrolytes of gastric and intestinal juices (**23**). Other transporters are involved in the uptake of amino acids or peptides from the environment, which may be an adaption allowing the cell to use the peptides made available as a result of the enzymatic action of pepsin in gastric juices (**15**).

This analysis of functional plasmid coding capacity for Lb464, KB290 and Pc344 confirms that plasmid acquisition is critical for adaptation to niche-environments in general. These niche-specific adaptations appear to be often involved in membrane transport and niche-specific nutrient acquisition. This underscores the finding of COG enrichment analysis that suggested membrane transport mechanisms to be a defining trait for adaptation to specific environments. Here is must be recalled that there is a high level of redundancy for membrane transport mechanisms at coded chromosomally for Lb464, Pc344 and KB290, Thus, while plasmid-coding capacity can exert a strong influence on the beer-spoilage ability of an organism and brewery/niche-specific genes can be found on plasmids of BSR LAB, the total genetic response of LAB to the beer environment is most certainly complex. Together with the finding that plasmid transmission of hop-tolerance genes is not a conserved, or error-free process (Chapter 3), the present data provides strong impetus to further analyze how plasmids are maintained and transmitted throughout the brewery. Yet again, the present data supports the concept that LAB beer-spoilage and overall virulence is not mediated by the absence or presence of solely hop-tolerance genes, and is instead an adaptive phenotype, borne of multiple genes that work in synergy to increase survival in beer.

8.5. CONCLUSIONS

Beer-spoilage is a problem to which there could be dozens of “correct answers” or adaptations – used in various combinations to produce a scale of virulence across BSR LAB. Comparative analysis within a given species, and then within a genus of LAB is likely to yield greatest resolution of common genes linked to beer-spoilage ability, and an increased number of BSR LAB genomes made available will only increase the robustness of these analyses. As an important caveat to this analysis, it should be noted that the use of a subset of isolates from non-diverse locations is proven to skew marker discovery towards brewery/niche-specific genetic anomalies, and not species-level beer-spoilage markers, as seen by using specific transcripts suggested previously in (**5**). The ability to described beer-spoilage ability genetically, therefore, is most greatly influenced at the brewery-level and secondly at the species-level.

Two chromosomal genetic markers discovered at the brewery-level are scalable to the general species level, specifically, polygalacturonase and coniferyl aldehyde dehydrogenase, both of

which are involved in breaking down plant materials. One gene, discovered at the species level, a sorbose-specific PTS component IIC, has the strongest correlation with beer-spoilage ability across the *Lactobacillus* genus. Thus, nutrient scavenging and plant-breakdown enzymes are broad categories of genes that can be investigated further in future research. Armed with the knowledge that BSR LAB accumulates beer-spoilage adaptations from their environment to create a Swiss-army knife-like collection of genetic responses, we must now utilize comparative genomics to understand how the total microflora of the brewery can drive BSR LAB development and maintenance.

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Chapter 9: Concluding remarks and the future

9. INTERACE

Section 9.2.2 and 9.2.3 include material adapted from "Investigation of beer-spoilage lactic acid bacteria using omic approaches" authored by Jordyn Bergsveinson and Barry Ziola for inclusion in the upcoming book *Omics in Brewing Microbiology* (edited by Charlie Bamforth and Nick Bokulich; to be published by Caister Academic Press, Poole, United kingdom, in 2016). Material from this book chapter is also presented in Chapter 1.

9.1. CONCLUDING REMARKS

Beer-spoilage-related lactic acid bacteria (BSR LAB) are underappreciated as complex and adaptive organisms. The original paradigm of thought governing BSR LAB research is that these isolates must be uniquely adapted to the presence of hops, and as a result, hop-tolerance genes have been solely attributed as conferring beer-spoilage ability. In order to more adequately appreciate the problem of BSR LAB contamination, and improve upon current screening methods, evidence must be gathered to enforce the notion that hop-tolerance, while an important component of BSR LAB physiology, is not the entire narrative of beer-spoilage ability. Thus, the goal of this thesis was to investigate the effect that the poorly characterized stress of dissolved CO_2 (dCO_2) has on the physiology of BSR LAB and the role that mobile genetic elements (MGEs) such as plasmids have on determining beer-spoilage phenotype. The ultimate goal of this thesis is to elucidate specific physiological responses and/or BSR LAB-common genes that could be candidates for genetic markers for beer-spoilage ability.

The work presented in Chapters 2 and 3 were the raised important questions regarding the utility of using only one to three hop-tolerance genes as indicators of LAB beer-spoilage ability. Specifically, it was found that hop-tolerance genes are transcribed to different extents in different BSR LAB (Chapter 2), and that the conserved nature of these genes (and thus their proposed "universal" nature) is highly overestimated (Chapter 3). This suggests that current routine screening methods (polymerase chain reaction (PCR) or multiplex-PCR-based screening) are inadequate to ensure that these genes are complete and functional (Chapter 3). The collective data from these two chapters begin to uncouple the notion that presence of known hop-tolerance

mechanisms is strongly predictive of overall-beer-spoilage ability. The isolates analyzed in those two studies provide indication that just because one or more of the three earlier described hop-tolerance genes (*hitA*, *horA*, *horC*) are present does not mean that they are being utilized uniformly by all BSR LAB and, this in turn means that these genes do not necessarily correlate to relative increased beer-spoilage ability. Overall, this data highlights that the horizontal gene-transfer occurring within LAB in the brewery environment is potentially error-prone, which indirectly suggests that there are redundant hop-tolerance mechanisms within a given LAB (Fig. 9.1).

To further investigate the role that the overall plasmid profile of a BSR (and not-solely just plasmid-encoded hop-tolerance genes) have on beer-spoilage ability, plasmids were intentionally cured from beer-spoiling isolate *Lactobacillus brevis* BSO 464 (Lb464) (Chapter 4). Analysis of the resultant plasmid variants clearly demonstrate that the total plasmid coding capacity is a major driver of the Lb464 beer-spoilage phenotype and that there is redundancy of function for not only hop-tolerance, but also for survival in beer. Indeed, several other stress-related genes and/or mechanisms were found localized on the Lb464 plasmids that appear to contribute in a synergistic manner to aid in survival in beer. Thus, the loss or acquisition of plasmids in the brewery environment in specific combinations can greatly influence the virulence of a LAB and the ability for it to adapt to different beer styles and/or environments it may encounter. Similar conclusions were drawn from the data presented in Chapter 7, which examined the effect of plasmid loss on the beer-spoilage ability of *Pediococcus claussenii* ATCC BAA-344^{T} (Pc344). Here it was found that the combinatorial loss of plasmids alters its growth kinetics in beer, and to a lesser extent Pc344 hop-tolerance. Plasmid-variant analysis of these two isolates has interesting implications for breweries performing novel fermentations using LAB, since this data clearly emphasizes the necessity of describing, and maintaining an isolate's plasmid profile in order to maintain expected function and metabolism. In this sense, the results presented in Chapter 4 and 7 are an open invitation for the industry to perform more genomic sequencing of BSR LAB, specifically targeting and researching brewing-specific plasmids.

Understanding the role that plasmids and plasmid transfer has in LAB beer-spoilage capability is central to developing improved beer-spoilage screening methods for these bacteria. Such

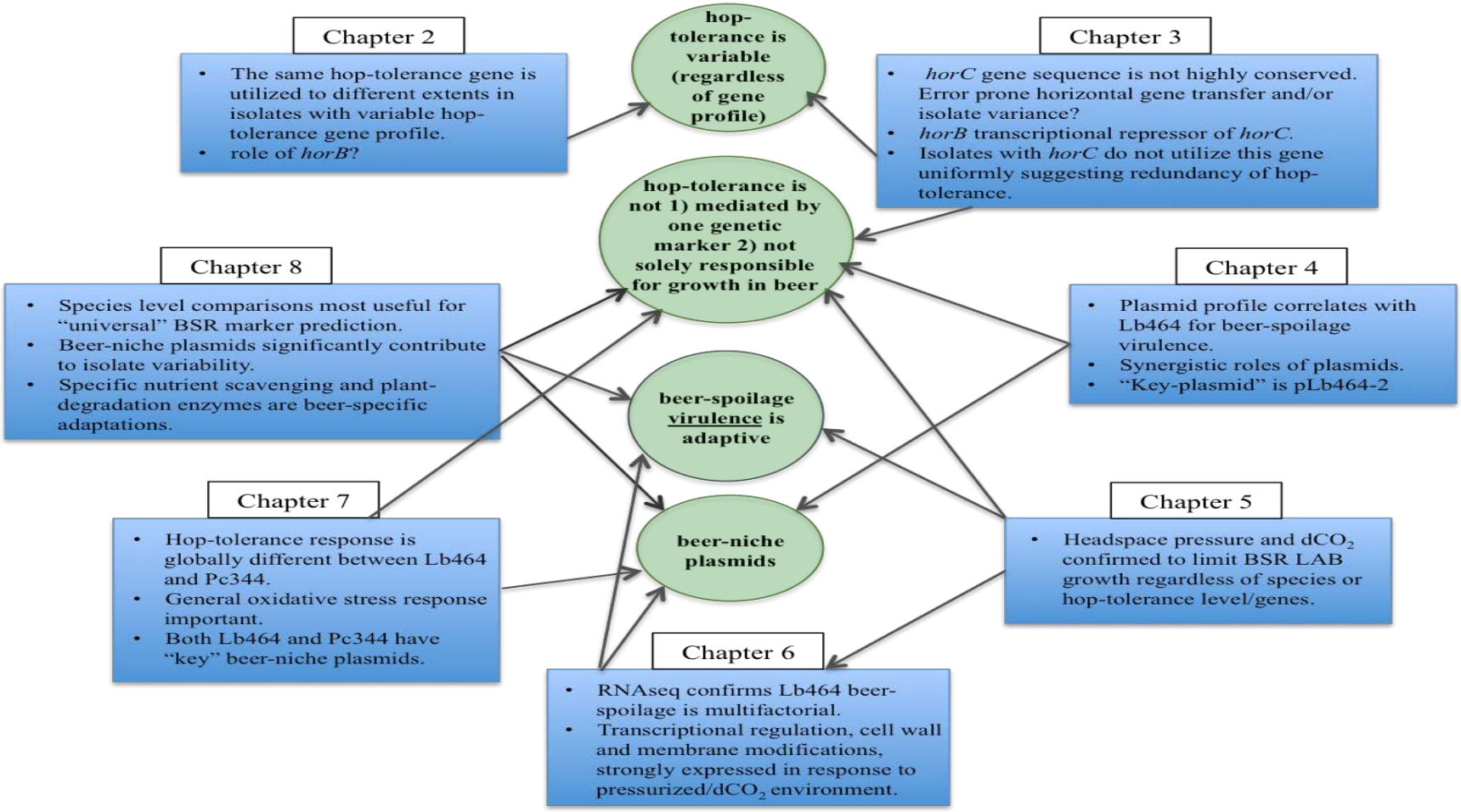

Fig. 9.1. Summary of thesis content.

Main conclusions of each data chapter are provided in blue boxes, connected by arrows to the central theories or general concepts (green circles) they support. This thesis ultimately concludes, using Lb464 and Pc344 as model BSR LAB that hop-tolerance genes are not sole determinants of beer-spoilage ability and that beer-spoilage phenotype can be acquired as a result of a multitude of genes. Plasmids, in general, greatly influence the beer-spoilage phenotype of these BSR LAB isolates.

screening methods are critical for the success of a brewery, not only in monitoring the hygiene and quality of all material throughout the brewing process - from the mash and wort, to the final, packaged product that reaches the consumer. At the final packaged product stage, however, there is the presence of headspace pressure and dissolved CO_2 (dCO_2), which reasonably would be expected to present new and significant stress to any BSR LAB present in the final beer. The effect that these two stresses have on the beer-spoilage physiology of BSR LAB previously was not characterized. Consequently, the investigations reported in Chapter 5 are the first to explore the ability of supposed beer-spoilage isolates to survive in a packaged product beer. It was found that the presence of headspace pressure and the resultant dCO_2 limits the ability of all but four of the 20 "beer-spoilage" LAB isolates tested. The data indicate that non-dCO_2-tolerant organisms can enter a viable, but not culturable (VBNC) state while tolerant isolates were able to adapt to avoid entry into this phase. Importantly, dCO_2-tolerance had no correlation to the presence/absence of hop-tolerance related genes, underscoring the notion that hop-tolerance genes are not wholly useful for predicting true LAB beer-spoilage ability (Fig. 9.1). It should also be cautioned that analysis of BSR LAB isolates recovered from sources in the brewery free of dCO_2 content (i.e., prior to packaging) should be performed from the perspective that these isolates may not be truly adapted to the total beer environment, but of course can still be problematic and spoil unfinished or unpackaged beer (i.e., which are minus the stress of dCO_2 and headspace pressure).

The finding that the packaged beer environment can limit the ability of BSR LAB to establish growth in beer raises the question of how best to screen isolates for dCO_2-tolerance? Although it may cause initial elation for brewers to find that headspace pressure will limit the growth of contaminating BSR LAB, it is the organisms that can mediate this environment and survive to greet the consumer, which are truly problematic. Further, imagine a situation where a potential BSR LAB is detected at some location in the production process, and the decision is made to shut down this machinery to clean and delay production, only to have it revealed by subsequent analysis that this isolate posed no threat to the packaged product and the consumer's experience. From a brewery quality control viewpoint, would it not be ideal to screen for "dangerous" LAB during the production process and within the brewery's normal microflora?

Whole transcriptome analysis (RNAseq) was performed in order to broadly assess what genetics are critical for the growth of model organism Lb464 in both degassed and pressurized/gassed beer (Chapter 6). The transcriptional response of Lb464 was similar to that observed in previous analysis of Pc344 in terms of responses to general oxidative stress mechanisms; however, Lb464 differed notably in that it appeared to rely on nitrogen source uptake and metabolism as a main energy source during exponential growth. This data also confirmed that transcripts (hops and non-hop-related) from specific plasmids (which were indicated to play a role during growth in beer in Chapter 4) were important for growth in beer, enforcing the notion of beer-niche-specific plasmids (Fig. 9.1). This transcriptional data suggests that DNA metabolism, recombination, and overall cellular transcriptional regulation are critical for Lb464 growth in beer, specifically with respect to growth in pressurized/gassed beer. This provides strong support for the theory postulated in Chapter 5 that cellular regulation is critical for avoidance of the VNBC state in the beer environment. Overall, alterations to both the constituents of the cell membrane (fatty acid metabolism) and cell wall are made by Lb464 in response to the beer environment.

RNAseq was also used to interrogate the transcriptional behavior of Lb464 and Pc344 during growth in hops in an attempt to separate of the genetics involved in hop-tolerance relative to those involved in beer-spoilage ability (Chapter 7). This data corroborated previous transcriptional data that hop-tolerance genes from both these isolates are transcribed to different extents (Chapter 2). Further, the RNAseq analysis of the response to hops alone demonstrates that the presence of hops elicits a notably different *total* response and that the strength of the oxidative stress response in Lb464 and Pc344 differs considerably, suggesting that even this basic physiological response can involve redundant genetic mechanisms and thus be variable in different BSR LAB. Overall, the sum total of transcriptional analysis performed in this thesis and a previous study involving Pc344 (**10**), indicates that the response to hops is largely one of mediating oxidative stress and maintaining cellular proton motive force (PMF), with this being but a component of the *total* response elicited in response to the beer environment (Fig. 9.2).

The variability observed in the in BSR LAB transcriptional data presented in Chapters 6, 7, and (**10**) led to a comparative genomics study of available BSR LAB genomes (Chapter 8). This was done with the goal to better understand if it is possible to resolve common “beer-spoilage genes”

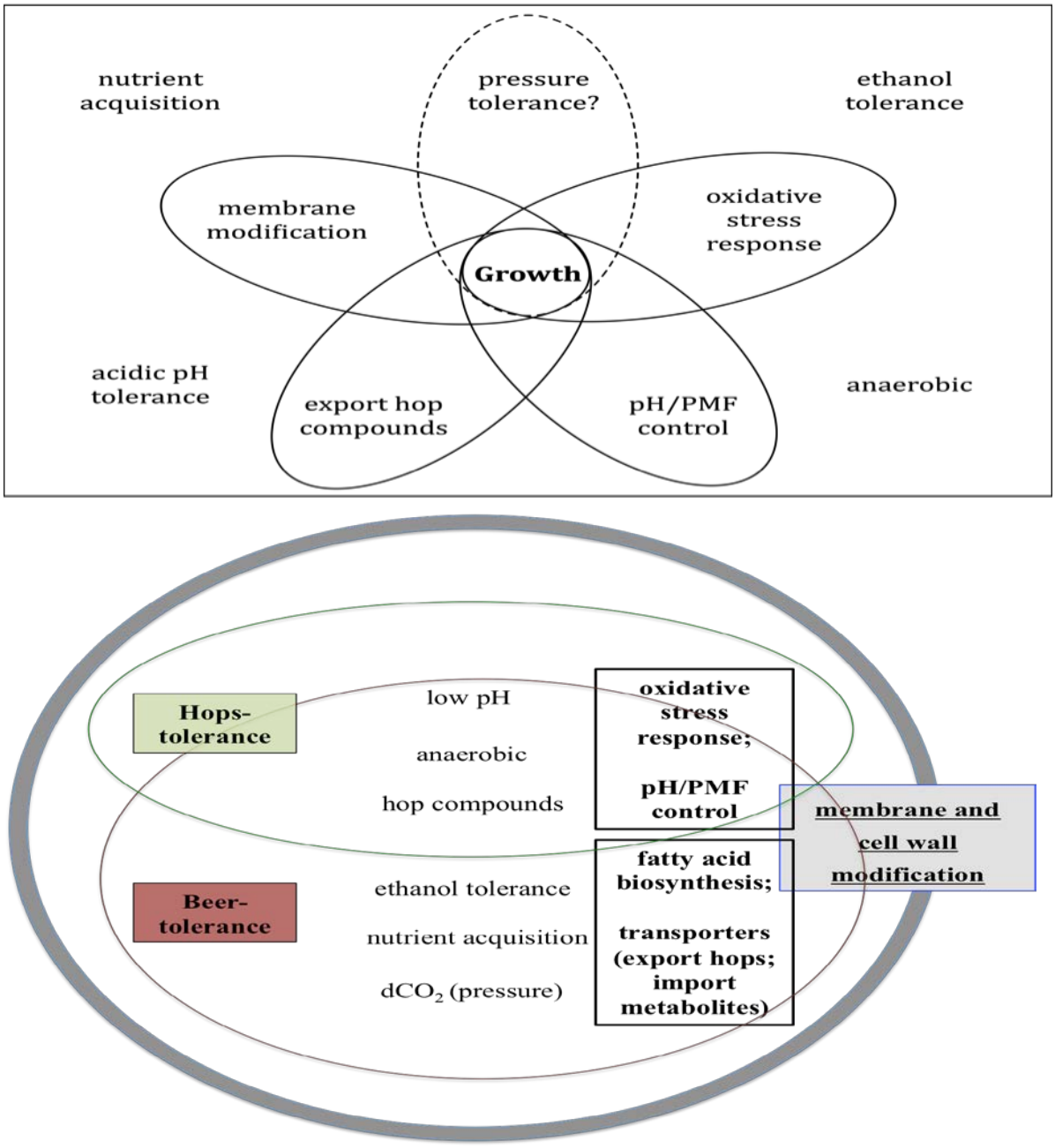

Fig. 9.2. Modified depiction of BSR LAB adaptations to beer environment.

The top panel is Fig. 7.1. in the Pittet, 2012 Doctoral thesis (**9**). The bottom panel is the adapted and modified summary based on data presented in this thesis. Black-outlined boxes describe mechanisms critical for growth in beer and overlap between beer- and hops-tolerance to degrees reflective of their importance for each respective tolerance.

at various levels of relatedness (i.e., similar breweries, species, genus). What was found is that there is considerable difference between BSR LAB isolates of different genera and thus elucidation of beer-spoilage markers is optimally done at the species level. The data also suggest that comparative genomics can be used to elucidate genetic features, both chromosomal and plasmid-localized, specific to a given brewery or isolation source. This analysis determined that genes involved in nutrient scavenging for carbohydrate sources not used by brewery yeast, specifically sorbose and xylose, are advantageous for LAB growth in the beer environment, and a gene for the sorbose-specific phosphotransferase system subunit IIC shows potential for use in screening for beer-spoilage ability in both *Lactobacillus* and *Pediococcus* genera. Other BSR genes of interest include enzymes related to the breakdown of plant materials such as coniferyl aldehyde dehydrogenase and polygalacturonase, which are indicated as useful for discriminating BSR *L. brevis* from non-BSR *L. brevis* isolates.

The finding that plant-specific enzymes are contained in BSR LAB indicates that these isolates are likely once plant-adapted organisms prior to their introduction into the brewery. This may suggest that plant-adapted LAB are predisposed survival in or adaptation to the brewery environment. Once in the brewery, the acquisition of other genetic elements further develops a given isolates beer-spoilage virulence. This scenario outlines the future frontier of BSR LAB research – discovery and tracking of BSR genes into and throughout the brewery, in addition to community interactions between and among the microflora of a brewery. Though the data presented in this thesis culminates to refocus research efforts to areas apart from hop-tolerance through use of –omics approaches, it does so by analyzing BSR LAB in isolation and in focused parameters (time points, beer styles). Thus, to have the most complete understanding of BSR LAB, the investigation horizon must be expanded and "taken to the brewery".

The ultimate conclusions of this thesis are depicted in Figures 9.1 and 9.2. Specifically, both hop-tolerance and beer-spoilage ability/tolerance are variable among BSR LAB and are largely mediated by the acquisition of "brewery/niche"-specific plasmids. Thus, beer-spoilage is not a binary phenotype, but exists on a continuum of ability that is influenced by natural LAB genetic variation, the environment they are harbored in and the stresses they encounter (Fig. 9.1). Beer-spoilage by LAB is thus borne of a "Swiss-army knife" approach to mediating specific nutrient

depletions and/or environmental factors, and the characterization of the elements (genetics) that comprise this knife are most likely to be done at the species and brewery-level. The common adaptations that BSR LAB appears to share is ability scavenge not only for nutrients through membrane transport, but also active membrane and cell wall modification. Further, this data concludes that hop-tolerance is but ne component of the LAB physiological response to beer and that pressure and dCO_2–tolerance is the last and, perhaps from a brewer's perspective, most significant influence on the spoilage-ability of an isolate. Thus, dCO_2–tolerant organisms are the "true" ultimate beer-spoilers and such isolates should be the focus of further characterization.

9.2. THE FUTURE

9.2.1. Fatty acid analysis

The data presented in this thesis indicates that investigations should be done of the LAB cell membrane and cell wall composition and modification in response to growth in beer. Transcriptome data of BSR LAB in beer indicate that genes related to lipid and fatty acid metabolism are important to mediate cellular membrane damage from oxidative stress. Genes related to cell wall metabolism appear to be induced by growth in pressurized/gassed beer, as transcripts for genes related to cell-wall products appear to be enriched in dCO_2–tolerant Lb464 when grown in packaged beer. Thus, the investigation of particular BSR LAB fatty-acid signatures and/or tracking the modification of these products in response to beer may elucidate a "signature" fatty acid profile of BSR LAB that may allow for the development of further screening methods for BSR LAB.

9.2.2. "Omics" as the future and microbial community profiling

The demonstrated variability of LAB as to genetics, niche-adaptations, and stress tolerance, together with the individuality of brewery environments leads to the conclusion that single episodes of BSR LAB contamination cannot be considered exemplary. Unfortunately, historical preoccupation with a select few *exemplary* spoilage isolates, brew styles, and physiological stresses means that the sum total of current BSR LAB research does not adequately describe the dynamic event that is LAB-related beer spoilage. Examples of shortsightedness or incomplete information about BSR LAB include the following. (i) Although the *total* beer environment is

recognized as stressful for microbial growth, the effect of dissolved CO_2 on LAB growth has not been studied to the same extent as for the other described stresses in beer, particularly hops. (ii) There has been virtually no investigation of the multiple beer stresses simultaneously as to their affect on BSR LAB physiology or gene transcription. (iii) The presence and role that pervasive or common bacteriophages might have in establishing BSR LAB communities as well as patterns of bacteriophage resistance remain unknown. (iv) No data are available on the role of redundant genetic mechanisms that may operate in BSR LAB to deal with the individual, let alone the simultaneous growth stresses found in beer. (v) Only minimal efforts have been made to describe in literature *non-traditional* (i.e., unexpected) BSR LAB or to discover the full spectrum of microbial diversity in breweries. (vi) We have limited understanding of how shifts in the composition of beer (nutrient levels, processing conditions, changes in ethanol or hops) affect the survival of BSR LAB or microbial communities. Further, when considering these examples of where research on BSR LAB is needed, it must be emphasized that the brewing industry lags behind other LAB-fermentation industries such as the dairy/cheese and wine industry in understanding of both product-fermenting and product-spoiling LAB isolates, and how they behave within a microbial community, and if these interactions are influenced by raw substrates as well as the brewing process.

To address these issues and enlarge the data available on BSR LAB, the brewing industry must align more closely with advances made in other LAB industries. Concurrently, omics-based technologies must be applied to the study of BSR LAB within both academia and the brewing industry. Not only is the power and versatility of these technologies well demonstrated for interrogating microbial processes, but the simple utilitarian perspective remains that these methods clearly provide *more* information through *fewer* experimental trials than other approaches. For example, as opposed to using classic methods (laborious processes with need for excessive replication) to investigate the presence or absence of a gene and the correlation to a specific physiological trait, the use of metatranscriptomics together with metagenomics can reveal patterns of gene activity in relation to growth environment and concurrently evident phenotype – and do so for multiple isolates with fewer procedural steps.

Support for application of such broad scale omics analyses to brewery LAB contamination has come from this thesis, which strongly supports the call for a shift in research paradigm from targeted-analysis to the perspective of community-analysis. To begin with, and most importantly, genomic and transcriptomic analyses of BSR LAB isolates have firmly called into question the utility of the few known hop-tolerance genes as indicators of LAB beer-spoilage potential, and have begun to indicate other potential genetic markers of beer spoilage that could be potentially exploited for detection and thus monitoring of BSR LAB. Further, microbial community analysis using next-generation PCR applications and sensitive molecular methods has indicated that this type of analysis is critical to perform for breweries producing multiple brew styles, particularly to determine and then modify personnel-driven contamination patterns within the brewery. Additionally, omics analyses have begun to reveal the presence of unique microbes not yet described in brewery settings that must be explored for their potential to contribute via HGT beer-spoilage resistance genes to LAB found in the brewery setting.

Though available omics data on BSR LAB is sparse to date, what is available is sufficiently broad and of impact that reservations as to the efficacy and necessity of omics for the brewing industry are removed. Concerns of accessibility of omics technologies for those that work within brewery settings expose an interesting developing niche within the industry – for commercial or academic laboratories that can provide omics services to breweries and assist with interpretation of omics data. The emergence of such an approach represents an important evolution in the intersection of academia and the brewing industry. Based on the established progression of omics accessibility (i.e., decreasing cost, with increasing ease of use) there is a foreseeable future wherein brewers readily rely on these technologies for investigating incidences of spoilage, as well as for obtaining information needed for product innovation.

9.2.3. “Designing” Beer

It is obvious that beer-spoilage is not a binary phenotype mediated by the presence/absence of one or even a few genes, as antibiotic resistance or other phenotypes like motility most often are (**1**, **12**). Instead, BSR LAB isolates exist on a scale of beer-spoiling virulence, just as LAB isolates in other industries sit on a continuum of capabilities and efficiency in performing a task (**8**). Indeed the extent to which a LAB isolate can grow and metabolize is important for its

classification as either a contaminant or fermentor. Contaminants are considered so because their growth is at once unexpected, and *uncontrollable* by the environmental conditions posed by production of a given beer, however, the damage they impose on the beer product is relative across isolates as a result of their growth ability and metabolic byproducts. On the other hand, ideal LAB participants in sour beer fermentations contribute some specific flavor component(s), however, do not over-produce these compounds through cellular overgrowth, as this leads to flavor imbalance (i.e., spoilage). Thus, to prevent a helpful LAB isolate from being considered a contaminant, either the genetic makeup or surrounding environment of an isolate must limit its own growth and metabolism.

The relative ability for LAB to establish rapid growth in beer or produce flavor compounds in a moderate fashion is genetically based, which strongly points to subtle differences in the genetics and metabolism of BSR LAB and fermenting LAB, however, these changes are not readily interrogated using targeted analytical methods such as multilocus sequence typing. To distinguish contaminant from helpful isolate, the analytical method must be able to take into account the influence of the total beer environment (i.e., available fermentable sugars and other nutrients, ethanol levels, hop levels, pH, dCO_2) when describing the beer-spoilage virulence of a given LAB. Thus it is only through the use of meta-genomics, global transcriptomics and phenotype correlation, that researchers and the brewery industry will be able to effectively profile helpful, fermenting LAB for development of new beer product, as has been done in other non-brewing industries. For example, efforts are underway to perform en masse genome analysis of LAB *Oenococcus oenii* isolates relevant to the wine industry, in order to link genotype with isolate's winemaking properties and wine characteristics (**2 – 5**). Linkage analysis allows for the distinction between content diversity (specifically, gene presence/absence) and genome diversity (organization, regulation, plasmid and phage presence) and their link to overall isolate phenotype such as flavor profile produced. Importantly, this large-scale analysis ameliorates the potential bias that isolate-selection has on between-isolate comparisons (**6**, **11**, **13**).

Within the brewing industry, non-academic institutions are beginning to conduct analyses similar to that of White Labs Ltd. (San Diego, CA) who are analyzing brewing yeast genomes in relation to the flavor profile of beers the yeasts produce (**7**). It is reasonable to expect that comparable

analyses could be performed not only for LAB in relation to the styles and composition of beer they are able to spoil, but also for the characterization of helpful, fermenting LAB for sour beer production and for defining LAB exhibiting beneficial interactions with yeast in specialty brews.

9.3. SUMMARY

The two main issues that remain endemic to brewers and brewery quality control laboratories is the concept that a few select genes are responsible for conferring beer-spoilage ability and that a handful of BSR LAB species can be used as models for investigating all matters of LAB spoilage. Current omics results presented in this thesis point the fallacy of these two dogmas, and future omics research is expected to provide confirmation. In this context, it is important to note that omics approaches can be used going forward to search answers to critical remaining questions. Specifically, are there are other conserved plasmid sequences transferred in brewery HGT events? How do BSR LAB interact with one another within aerobic and anaerobic microbial communities (metabolome, quorum sensing), and with brewing or wild yeasts, and how do these interactions influence the relative proportions of the microbes present? How or what is the connection between genomic content and the good, or the bad or ugly, physiological capacities of BSR LAB? Finally, how can omics technologies be exploited to screen for helpful brewing LAB, and help us understand as well as control their contribution to beer flavor and interaction with brewing yeasts? Applications of omics approaches to BSR LAB truly have the potential to quickly and exponentially expand our understanding of these bacteria.

The reality of the highly adaptable and variable nature of LAB, and the selective and individual environment of a given brewery, guarantee that LAB-contamination will continue to pose a threat to the global beer industry. Undoubtedly, improved brewery hygiene has increased the general stability of beer products, however, the current industry environment fosters competition and innovation, thus necessitating not only the production of new beers, but often of different types of beer simultaneously within a given physical plant. This means there are developing layers of complexity and new challenges to maintaining a known, stable, and controlled microbial brewing environment. Community-scale analysis together with increased application of omics approaches for troubleshooting, general research, and innovation is the only way to keep pace with these demands.

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Appendices

Table S6.1-a, b, c and Table S7.1-a, b,c were originally uploaded as separate .xlsx supplementary files accompanying the main thesis. For brevity, these files have been combined within one master table below – Appendix 1: Table S6.1-7.1 – DESeq 2 analysis of Lb464 gene expression.

Black indicates Log_2 Fold Change (FC) of expression in first listed condition. **BOLD** indicates significant FC (< 0.1 FDR).
Blue (-) indicates Log_2 Fold Change (FC) of expression in second listed condition. **BOLD** indicates significant FC (< 0.1 FDR).

[a] Data originally found in Table S7.1-a (supplementary .xlsx file)
[b] Data originally found in Table S6.1-a (supplementary .xlsx file)
[c] Data originally found in Table S6.1-b (supplementary .xlsx file)
[d] Data originally found in Table S6.1-c (supplementary .xlsx file)

Appendix 1: Table S6.1-7.1 – DESeq 2 analysis of Lb464 gene expression.

Gene	*Product*	*50 BU Hops v. mMRS* [a]	*Deg v. mMRS* [b]	*Gas v. mMRS* [c]	*Gas v. Deg* [d]	*Locus_tag*	*Location*
0	chromosomal replication protein DnaA	-0.05	0.03	**0.86**	**0.87**	L747_01325	xsome
1	hypothetical protein	-0.18	-0.03	**0.78**	**0.84**	L747_01330	xsome
2	DNA polymerase III subunit beta	0.21	**0.51**	**1.13**	**0.65**	L747_01335	xsome
3	S4-like RNA binding protein	**0.81**	**0.63**	**-0.42**	**-0.99**	L747_01340	xsome
4	recombinase RecF	**0.68**	**0.56**	**-0.44**	**-0.95**	L747_01345	xsome
5	DNA gyrase subunit B; gyrB	0.00	**0.60**	0.21	**-0.35**	L747_01350	xsome
6	DNA gyrase subunit A	-0.03	**0.67**	**0.56**	-0.06	L747_01355	xsome
7	30S ribosomal protein S6	**0.27**	**0.61**	**1.08**	**0.51**	L747_01360	xsome
8	single-stranded DNA-binding protein	**0.35**	**0.93**	**1.38**	**0.49**	L747_01365	xsome
9	30S ribosomal protein S18	**0.38**	**0.98**	**1.41**	**0.47**	L747_01370	xsome
10	peptide ABC transporter substrate-binding protein	-0.18	**1.97**	**2.21**	0.27	L747_01375	xsome
11	hypothetical protein	-0.12	**-0.68**	**-3.17**	**-2.39**	L747_01380	xsome
12	imidazolonepropionase	-0.12	**3.41**	**3.21**	-0.17	L747_01385	xsome
13	peptide ABC transporter substrate-binding protein	-0.43	**1.45**	**1.60**	0.18	L747_01390	xsome

14	hypothetical protein	-0.16	-0.67	-0.50	0.20	L747_01395	xsome
15	permease	-0.65	**-1.27**	**-2.12**	-0.64	L747_01400	xsome
16	hypothetical protein	-0.07	0.07	**-0.52**	**-0.55**	L747_01405	xsome
17	50S ribosomal protein L9	**0.45**	**0.31**	0.25	-0.02	L747_01410	xsome
18	hypothetical protein	-0.41	**-1.40**	**-2.12**	-0.54	L747_01415	xsome
19	hypothetical protein	0.07	0.12	-0.54	-0.50	L747_01420	xsome
20	DNA helicase	**0.52**	**1.64**	**1.53**	-0.07	L747_01425	xsome
21	hypothetical protein	0.75	**6.64**	**8.08**	**1.44**	L747_01430	xsome
22	hypothetical protein	-0.57	**1.22**	0.29	**-0.88**	L747_01435	xsome
23	descriptionMFS transporter;distrupted(psuedo)	0.05	0.06	**-0.72**	**-0.74**	L747_01440	xsome
24	hypothetical protein	-0.30	0.05	**-0.89**	**-0.89**	L747_01445	xsome
25	peptide ABC transporter substrate-binding protein	**-1.44**	**-2.00**	**-2.04**	0.01	L747_01450	xsome
26	hypothetical protein	-0.47	-0.14	-0.78	**-0.57**	L747_01455	xsome
27	hypothetical protein	-0.80	**-0.82**	0.25	**1.09**	L747_01460	xsome
29	PhoP family transcriptional regulator	-0.04	**1.97**	**1.86**	-0.08	L747_01470	xsome
30	sensor histidine kinase	0.12	**1.90**	**1.39**	**-0.46**	L747_01475	xsome
31	hypothetical protein	**0.50**	**1.31**	**0.42**	**-0.84**	L747_01480	xsome
32	hypothetical protein	**0.57**	**1.89**	**1.86**	0.00	L747_01485	xsome
33	transposase ISL3	-0.40	-0.58	-0.01	**0.59**	L747_01490	xsome
34	metallo-hydrolase	0.42	**0.57**	**-0.32**	**-0.85**	L747_01495	xsome
35	serine protease	**0.72**	**3.54**	**3.68**	0.18	L747_01500	xsome
36	hypothetical protein	0.31	0.06	-0.94	**-0.88**	L747_01505	xsome
37	50S rRNA methyltransferase	**0.86**	-0.43	**-1.90**	**-1.38**	L747_01510	xsome
38	hypothetical protein	0.13	0.91	-0.28	**-1.01**	L747_01515	xsome
39	integrase	0.33	**1.47**	**0.59**	**-0.83**	L747_01520	xsome
40	adenine methyltransferase	0.42	**-1.10**	**-1.33**	-0.19	L747_01525	xsome
41	hypothetical protein	NA	0.68	1.32	0.28	L747_01530	xsome
42	integrase	0.33	**0.63**	**1.35**	**0.75**	L747_01535	xsome
43	hypothetical protein	-0.38	0.34	**-1.43**	**-1.69**	L747_01540	xsome
44	hypothetical protein	-0.20	-1.69	-1.85	0.02	L747_01545	xsome
45	transposase	-0.45	0.00	0.30	**0.33**	L747_01550	xsome

46	hypothetical protein	-0.44	-1.27	-0.76	0.48	L747_01555	xsome
47	hypothetical protein	-0.38	**-2.20**	**-2.10**	0.14	L747_01560	xsome
48	hypothetical protein	-0.07	**-1.83**	-1.03	0.65	L747_01565	xsome
49	hypothetical protein	0.17	-0.25	0.14	**0.41**	L747_01570	xsome
50	hypothetical protein	-0.15	**-1.54**	**-1.24**	0.33	L747_01575	xsome
51	integrase	0.26	**0.74**	**0.50**	-0.20	L747_01580	xsome
52	membrane protein	**0.59**	-0.27	**-0.87**	**-0.55**	L747_01585	xsome
53	peptidase	**-0.70**	**-2.11**	**-1.67**	**0.47**	L747_01590	xsome
54	pilus biosynthesis protein HicB	-0.66	**-3.35**	**-3.38**	0.01	L747_01595	xsome
55	hypothetical protein	-0.24	-0.82	-0.81	0.06	L747_01600	xsome
56	nucleotidyltransferase	-0.43	-0.57	-0.59	0.03	L747_01605	xsome
57	membrane protein	-0.10	**2.74**	**3.65**	**0.94**	L747_01610	xsome
58	thioesterase	-0.28	0.63	0.54	-0.06	L747_01615	xsome
59	dihydroxynaphthoic acid synthetase	0.10	**1.41**	**1.07**	**-0.30**	L747_01620	xsome
60	O-succinylbenzoic acid--CoA ligase	**0.45**	**1.41**	**1.05**	**-0.32**	L747_01625	xsome
61	hypothetical protein	-0.22	**-0.77**	**-0.42**	**0.38**	L747_01630	xsome
62	hypothetical protein	-0.40	**1.01**	**0.70**	**-0.27**	L747_01635	xsome
63	UDP-N-acetylmuramoylalanyl-D-glutamate-2C6-diaminopimelate ligase	0.22	**0.91**	**1.29**	**0.42**	L747_01640	xsome
64	lysyl-tRNA synthetase;lysS	-0.23	**0.71**	**1.08**	**0.40**	L747_01645	xsome
65	hypothetical protein	-0.16	**0.61**	**0.71**	0.14	L747_01650	xsome
68	hypothetical protein	-0.14	**1.22**	**1.88**	**0.69**	L747_01665	xsome
69	hypothetical protein	-0.17	**0.71**	**1.08**	**0.40**	L747_01670	xsome
70	hypothetical protein	0.00	**-1.84**	**-1.55**	0.32	L747_01675	xsome
71	hypothetical protein	0.39	**-3.08**	**-1.94**	0.87	L747_01680	xsome
72	glutamate:gamma-aminobutyrate antiporter	**2.64**	**-2.59**	**-1.85**	**0.73**	L747_01685	xsome
73	glutamate decarboxylase	**3.03**	**-2.45**	**-1.86**	**0.60**	L747_01690	xsome
74	glutamyl-tRNA synthetase	**2.68**	**-1.62**	**-0.98**	**0.67**	L747_01695	xsome
75	hypothetical protein	0.34	**0.73**	0.22	**-0.47**	L747_01700	xsome
81	transposase IS204	-0.14	-0.43	-0.01	**0.45**	L747_01730	xsome
82	hypothetical protein	-0.30	0.33	**1.44**	**1.08**	L747_01735	xsome

83	peptide ABC transporter permease	-0.03	**2.48**	**3.11**	**0.66**	L747_01740	xsome
84	phosphoesterase	**0.95**	**0.84**	**0.38**	**-0.42**	L747_01745	xsome
87	pyrroline-5-carboxylate reductase	0.04	**-1.21**	**-1.48**	**-0.23**	L747_01760	xsome
88	N-acetylglucosamine-6-phosphate deacetylase	**0.39**	**-0.90**	**-1.00**	-0.06	L747_01765	xsome
89	GntR family transcriptional regulator	0.19	**-1.08**	**-1.05**	0.07	L747_01770	xsome
90	hypothetical protein	0.05	**-0.71**	-0.32	**0.43**	L747_01775	xsome
91	acetylglucosaminyldiphosphoundecaprenol acetyl-beta-D-mannosaminyltransferase	0.04	**-0.46**	0.22	**0.71**	L747_01780	xsome
92	nicotinate phosphoribosyltransferase	0.06	**-0.98**	**-1.14**	-0.12	L747_01785	xsome
93	NAD synthetase;nadE	**0.35**	**-1.22**	**-1.70**	**-0.44**	L747_01790	xsome
94	hypothetical protein	0.42	**0.46**	**0.45**	0.03	L747_01795	xsome
95	S1 RNA-binding protein	0.14	**0.42**	0.15	**-0.23**	L747_01800	xsome
96	hypothetical protein	-0.05	0.10	-0.03	-0.09	L747_01805	xsome
97	homoserine kinase	-0.08	**0.45**	**0.36**	-0.04	L747_01810	xsome
99	amidohydrolase	-0.52	**1.21**	**1.82**	**0.64**	L747_01820	xsome
100	hypothetical protein	-0.30	**-1.72**	**-1.66**	0.09	L747_01825	xsome
101	membrane protein	0.08	0.47	**1.10**	**0.62**	L747_01830	xsome
102	transcriptional regulator	0.12	0.24	0.21	0.00	L747_01835	xsome
103	diguanylate cyclase	-0.15	**1.67**	**2.23**	**0.59**	L747_01840	xsome
104	hypothetical protein	-0.15	-0.17	**-0.60**	**-0.38**	L747_01845	xsome
105	hypothetical protein	**0.64**	**-1.35**	**-1.28**	0.11	L747_01850	xsome
106	hypothetical protein	**0.44**	**-1.20**	**-0.73**	**0.51**	L747_01855	xsome
107	PTS mannose transporter subunit IIC	0.24	**-1.32**	**-1.98**	**-0.59**	L747_01860	xsome
108	PTS mannose transporter subunit IID	0.24	**-0.85**	**-1.66**	**-0.76**	L747_01865	xsome
109	membrane protein	0.23	-0.31	**0.45**	**0.79**	L747_01870	xsome
110	hypothetical protein	-0.02	0.34	**0.95**	**0.64**	L747_01875	xsome
111	transposase ISL3	-0.25	-0.51	0.00	**0.54**	L747_01880	xsome
112	diguanylate cyclase	0.05	**0.98**	**1.45**	**0.50**	L747_01885	xsome
113	TetR family transcriptional regulator	0.08	**1.28**	**1.37**	0.12	L747_01890	xsome
114	hypothetical protein	-0.02	**-0.75**	**-1.47**	**-0.64**	L747_01895	xsome
115	metal ABC transporter substrate-binding protein	-0.72	-0.46	**1.71**	**2.13**	L747_01900	xsome

116	LytR family transcriptional regulator	**0.42**	**1.62**	**2.01**	**0.43**	L747_01905	xsome
117	malate transporter	-0.05	**0.99**	**1.44**	**0.48**	L747_01910	xsome
118	membrane protein	0.08	**0.85**	**1.19**	**0.37**	L747_01915	xsome
119	phosphoglycerate mutase	0.06	**-0.75**	**-2.24**	**-1.41**	L747_01920	xsome
120	hypothetical protein	-0.17	**-0.28**	**-1.33**	**-1.01**	L747_01925	xsome
121	aminopeptidase C	**0.37**	**3.28**	**3.76**	**0.51**	L747_01930	xsome
122	hypothetical protein	0.08	-0.63	-0.64	0.04	L747_01935	xsome
123	ribose 5-phosphate isomerase	**0.42**	**0.73**	**0.99**	**0.30**	L747_01940	xsome
124	hypothetical protein	**0.54**	**0.61**	**0.56**	-0.01	L747_01945	xsome
125	dUTPase	-0.30	**-0.98**	**-1.75**	**-0.72**	L747_01950	xsome
126	DNA repair protein RadA	0.12	**0.26**	**-0.51**	**-0.72**	L747_01955	xsome
127	twitching motility protein PilT	-0.01	0.00	**-0.47**	**-0.43**	L747_01960	xsome
128	glutamyl-tRNA synthase	**0.28**	**0.56**	**1.09**	**0.57**	L747_01965	xsome
129	cysteinyl-tRNA synthetase	-0.25	-0.01	**-0.34**	**-0.29**	L747_01970	xsome
130	Mini-ribonuclease 3	0.17	**-0.51**	**-1.39**	**-0.84**	L747_01975	xsome
131	rRNA methyltransferase	0.21	**0.30**	**-0.30**	**-0.56**	L747_01980	xsome
132	hypothetical protein	-0.02	**0.40**	0.18	-0.18	L747_01985	xsome
133	hypothetical protein	-0.75	-0.39	0.09	**0.49**	L747_01990	xsome
134	50S ribosomal protein L33	0.22	**0.32**	-0.20	**-0.48**	L747_01995	xsome
135	preprotein translocase subunit SecE	**0.55**	**2.00**	**2.14**	0.18	L747_02000	xsome
136	transcription antitermination protein NusG	**-0.62**	**-0.99**	**-0.51**	**0.51**	L747_02005	xsome
137	50S ribosomal protein L11	**-0.78**	**-1.95**	**-1.95**	0.04	L747_02010	xsome
138	50S ribosomal protein L1	**-0.53**	**-0.94**	**-0.72**	0.26	L747_02015	xsome
139	50S ribosomal protein L10	-0.05	**-0.41**	-0.12	**0.32**	L747_02020	xsome
140	50S ribosomal protein L7/L12; rpllL	0.05	**1.00**	**1.96**	**0.99**	L747_02025	xsome
141	PhoB family transcriptional regulator	0.38	**0.76**	**0.68**	-0.05	L747_02030	xsome
142	hypothetical protein	**0.84**	**1.44**	**1.99**	**0.57**	L747_02035	xsome
143	bactoprenol glucosyl transferase	-0.41	**1.13**	**3.35**	**2.18**	L747_02040	xsome
144	hypothetical protein	-0.45	0.52	**2.77**	**2.21**	L747_02045	xsome
145	lysyl-tRNA synthetase	**-0.80**	**-1.61**	**-2.51**	**-0.85**	L747_02050	xsome
146	transposase IS30	-0.08	**0.66**	**0.35**	**-0.27**	L747_02055	xsome

147	decarboxylase	-0.30	**-1.30**	**-0.85**	**0.48**	L747_02060	xsome
148	3-hydroxybutyryl-CoA dehydrogenase	0.21	**0.73**	**0.92**	0.22	L747_02065	xsome
149	ribonucleoside-diphosphate reductase	**0.37**	**1.07**	**1.33**	**0.30**	L747_02070	xsome
150	ribonucleotide-diphosphate reductase subunit alpha	**-0.35**	**0.93**	**1.35**	**0.46**	L747_02075	xsome
151	glutaredoxin	0.13	**1.98**	**2.38**	**0.43**	L747_02080	xsome
152	16S RNA G1207 methylase RsmC	-0.24	-0.30	**-0.99**	**-0.63**	L747_02085	xsome
153	deaminase	-0.16	0.28	0.02	-0.22	L747_02090	xsome
154	DNA polymerase III subunit gamma/tau	0.10	**0.75**	**0.65**	-0.06	L747_02095	xsome
155	hypothetical protein	**0.70**	**0.48**	-0.19	**-0.62**	L747_02100	xsome
156	recombinase RecR	**0.73**	**0.98**	**0.57**	**-0.36**	L747_02105	xsome
157	hypothetical protein	**0.58**	**1.20**	**1.04**	-0.13	L747_02110	xsome
158	thymidylate kinase	0.11	**-0.74**	**-1.16**	**-0.37**	L747_02115	xsome
159	protein from nitrogen regulatory protein P-II (GLNB) family	0.22	**-0.55**	**-1.51**	**-0.90**	L747_02120	xsome
160	hypothetical protein	0.23	**-0.77**	**-1.73**	**-0.90**	L747_02125	xsome
161	Initiation-control protein yabA	0.12	0.02	-0.60	**-0.57**	L747_02130	xsome
162	16S rRNA methyltransferase	0.14	**-0.76**	**-1.63**	**-0.81**	L747_02135	xsome
163	acyl-ACP thioesterase	0.20	**-0.77**	**-1.40**	**-0.59**	L747_02140	xsome
164	endopeptidase	0.15	**1.63**	**1.13**	**-0.46**	L747_02145	xsome
165	alanine acetyltransferase	0.34	**1.06**	**0.41**	**-0.60**	L747_02150	xsome
166	tRNA threonylcarbamoyladenosine biosynthesis protein Gcp	0.12	**0.90**	0.24	**-0.62**	L747_02155	xsome
167	MFS transporter	-0.17	-0.44	-0.04	**0.43**	L747_02160	xsome
168	ArsR family transcriptional regulator	0.80	0.14	**0.97**	**0.85**	L747_02165	xsome
169	multidrug ABC transporter ATP-binding protein	-0.39	**-0.39**	**0.32**	**0.74**	L747_02170	xsome
170	redox-sensing transcriptional repressor Rex	0.30	**-0.91**	**-1.28**	**-0.32**	L747_02175	xsome
171	molecular chaperone GroES	**-1.27**	**-1.34**	**-1.62**	-0.23	L747_02180	xsome
172	molecular chaperone GroEL;groEL	**-0.69**	**-0.36**	**-0.28**	0.12	L747_02185	xsome
173	amino acid permease	**0.33**	**0.85**	**0.73**	-0.09	L747_02190	xsome
174	antiholin	**-0.79**	**-2.43**	**-2.01**	**0.45**	L747_02195	xsome

175	LrgA	**-0.98**	**-2.89**	**-2.35**	**0.57**	L747_02200	xsome
176	integrase	0.06	0.27	**0.29**	0.06	L747_02205	xsome
177	UDP-phosphate N-acetyl-glucosaminyl transferase	0.39	**1.00**	**1.69**	**0.72**	L747_02210	xsome
178	hypothetical protein	0.31	**0.62**	**0.82**	0.23	L747_02215	xsome
179	hypothetical protein	0.18	-0.18	-0.16	0.06	L747_02220	xsome
180	hypothetical protein	-0.24	0.23	0.53	0.31	L747_02225	xsome
181	sigma-54 modulation protein	**0.23**	**-0.21**	-0.17	0.08	L747_02230	xsome
182	preprotein translocase subunit SecA	0.23	**1.21**	**1.19**	0.03	L747_02235	xsome
183	peptide chain release factor 2	0.13	**0.29**	**0.67**	**0.41**	L747_02240	xsome
184	hypothetical protein	-0.34	**-1.02**	**-1.18**	-0.12	L747_02245	xsome
185	PhoP family transcriptional regulator	-0.13	0.08	-0.05	-0.09	L747_02250	xsome
186	hypothetical protein	0.10	0.20	0.13	-0.03	L747_02255	xsome
187	phosphate-binding protein	-0.25	**-0.77**	**-1.56**	**-0.71**	L747_02260	xsome
188	phosphate ABC transporter permease	-0.49	0.06	-0.23	-0.24	L747_02265	xsome
189	phosphate ABC transporter permease	-0.38	-0.08	-0.44	-0.32	L747_02270	xsome
190	phosphate ABC transporter ATP-binding protein;pstB	-0.26	**-0.82**	**-1.88**	**-0.98**	L747_02275	xsome
191	phosphate ABC transporter ATP-binding protein	0.14	-0.34	**-2.12**	**-1.70**	L747_02280	xsome
192	PhoU family transcriptional regulator	**0.55**	**0.84**	**-0.37**	**-1.16**	L747_02285	xsome
193	stress-responsive transcription regulator	0.33	**0.36**	-0.33	**-0.64**	L747_02290	xsome
194	membrane protein	0.09	**1.19**	**1.14**	-0.01	L747_02295	xsome
195	HPr kinase/phosphorylase	0.06	0.22	-0.22	**-0.39**	L747_02300	xsome
196	prolipoprotein diacylglyceryl transferase	0.15	0.09	**-0.29**	**-0.34**	L747_02305	xsome
197	glycerol-3-phosphate dehydrogenase	**0.38**	**0.32**	-0.18	**-0.45**	L747_02310	xsome
198	UTP--glucose-1-phosphate uridylyltransferase	**0.31**	**-0.27**	**-0.95**	**-0.64**	L747_02315	xsome
199	thioredoxin reductase	**0.41**	**1.23**	**0.41**	**-0.78**	L747_02320	xsome
200	hydrolase	0.06	**-0.74**	**-1.68**	**-0.87**	L747_02325	xsome
201	hypothetical protein	0.19	-0.19	**-0.90**	**-0.65**	L747_02330	xsome
202	excinuclease ABC subunit B	0.04	0.17	-0.19	**-0.32**	L747_02335	xsome
203	excinuclease ABC subunit A;uvrA	0.13	-0.12	**-0.32**	-0.15	L747_02340	xsome

204	membrane protein	0.19	**-0.37**	**0.45**	**0.85**	L747_02345	xsome
205	ATPase P	-0.30	**0.68**	**0.88**	**0.24**	L747_02350	xsome
206	CofD protein	0.26	0.05	**-0.24**	**-0.25**	L747_02355	xsome
207	sporulation regulator WhiA	**0.55**	**0.45**	0.20	**-0.21**	L747_02360	xsome
208	ATP-dependent Clp protease proteolytic subunit	**0.81**	**0.87**	**1.04**	**0.21**	L747_02365	xsome
209	hypothetical protein	-0.40	**-1.48**	**-1.76**	-0.24	L747_02370	xsome
210	hypothetical protein	-0.36	**-1.02**	**-1.12**	-0.06	L747_02375	xsome
212	membrane protein	0.49	**1.54**	**2.06**	**0.55**	L747_02385	xsome
214	SorC family transcriptional regulator	**0.74**	**1.86**	**2.14**	**0.31**	L747_02395	xsome
215	glyceraldehyde-3-phosphate dehydrogenase	0.19	**0.30**	**0.91**	**0.65**	L747_02400	xsome
216	phosphoglycerate kinase	**0.64**	**0.70**	**1.12**	**0.46**	L747_02405	xsome
217	triosephosphate isomerase	**1.16**	**1.37**	**0.69**	**-0.63**	L747_02410	xsome
218	enolase;eno	**0.63**	**0.61**	**0.89**	**0.32**	L747_02415	xsome
219	hypothetical protein	0.18	-0.18	0.37	**0.57**	L747_02420	xsome
220	ATP synthase F0 subunit A	**-0.77**	**-1.11**	**-0.76**	**0.38**	L747_02425	xsome
221	preprotein translocase subunit SecG	0.34	**0.71**	**1.25**	**0.57**	L747_02430	xsome
222	carboxylesterase	0.27	**0.34**	-0.14	**-0.44**	L747_02435	xsome
223	exoribonuclease R	0.12	**0.24**	**0.31**	0.11	L747_02440	xsome
224	single-stranded DNA-binding protein	0.23	**-0.37**	-0.05	**0.36**	L747_02445	xsome
225	transposase ISL3	-0.44	-0.34	0.04	**0.42**	L747_02450	xsome
226	arginine ABC transporter ATP-binding protein;artP	0.24	0.19	**1.81**	**1.52**	L747_02455	xsome
227	hypothetical protein	**1.02**	**1.51**	**2.03**	**0.56**	L747_02460	xsome
228	uracil-DNA glycosylase	**0.44**	0.10	**-0.46**	**-0.51**	L747_02465	xsome
229	phosphotransacetylase;eutD	0.18	0.01	**-0.35**	**-0.32**	L747_02470	xsome
230	ATP/GTP hydrolase	0.12	**-1.11**	**-2.38**	**-1.21**	L747_02475	xsome
231	hypothetical protein	0.01	-0.37	**-1.04**	**-0.61**	L747_02480	xsome
232	L-glyceraldehyde 3-phosphate reductase	0.06	-0.07	**-0.35**	**-0.24**	L747_02485	xsome
233	DNA polymerase III subunit alpha	-0.31	**0.93**	**1.08**	0.19	L747_02490	xsome
234	exodeoxyribonuclease III	0.08	0.33	0.27	-0.02	L747_02495	xsome
235	hypothetical protein	0.36	**-2.03**	**-3.96**	**-0.99**	L747_02500	xsome

236	UDP-N-acetylenolpyruvoylglucosamine reductase; murB	**0.36**	-0.07	**-0.45**	**-0.34**	L747_02505	xsome
237	sodium:proton antiporter	0.32	**0.49**	**0.54**	0.09	L747_02510	xsome
238	membrane protein	**0.56**	**1.53**	**2.89**	**1.38**	L747_02515	xsome
239	membrane protein	-0.10	**-0.41**	**-0.94**	**-0.48**	L747_02520	xsome
240	cell surface protein	-0.41	0.10	-0.07	-0.13	L747_02525	xsome
241	phosphoglucosamine mutase; glmM	0.11	**0.61**	**0.90**	**0.33**	L747_02530	xsome
242	glucosamine--fructose-6-phosphate aminotransferase	**-0.43**	**0.38**	0.06	**-0.28**	L747_02535	xsome
243	hypothetical protein	0.47	0.23	0.37	0.18	L747_02540	xsome
244	glucosamine-6-phosphate deaminase	**1.38**	**-0.74**	-0.09	**0.69**	L747_02545	xsome
245	transposase IS4	**0.89**	-0.01	**0.79**	**0.83**	L747_02550	xsome
246	transposase	0.19	**-0.60**	0.15	**0.77**	L747_02555	xsome
247	muramidase	0.23	**-1.95**	**-1.22**	**0.77**	L747_02560	xsome
248	HAD family hydrolase	-0.33	-0.17	0.00	0.20	L747_02565	xsome
249	DNA-binding protein	0.67	**1.24**	**1.08**	-0.13	L747_02570	xsome
250	copper chaperone	0.27	0.15	-0.29	-0.39	L747_02575	xsome
251	teichoic acid ABC transporter ATP-binding protein	-0.04	**0.81**	**0.46**	**-0.30**	L747_02580	xsome
252	hypothetical protein	-0.14	0.25	**-2.22**	**-2.36**	L747_02585	xsome
253	hypothetical protein	**-0.63**	**-1.05**	**-2.39**	**-1.29**	L747_02590	xsome
254	hypothetical protein	**-0.68**	**-2.32**	**-3.41**	**-1.03**	L747_02595	xsome
255	hypothetical protein	**-0.60**	**-1.48**	**-2.43**	**-0.88**	L747_02600	xsome
256	iron ABC transporter substrate-binding protein	-0.04	**-1.28**	**-2.13**	**-0.79**	L747_02605	xsome
257	iron ABC transporter permease	0.40	**-1.09**	**-1.69**	**-0.55**	L747_02610	xsome
258	cobalamin ABC transporter ATPase	0.47	**-0.66**	**-1.06**	**-0.36**	L747_02615	xsome
259	exopolyphosphatase	0.00	**0.66**	**0.60**	-0.02	L747_02620	xsome
260	polyphosphate kinase	-0.43	0.02	0.31	**0.32**	L747_02625	xsome
261	exopolyphosphatase; distrupted(psuedo)	**-0.65**	**-0.62**	**-0.51**	0.15	L747_02630	xsome
262	lipopolysaccharide cholinephosphotransferase	**-0.92**	**-0.70**	**-0.55**	0.18	L747_02635	xsome
263	hypothetical protein	-0.65	-0.47	-0.19	0.31	L747_02640	xsome

264	MarR family transcriptional regulator	**0.46**	**0.96**	0.10	**-0.80**	L747_02645	xsome
265	hypothetical protein	0.41	**1.57**	**1.16**	**-0.36**	L747_02650	xsome
266	dihydroneopterin aldolase	0.10	**1.41**	**1.33**	-0.04	L747_02655	xsome
267	hypothetical protein	0.17	**-0.97**	**-1.69**	**-0.67**	L747_02660	xsome
268	hypothetical protein	0.02	**-1.25**	**-1.17**	0.12	L747_02665	xsome
269	membrane protein	-0.31	-0.46	0.02	**0.50**	L747_02670	xsome
270	hypothetical protein	-0.42	**1.85**	**1.46**	**-0.35**	L747_02675	xsome
271	fumarate hydratase	0.05	**1.54**	**1.09**	**-0.41**	L747_02680	xsome
272	hypothetical protein	**-0.96**	0.13	**-3.11**	**-3.13**	L747_02685	xsome
273	hypothetical protein	**-1.32**	0.27	**-1.30**	**-1.51**	L747_02690	xsome
274	acetylornithine deacetylase	**-0.91**	**2.43**	**1.18**	**-1.21**	L747_02695	xsome
275	hypothetical protein	**-0.87**	**-0.92**	**-1.68**	**-0.70**	L747_02700	xsome
276	hypothetical protein	0.13	-0.66	-0.89	-0.16	L747_02705	xsome
277	membrane protein	-0.02	**-1.31**	**-2.26**	**-0.83**	L747_02710	xsome
278	hypothetical protein	0.09	**-1.34**	**-1.89**	**-0.50**	L747_02715	xsome
279	arginine deiminase	**0.49**	**-1.49**	**-1.71**	-0.18	L747_02720	xsome
280	hypothetical protein	-0.09	-0.81	0.33	**1.11**	L747_02725	xsome
281	integrase	0.47	**0.53**	**1.30**	**0.80**	L747_02730	xsome
282	hypothetical protein	0.66	0.73	0.55	-0.14	L747_02735	xsome
283	hypothetical protein	**-0.97**	**-1.63**	**-2.12**	-0.36	L747_02740	xsome
284	alcohol dehydrogenase	-0.17	**-0.65**	**-0.95**	-0.25	L747_02745	xsome
285	hypothetical protein	-0.39	**-2.44**	**-2.06**	0.36	L747_02750	xsome
286	hypothetical protein	-0.21	**-1.99**	-0.69	0.90	L747_02755	xsome
287	hypothetical protein	-0.06	-0.05	0.20	**0.29**	L747_02760	xsome
288	PhoP family transcriptional regulator	-0.09	**1.22**	**0.78**	**-0.40**	L747_02765	xsome
289	signal transduction histidine kinase	0.14	**1.07**	**0.49**	**-0.54**	L747_02770	xsome
290	hypothetical protein	**0.58**	**0.98**	**0.80**	-0.14	L747_02775	xsome
291	glucose transporter GlcU	-0.13	**-1.06**	**-1.10**	0.00	L747_02780	xsome
292	glucose-6-phosphate isomerase; pgi	-0.06	**-0.35**	0.05	**0.44**	L747_02785	xsome
293	hypothetical protein	-0.21	0.03	**-0.39**	**-0.37**	L747_02790	xsome
294	hypothetical protein	**-0.35**	**-0.40**	**-0.60**	-0.16	L747_02795	xsome

295	carotenoid biosynthetic protein CrtK	-0.20	**1.58**	**1.27**	**-0.27**	L747_02800	xsome
296	membrane protein	-0.56	**-1.04**	-0.42	0.61	L747_02805	xsome
299	hypothetical protein	NA	NA	NA	NA	L747_02820	xsome
300	DeoR family transcriptional regulator	-0.86	-0.38	-0.21	0.20	L747_02825	xsome
301	diguanylate cyclase	**-0.92**	**-1.46**	-0.33	**1.15**	L747_02830	xsome
302	transposase	-0.05	-0.10	0.27	**0.40**	L747_02835	xsome
303	hypothetical protein	-0.19	-0.50	-0.20	0.33	L747_02840	xsome
304	hypothetical protein	-0.04	0.31	**1.25**	**0.91**	L747_02845	xsome
305	hypothetical protein	0.24	**1.48**	**1.36**	-0.09	L747_02850	xsome
306	multidrug ABC transporter ATP-binding protein	0.00	0.20	**0.26**	0.10	L747_02855	xsome
307	multidrug ABC transporter ATP-binding protein	0.24	0.05	**0.29**	**0.28**	L747_02860	xsome
308	beta-lactamase	0.02	**0.59**	**0.68**	0.12	L747_02865	xsome
309	glutamine amidotransferase	0.30	**1.26**	**1.15**	-0.07	L747_02870	xsome
310	UDP-N-acetylmuramyl peptide synthase	-0.22	**1.27**	**1.31**	0.08	L747_02875	xsome
311	thymidine kinase	**-0.56**	**-0.36**	**-0.63**	-0.22	L747_02880	xsome
312	peptide chain release factor 1	-0.30	**-0.39**	**-0.77**	**-0.34**	L747_02885	xsome
313	hypothetical protein	0.27	-0.21	**-0.52**	**-0.27**	L747_02890	xsome
314	translation factor Sua5	0.16	0.18	0.21	0.07	L747_02895	xsome
315	hypothetical protein	-0.21	-0.58	-0.28	0.33	L747_02900	xsome
316	hypothetical protein	-0.31	-0.53	0.46	**0.90**	L747_02905	xsome
317	uracil phosphoribosyltransferase	-0.06	**0.66**	**1.19**	**0.56**	L747_02910	xsome
318	F0F1 ATP synthase subunit A	**0.38**	**0.38**	**0.25**	-0.09	L747_02915	xsome
319	F0F1 ATP synthase subunit C	**0.41**	**0.64**	0.06	**-0.54**	L747_02920	xsome
320	F0F1 ATP synthase subunit B	**0.54**	**1.24**	**1.27**	0.07	L747_02925	xsome
321	F0F1 ATP synthase subunit delta	**0.37**	**0.50**	**0.40**	-0.06	L747_02930	xsome
322	F0F1 ATP synthase subunit alpha	**0.40**	**0.59**	**0.47**	-0.08	L747_02935	xsome
323	F0F1 ATP synthase subunit gamma	**0.52**	-0.11	**-0.39**	**-0.23**	L747_02940	xsome
324	F0F1 ATP synthase subunit beta	**0.50**	**0.46**	**0.52**	0.09	L747_02945	xsome
325	F0F1 ATP synthase subunit epsilon	**0.52**	**1.13**	**1.14**	0.05	L747_02950	xsome
326	membrane protein	0.47	**2.11**	**1.57**	**-0.50**	L747_02955	xsome
327	UDP-N-acetylglucosamine 1-	0.17	**1.52**	**0.31**	**-1.16**	L747_02960	xsome

	carboxyvinyltransferase						
328	rod shape-determining protein MreB	**-0.52**	-0.03	**-0.93**	**-0.85**	L747_02965	xsome
329	membrane protein insertion efficiency factor	-0.10	-0.02	**-0.87**	**-0.80**	L747_02970	xsome
330	hypothetical protein	-0.22	0.21	**-0.51**	**-0.67**	L747_02975	xsome
331	cell division protein FtsW	**0.41**	**1.43**	**1.26**	-0.13	L747_02980	xsome
332	glycine cleavage system protein H	0.47	**1.87**	**2.19**	**0.35**	L747_02985	xsome
333	membrane protein	0.19	**0.68**	-0.22	**-0.85**	L747_02990	xsome
334	hypothetical protein	0.13	**1.01**	0.24	**-0.72**	L747_02995	xsome
335	D-alanine--D-alanine ligase	**0.55**	**1.03**	**0.93**	-0.06	L747_03000	xsome
336	hypothetical protein	-0.10	**-0.47**	**-1.38**	**-0.84**	L747_03005	xsome
337	hypothetical protein	0.04	**-1.48**	**-1.80**	-0.24	L747_03010	xsome
338	universal stress protein UspA	0.12	-0.06	-0.05	0.05	L747_03015	xsome
339	hypothetical protein	-0.51	0.00	0.29	**0.32**	L747_03020	xsome
340	recombinase RarA	-0.05	**2.16**	**1.95**	-0.17	L747_03025	xsome
341	hypothetical protein	-0.37	**1.43**	**1.72**	**0.33**	L747_03030	xsome
342	hypothetical protein	0.16	**-0.87**	**-1.07**	-0.14	L747_03035	xsome
343	ribonuclease G or E	**-0.83**	**-5.21**	**-4.88**	0.35	L747_03040	xsome
344	30S ribosomal protein S4	0.05	**0.73**	**1.64**	**0.94**	L747_03045	xsome
345	sensory histidine kinase	**-0.65**	**0.40**	**1.16**	**0.80**	L747_03050	xsome
346	selenide-water dikinase	-0.07	0.21	-0.16	**-0.33**	L747_03055	xsome
347	aminotransferase V	-0.26	**0.68**	**0.72**	0.08	L747_03060	xsome
348	thiamine biosynthesis protein ThiI	-0.18	**0.43**	**0.58**	**0.19**	L747_03065	xsome
349	thiol peroxidase	-0.13	**0.39**	0.03	**-0.32**	L747_03070	xsome
350	valyl-tRNA synthase; valS	0.03	**-0.90**	**-0.88**	0.06	L747_03075	xsome
351	folylpolyglutamate synthase	-0.14	**-0.78**	**-0.91**	-0.09	L747_03080	xsome
352	hypothetical protein	0.08	**-1.06**	**-1.40**	-0.28	L747_03085	xsome
353	rod shape-determining protein Mbl	**0.58**	**1.10**	**1.31**	**0.25**	L747_03090	xsome
354	rod shape-determining protein MreC	0.20	**0.24**	**-0.29**	**-0.49**	L747_03095	xsome
355	rod shape-determining protein MreD	**0.44**	**-0.32**	**-1.64**	**-1.27**	L747_03100	xsome
356	selenocysteine lyase	**0.42**	**0.32**	**-0.55**	**-0.82**	L747_03105	xsome
357	septum site-determining protein MinD	**0.47**	**0.41**	0.01	**-0.35**	L747_03110	xsome

358	amino acid ABC transporter permease	-0.58	**-2.31**	**-1.92**	0.41	L747_03115	xsome
359	peptide ABC transporter ATP-binding protein	-0.44	**-2.08**	**-1.75**	0.37	L747_03120	xsome
360	amino acid ABC transporter substrate-binding protein	-0.10	**-2.18**	**-1.68**	**0.52**	L747_03125	xsome
361	hypothetical protein	-0.11	**0.32**	**0.51**	**0.23**	L747_03130	xsome
362	zinc protease	0.24	0.18	0.09	-0.04	L747_03135	xsome
363	XRE family transcriptional regulator	**0.45**	**0.75**	**0.98**	**0.27**	L747_03140	xsome
364	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	0.14	-0.15	**-0.34**	-0.15	L747_03145	xsome
365	damage-inducible protein A	0.21	0.00	-0.02	0.01	L747_03150	xsome
366	protein RecA	0.21	-0.10	0.07	**0.20**	L747_03155	xsome
367	hypothetical protein	-0.07	-1.05	-0.60	0.43	L747_03160	xsome
368	ribonuclease	**0.49**	0.15	0.08	-0.03	L747_03165	xsome
369	hypothetical protein	-0.25	-0.06	**-0.37**	**-0.26**	L747_03170	xsome
370	DNA mismatch repair protein MutS	0.01	0.24	0.03	-0.17	L747_03175	xsome
371	DNA mismatch repair protein MutL	0.02	**0.34**	0.18	-0.13	L747_03180	xsome
372	Holliday junction DNA helicase RuvA	0.02	**-0.68**	**-1.06**	**-0.34**	L747_03185	xsome
373	Holliday junction DNA helicase RuvB	0.04	0.17	-0.05	-0.18	L747_03190	xsome
374	S-adenosylmethionine tRNA ribosyltransferase	0.01	**0.26**	0.11	-0.11	L747_03195	xsome
375	queuine tRNA-ribosyltransferase; tgt	0.21	0.05	-0.10	-0.11	L747_03200	xsome
376	preprotein translocase subunit YajC	**1.08**	**0.74**	**1.03**	**0.32**	L747_03205	xsome
377	geneDNA polymerase IV; distrupted(psuedo)	0.19	-0.04	-0.06	0.02	L747_03210	xsome
378	phosphoesterase	-0.22	-0.16	**-0.36**	-0.16	L747_03215	xsome
379	DEAD/DEAH box helicase	0.12	**0.49**	0.20	**-0.26**	L747_03220	xsome
380	alanyl-tRNA synthase; alaS	-0.03	**-0.82**	**-0.81**	0.06	L747_03225	xsome
381	hypothetical protein	-0.05	**1.05**	**1.29**	**0.27**	L747_03230	xsome
382	Holliday junction resolvase	0.30	**0.26**	-0.18	**-0.40**	L747_03235	xsome
383	hypothetical protein	**0.74**	**0.93**	**1.13**	0.23	L747_03240	xsome
384	cell division protein FtsZ	-0.12	-0.06	**-0.37**	**-0.27**	L747_03245	xsome
385	hypothetical protein	-0.29	**0.64**	**1.21**	**0.59**	L747_03250	xsome
386	DNA mismatch repair protein MutS	-0.21	0.11	0.11	0.04	L747_03255	xsome

387	thioredoxin	0.22	**1.35**	**0.69**	**-0.61**	L747_03260	xsome
388	hypothetical protein	-0.03	**1.13**	0.25	**-0.84**	L747_03265	xsome
389	glutamate racemase	0.23	**0.71**	-0.25	**-0.91**	L747_03270	xsome
390	nucleoside-triphosphate diphosphatase	**0.37**	-0.14	**-1.72**	**-1.52**	L747_03275	xsome
391	hypothetical protein	0.29	0.30	**-0.73**	**-0.98**	L747_03280	xsome
392	hypothetical protein	**0.54**	**0.45**	0.06	**-0.34**	L747_03285	xsome
393	hypothetical protein	0.05	**-1.11**	0.19	**1.27**	L747_03290	xsome
394	hypothetical protein	-0.39	**-1.47**	**-1.75**	-0.20	L747_03295	xsome
395	transposase	-0.06	**0.61**	**0.42**	-0.16	L747_03300	xsome
396	hypothetical protein	-0.30	**-0.96**	**-2.00**	**-0.88**	L747_03305	xsome
397	hypothetical protein	-0.20	**-1.84**	**-3.07**	**-1.08**	L747_03310	xsome
398	hypothetical protein	-0.15	**-0.99**	**-2.15**	**-1.05**	L747_03315	xsome
399	hypothetical protein	0.51	0.19	0.50	0.31	L747_03320	xsome
400	transcriptional regulator	-0.04	**-0.73**	-0.09	**0.65**	L747_03325	xsome
401	major facilitator transporter	-0.52	**-0.80**	**-1.96**	**-1.01**	L747_03330	xsome
402	hypothetical protein	0.38	0.76	**1.09**	0.33	L747_03335	xsome
403	small-conductance mechanosensitive channel protein MscS	0.16	-0.46	-0.45	0.06	L747_03340	xsome
404	chemotaxis protein	**0.39**	-0.27	**-1.25**	**-0.93**	L747_03345	xsome
405	hypothetical protein	**0.62**	**0.84**	**0.73**	-0.07	L747_03350	xsome
406	dipeptidase	-0.07	**0.68**	**1.37**	**0.73**	L747_03355	xsome
407	catabolite control protein A	**-0.66**	0.03	**0.34**	**0.34**	L747_03360	xsome
408	hypothetical protein	0.36	**2.52**	**2.00**	**-0.47**	L747_03365	xsome
409	ABC transporter ATP-binding protein	**-1.79**	**-1.83**	-0.18	**1.67**	L747_03370	xsome
410	ABC transporter permease	**-2.30**	**-1.93**	**-0.51**	**1.44**	L747_03375	xsome
411	transcriptional regulator	0.16	**-0.42**	-0.15	**0.31**	L747_03380	xsome
412	hypothetical protein	0.15	-0.45	-0.77	-0.23	L747_03385	xsome
413	hypothetical protein	0.14	-0.23	-0.49	-0.20	L747_03390	xsome
414	competence protein ComGC	0.13	-0.23	-1.06	-0.52	L747_03395	xsome
415	hypothetical protein	-0.06	-0.95	-1.28	-0.16	L747_03400	xsome
416	hypothetical protein	NA	0.24	1.41	0.57	L747_03405	xsome

417	hypothetical protein	-0.28	-0.79	**-1.63**	-0.68	L747_03410	xsome
418	DNA methyltransferase	**-0.62**	**-0.92**	**-1.12**	-0.15	L747_03415	xsome
419	acetate kinase	**-0.37**	**-0.28**	-0.16	0.16	L747_03420	xsome
420	alanine glycine permease	**0.76**	-0.32	**1.79**	**2.13**	L747_03425	xsome
421	aldo/keto reductase	0.51	0.41	-0.32	**-0.68**	L747_03430	xsome
422	metallophosphatase	0.31	0.45	**-0.58**	**-0.96**	L747_03435	xsome
423	5'-nucleotidase	0.39	**0.65**	-0.13	**-0.73**	L747_03440	xsome
424	hypothetical protein	0.39	0.27	**-1.29**	**-1.50**	L747_03445	xsome
425	haloacid dehalogenase	0.17	0.26	**-1.27**	**-1.47**	L747_03450	xsome
426	hypothetical protein	0.19	-0.11	**-1.01**	**-0.85**	L747_03455	xsome
427	hypothetical protein	-0.68	**-1.25**	**-2.29**	**-0.81**	L747_03460	xsome
428	hypothetical protein	-0.82	**-1.03**	**-2.20**	**-1.03**	L747_03465	xsome
429	hypothetical protein	0.10	**0.99**	-0.20	**-1.12**	L747_03470	xsome
430	amino acid permease	**-0.53**	0.11	**-0.64**	**-0.70**	L747_03475	xsome
431	oxidoreductase	0.42	**1.98**	**2.07**	0.12	L747_03480	xsome
432	glyoxal reductase	0.38	**1.30**	**1.55**	0.28	L747_03485	xsome
433	hypothetical protein	-0.24	-0.32	**0.73**	**1.06**	L747_03490	xsome
434	hypothetical protein	-0.05	-0.63	-0.64	0.03	L747_03495	xsome
435	membrane protein	**0.68**	**2.84**	**3.42**	**0.61**	L747_03500	xsome
436	hypothetical protein	0.24	**0.87**	**1.29**	**0.45**	L747_03505	xsome
437	peptidyl-prolyl cis-trans isomerase	**0.48**	**0.30**	**0.27**	0.01	L747_03510	xsome
438	general stress protein	**0.54**	**0.95**	**1.52**	**0.60**	L747_03515	xsome
463	hypothetical protein	-0.28	0.10	**0.59**	**0.52**	L747_03640	xsome
464	hypothetical protein	-0.43	**0.84**	**0.69**	-0.11	L747_03645	xsome
465	hypothetical protein	0.15	0.44	**0.75**	**0.34**	L747_03650	xsome
466	ArsR family transcriptional regulator	**0.54**	0.04	**0.95**	**0.94**	L747_03655	xsome
467	competence negative regulator MecA	**0.34**	**0.28**	**0.51**	**0.27**	L747_03660	xsome
468	hypothetical protein	-0.11	-0.22	-0.33	-0.07	L747_03665	xsome
469	hypothetical protein	-0.20	-0.54	0.23	0.71	L747_03670	xsome
470	hypothetical protein	-0.44	-0.93	-0.28	0.63	L747_03675	xsome
471	hypothetical protein	-0.05	0.80	0.87	-0.02	L747_03680	xsome

472	transposase IS30	-0.45	**0.50**	0.27	-0.19	L747_03685	xsome
473	hypothetical protein	-0.05	**-0.67**	**-0.63**	0.08	L747_03690	xsome
474	XRE family transcriptional regulator	-0.04	**-1.36**	**-1.63**	-0.23	L747_03695	xsome
475	hypothetical protein	0.23	0.28	1.11	**0.79**	L747_03700	xsome
476	hypothetical protein	-0.39	-0.14	-0.19	0.00	L747_03715	xsome
477	integrase	0.21	0.36	**1.16**	**0.82**	L747_03720	xsome
478	hypothetical protein	-0.23	-1.48	0.02	**1.24**	L747_03725	xsome
479	hypothetical protein	NA	0.53	1.04	0.13	L747_03730	xsome
480	hypothetical protein	-0.10	-2.04	-0.81	0.52	L747_03735	xsome
481	hypothetical protein	0.04	-0.46	0.67	0.74	L747_03740	xsome
482	hypothetical protein	-0.06	-1.25	**-3.15**	-0.44	L747_03745	xsome
483	hypothetical protein	-0.63	**-1.52**	**-1.49**	0.08	L747_03750	xsome
484	hypothetical protein	**-1.02**	-0.05	0.24	0.33	L747_03755	xsome
485	hypothetical protein	**-1.07**	**-1.07**	**-1.02**	0.09	L747_03760	xsome
486	transposase	0.35	**0.84**	**0.63**	-0.18	L747_03765	xsome
487	hypothetical protein	-0.33	-0.13	0.42	0.55	L747_03770	xsome
488	hypothetical protein	-0.09	0.12	0.45	0.27	L747_03775	xsome
489	membrane protein	-0.18	-1.01	-1.06	0.03	L747_03780	xsome
490	glycosyl transferase; distrupted(psuedo)	-0.11	-0.01	-0.63	**-0.55**	L747_03785	xsome
491	hypothetical protein	-0.14	**0.59**	0.26	-0.29	L747_03790	xsome
492	rRNA methyltransferase	-0.46	**0.75**	**0.81**	0.09	L747_03795	xsome
493	hypothetical protein	0.01	**0.65**	**0.88**	0.27	L747_03800	xsome
494	leucyl-tRNA synthetase	-0.13	**-1.17**	**-0.97**	**0.24**	L747_03805	xsome
495	hypothetical protein	0.19	**3.64**	0.25	**-3.26**	L747_03810	xsome
496	hypothetical protein	-0.37	**2.53**	**-1.48**	**-3.82**	L747_03815	xsome
497	hypothetical protein	0.36	**4.30**	**1.69**	**-2.57**	L747_03820	xsome
498	NADPH-dependent FMN reductase	-0.23	**0.58**	0.34	-0.21	L747_03825	xsome
499	hypothetical protein	0.01	**0.51**	**0.88**	**0.40**	L747_03830	xsome
500	transporter	**0.46**	**0.92**	**1.03**	0.15	L747_03835	xsome
501	hypothetical protein	**0.94**	**1.64**	**1.99**	**0.38**	L747_03840	xsome
502	hypothetical protein	0.12	-0.01	0.47	**0.51**	L747_03845	xsome

503	hypothetical protein	-0.09	0.13	1.13	**0.88**	L747_03850	xsome
504	carbohydrate kinase	-0.20	-0.29	**-0.56**	-0.23	L747_03855	xsome
505	dipeptidase PepV	0.16	-0.10	**-1.10**	**-0.96**	L747_03860	xsome
506	universal stress protein UspA	0.20	0.03	**-1.33**	**-1.32**	L747_03865	xsome
507	alanine dehydrogenase	-0.67	**-1.39**	**-2.83**	**-1.21**	L747_03870	xsome
508	alanine glycine permease	0.24	0.08	-0.46	-0.47	L747_03875	xsome
509	hypothetical protein	**0.44**	**0.74**	**0.69**	-0.01	L747_03880	xsome
510	thioredoxin	-0.34	**1.39**	**1.32**	-0.02	L747_03885	xsome
511	DSBA oxidoreductase	**-0.72**	**1.43**	**1.43**	0.04	L747_03890	xsome
512	cell division protein FtsK	0.01	0.08	**-0.83**	**-0.87**	L747_03895	xsome
513	UDP-N-acetylmuramate--alanine ligase	-0.05	-0.07	**-0.38**	**-0.27**	L747_03900	xsome
514	nicotinamide mononucleotide transporter; distrupted(pseudo)	-0.58	-0.63	**-2.03**	**-1.26**	L747_13715	xsome
515	membrane protein	0.14	**0.25**	**0.70**	**0.49**	L747_03905	xsome
516	xanthine/uracil permease	-0.10	-0.72	-0.38	0.32	L747_03910	xsome
517	transposase IS30	-0.14	**0.64**	**0.47**	-0.13	L747_03915	xsome
518	hypothetical protein	-0.15	0.13	**1.02**	**0.87**	L747_03920	xsome
519	chlorohydrolase	-0.25	0.47	**1.35**	**0.89**	L747_03925	xsome
520	DNA polymerase I	0.06	**-0.51**	**-1.43**	**-0.87**	L747_03930	xsome
521	5-hydroxymethyluracil DNA glycosylase	0.14	-0.22	**-1.02**	**-0.75**	L747_03935	xsome
522	dephospho-CoA kinase	0.02	**-0.48**	**-1.26**	**-0.73**	L747_03940	xsome
523	NrdR family transcriptional regulator; nrdR	0.30	**-0.30**	**-0.66**	**-0.32**	L747_03945	xsome
524	hypothetical protein	-0.18	0.04	**-0.51**	**-0.51**	L747_03950	xsome
525	primosomal protein DnaI	-0.02	**0.50**	**0.42**	-0.03	L747_03955	xsome
526	threonyl-tRNA synthase	0.15	**1.00**	**0.71**	**-0.25**	L747_03960	xsome
527	translation initiation factor IF-3	0.14	**0.64**	**0.99**	**0.38**	L747_03965	xsome
528	50S ribosomal protein L35	0.11	**-0.71**	**-0.77**	-0.02	L747_03970	xsome
529	50S ribosomal protein L20	0.29	0.08	**0.69**	**0.64**	L747_03975	xsome
530	hypothetical protein	0.02	**-2.17**	**-1.93**	0.25	L747_03980	xsome
531	hypothetical protein	-0.40	**-0.74**	**-0.86**	-0.08	L747_03985	xsome
532	hypothetical protein	-0.08	-0.39	0.03	0.42	L747_03990	xsome

533	HAD family hydrolase	-0.10	0.02	**-0.42**	**-0.39**	L747_03995	xsome
534	GTPase	-0.11	**0.37**	0.12	**-0.20**	L747_04000	xsome
535	RNA-binding protein	0.00	0.14	**-0.46**	**-0.55**	L747_04005	xsome
536	nicotinic acid mononucleotide adenylyltransferase; nadD	0.09	**0.36**	**0.28**	-0.04	L747_04010	xsome
537	hypothetical protein	0.07	-0.25	**-0.48**	-0.19	L747_04015	xsome
538	Iojap family protein	0.05	**0.58**	**0.85**	**0.30**	L747_04020	xsome
539	SAM-dependent methyltransferase	0.20	**-0.74**	**-0.81**	-0.04	L747_04025	xsome
540	hypothetical protein	0.05	**-0.36**	-0.12	**0.28**	L747_04030	xsome
541	DNA-binding protein	-0.08	**0.80**	**1.44**	**0.67**	L747_04035	xsome
542	50S ribosomal protein L32; rpmF	**0.41**	**1.01**	**2.52**	**1.53**	L747_04040	xsome
543	HAD family hydrolase	0.00	0.27	**0.85**	**0.61**	L747_04045	xsome
544	ArsR family transcriptional regulator	0.14	0.31	**1.19**	**0.90**	L747_04050	xsome
545	hypothetical protein	0.35	**2.11**	**1.75**	**-0.32**	L747_04055	xsome
546	alcohol dehydrogenase	-0.10	**-3.43**	**-2.15**	**1.31**	L747_04060	xsome
547	PhoB family transcriptional regulator	-0.05	**0.77**	**0.92**	0.18	L747_04065	xsome
548	sensor histidine kinase	-0.19	**0.70**	**0.67**	0.00	L747_04070	xsome
549	hypothetical protein	**-0.73**	**-2.54**	**-3.33**	**-0.66**	L747_04075	xsome
550	membrane protein	0.00	**1.35**	**1.96**	**0.65**	L747_04080	xsome
551	acylphosphatase	-0.55	-0.64	-0.41	0.25	L747_04085	xsome
552	23S rRNA methyltransferase	-0.46	**-0.58**	**-1.26**	**-0.63**	L747_04090	xsome
553	hydrolase	**0.66**	**2.12**	**1.50**	**-0.57**	L747_04095	xsome
554	HxlR family transcriptional regulator	**0.54**	**2.46**	**2.08**	**-0.33**	L747_04100	xsome
555	phenylalanyl-tRNA synthase subunit alpha; pheS	0.02	**-0.40**	**-0.54**	-0.10	L747_04105	xsome
556	phenylalanyl-tRNA synthase subunit beta	0.17	**-0.78**	**-0.89**	-0.08	L747_04110	xsome
557	aminodeoxychorismate lyase	-0.17	0.18	-0.12	**-0.26**	L747_04115	xsome
558	uridine/cytidine kinase	-0.08	0.01	**-0.38**	**-0.34**	L747_04120	xsome
559	transcription elongation factor GreA	0.17	**-0.26**	**-0.73**	**-0.43**	L747_04125	xsome
560	hypothetical protein	-0.18	**-0.79**	-0.22	**0.58**	L747_04130	xsome
561	hypothetical protein	0.03	1.76	**2.76**	0.80	L747_04135	xsome
562	iron-sulfur cluster biosynthesis protein	**0.56**	**2.43**	**1.84**	**-0.55**	L747_04140	xsome

563	hypothetical protein	-0.32	**-0.39**	**0.33**	**0.76**	L747_04145	xsome
564	membrane protein	**1.27**	**2.76**	**2.41**	**-0.32**	L747_04150	xsome
565	penicillin-binding protein	0.20	**0.90**	**0.79**	-0.07	L747_04155	xsome
566	50S ribosomal protein L33	-0.44	**-1.55**	-0.26	**1.30**	L747_04160	xsome
567	hypothetical protein	0.08	**-0.75**	**-0.73**	0.06	L747_04165	xsome
568	membrane protein	0.39	**0.72**	0.30	**-0.38**	L747_04170	xsome
569	hypothetical protein	0.17	**-0.97**	**-1.32**	**-0.30**	L747_04175	xsome
570	glucokinase	0.16	**-0.61**	**-1.19**	**-0.54**	L747_04180	xsome
571	sulfurtransferase	0.01	**-0.62**	**-1.04**	**-0.37**	L747_04185	xsome
572	hypothetical protein	0.40	**1.36**	**1.89**	**0.57**	L747_04190	xsome
573	glycerophosphoryl diester phosphodiesterase	0.20	**-0.50**	**-0.82**	**-0.28**	L747_04195	xsome
574	tRNA delta(2)-isopentenylpyrophosphate transferase	**0.54**	**-0.46**	**-1.11**	**-0.60**	L747_04200	xsome
575	MerR family transcriptional regulator	**0.42**	**2.12**	**2.51**	**0.42**	L747_04205	xsome
576	glutamine synthetase	**0.41**	**2.20**	**2.87**	**0.70**	L747_04210	xsome
577	exonuclease SbcC	0.05	**0.49**	**0.33**	-0.12	L747_04215	xsome
578	hypothetical protein	0.18	**0.59**	**0.56**	0.01	L747_04220	xsome
579	2-deoxyuridine 5-triphosphate nucleotidohydrolase	**0.53**	0.18	-0.19	**-0.33**	L747_04225	xsome
580	hypothetical protein	**0.56**	-0.13	**-0.38**	-0.21	L747_04230	xsome
581	50S ribosomal protein L21	**0.24**	0.10	-0.13	**-0.19**	L747_04235	xsome
582	ribosomal protein	0.23	**-0.32**	**-0.73**	**-0.37**	L747_04240	xsome
583	50S ribosomal protein L27	0.04	**0.34**	**0.51**	**0.21**	L747_04245	xsome
584	Xaa-Pro aminopeptidase	-0.20	**-0.95**	**-1.31**	**-0.31**	L747_04250	xsome
585	elongation factor P	**-0.31**	**-0.89**	**-1.45**	**-0.51**	L747_04255	xsome
586	alkaline shock protein	-0.23	**-0.25**	**-0.70**	**-0.41**	L747_04260	xsome
587	transcription antitermination protein NusB	-0.15	0.18	0.12	-0.03	L747_04265	xsome
588	bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase	-0.11	**-0.50**	**-0.85**	**-0.31**	L747_04270	xsome
589	exodeoxyribonuclease VII large subunit	**-0.41**	**-0.60**	**-0.99**	**-0.35**	L747_04275	xsome

590	exodeoxyribonuclease VII small subunit	-0.15	-0.47	**-1.19**	**-0.66**	L747_04280	xsome
591	farnesyl-diphosphate synthase	0.12	-0.19	**-0.89**	**-0.64**	L747_04285	xsome
592	cell division protein FtsJ	-0.11	-0.18	**-0.87**	**-0.64**	L747_04290	xsome
593	ArgR family transcriptional regulator	-0.21	**0.36**	0.07	**-0.24**	L747_04295	xsome
594	DNA repair protein RecN	-0.20	0.02	**-1.10**	**-1.07**	L747_04300	xsome
595	hypothetical protein	**-0.40**	**-2.07**	**-3.91**	**-1.76**	L747_04305	xsome
596	hypothetical protein	-0.06	-0.49	0.32	**0.83**	L747_04310	xsome
597	hypothetical protein	**1.02**	**1.23**	**2.36**	**1.16**	L747_04315	xsome
598	guanylate kinase	0.22	0.06	-0.17	-0.19	L747_04320	xsome
599	DNA-directed RNA polymerase subunit omega	0.02	**-0.81**	**-0.60**	0.25	L747_04325	xsome
600	DNA/pantothenate metabolism flavoprotein	-0.05	**-0.59**	**-1.37**	**-0.73**	L747_04330	xsome
601	primosomal protein N'	-0.03	**-1.08**	**-1.15**	-0.02	L747_04335	xsome
602	methionyl-tRNA formyltransferase	-0.19	**-1.29**	**-1.33**	0.00	L747_04340	xsome
603	16S rRNA methyltransferase	-0.29	**-1.14**	**-0.98**	0.20	L747_04345	xsome
604	protein phosphatase	-0.06	**-0.85**	**-0.89**	0.00	L747_04350	xsome
605	protein kinase	0.04	**-0.65**	**-0.40**	**0.28**	L747_04355	xsome
606	ribosome biogenesis GTPase RsgA	-0.16	**-0.72**	**-1.12**	**-0.36**	L747_04360	xsome
607	ribulose-phosphate 3-epimerase	-0.21	**-0.48**	**-0.66**	-0.13	L747_04365	xsome
608	thiamine pyrophosphokinase	-0.10	**-0.27**	**-0.50**	-0.19	L747_04370	xsome
609	50S ribosomal protein L28	0.00	**-0.47**	**0.76**	**1.24**	L747_04375	xsome
610	hypothetical protein	-0.23	**-0.44**	**-0.93**	**-0.45**	L747_04380	xsome
611	hypothetical protein	-0.19	**-0.26**	**-0.62**	**-0.32**	L747_04385	xsome
612	ATP-dependent DNA helicase	0.15	**-0.37**	**-0.92**	**-0.51**	L747_04390	xsome
613	phosphate acyltransferase	0.18	-0.17	**-0.81**	**-0.60**	L747_04395	xsome
614	acyl carrier protein	0.16	0.12	0.11	0.03	L747_04400	xsome
615	ribonuclease III	0.03	**-0.57**	**-1.59**	**-0.96**	L747_04405	xsome
616	hypothetical protein	0.26	-0.18	**-0.93**	**-0.70**	L747_04410	xsome
617	cell division protein FtsY	0.21	**-0.27**	**-1.01**	**-0.69**	L747_04415	xsome
618	DNA-binding protein	0.01	0.12	-0.06	-0.13	L747_04420	xsome
619	signal recognition particle	0.23	**0.33**	**-0.38**	**-0.67**	L747_04425	xsome
620	ATPase	**-0.64**	**0.80**	**0.43**	**-0.33**	L747_04430	xsome

621	hypothetical protein	**0.97**	**0.93**	**0.83**	-0.06	L747_04435	xsome
622	hypothetical protein	**1.00**	**0.25**	-0.01	**-0.22**	L747_04440	xsome
623	hypothetical protein	**0.89**	-0.11	-0.17	-0.02	L747_04445	xsome
624	30S ribosomal protein S16; rpsP	0.12	**-0.37**	**0.58**	**0.98**	L747_04450	xsome
625	RNA-binding protein	0.16	0.07	**1.03**	**0.99**	L747_04455	xsome
626	16S rRNA-processing protein RimM	-0.43	**-1.11**	0.00	**1.13**	L747_04460	xsome
627	tRNA (guanine-N1)-methyltransferase	-0.19	**-0.78**	0.28	**1.09**	L747_04465	xsome
628	50S ribosomal protein L19; rplS	-0.11	-0.02	**1.49**	**1.52**	L747_04470	xsome
629	integrase	0.01	**0.55**	**0.36**	-0.14	L747_04475	xsome
630	hypothetical protein	0.18	-0.20	-0.20	0.04	L747_04480	xsome
631	3-hydroxyacyl-ACP dehydratase	**0.38**	**0.62**	**-0.25**	**-0.82**	L747_04485	xsome
632	FabT; transcriptional regulator of fatty acid biosynthesis	0.17	**0.39**	**-0.26**	**-0.60**	L747_04490	xsome
633	3-oxoacyl-ACP synthase	**0.37**	**0.27**	**-0.68**	**-0.90**	L747_04495	xsome
634	acyl carrier protein	**0.39**	-0.16	**-1.99**	**-1.78**	L747_04500	xsome
635	hypothetical protein	**0.31**	**-0.24**	**-1.82**	**-1.53**	L747_04505	xsome
636	3-ketoacyl-ACP reductase	0.13	0.10	**-1.26**	**-1.31**	L747_04510	xsome
637	3-oxoacyl-ACP synthase	0.03	**-0.84**	**-2.33**	**-1.44**	L747_04515	xsome
638	acetyl-CoA biotin carboxyl carrier	-0.19	**-0.87**	**-1.92**	**-1.00**	L747_04520	xsome
639	3-hydroxyacyl-ACP dehydratase	-0.13	**-0.83**	**-2.14**	**-1.26**	L747_04525	xsome
640	acetyl-CoA carboxylase biotin carboxylase subunit	**-0.26**	**-1.16**	**-2.36**	**-1.16**	L747_04530	xsome
641	acetyl-CoA carboxylase carboxyl transferase subunit beta	**-0.29**	**-1.95**	**-3.22**	**-1.22**	L747_04535	xsome
642	acetyl-CoA carboxylase	**-0.40**	**-1.03**	**-2.05**	**-0.98**	L747_04540	xsome
643	enoyl-ACP reductase	**-0.70**	**-1.72**	**-3.08**	**-1.31**	L747_04545	xsome
644	hypothetical protein	**-0.68**	**-1.42**	**-2.61**	**-1.15**	L747_04550	xsome
645	hypothetical protein	**-0.89**	**-1.71**	**-2.87**	**-1.11**	L747_04555	xsome
646	transposase IS30	0.53	0.31	**1.22**	**0.93**	L747_04560	xsome
647	hypothetical protein	-0.57	**-1.68**	**-1.26**	0.44	L747_04565	xsome
648	membrane protein	0.14	-0.34	0.29	0.55	L747_04570	xsome

649	hypothetical protein	0.21	-0.77	-0.87	-0.02	L747_04575	xsome
650	hypothetical protein	-0.71	**-1.39**	-0.64	**0.75**	L747_04580	xsome
651	multidrug MFS transporter	-0.57	-0.25	-0.47	-0.17	L747_04585	xsome
652	hypothetical protein	0.22	0.97	1.10	0.14	L747_04590	xsome
653	integrase	-0.08	**-0.35**	**0.79**	**1.17**	L747_04595	xsome
654	hypothetical protein	**1.04**	**0.78**	**1.78**	**1.01**	L747_04600	xsome
655	alcohol dehydrogenase	0.27	**-1.13**	-0.46	**0.69**	L747_04605	xsome
656	hypothetical protein	-0.08	**-0.71**	**-0.59**	0.16	L747_04610	xsome
657	transcriptional regulator	**0.52**	**0.70**	**1.41**	**0.74**	L747_04615	xsome
658	NADP-dependent aryl-alcohol dehydrogenase	-0.08	-0.68	-0.30	0.38	L747_04620	xsome
659	nitrobenzoate reductase	**0.95**	**2.09**	**1.78**	**-0.27**	L747_04625	xsome
660	hypothetical protein	0.28	**1.73**	**1.66**	-0.03	L747_04630	xsome
661	catalase	0.23	0.21	**-0.59**	**-0.75**	L747_04635	xsome
662	hypothetical protein	0.34	**2.23**	**2.45**	0.24	L747_04640	xsome
663	hypothetical protein	0.15	**0.66**	**1.12**	**0.49**	L747_04645	xsome
664	hypothetical protein	0.32	**1.16**	**1.75**	**0.61**	L747_04650	xsome
665	hypothetical protein	-0.19	**-0.63**	**-0.99**	-0.31	L747_04655	xsome
666	hypothetical protein	-0.43	-0.85	-0.17	0.67	L747_04660	xsome
667	hypothetical protein	-0.07	0.27	0.85	0.55	L747_04665	xsome
668	hypothetical protein	-0.43	-0.46	-0.05	0.44	L747_04670	xsome
669	amino acid ABC transporter permease	**-1.14**	**-2.67**	**-0.72**	**1.96**	L747_04675	xsome
670	peptidase U34	**-0.72**	**-2.74**	**-1.05**	**1.72**	L747_04680	xsome
671	membrane protein	**-0.65**	**-0.83**	**-1.22**	**-0.34**	L747_04685	xsome
672	4-oxalocrotonate tautomerase	0.32	**-0.33**	**-1.35**	**-0.96**	L747_04690	xsome
673	glycerol kinase; glpK	-0.04	**-0.36**	**-1.07**	**-0.66**	L747_04695	xsome
674	hypothetical protein	**-1.00**	**1.14**	**0.72**	**-0.38**	L747_04700	xsome
675	Cro/Cl family transcriptional regulator	-0.14	**1.71**	**1.43**	-0.25	L747_04705	xsome
676	hypothetical protein	-0.15	-0.99	0.35	**1.15**	L747_04710	xsome
677	hypothetical protein	0.16	0.88	**2.10**	**1.05**	L747_04715	xsome
678	hypothetical protein	**-0.90**	-0.19	0.75	**0.95**	L747_04720	xsome
679	HAD family hydrolase	**-0.47**	-0.13	**0.42**	**0.59**	L747_04725	xsome

680	23S rRNA methyltransferase	**-0.57**	**-0.62**	-0.30	**0.36**	L747_04730	xsome
681	isopentenyl pyrophosphate isomerase	0.13	**0.78**	**1.14**	**0.40**	L747_04735	xsome
682	phosphomevalonate kinase	0.10	**0.82**	**1.09**	**0.31**	L747_04740	xsome
683	diphosphomevalonate decarboxylase	**-0.56**	**-0.39**	-0.05	**0.38**	L747_04745	xsome
684	mevalonate kinase	**-0.74**	0.15	**0.63**	**0.51**	L747_04750	xsome
685	hypothetical protein	-0.08	-0.23	0.03	**0.30**	L747_04755	xsome
686	hypothetical protein	0.06	**1.44**	**2.00**	**0.59**	L747_04760	xsome
687	aspartate aminotransferase	-0.09	**1.00**	**1.30**	**0.34**	L747_04765	xsome
688	XRE family transcriptional regulator	0.30	0.29	-0.23	**-0.47**	L747_04770	xsome
689	hypothetical protein	-0.48	-0.02	**-0.60**	**-0.52**	L747_04775	xsome
690	sulfurtransferase	-0.11	0.35	0.14	-0.17	L747_04780	xsome
691	hypothetical protein	NA	0.33	0.71	0.15	L747_04785	xsome
692	penicillin-binding protein 1A	**-0.48**	**0.64**	**1.37**	**0.76**	L747_04790	xsome
693	recombinase RecU; recU	**-0.58**	-0.05	**0.55**	**0.64**	L747_04795	xsome
694	hypothetical protein	0.36	0.05	**0.68**	**0.66**	L747_04800	xsome
695	cell division protein GpsB	-0.06	**0.81**	**2.01**	**1.23**	L747_04805	xsome
696	hypothetical protein	**-0.39**	**-1.37**	0.67	**1.57**	L747_04810	xsome
697	RNA methyltransferase	-0.23	**-0.80**	**-0.61**	**0.23**	L747_04815	xsome
698	HIT family hydrolase	-0.07	0.36	**1.04**	**0.70**	L747_04820	xsome
699	ammonia permease	0.10	**2.39**	**3.80**	**1.44**	L747_04825	xsome
700	hypothetical protein	**0.47**	**1.31**	**1.39**	0.12	L747_04830	xsome
701	NUDIX hydrolase	0.30	**-0.55**	**-0.63**	-0.03	L747_04835	xsome
702	hypothetical protein	-0.33	**-0.43**	**-0.76**	**-0.28**	L747_04840	xsome
703	hypothetical protein	-0.33	**1.44**	**2.25**	**0.83**	L747_04845	xsome
704	hypothetical protein	-0.10	**-1.16**	**-1.98**	**-0.73**	L747_04850	xsome
705	hypothetical protein	**0.46**	**1.97**	**1.33**	**-0.60**	L747_04855	xsome
706	oxidoreductase	-0.09	**1.38**	**1.47**	0.13	L747_04860	xsome
707	ribonuclease HI	0.07	**1.39**	**1.82**	**0.46**	L747_04865	xsome
708	hypothetical protein	-0.05	0.14	0.13	0.02	L747_04870	xsome
709	formate--tetrahydrofolate ligase	-0.05	**0.91**	**0.69**	-0.17	L747_04875	xsome
710	signal peptidase II	-0.22	**-1.06**	**-1.79**	**-0.68**	L747_04880	xsome

711	RNA pseudouridine synthase	-0.19	-0.27	**-0.46**	-0.15	L747_04885	xsome
712	uracil phosphoribosyltransferase	-0.35	**-1.62**	**-1.62**	0.04	L747_04890	xsome
713	carbamoyl phosphate synthase small subunit	-0.34	**-1.68**	**-1.83**	-0.11	L747_04895	xsome
714	carbamoyl phosphate synthase	-0.12	**-1.11**	**-0.90**	**0.26**	L747_04900	xsome
715	fibronectin-binding protein A	-0.45	**-0.60**	-0.22	**0.41**	L747_04905	xsome
716	MarR family transcriptional regulator	**-0.49**	**-1.51**	**-1.70**	-0.14	L747_04910	xsome
717	hypothetical protein	**-0.88**	**-1.25**	**-1.24**	0.05	L747_04915	xsome
718	MFS transporter	**-0.35**	**-0.98**	**-1.14**	-0.12	L747_04920	xsome
719	hypothetical protein	**0.38**	**0.66**	**0.93**	**0.31**	L747_04925	xsome
720	carbon starvation protein%2C membrane protein	0.24	**0.67**	**1.02**	**0.38**	L747_04930	xsome
721	transposase IS204	-0.48	-0.22	-0.16	0.10	L747_04935	xsome
722	membrane protein	0.30	**0.54**	0.19	**-0.31**	L747_04940	xsome
723	hypothetical protein	-0.08	**2.50**	**3.20**	**0.61**	L747_04945	xsome
724	glycopeptide antibiotics resistance protein	0.76	**4.97**	**5.87**	**0.92**	L747_04950	xsome
725	glycopeptide antibiotics resistance protein	-0.14	**3.94**	**3.97**	0.06	L747_04955	xsome
726	endonuclease III	-0.10	-0.28	**-0.61**	-0.28	L747_04960	xsome
727	hypothetical protein	0.31	**0.82**	**0.82**	0.04	L747_04965	xsome
728	NADPH:quinone reductase	0.29	0.24	0.22	0.01	L747_04970	xsome
729	hypothetical protein	0.74	**2.26**	**2.73**	**0.48**	L747_04975	xsome
730	hypothetical protein	**-0.57**	**-0.41**	**-1.07**	**-0.60**	L747_04980	xsome
731	hypothetical protein	-0.49	0.02	**-0.60**	**-0.57**	L747_04985	xsome
732	permease	-0.23	-0.05	-0.07	0.02	L747_04990	xsome
733	hypothetical protein	-0.28	**-0.96**	**-0.87**	0.14	L747_04995	xsome
734	hypothetical protein	**-0.82**	**-1.15**	**-1.46**	-0.25	L747_05000	xsome
735	hypothetical protein	-0.47	**-1.16**	**-1.43**	-0.22	L747_05005	xsome
736	short-chain dehydrogenase	-0.44	**-1.56**	**-1.96**	**-0.35**	L747_05010	xsome
737	hypothetical protein	-0.62	**-0.89**	**-0.53**	**0.39**	L747_05015	xsome
738	hypothetical protein	-0.17	0.00	0.18	0.16	L747_05020	xsome
739	integrase	-0.01	0.34	**1.09**	**0.78**	L747_05025	xsome
740	integrase	0.20	**0.71**	**0.54**	-0.13	L747_05030	xsome
741	LysR family transcriptional regulator	-0.14	0.13	0.08	-0.02	L747_05035	xsome

742	oxidoreductase	0.15	-0.26	0.26	0.52	L747_05040	xsome
743	NAD-dependent dehydratase	0.16	0.53	0.46	-0.04	L747_05045	xsome
744	hypothetical protein	0.16	-0.15	0.01	0.18	L747_05050	xsome
745	hypothetical protein	-0.02	-0.35	-0.57	-0.16	L747_05055	xsome
746	membrane protein	**1.45**	**0.89**	**0.99**	0.14	L747_05060	xsome
747	recombinase XerS	-0.01	-0.46	0.22	**0.70**	L747_05065	xsome
748	hypothetical protein	-0.08	0.08	**-0.31**	**-0.35**	L747_05070	xsome
749	phosphohydrolase	**0.92**	**2.39**	**1.23**	**-1.10**	L747_05075	xsome
750	methionine sulfoxide reductase A	**0.99**	**3.09**	**1.82**	**-1.23**	L747_05080	xsome
751	methionine sulfoxide reductase B	**1.07**	**1.55**	0.00	**-1.50**	L747_05085	xsome
752	transposase IS4	0.16	0.11	**0.43**	**0.36**	L747_05090	xsome
753	transposase	0.47	**1.05**	**1.44**	**0.42**	L747_05095	xsome
754	hypothetical protein	0.02	**0.74**	-0.55	**-1.22**	L747_05100	xsome
755	pyrophosphatase	-0.15	**0.69**	**1.20**	**0.55**	L747_05105	xsome
756	productLysR family transcriptional regulator; dirstrupted(pseudo)	-0.17	-0.10	-0.13	0.01	L747_05110	xsome
757	productDNA topoisomerase IV subunit A	**-0.32**	**-0.90**	**-0.94**	0.00	L747_05115	xsome
758	DNA topoisomerase IV subunit B	**-0.65**	**-1.50**	**-1.60**	-0.06	L747_05120	xsome
759	membrane protein	**0.88**	**1.18**	**1.53**	**0.39**	L747_05125	xsome
760	galactose mutarotase	0.16	0.05	0.31	**0.29**	L747_05130	xsome
761	ATP-dependent protease	**0.53**	**-0.32**	**-0.58**	**-0.22**	L747_05135	xsome
762	ATP-dependent protease	**0.57**	-0.24	**-0.50**	-0.21	L747_05140	xsome
763	tyrosine recombinase XerC	**0.62**	**-0.25**	**-1.18**	**-0.89**	L747_05145	xsome
764	DNA topoisomerase I	**-0.46**	0.09	**0.43**	**0.38**	L747_05150	xsome
765	hypothetical protein	-0.77	**-1.99**	**-1.47**	0.52	L747_05155	xsome
766	ribonuclease HII	-0.15	**0.54**	**0.76**	**0.26**	L747_05160	xsome
767	ribosome biogenesis GTPase A; rbgA	-0.41	-0.09	-0.08	0.04	L747_05165	xsome
768	hypothetical protein	-0.15	-0.53	0.15	0.64	L747_05170	xsome
769	hypothetical protein	-0.50	**-1.16**	-0.24	**0.91**	L747_05175	xsome
770	hypothetical protein	-0.09	-0.32	0.20	0.48	L747_05190	xsome
771	producthypothetical protein	-0.05	0.46	0.53	0.04	L747_05195	xsome

772	hypothetical protein	-0.30	-0.44	-0.92	-0.39	L747_05200	xsome
773	hypothetical protein	0.03	0.52	**1.24**	**0.74**	L747_05205	xsome
774	hypothetical protein	0.03	-0.19	0.15	**0.38**	L747_05210	xsome
775	hypothetical protein	**-0.46**	**-0.61**	**-0.46**	0.19	L747_05215	xsome
776	hypothetical protein	0.12	**0.71**	**0.87**	0.20	L747_05220	xsome
777	hemolysin III	0.05	**0.52**	**0.85**	**0.36**	L747_05225	xsome
778	dihydrofolate reductase	0.17	0.16	-0.29	**-0.41**	L747_05230	xsome
779	thymidylate synthase	**0.44**	**0.54**	0.08	**-0.41**	L747_05235	xsome
780	ABC transporter ATP-binding protein	0.32	**-0.43**	**-0.85**	**-0.38**	L747_05240	xsome
781	tRNA CCA-pyrophosphorylase	0.03	-0.06	-0.22	-0.11	L747_05245	xsome
782	membrane protein	-0.44	**-1.54**	**-1.35**	0.23	L747_05250	xsome
783	hypothetical protein	**-0.32**	**-1.47**	**-1.41**	0.10	L747_05255	xsome
784	transcriptional regulator	**0.39**	0.04	**0.64**	**0.63**	L747_05260	xsome
785	GTP-binding protein Der; engA	0.16	-0.14	-0.11	0.07	L747_05265	xsome
786	30S ribosomal protein S1	**0.28**	**0.94**	**0.56**	**-0.34**	L747_05270	xsome
787	cytidylate kinase	0.01	0.20	0.11	-0.05	L747_05275	xsome
788	peptidoglycan-binding protein	-0.27	**0.86**	**0.71**	-0.11	L747_05280	xsome
789	ATP-dependent DNA helicase RecQ	0.24	**-0.77**	**-1.32**	**-0.50**	L747_05285	xsome
790	hypothetical protein	0.29	**-0.69**	**-1.13**	**-0.39**	L747_05290	xsome
791	hypothetical protein	-0.20	-0.22	**-1.56**	**-1.27**	L747_05295	xsome
792	ribosomal large subunit pseudouridine synthase B	0.37	0.26	**0.29**	0.07	L747_05300	xsome
793	segregation protein A	0.33	**0.68**	**0.56**	-0.08	L747_05305	xsome
794	hypothetical protein	**0.54**	**0.77**	**0.59**	-0.14	L747_05310	xsome
795	hypothetical protein	0.34	-0.27	**-0.69**	**-0.38**	L747_05315	xsome
796	tyrosine recombinase XerD	0.31	-0.12	**-0.46**	**-0.29**	L747_05320	xsome
797	S1 RNA-binding protein	**0.39**	**0.32**	0.00	**-0.27**	L747_05325	xsome
798	pyruvate kinase	0.11	**0.90**	**-0.45**	**-1.30**	L747_05330	xsome
799	transaldolase	**-1.10**	**3.04**	**3.68**	**0.67**	L747_05335	xsome
800	DNA polymerase III subunit epsilon	**-0.48**	**-0.59**	**-0.57**	0.05	L747_05340	xsome
801	ATP-dependent Clp protease ATP-binding protein; distrupted(psuedo)	**-0.34**	**-1.10**	**-1.10**	0.03	L747_05345	xsome

802	integrase	-0.35	**-0.53**	**0.83**	**1.39**	L747_05350	xsome
803	peptidase T	0.15	-0.23	**-3.31**	**-2.99**	L747_05355	xsome
804	hypothetical protein	-0.37	**-0.70**	**-2.21**	**-1.45**	L747_05360	xsome
805	SAM-dependent methyltransferase	-0.32	**-0.74**	**-1.78**	**-0.99**	L747_05365	xsome
806	membrane protein	**-1.28**	**-0.35**	**-0.43**	-0.04	L747_05370	xsome
807	hypothetical protein	**-1.38**	**-0.58**	**-1.02**	-0.38	L747_05375	xsome
808	RNA polymerase sigma factor RpoD; distrupted(psuedo)	0.09	0.24	0.04	-0.16	L747_05380	xsome
809	DNA primase	-0.01	**-0.72**	**-1.64**	**-0.87**	L747_05385	xsome
810	glycine-tRNA synthetase subunit beta	**0.32**	0.17	**0.33**	**0.20**	L747_05390	xsome
811	glycyl-tRNA synthase subunit alpha; glyQ	0.03	-0.06	-0.23	-0.13	L747_05395	xsome
812	DNA repair protein RecO	-0.13	-0.01	**-0.84**	**-0.78**	L747_05400	xsome
813	GTPase Era; era	-0.08	**-0.41**	**-1.87**	**-1.40**	L747_05405	xsome
814	UDP kinase	-0.23	0.11	**-0.86**	**-0.91**	L747_05410	xsome
815	rRNA maturation factor	-0.33	**-0.70**	**-2.18**	**-1.43**	L747_05415	xsome
816	phosphate starvation-inducible protein PhoH	**-0.71**	**-0.52**	**-1.37**	**-0.80**	L747_05420	xsome
817	hypothetical protein	0.02	**1.38**	**1.41**	0.07	L747_05425	xsome
818	HxlR family transcriptional regulator	0.45	**1.52**	0.44	**-1.02**	L747_05430	xsome
819	NADPH:quinone reductase	**0.61**	**1.11**	**0.75**	**-0.32**	L747_05435	xsome
820	30S ribosomal protein S21	**0.35**	**0.54**	**1.72**	**1.19**	L747_05440	xsome
821	phosphotransferase	-0.66	-0.38	0.26	**0.66**	L747_05445	xsome
822	endonuclease IV	**0.52**	**0.46**	**0.74**	**0.31**	L747_05450	xsome
823	CDP-glycerol glycerophosphotransferase	0.13	0.23	**0.56**	**0.36**	L747_05455	xsome
824	teichoic acid ABC transporter permease	0.12	**0.31**	**1.16**	**0.89**	L747_05460	xsome
825	glycosyl transferase	0.00	**0.74**	**1.07**	**0.36**	L747_05465	xsome
826	membrane protein	0.16	**2.12**	**1.50**	**-0.58**	L747_05470	xsome
827	methionine sulfoxide reductase A	-0.38	**3.35**	**2.69**	**-0.63**	L747_05475	xsome
828	aspartyl-tRNA synthase; aspS	0.05	**-0.96**	**-0.74**	**0.26**	L747_05480	xsome
829	histidyl-tRNA synthase	-0.03	**-1.55**	**-1.54**	0.05	L747_05485	xsome
830	hypothetical protein	-0.25	**-0.78**	0.07	**0.85**	L747_05490	xsome
831	N-acetylmuramoyl-L-alanine amidase	-0.13	**0.57**	**0.40**	-0.13	L747_05495	xsome

832	aminoglycoside phosphotransferase	-0.30	**1.10**	**1.31**	**0.25**	L747_05500	xsome
833	hypothetical protein	0.46	-0.05	**0.41**	**0.50**	L747_05505	xsome
834	D-tyrosyl-tRNA(Tyr) deacylase	0.12	-0.07	0.02	0.13	L747_05510	xsome
835	GTP pyrophosphokinase	-0.02	**-0.56**	**-0.96**	**-0.36**	L747_05515	xsome
836	hypothetical protein	0.20	**-1.04**	**-1.65**	**-0.56**	L747_05520	xsome
837	16S rRNA methyltransferase	0.12	-0.16	**0.50**	**0.69**	L747_05525	xsome
838	ribosomal protein L11 methyltransferase	0.03	**-0.35**	**0.40**	**0.78**	L747_05530	xsome
839	hypothetical protein	**0.49**	**0.37**	**1.00**	**0.66**	L747_05535	xsome
840	large conductance mechanosensitive channel protein MscL	0.40	0.32	**0.43**	0.14	L747_05540	xsome
841	hypothetical protein	**0.56**	0.29	**0.48**	**0.23**	L747_05545	xsome
842	hypothetical protein	**0.73**	**0.52**	**0.74**	0.26	L747_05550	xsome
843	integrase	0.28	0.36	**1.12**	**0.79**	L747_05555	xsome
844	hypothetical protein	-0.08	-0.40	-0.06	0.33	L747_05560	xsome
845	cyclopropane-fatty-acyl-phospholipid synthase	0.10	**-0.64**	**-1.95**	**-1.26**	L747_05565	xsome
846	hypothetical protein	-0.30	**-0.89**	**-0.78**	0.14	L747_05570	xsome
847	GTP-binding protein LepA	**-0.32**	-0.05	-0.13	-0.04	L747_05580	xsome
848	cytochrome C552	0.11	**-2.23**	**-2.48**	**-0.20**	L747_05585	xsome
849	D-alanine--poly(phosphoribitol) ligase	0.52	**-1.47**	**-2.13**	**-0.59**	L747_05590	xsome
850	alanine transporter	0.28	**-2.16**	**-2.52**	**-0.31**	L747_05595	xsome
851	cytochrome C553	0.33	**-1.83**	**-2.40**	**-0.52**	L747_05600	xsome
852	transposase	0.21	0.20	**0.59**	**0.42**	L747_05605	xsome
853	transposase IS4	0.30	0.08	**0.59**	**0.55**	L747_05610	xsome
854	hypothetical protein	-0.25	-0.42	0.03	0.47	L747_05615	xsome
855	molecular chaperone DnaJ	**-0.36**	**-1.39**	**-3.17**	**-1.72**	L747_05620	xsome
856	molecular chaperone DnaK; dnaK	-0.24	**-0.89**	**-1.06**	-0.13	L747_05625	xsome
857	heat shock protein GrpE	**-0.56**	**-1.87**	**-2.61**	**-0.69**	L747_05630	xsome
858	HrcA family transcriptional regulator	**-0.52**	**-1.95**	**-2.11**	-0.12	L747_05635	xsome
859	riboflavin biosynthesis protein RibF	0.09	**-1.03**	**-1.10**	-0.03	L747_05640	xsome
860	tRNA pseudouridine synthase B	0.01	**-1.01**	**-0.96**	0.09	L747_05645	xsome
861	ribosome-binding factor A	0.30	-0.07	**0.69**	**0.79**	L747_05650	xsome

862	translation initiation factor IF-2	0.03	**-0.56**	**-0.42**	**0.18**	L747_05655	xsome
863	50S ribosomal protein L7	0.18	0.06	-0.09	-0.10	L747_05660	xsome
864	hypothetical protein	-0.08	**-0.60**	**-0.69**	-0.04	L747_05665	xsome
865	transcription elongation factor NusA	-0.31	**-0.63**	**-0.29**	**0.38**	L747_05670	xsome
866	ribosome maturation protein RimP	-0.28	**-1.38**	**-1.03**	**0.39**	L747_05675	xsome
867	hypothetical protein	**-0.75**	**-1.70**	**-2.03**	-0.28	L747_05680	xsome
868	DNA polymerase III subunit alpha; polC	**-0.53**	**-0.77**	**-0.57**	**0.24**	L747_05685	xsome
869	prolyl-tRNA synthetase	-0.17	**-0.27**	-0.17	0.14	L747_05690	xsome
870	metalloprotease RseP	0.02	-0.18	**-0.38**	-0.16	L747_05695	xsome
871	CDP-diglyceride synthetase	-0.17	-0.05	**-0.41**	**-0.31**	L747_05700	xsome
872	UDP pyrophosphate synthase	-0.19	**0.50**	-0.22	**-0.67**	L747_05705	xsome
873	hypothetical protein	0.01	**0.94**	**1.19**	**0.29**	L747_05710	xsome
874	ribosome recycling factor	0.09	**0.36**	0.09	**-0.22**	L747_05715	xsome
875	uridylate kinase; pyrH	-0.14	**-0.50**	**-1.02**	**-0.48**	L747_05720	xsome
876	elongation factor Ts	0.13	**-0.29**	-0.19	0.14	L747_05725	xsome
877	30S ribosomal protein S2	0.17	**0.35**	**0.72**	**0.41**	L747_05730	xsome
878	hypothetical protein	-0.39	**0.91**	**1.64**	**0.76**	L747_05735	xsome
879	D-lactate dehydrogenase	-0.16	0.02	**-0.49**	**-0.46**	L747_05740	xsome
880	hypothetical protein	-0.58	**-1.11**	**-1.54**	-0.37	L747_05745	xsome
881	O-methyltransferase	-0.42	-0.11	0.42	**0.56**	L747_05750	xsome
882	1-acyl-sn-glycerol-3-phosphate acyltransferase	-0.03	-0.26	0.13	**0.42**	L747_05755	xsome
883	ABC transporter ATP-binding protein	**-0.78**	**-0.47**	**-0.74**	-0.22	L747_05760	xsome
884	multidrug ABC transporter ATP-binding protein	**-0.85**	**-0.36**	**-1.29**	**-0.88**	L747_05765	xsome
885	hypothetical protein	0.03	**-1.35**	**-1.54**	-0.14	L747_05770	xsome
886	hypothetical protein	-0.09	-0.29	0.14	**0.46**	L747_05775	xsome
887	LexA family transcriptional regulator	0.11	0.27	**1.31**	**1.07**	L747_05780	xsome
888	nucleoside 2-deoxyribosyltransferase	-0.30	**-1.04**	**-0.40**	**0.67**	L747_05785	xsome
889	hypothetical protein	-0.08	0.60	**0.82**	0.26	L747_05790	xsome
890	hydroxymethylglutaryl-CoA synthase	0.28	**0.37**	**0.46**	0.13	L747_05795	xsome
891	polyphosphate:AMP phosphotransferase	0.28	0.25	**-0.86**	**-1.06**	L747_05800	xsome
892	fructose-2,6-bisphosphatase	0.30	-0.22	**-0.77**	**-0.50**	L747_05805	xsome

893	stress-responsive transcription regulator	0.60	**1.46**	**1.73**	0.30	L747_05810	xsome
894	nucleoside diphosphate kinase	-0.44	**-0.91**	**-1.33**	**-0.38**	L747_05815	xsome
895	hypothetical protein	-0.51	**-1.27**	**-2.00**	**-0.66**	L747_05820	xsome
896	hypothetical protein	-0.59	**-2.27**	**-2.20**	0.11	L747_05825	xsome
897	hypothetical protein	**-0.67**	**-1.23**	**-0.94**	**0.33**	L747_05830	xsome
898	hypothetical protein	-0.59	-1.12	-0.83	0.30	L747_05835	xsome
899	transposase IS204	0.24	-0.07	0.17	0.27	L747_05840	xsome
901	hypothetical protein	-0.04	-0.16	**1.71**	**1.85**	L747_05850	xsome
902	diacylglycerol kinase	-0.18	**-0.87**	**-1.50**	**-0.55**	L747_05855	xsome
903	hypothetical protein	-0.19	-0.46	**-1.29**	**-0.73**	L747_05860	xsome
904	hypothetical protein	0.10	**2.18**	**1.28**	**-0.85**	L747_05865	xsome
905	universal stress protein UspA	0.24	**2.62**	**1.89**	**-0.69**	L747_05870	xsome
906	adenine phosphoribosyltransferase	0.09	0.21	**0.79**	**0.62**	L747_05875	xsome
907	recombination protein RecJ	-0.10	-0.07	-0.19	-0.07	L747_05880	xsome
908	hypothetical protein	-0.01	**0.75**	0.23	**-0.47**	L747_05885	xsome
909	ribonuclease Z	-0.27	**0.50**	-0.17	**-0.63**	L747_05890	xsome
910	hypothetical protein	0.09	**-1.26**	**-0.98**	0.31	L747_05895	xsome
911	hypothetical protein	-0.12	**-1.43**	**-0.99**	**0.47**	L747_05900	xsome
912	GTPase CgtA; obgE	0.20	**0.73**	**-1.24**	**-1.91**	L747_05905	xsome
913	excinuclease ABC subunit C	-0.08	0.07	**-0.97**	**-0.99**	L747_05910	xsome
914	hypothetical protein	**0.51**	-0.02	**0.30**	**0.36**	L747_05915	xsome
915	nucleotide pyrophosphohydrolase	0.10	**1.24**	**1.67**	**0.47**	L747_05920	xsome
916	GTP-binding protein YsxC	0.29	**1.35**	**1.38**	0.07	L747_05925	xsome
917	ATP-dependent protease	**0.33**	**1.98**	**0.96**	**-0.98**	L747_05930	xsome
918	producttrigger factor; tig	-0.22	-0.18	**-0.21**	0.01	L747_05935	xsome
919	elongation factor Tu; tuf	0.07	**0.50**	**1.04**	**0.58**	L747_05940	xsome
920	hypothetical protein	-0.20	-0.25	0.23	**0.51**	L747_05945	xsome
921	Zn-dependent hydrolase	**0.68**	**0.82**	**0.51**	**-0.27**	L747_05950	xsome
922	30S ribosomal protein S15	0.03	-0.18	**1.67**	**1.72**	L747_05955	xsome
923	30S ribosomal protein S20	0.14	**0.71**	**2.14**	**1.44**	L747_05960	xsome
924	hypothetical protein	-0.53	-0.33	0.02	0.37	L747_05965	xsome

925	hypothetical protein	-0.25	0.14	**1.77**	**1.59**	L747_05970	xsome
926	DNA polymerase III subunit delta	0.05	-0.04	**-0.34**	**-0.26**	L747_05975	xsome
927	hypothetical protein	-0.02	**0.32**	-0.20	**-0.47**	L747_05980	xsome
928	competence protein ComE	0.04	0.04	0.21	0.20	L747_05985	xsome
929	hypothetical protein	0.17	0.15	-0.66	**-0.72**	L747_05990	xsome
930	peptidase	0.12	0.18	-0.14	**-0.28**	L747_05995	xsome
931	productphosphopantetheine adenylyltransferase	0.02	**0.88**	**0.85**	0.00	L747_06000	xsome
932	rRNA methyltransferase	0.05	**0.72**	**0.37**	**-0.32**	L747_06005	xsome
933	hypothetical protein	0.20	0.41	-0.09	**-0.46**	L747_06010	xsome
934	cell division protein FtsW	-0.16	**0.36**	-0.16	**-0.48**	L747_06015	xsome
935	GTP-binding protein	-0.22	0.21	**0.60**	**0.42**	L747_06020	xsome
936	fructose-1 6-bisphosphatase	0.25	0.15	0.01	-0.10	L747_06025	xsome
937	hypothetical protein	0.24	-0.25	**-1.88**	**-1.55**	L747_06030	xsome
938	hypothetical protein	**0.63**	**0.29**	**-0.83**	**-1.07**	L747_06035	xsome
939	dihydrolipoamide dehydrogenase	**0.52**	**2.43**	**2.56**	0.17	L747_06040	xsome
940	branched-chain alpha-keto acid dehydrogenase subunit E2	**0.39**	**1.85**	**1.88**	0.07	L747_06045	xsome
941	2-oxoisovalerate dehydrogenase subunit beta	**0.33**	**1.90**	**1.96**	0.09	L747_06050	xsome
942	productpyruvate dehydrogenase E1 subunit alpha	**0.35**	**1.09**	**1.05**	0.00	L747_06055	xsome
943	peptide deformylase; def	-0.04	0.26	**0.98**	**0.75**	L747_06060	xsome
944	hypothetical protein	0.32	-0.19	0.40	**0.62**	L747_06065	xsome
945	hypothetical protein	0.38	**1.07**	**2.06**	**1.03**	L747_06070	xsome
946	ribonuclease J	0.26	**0.80**	**1.61**	**0.85**	L747_06075	xsome
947	diacylglycerol kinase	**-0.47**	0.03	0.22	**0.22**	L747_06080	xsome
948	hypothetical protein	0.34	0.24	**1.63**	**1.29**	L747_06085	xsome
949	hypothetical protein	-0.38	**0.58**	**0.52**	-0.02	L747_06090	xsome
950	hypothetical protein	0.09	**1.27**	**1.01**	**-0.22**	L747_06095	xsome
951	hypothetical protein	-0.12	**-0.31**	**-0.75**	**-0.40**	L747_06100	xsome
952	transketolase	**-0.45**	**1.94**	**2.73**	**0.83**	L747_06105	xsome
953	transposase IS204	-0.28	-0.27	-0.11	0.20	L747_06110	xsome
954	ribokinase	0.04	**-2.60**	**-2.29**	**0.35**	L747_06115	xsome

955	LacI family transcription regulator	0.05	**1.26**	**0.95**	**-0.27**	L747_06120	xsome
956	L-glyceraldehyde 3-phosphate reductase	0.12	**-0.51**	-0.11	**0.44**	L747_06125	xsome
957	N-acetylmuramic acid-6-phosphate etherase	-0.13	**-1.84**	**-3.03**	**-1.13**	L747_06130	xsome
958	peptide ABC transporter substrate-binding protein	-0.17	0.07	**-0.94**	**-0.97**	L747_06135	xsome
959	amidase	-0.13	0.09	**-0.58**	**-0.62**	L747_06140	xsome
960	hypothetical protein	0.03	**-1.54**	-0.73	**0.77**	L747_06145	xsome
961	transposase	-0.27	0.07	**0.46**	**0.43**	L747_06150	xsome
962	amino acid ABC transporter substrate-binding protein	-0.21	**-3.01**	**-2.81**	0.23	L747_06155	xsome
963	amino acid ABC transporter permease	-0.19	**-2.24**	**-2.03**	0.24	L747_06160	xsome
964	membrane protein	-0.49	**-1.11**	**-1.56**	-0.39	L747_06165	xsome
965	ribose-phosphate pyrophosphokinase	0.20	-0.06	0.02	0.12	L747_06170	xsome
966	hypothetical protein	**0.63**	**0.38**	**-0.44**	**-0.78**	L747_06175	xsome
967	hypothetical protein	0.10	-0.19	**-1.41**	**-1.16**	L747_06180	xsome
968	phosphoglycerate mutase	-0.27	**0.64**	0.31	**-0.30**	L747_06185	xsome
969	thiouridylase	**0.28**	**1.19**	**1.25**	0.10	L747_06190	xsome
970	cysteine desulfurase	0.06	**0.63**	-0.12	**-0.71**	L747_06195	xsome
971	cysteine desulfurase	0.13	**0.88**	**0.41**	**-0.42**	L747_06200	xsome
972	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase	0.13	**1.17**	**0.51**	**-0.62**	L747_06205	xsome
973	hypothetical protein	-0.13	**0.47**	-0.23	**-0.65**	L747_06210	xsome
974	ADP-ribose pyrophosphatase	0.28	**1.37**	**0.90**	**-0.43**	L747_06215	xsome
975	cold-shock protein	-0.08	**0.95**	**0.54**	**-0.37**	L747_06220	xsome
976	isoleucyl-tRNA synthetase; ileS	0.22	-0.09	**0.31**	**0.44**	L747_06225	xsome
977	cell division protein DivIVA	**0.60**	**0.46**	0.19	**-0.23**	L747_06230	xsome
978	RNA-binding protein	**0.70**	0.18	**-0.68**	**-0.81**	L747_06235	xsome
979	cell division protein	**0.59**	**0.34**	**-0.56**	**-0.85**	L747_06240	xsome
980	cell division protein SepF	**0.54**	**0.60**	-0.06	**-0.61**	L747_06245	xsome
981	cell division protein FtsZ	0.26	**0.44**	-0.09	**-0.49**	L747_06250	xsome
982	cell division protein FtsA	0.13	**-0.37**	**-1.01**	**-0.60**	L747_06255	xsome
983	hypothetical protein	**0.42**	**0.78**	**0.31**	**-0.42**	L747_06260	xsome

984	UDP-diphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	**0.59**	**0.65**	0.00	**-0.61**	L747_06265	xsome
985	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase; murD	**0.61**	0.20	**-0.63**	**-0.78**	L747_06270	xsome
986	phospho-N-acetylmuramoyl-pentapeptide-transferase	**0.37**	**0.85**	**0.39**	**-0.41**	L747_06275	xsome
987	penicillin-binding protein 2B	**-0.64**	0.21	-0.16	**-0.33**	L747_06280	xsome
988	hypothetical protein	**-0.84**	**-1.65**	**-1.86**	-0.16	L747_06285	xsome
989	16S rRNA methyltransferase	-0.27	**-0.77**	**-0.64**	0.17	L747_06290	xsome
990	cell division protein MraZ	-0.10	-0.24	0.23	**0.50**	L747_06295	xsome
991	hypothetical protein	-0.60	**-1.00**	-0.25	**0.75**	L747_06300	xsome
992	hypothetical protein	-0.51	**-0.57**	0.42	**1.01**	L747_06305	xsome
993	cell division protein FtsK	-0.16	**-0.71**	**-0.72**	0.03	L747_06310	xsome
994	RNA methyltransferase	-0.43	**-1.62**	**-1.61**	0.06	L747_06315	xsome
995	23S rRNA methyltransferase	-0.39	-0.25	**-0.40**	-0.12	L747_06320	xsome
996	membrane protein	-0.09	0.08	0.22	0.18	L747_06325	xsome
997	hypothetical protein	-0.69	0.39	**1.06**	**0.69**	L747_06330	xsome
998	hypothetical protein	0.09	0.61	-1.48	**-1.71**	L747_06335	xsome
999	poly(glycerol-phosphate) alpha-glucosyltransferase	0.18	**-1.64**	**-1.60**	0.09	L747_06340	xsome
1000	poly(glycerol-phosphate) alpha-glucosyltransferase; distrupted(psuedo)	0.09	**-0.99**	**-0.99**	0.05	L747_06345	xsome
1001	hypothetical protein	0.17	-0.60	-0.43	0.19	L747_06350	xsome
1002	hypothetical protein	-0.61	-1.24	-0.23	**0.90**	L747_06355	xsome
1003	23S rRNA pseudouridine synthase	0.16	-0.14	**-0.48**	**-0.29**	L747_06360	xsome
1004	inorganic polyphosphate/ATP-NAD kinase	**0.72**	**0.83**	**0.72**	-0.08	L747_06365	xsome
1005	GTP pyrophosphokinase	**0.78**	**0.81**	**0.74**	-0.03	L747_06370	xsome
1006	hypothetical protein	-0.39	**1.01**	**1.04**	0.06	L747_06375	xsome
1007	hypothetical protein; dirstrupted(psuedo)	-0.51	**-0.83**	0.19	**1.05**	L747_13690	xsome
1008	hypothetical protein	-0.28	**-0.81**	-0.13	**0.68**	L747_06380	xsome
1009	hypothetical protein	-0.44	-0.37	-0.22	0.17	L747_06385	xsome
1010	oligopeptidase PepB	-0.36	**2.82**	**2.29**	**-0.49**	L747_06390	xsome

1011	hypothetical protein	0.07	0.18	0.58	**0.42**	L747_06395	xsome
1012	tRNA (guanine-N(7)-)-methyltransferase	0.03	-0.18	-0.06	0.16	L747_06400	xsome
1013	phosphotransferase	0.21	**0.92**	**1.45**	**0.56**	L747_06405	xsome
1014	hypothetical protein	0.05	**0.50**	**0.32**	-0.14	L747_06410	xsome
1015	multidrug ABC transporter ATP-binding protein	0.06	**0.89**	**0.72**	-0.13	L747_06415	xsome
1016	histidine triad protein	-0.40	**0.91**	**0.78**	-0.09	L747_06420	xsome
1017	hypothetical protein	-0.29	**1.13**	**0.92**	-0.17	L747_06425	xsome
1018	peptidylprolyl isomerase	**0.55**	**2.07**	**2.92**	**0.88**	L747_06430	xsome
1019	3'-5' exoribonuclease	0.16	**-0.61**	**-0.80**	-0.15	L747_06435	xsome
1020	hypothetical protein	-0.07	**-0.62**	**-1.10**	**-0.43**	L747_06440	xsome
1021	hypothetical protein	0.21	0.18	0.03	-0.11	L747_06445	xsome
1022	hypothetical protein	**0.35**	-0.05	0.01	0.09	L747_06450	xsome
1023	penicillin-binding protein 1A	0.19	**1.27**	**1.07**	-0.16	L747_06455	xsome
1024	ArgR family transcriptional regulator	-0.32	**-0.67**	0.03	**0.73**	L747_06460	xsome
1025	arginyl-tRNA synthase	0.04	**-0.89**	**-1.10**	-0.17	L747_06465	xsome
1027	hypothetical protein	-0.25	**1.05**	**1.05**	0.05	L747_06475	xsome
1028	N-acetylmuramoyl-L-alanine amidase	**-0.67**	**1.15**	**2.34**	**1.22**	L747_06480	xsome
1029	hypothetical protein	-0.17	**1.10**	**1.79**	**0.69**	L747_06485	xsome
1030	hypothetical protein	**0.61**	0.15	0.31	0.19	L747_06490	xsome
1031	hypothetical protein	0.22	**-1.35**	**-1.68**	-0.29	L747_06495	xsome
1032	phosphoenolpyruvate-protein phosphotransferase	-0.17	**-1.10**	**-1.99**	**-0.83**	L747_06500	xsome
1033	transketolase	-0.19	**-1.35**	**-2.49**	**-1.08**	L747_06505	xsome
1034	PTS ascorbate transporter subunit IIC	**-0.59**	**-1.47**	**-2.95**	**-1.41**	L747_06510	xsome
1035	hypothetical protein	-0.31	**-1.80**	**-2.79**	**-0.86**	L747_06515	xsome
1036	hypothetical protein	-0.39	**-1.27**	**-2.54**	**-1.18**	L747_06520	xsome
1037	integrase	0.14	-0.04	**1.34**	**1.40**	L747_06525	xsome
1038	aspartate racemase	0.01	**0.28**	**0.43**	**0.19**	L747_06530	xsome
1039	carboxylate--amine ligase	-0.12	0.05	0.11	0.09	L747_06535	xsome
1040	asparagine synthase	**-0.32**	0.07	0.11	0.08	L747_06540	xsome
1041	hypothetical protein	0.65	0.29	-0.36	**-0.61**	L747_06545	xsome
1042	peptidase C69	**-0.73**	**-0.34**	-0.04	**0.33**	L747_06550	xsome

1043	hypothetical protein	**0.36**	**0.54**	**0.52**	0.02	L747_06555	xsome
1044	hypothetical protein	0.39	-0.39	0.92	**1.15**	L747_06560	xsome
1045	hypothetical protein	-0.10	-0.52	-0.03	**0.52**	L747_06565	xsome
1046	hypothetical protein	-0.65	**-1.40**	-0.44	**0.94**	L747_06570	xsome
1047	integrase	0.03	**-0.38**	**0.88**	**1.29**	L747_06575	xsome
1048	hypothetical protein	0.26	**-1.38**	**-2.41**	**-0.94**	L747_06580	xsome
1049	excinuclease ABC subunit A	0.01	-0.21	0.17	**0.42**	L747_06585	xsome
1051	hypothetical protein	0.35	**-0.96**	**-3.84**	**-2.49**	L747_06595	xsome
1052	hypothetical protein	0.15	**-1.38**	**-4.13**	**-2.54**	L747_06600	xsome
1053	MarR family transcriptional regulator	0.25	**1.87**	**1.92**	0.09	L747_06605	xsome
1054	hypothetical protein	0.45	-0.03	**-0.84**	**-0.76**	L747_06610	xsome
1055	hypothetical protein	-0.05	**-0.94**	**-2.00**	**-0.99**	L747_06615	xsome
1056	2-hydroxyacid dehydrogenase	0.28	**1.07**	**1.00**	-0.03	L747_06620	xsome
1057	kojibiose phosphorylase	0.09	**-0.83**	**-0.36**	**0.50**	L747_06625	xsome
1058	beta-phosphoglucomutase	**0.62**	-0.17	0.04	**0.25**	L747_06630	xsome
1059	HAD family hydrolase	-0.06	**0.53**	0.23	**-0.27**	L747_06635	xsome
1060	productdeoxyadenosine kinase	**-1.20**	**-0.97**	**-1.01**	0.00	L747_06640	xsome
1061	GTP cyclohydrolase	**0.84**	**1.52**	**1.56**	0.08	L747_06645	xsome
1062	multidrug MFS transporter	0.40	**1.70**	**1.51**	-0.15	L747_06650	xsome
1063	S-adenosylmethionine synthetase	-0.02	**2.13**	**2.33**	**0.24**	L747_06655	xsome
1064	hypothetical protein	-0.55	**-0.54**	0.17	**0.74**	L747_06660	xsome
1065	hypothetical protein	**0.97**	**2.02**	**2.93**	**0.94**	L747_06665	xsome
1066	hypothetical protein	0.47	-0.27	-0.23	0.07	L747_06670	xsome
1067	hypothetical protein	0.13	**-3.48**	**-4.25**	**-0.71**	L747_06675	xsome
1068	productintegrase; distrupted(psuedo)	0.20	**0.69**	**0.64**	-0.01	L747_13740	xsome
1074	glycerol phosphate lipoteichoic acid synthase	0.20	**0.67**	**0.48**	-0.15	L747_06705	xsome
1075	integrase	-0.12	**0.48**	**0.34**	-0.10	L747_06710	xsome
1076	hypothetical protein	**0.74**	-0.13	-0.04	0.13	L747_06715	xsome
1077	membrane protein	**0.56**	**-1.15**	**-1.44**	**-0.25**	L747_06720	xsome
1078	glycosyltransferase	**0.45**	**-0.27**	**-0.51**	-0.19	L747_06725	xsome
1079	glycosyl transferase	-0.03	**-0.59**	**-0.33**	**0.30**	L747_06730	xsome

1080	phosphoenolpyruvate-protein phosphotransferase	0.21	-0.03	0.15	**0.22**	L747_06735	xsome
1081	phosphocarrier protein HPr	0.12	**-0.38**	0.04	**0.46**	L747_06740	xsome
1082	hypothetical protein	0.28	**0.48**	**-0.39**	**-0.83**	L747_06745	xsome
1083	ATP-dependent Clp protease ATP-binding protein	**0.46**	**0.86**	**0.81**	-0.01	L747_06750	xsome
1084	hypothetical protein	0.06	-0.04	**0.51**	**0.58**	L747_06755	xsome
1085	ATPase	**1.88**	**4.13**	**4.04**	-0.06	L747_06760	xsome
1086	NADH oxidase	-0.16	**-2.20**	**-2.55**	**-0.31**	L747_06765	xsome
1087	hypothetical protein	-0.60	-0.18	**-1.40**	**-1.09**	L747_06770	xsome
1088	peptide chain release factor 3	0.01	**-0.46**	**-0.55**	-0.05	L747_06775	xsome
1089	hypothetical protein	-0.06	0.24	**0.45**	0.25	L747_06780	xsome
1090	glycosyl transferase	-0.12	**-1.01**	**-0.74**	**0.30**	L747_06785	xsome
1091	hypothetical protein	**-0.38**	**-0.50**	**-0.30**	**0.25**	L747_06790	xsome
1092	glycerol-3-phosphate cytidylyltransferase	-0.22	**0.32**	**0.32**	0.04	L747_06795	xsome
1093	membrane protein	**-0.43**	**0.49**	**0.70**	**0.24**	L747_06800	xsome
1094	hypothetical protein	**0.39**	0.01	**0.38**	**0.40**	L747_06805	xsome
1095	laaC	-0.60	0.07	**1.03**	**0.98**	L747_06810	xsome
1096	hypothetical protein	**0.85**	**1.59**	**1.83**	**0.27**	L747_06815	xsome
1097	recombination protein RecX	**0.89**	**1.03**	**0.28**	**-0.71**	L747_06820	xsome
1098	flavodoxin	0.15	**-0.67**	-0.17	**0.52**	L747_06825	xsome
1099	ribonuclease BN	-0.15	-0.16	**-0.71**	**-0.50**	L747_06830	xsome
1100	hypothetical protein	0.14	**-0.70**	**-1.43**	**-0.68**	L747_06835	xsome
1101	UDP-N-acetylglucosamine 2-epimerase	-0.16	0.08	**-0.39**	**-0.43**	L747_06840	xsome
1102	bactoprenol glucosyl transferase	**-0.73**	**0.71**	**1.67**	**0.99**	L747_06845	xsome
1103	gluconate permease	**0.36**	**-0.56**	**0.38**	**0.97**	L747_06850	xsome
1104	universal stress protein UspA	0.08	**-0.33**	**-1.37**	**-0.98**	L747_06855	xsome
1105	hypothetical protein	0.10	**-2.96**	**-4.31**	**-1.14**	L747_06860	xsome
1107	helicase	0.09	**0.73**	**0.60**	-0.09	L747_06870	xsome
1108	oxidoreductase	-0.11	0.21	0.29	0.11	L747_06875	xsome
1109	hypothetical protein	**0.96**	**1.98**	**1.92**	-0.01	L747_06880	xsome
1110	hypothetical protein	**0.42**	**-1.44**	**-1.74**	-0.26	L747_06885	xsome
1111	RpiR family transcriptional regulator	0.29	**-3.49**	**-3.44**	0.09	L747_06890	xsome

1112	gluconate permease	-0.22	**-1.08**	**-0.76**	**0.36**	L747_06895	xsome
1113	amino acid permease	0.09	**1.05**	**1.29**	**0.27**	L747_06900	xsome
1114	sugar:proton symporter	**-0.54**	**-4.95**	**-6.32**	**-1.31**	L747_06905	xsome
1115	ribose pyranase	**-0.70**	**-4.86**	**-6.57**	**-1.63**	L747_06910	xsome
1116	gamma-glutamylcysteine synthetase; distrupted(psuedo)	0.31	**0.41**	0.16	**-0.21**	L747_13650	xsome
1117	pyrimidine-nucleoside phosphorylase	-0.08	**-0.94**	**-1.94**	**-0.93**	L747_06920	xsome
1118	DeoR family transcriptional regulator	-0.12	**-1.68**	**-3.17**	**-1.41**	L747_06925	xsome
1119	purine nucleoside phosphorylase	-0.27	**-1.38**	**-1.83**	**-0.40**	L747_06930	xsome
1120	phosphopentomutase	0.00	**-1.74**	**-2.35**	**-0.55**	L747_06935	xsome
1121	deoxyribose-phosphate aldolase	0.16	**-3.25**	**-4.26**	**-0.94**	L747_06940	xsome
1122	integrase	-0.09	**-0.35**	**0.82**	**1.20**	L747_06945	xsome
1123	XRE family transcriptional regulator	-0.36	-0.43	-0.01	0.41	L747_06950	xsome
1124	hypothetical protein	-0.47	-0.41	-0.37	0.08	L747_06955	xsome
1125	hypothetical protein	-0.26	**-1.45**	**-1.76**	-0.25	L747_06960	xsome
1126	NADH:flavin oxidoreductase	**-0.80**	**-2.80**	**-3.54**	**-0.63**	L747_06965	xsome
1127	propanediol utilization protein PduU	-0.60	**-3.07**	**-3.85**	-0.42	L747_06970	xsome
1128	acetate kinase	-0.47	**-2.37**	**-2.95**	-0.49	L747_06975	xsome
1129	alcohol dehydrogenase	-0.46	**-2.02**	**-2.64**	-0.53	L747_06980	xsome
1130	aldehyde dehydrogenase	**-0.61**	**-2.26**	**-2.75**	-0.42	L747_06985	xsome
1131	ATP:cob(I)alamin adenosyltransferase	-0.13	-0.98	**-1.50**	-0.38	L747_06990	xsome
1132	ATP--cobalamin adenosyltransferase	-0.78	**-2.50**	**-3.36**	**-0.70**	L747_06995	xsome
1133	ethanolamine utilization protein EutN	-0.40	**-3.52**	**-5.41**	**-1.29**	L747_07000	xsome
1134	propanediol utilization protein	-0.37	**-2.53**	**-3.67**	-0.82	L747_07005	xsome
1135	propanediol utilization phosphotransacylase	-0.56	**-1.83**	**-2.76**	-0.74	L747_07010	xsome
1136	productcarboxysome shell protein	-0.59	**-2.50**	**-2.62**	-0.03	L747_07015	xsome
1137	carboxysome shell protein	-0.10	**-2.95**	**-3.67**	-0.55	L747_07020	xsome
1138	propanediol dehydratase reactivation protein PduH	-0.25	**-2.93**	**-2.06**	0.49	L747_07025	xsome
1139	glycerol dehydratase	-0.37	**-2.66**	**-3.06**	-0.32	L747_07030	xsome
1140	propanediol dehydratase small subunit; pduE	-0.22	**-2.10**	**-2.59**	-0.30	L747_07035	xsome

1141	propanediol dehydratase	-0.35	**-2.81**	**-3.98**	-0.82	L747_07040	xsome
1142	propanediol dehydratase large subunit; pduC	-0.16	**-2.44**	**-3.29**	**-0.69**	L747_07045	xsome
1143	propanediol utilization protein PduB	-0.01	**-3.93**	**-4.45**	-0.32	L747_07050	xsome
1144	carboxysome shell protein	0.15	**-2.93**	**-2.77**	0.18	L747_07055	xsome
1145	hypothetical protein	-0.14	**-4.20**	**-3.98**	0.25	L747_07060	xsome
1146	glycerol transporter	**-0.83**	**-2.67**	**-3.02**	-0.28	L747_07065	xsome
1147	hypothetical protein	**-0.88**	**-4.63**	**-6.02**	**-1.10**	L747_07070	xsome
1148	RNA methyltransferase	-0.17	**-0.78**	0.18	**0.99**	L747_07075	xsome
1149	lipid kinase	0.17	**-0.54**	-0.20	**0.38**	L747_07080	xsome
1150	aspartyl/glutamyl-tRNA amidotransferase subunit B	0.13	**-1.01**	**-0.96**	0.10	L747_07085	xsome
1151	aspartyl/glutamyl-tRNA amidotransferase subunit A; gatA	-0.12	**-0.59**	**-0.42**	**0.21**	L747_07090	xsome
1152	glutamyl-tRNA amidotransferase subunit C	-0.13	**-0.58**	-0.19	**0.43**	L747_07095	xsome
1153	calcium-transporting ATPase	-0.11	0.10	**0.42**	**0.36**	L747_07100	xsome
1154	DNA ligase LigA	0.00	-0.08	-0.15	-0.04	L747_07105	xsome
1155	ATP-dependent DNA helicase PcrA	**-0.30**	**-0.66**	**-1.27**	**-0.57**	L747_07110	xsome
1156	phosphoribosylaminoimidazole carboxylase	**-0.48**	-0.27	-0.24	0.07	L747_07115	xsome
1157	xanthine phosphoribosyltransferase	-0.35	**-0.59**	-0.32	**0.30**	L747_07120	xsome
1158	1,4-dihydroxy-2-naphthoate prenyltransferase	-0.50	**-1.27**	**-1.54**	-0.23	L747_07125	xsome
1159	geranylgeranyl pyrophosphate synthase	-0.31	-0.24	**0.48**	**0.76**	L747_07130	xsome
1160	hypothetical protein	-0.06	0.78	**2.23**	**1.13**	L747_07135	xsome
1161	cysteine ABC transporter ATP-binding protein	-0.15	**-1.15**	**-1.00**	0.18	L747_07140	xsome
1162	hypothetical protein	-0.35	**-1.31**	**-1.07**	0.27	L747_07145	xsome
1163	cytochrome C oxidase assembly protein	-0.53	**-0.53**	-0.28	**0.28**	L747_07150	xsome
1164	cytochrome D ubiquinol oxidase subunit I	-0.40	**-1.21**	**-1.56**	**-0.30**	L747_07155	xsome
1165	hypothetical protein	**-0.60**	-0.16	**0.39**	**0.58**	L747_07160	xsome
1166	hypothetical protein	0.66	0.45	**0.81**	0.38	L747_07165	xsome
1167	LytR family transcriptional regulator	-0.62	**-1.54**	**-1.64**	-0.04	L747_07170	xsome
1168	hypothetical protein	-0.30	**-1.78**	**-2.28**	-0.36	L747_07175	xsome
1169	integrase	0.29	**0.48**	**1.17**	**0.72**	L747_07180	xsome

1170	integrase	-0.02	**-0.30**	**0.90**	**1.23**	L747_07185	xsome
1171	hypothetical protein	0.09	0.07	**-0.96**	**-0.95**	L747_07190	xsome
1172	hypothetical protein	0.07	-0.32	-0.05	0.29	L747_07195	xsome
1173	hypothetical protein	-0.06	0.39	1.57	**1.04**	L747_07200	xsome
1174	hypothetical protein	-0.23	0.47	**1.94**	**1.47**	L747_07205	xsome
1175	hypothetical protein	-0.17	**-0.97**	-0.21	**0.76**	L747_07210	xsome
1176	transposase; distrupted(psuedo)	0.12	**0.47**	0.31	-0.12	L747_13745	xsome
1177	hypothetical protein	-0.05	-0.34	1.27	**1.35**	L747_07215	xsome
1178	multidrug MFS transporter; distrupted(psuedo)	-0.22	-0.80	0.14	**0.91**	L747_13735	xsome
1179	hypothetical protein	-0.14	-0.99	0.11	**1.00**	L747_07220	xsome
1180	hypothetical protein	0.04	-0.52	0.03	0.32	L747_07225	xsome
1181	tyrosine protein phosphatase	-0.16	**-1.49**	0.51	**1.76**	L747_07230	xsome
1182	hypothetical protein	-0.13	0.34	1.32	**0.85**	L747_07235	xsome
1183	hypothetical protein	0.00	-0.23	0.72	0.77	L747_07240	xsome
1184	LytR family transcriptional regulator	0.13	**2.55**	**1.81**	**-0.70**	L747_07245	xsome
1185	lipoate-protein ligase A	0.12	**2.77**	**1.06**	**-1.65**	L747_07250	xsome
1186	amino acid permease	-0.45	**3.94**	**2.97**	**-0.92**	L747_07255	xsome
1187	productasparagine synthase	-0.26	**3.88**	**3.84**	0.01	L747_07260	xsome
1188	6,27-dimethyl-8-ribityllumazine synthase	0.16	-0.23	**-0.92**	**-0.61**	L747_07265	xsome
1189	3,4-dihydroxy-2-butanone 4-phosphate synthase	0.36	0.18	0.03	-0.11	L747_07270	xsome
1190	riboflavin synthase subunit alpha	0.22	0.68	0.39	-0.25	L747_07275	xsome
1191	pyrimidine reductase	0.14	**1.04**	**1.24**	0.23	L747_07280	xsome
1192	potassium transporter Trk	0.54	-0.10	-0.28	-0.13	L747_07285	xsome
1193	V-type sodium ATP synthase subunit J	0.19	0.27	0.38	0.15	L747_07290	xsome
1194	mannose-6-phosphate isomerase	0.00	-0.80	-0.53	0.30	L747_07295	xsome
1195	30S ribosomal protein S9	**0.41**	**1.41**	**2.13**	**0.75**	L747_07300	xsome
1196	50S ribosomal protein L13	0.20	**1.12**	**2.14**	**1.05**	L747_07305	xsome
1197	tRNA pseudouridine synthase A	**0.53**	**-0.41**	**-0.97**	**-0.52**	L747_07310	xsome
1198	cobalt ABC transporter ATP-binding protein	0.37	**-0.57**	**-1.16**	**-0.55**	L747_07315	xsome
1199	cobalt ABC transporter ATP-binding protein; cbiO	**0.52**	**0.60**	0.15	**-0.40**	L747_07320	xsome

	cobalt ABC transporter ATP-binding protein;						
1200	cbiO	0.35	-0.10	**-0.77**	**-0.62**	L747_07325	xsome
1201	50S ribosomal protein L17	0.15	**-0.40**	**-0.53**	-0.09	L747_07330	xsome
1202	DNA-directed RNA polymerase subunit alpha	0.08	**-0.45**	**-0.65**	-0.15	L747_07335	xsome
1203	30S ribosomal protein S11	0.06	**-0.90**	**-1.28**	**-0.33**	L747_07340	xsome
1204	30S ribosomal protein S13	0.01	**-1.39**	**-1.91**	**-0.47**	L747_07345	xsome
1205	50S ribosomal protein L36; rpmJ	0.17	**-1.42**	**-2.38**	**-0.90**	L747_07350	xsome
1206	translation initiation factor IF-1; infA	-0.04	**-1.51**	**-2.20**	**-0.64**	L747_07355	xsome
1207	adenylate kinase	-0.30	**-0.53**	-0.23	**0.33**	L747_07360	xsome
1208	preprotein translocase subunit SecY	**-0.28**	**-0.65**	**-0.73**	-0.04	L747_07365	xsome
1209	50S ribosomal protein L15	**-0.27**	**-0.35**	**-0.43**	-0.04	L747_07370	xsome
1210	50S ribosomal protein L30	**-0.36**	**-1.64**	**-2.34**	**-0.64**	L747_07375	xsome
1211	30S ribosomal protein S5	-0.21	**-1.25**	**-1.80**	**-0.51**	L747_07380	xsome
1212	50S ribosomal protein L18	**-0.27**	**-1.34**	**-1.59**	-0.20	L747_07385	xsome
1213	50S ribosomal protein L6	**-0.29**	**-0.85**	**-1.33**	**-0.43**	L747_07390	xsome
1214	30S ribosomal protein S8	**-0.40**	**-1.12**	**-1.51**	**-0.35**	L747_07395	xsome
1215	30S ribosomal protein S14; rpsN	-0.04	**-1.10**	**-1.71**	**-0.56**	L747_07400	xsome
1216	50S ribosomal protein L5	-0.19	**-0.99**	**-1.44**	**-0.41**	L747_07405	xsome
1217	50S ribosomal protein L24	-0.16	**-1.16**	**-1.62**	**-0.41**	L747_07410	xsome
1218	50S ribosomal protein L14	-0.22	**-0.92**	**-1.36**	**-0.39**	L747_07415	xsome
1219	30S ribosomal protein S17	**-0.26**	**-0.84**	**-1.27**	**-0.38**	L747_07420	xsome
1220	50S ribosomal protein L29	-0.11	**-1.40**	**-2.42**	**-0.97**	L747_07425	xsome
1221	50S ribosomal protein L16	-0.04	**-0.45**	**-0.84**	**-0.35**	L747_07430	xsome
1222	30S ribosomal protein S3	-0.20	**-1.17**	**-1.63**	**-0.42**	L747_07435	xsome
1223	50S ribosomal protein L22	0.13	**-1.24**	**-2.02**	**-0.73**	L747_07440	xsome
1224	30S ribosomal protein S19	-0.10	**-0.58**	**-0.74**	-0.12	L747_07445	xsome
1225	50S ribosomal protein L2; rplB	-0.16	**-0.42**	**-0.60**	-0.13	L747_07450	xsome
1226	50S ribosomal protein L23	-0.09	**-1.80**	**-2.59**	**-0.74**	L747_07455	xsome
1227	50S ribosomal protein L4	-0.14	**0.61**	**0.73**	0.16	L747_07460	xsome
1228	50S ribosomal protein L3	-0.16	**-1.36**	**-1.84**	**-0.43**	L747_07465	xsome
1229	30S ribosomal protein S10	-0.11	**-0.26**	**-0.98**	**-0.67**	L747_07470	xsome

1230	producthypothetical protein	-0.01	0.66	0.65	0.01	L747_07475	xsome
1231	transposase	-0.08	-0.01	**0.45**	**0.49**	L747_07480	xsome
1232	multidrug transporter MatE	-0.29	-0.04	-0.33	-0.25	L747_07485	xsome
1233	elongation factor P; fusA	-0.03	-0.17	**0.23**	**0.44**	L747_07490	xsome
1234	30S ribosomal protein S7	0.17	**0.93**	**1.54**	**0.64**	L747_07495	xsome
1235	30S ribosomal protein S12	0.08	**0.56**	**0.84**	**0.32**	L747_07500	xsome
1236	hypothetical protein	-0.58	-0.41	0.58	**1.01**	L747_07505	xsome
1237	DNA-directed RNA polymerase subunit beta'	**-0.56**	**-0.45**	**0.44**	**0.92**	L747_07510	xsome
1238	DNA-directed RNA polymerase subunit beta	**-0.73**	-0.02	**0.65**	**0.71**	L747_07515	xsome
1239	producthypothetical protein	0.06	0.28	0.24	0.00	L747_07520	xsome
1240	ATP-dependent Clp protease ATP-binding protein	**0.30**	**0.57**	**0.62**	0.10	L747_07525	xsome
1241	CtsR family transcriptional regulator	**0.46**	**0.35**	0.21	-0.10	L747_07530	xsome
1250	transposase IS204	-0.73	-0.43	-0.20	0.27	L747_07575	xsome
1251	seryl-tRNA synthetase	0.20	-0.12	-0.08	0.08	L747_07580	xsome
1252	deoxyadenosine kinase	**-0.95**	**-0.82**	**-0.50**	**0.35**	L747_07585	xsome
1253	gamma-aminobutyrate permease	0.74	**1.87**	**0.86**	**-0.97**	L747_07590	xsome
1254	hypothetical protein	0.44	-0.05	0.01	0.09	L747_07595	xsome
1255	lipase/esterase	-0.70	0.37	**1.03**	**0.68**	L747_07600	xsome
1256	phosphatase	0.24	**0.38**	0.22	-0.11	L747_07605	xsome
1257	membrane protein	-0.29	-0.17	**-1.40**	**-1.11**	L747_07610	xsome
1258	membrane protein	-0.37	**-0.60**	**-2.83**	**-2.10**	L747_07615	xsome
1259	Cro/Cl family transcriptional regulator	0.32	**0.31**	0.10	-0.17	L747_07620	xsome
1260	cobalt transporter	**0.75**	**-0.50**	**-1.37**	**-0.82**	L747_07625	xsome
1261	acetyltransferase	**-0.45**	**0.62**	**0.56**	-0.02	L747_07630	xsome
1262	hypothetical protein	**0.87**	**1.71**	**1.88**	0.21	L747_07635	xsome
1263	multidrug MFS transporter	**0.79**	**2.54**	**3.40**	**0.89**	L747_07640	xsome
1264	hypothetical protein	**0.86**	**2.34**	**3.18**	**0.87**	L747_07645	xsome
1265	hypothetical protein	-0.31	**1.30**	**0.44**	**-0.81**	L747_07650	xsome
1266	azaleucine resistance protein AzlC	-0.24	-0.04	**0.76**	**0.82**	L747_07655	xsome
1267	branched-chain amino acid ABC transporter	0.26	0.36	**1.66**	**1.27**	L747_07660	xsome
1268	uracil transporter	-0.03	**-1.31**	**-1.03**	**0.32**	L747_07665	xsome

1269	hypothetical protein	**1.04**	**-1.36**	**-1.33**	0.07	L747_07670	xsome
1270	GNAT family acetyltransferase	**2.09**	**-1.78**	**-2.85**	**-0.95**	L747_07675	xsome
1271	hypothetical protein	0.25	**-2.85**	**-3.70**	**-0.77**	L747_07680	xsome
1272	GNAT family acetyltransferase	**0.66**	**1.13**	**0.85**	**-0.24**	L747_07685	xsome
1273	cyclic nucleotide-binding protein	**-0.62**	**-0.33**	0.22	**0.59**	L747_07690	xsome
1274	pseudouridine synthase	**-0.85**	**-0.52**	**-0.87**	**-0.30**	L747_07695	xsome
1275	hypothetical protein	-0.65	**-1.03**	**-1.37**	-0.29	L747_07700	xsome
1276	hypothetical protein	**-0.76**	**-1.25**	-0.46	**0.80**	L747_07705	xsome
1277	hypothetical protein	-0.09	0.82	0.56	-0.23	L747_07710	xsome
1278	maltose O-acetyltransferase	-0.09	**6.12**	**5.75**	**-0.40**	L747_07715	xsome
1279	arabinose isomerase	0.58	**8.06**	**8.42**	**0.38**	L747_07720	xsome
1280	L-ribulose-5-phosphate 4-epimerase; sgbE	0.37	**7.52**	**7.55**	0.04	L747_07725	xsome
1281	ATPase	**0.79**	**7.30**	**6.85**	**-0.42**	L747_07730	xsome
1282	sugar:proton symporter	-0.03	**5.78**	**5.21**	**-0.57**	L747_07735	xsome
1283	GntR family transcriptional regulator	0.03	**-1.19**	**-1.60**	**-0.37**	L747_07740	xsome
1284	maltose O-acetyltransferase	**0.62**	**-1.04**	**-1.86**	**-0.76**	L747_07745	xsome
1285	integrase	-0.11	**0.53**	**0.31**	-0.17	L747_07750	xsome
1286	hypothetical protein	0.35	-0.47	**-0.59**	-0.08	L747_07755	xsome
1287	ArsR family transcriptional regulator	0.07	**-1.55**	**-2.20**	**-0.57**	L747_07760	xsome
1288	productalpha-N-arabinofuranosidase	0.03	0.61	0.60	0.01	L747_07765	xsome
1289	major facilitator transporter	0.12	**0.83**	**0.92**	0.11	L747_07770	xsome
1290	alpha-L-arabinofuranosidase	0.30	0.57	0.46	-0.07	L747_07775	xsome
1291	ATP-dependent DNA helicase RecQ	0.43	**0.36**	**-1.51**	**-1.80**	L747_07780	xsome
1292	Rrf2 family transcriptional regulator	-0.30	**-1.24**	**-1.79**	-0.47	L747_07785	xsome
1293	diguanylate cyclase	-0.29	**-1.02**	**-0.68**	0.36	L747_07790	xsome
1294	peptidase U34	-0.23	0.00	**0.58**	**0.61**	L747_07795	xsome
1295	amino acid APC transporter	**0.54**	**-1.09**	**-1.65**	**-0.51**	L747_07800	xsome
1296	aminopeptidase N	-0.29	**2.14**	**2.51**	**0.40**	L747_07805	xsome
1297	Fe-S cluster biosynthesis protein	0.54	**1.60**	**1.18**	**-0.39**	L747_07810	xsome
1298	alpha-galactosidase	-0.40	0.12	**-1.05**	**-1.10**	L747_07815	xsome
1299	hypothetical protein	-0.31	-0.87	**-1.04**	-0.11	L747_07820	xsome

1300	integrase	0.59	**0.81**	**1.72**	**0.93**	L747_07825	xsome
1301	hypothetical protein	-0.17	-0.60	**-1.01**	-0.35	L747_07830	xsome
1302	hypothetical protein	-0.28	-0.52	0.59	**1.00**	L747_07835	xsome
1303	GMP synthase	-0.18	-0.17	0.12	**0.32**	L747_07840	xsome
1304	hypothetical protein	0.21	**1.14**	**1.01**	-0.10	L747_07845	xsome
1305	pantothenate kinase	0.10	**-0.31**	**-1.03**	**-0.67**	L747_07850	xsome
1306	ATP-dependent DNA helicase	**-0.71**	-0.15	**-0.55**	**-0.36**	L747_07855	xsome
1307	hypothetical protein	**-1.19**	-0.41	-0.29	0.16	L747_07860	xsome
1308	fructose-2,6-bisphosphatase	-0.29	0.01	**0.74**	**0.76**	L747_07865	xsome
1309	peptide ABC transporter substrate-binding protein	0.31	**4.01**	**4.13**	0.17	L747_07870	xsome
1310	peptide ABC transporter ATP-binding protein	0.23	**3.03**	**2.67**	**-0.32**	L747_07875	xsome
1311	peptide ABC transporter permease	**-0.46**	**2.42**	**1.95**	**-0.43**	L747_07880	xsome
1312	peptide ABC transporter permease	**-0.59**	**2.28**	**2.29**	0.05	L747_07885	xsome
1313	peptide ABC transporter substrate-binding protein	**-1.23**	**1.12**	**1.46**	**0.38**	L747_07890	xsome
1314	peptide ABC transporter substrate-binding protein	-0.57	**2.67**	**2.58**	0.02	L747_07895	xsome
1315	GTP-binding protein	0.26	**0.64**	**0.69**	0.08	L747_07900	xsome
1316	glutamine ABC transporter permease	0.01	**0.90**	**0.89**	0.04	L747_07905	xsome
1317	glutamine ABC transporter permease	0.39	-0.04	**-0.62**	**-0.54**	L747_07910	xsome
1318	glutamine ABC transporter substrate-binding protein	-0.14	**0.28**	**-0.48**	**-0.71**	L747_07915	xsome
1319	arginine ABC transporter ATP-binding protein; artP	-0.11	0.00	**-0.44**	**-0.39**	L747_07920	xsome
1320	hypothetical protein	-0.26	**1.34**	**1.72**	**0.41**	L747_07925	xsome
1321	hypothetical protein	**-0.39**	0.05	**-0.44**	**-0.45**	L747_07930	xsome
1322	Fur family transcriptional regulator	**-0.58**	0.25	**-0.37**	**-0.57**	L747_07935	xsome
1323	hypothetical protein	-0.23	-0.20	**-0.72**	**-0.47**	L747_07940	xsome
1324	NUDIX hydrolase	-0.25	0.31	-0.16	**-0.43**	L747_07945	xsome
1325	amino acid permease	**-0.80**	**0.57**	**1.26**	**0.71**	L747_07950	xsome
1326	x-prolyl-dipeptidyl aminopeptidase	-0.10	**1.00**	**1.34**	**0.37**	L747_07955	xsome
1327	prolyl aminopeptidase	0.33	**1.08**	**1.43**	**0.39**	L747_07960	xsome
1328	cell surface protein	**0.71**	**1.48**	**2.13**	**0.68**	L747_07965	xsome

1329	ABC transporter ATP-binding protein	**0.45**	**0.35**	**0.35**	0.04	L747_07970	xsome
1330	glyoxalase	0.08	**0.85**	**1.11**	**0.30**	L747_07975	xsome
1331	peptidase	-0.12	**0.60**	**0.97**	**0.40**	L747_07980	xsome
1332	ABC transporter ATP-binding protein	-0.11	**1.06**	**1.37**	**0.33**	L747_07985	xsome
1333	ABC transporter permease	-0.21	**1.16**	**1.63**	**0.48**	L747_07990	xsome
1334	ABC transporter substrate-binding protein	-0.24	**1.31**	**1.33**	0.05	L747_07995	xsome
1335	hypothetical protein	0.16	**-0.41**	**-0.30**	0.14	L747_08000	xsome
1336	peptide ABC transporter substrate-binding protein	-0.19	**-2.05**	**-0.84**	**1.22**	L747_08005	xsome
1337	branched-chain amino acid ABC transporter permease	0.09	**-1.54**	**-0.64**	**0.92**	L747_08010	xsome
1338	phosphonate ABC transporter ATP-binding protein	-0.03	**-1.00**	-0.07	**0.95**	L747_08015	xsome
1339	hypothetical protein	-0.51	**-1.78**	**-1.07**	**0.70**	L747_08020	xsome
1340	hypothetical protein	-0.52	**-2.19**	**-1.85**	0.36	L747_08025	xsome
1341	producthypothetical protein	0.04	**-0.99**	**-1.06**	-0.01	L747_08030	xsome
1342	transposase	-0.01	**0.61**	**0.43**	-0.14	L747_08035	xsome
1343	hypothetical protein	-0.21	**-1.20**	**-0.88**	0.35	L747_08040	xsome
1344	hypothetical protein	-0.09	-0.98	-0.60	0.34	L747_08045	xsome
1345	hypothetical protein	**-0.94**	**-0.74**	-0.46	0.32	L747_08050	xsome
1346	hypothetical protein	0.52	0.52	0.34	-0.14	L747_08055	xsome
1347	oxidoreductase	-0.32	**0.38**	**-1.68**	**-1.99**	L747_08060	xsome
1348	membrane protein	0.40	**1.01**	**0.95**	-0.02	L747_08065	xsome
1349	hypothetical protein	**-0.64**	**2.22**	**4.14**	**1.93**	L747_08070	xsome
1350	N-acetylmuramidase	**0.65**	**2.50**	**2.45**	-0.02	L747_08075	xsome
1351	hypothetical protein	0.10	**0.46**	**1.38**	**0.95**	L747_08080	xsome
1352	choloylglycine hydrolase	-0.02	-0.41	-0.33	0.11	L747_08085	xsome
1353	diacetyl reductase	0.13	**1.48**	**0.40**	**-1.04**	L747_08090	xsome
1354	hypothetical protein	-0.69	**-1.75**	**-1.29**	0.45	L747_08095	xsome
1355	hypothetical protein	**-0.41**	**-0.44**	**0.37**	**0.85**	L747_08100	xsome
1356	multidrug MFS transporter	**-0.62**	**-0.64**	**-1.02**	**-0.33**	L747_08105	xsome
1357	PadR family transcriptional regulator	-0.49	-0.54	**-1.23**	**-0.63**	L747_08110	xsome

1358	hypothetical protein	**0.95**	-0.19	-0.01	0.22	L747_08115	xsome
1359	hydrolase	**-1.06**	**1.49**	**3.24**	**1.76**	L747_08120	xsome
1360	acetyltransferase	0.12	0.36	0.26	-0.06	L747_08125	xsome
1361	fructose-2,6-bisphosphatase	0.35	**-0.72**	**-1.22**	**-0.46**	L747_08130	xsome
1362	alkaline phosphatase	**-0.42**	**0.96**	**1.81**	**0.88**	L747_08135	xsome
1363	ferredoxin--NADP reductase	0.10	**1.93**	**1.85**	-0.04	L747_08140	xsome
1364	hypothetical protein	0.02	**1.10**	**1.03**	-0.04	L747_08145	xsome
1365	hypothetical protein	-0.10	**0.87**	**0.61**	-0.22	L747_08150	xsome
1366	inosine-uridine nucleoside N-ribohydrolase	**-0.59**	**-1.96**	**-1.78**	**0.22**	L747_08155	xsome
1367	hypothetical protein	-0.08	-0.40	**-0.61**	-0.16	L747_08160	xsome
1368	hypothetical protein	-0.36	0.15	-0.22	**-0.33**	L747_08165	xsome
1369	COF family hydrolase	0.17	0.31	-0.20	**-0.47**	L747_08170	xsome
1370	acyltransferase	0.50	**1.87**	0.24	**-1.56**	L747_08175	xsome
1371	hypothetical protein	0.69	**1.85**	0.49	**-1.30**	L747_08180	xsome
1372	hypothetical protein	0.34	**2.63**	**1.35**	**-1.23**	L747_08185	xsome
1373	transcriptional regulator	**-1.18**	**-1.16**	**-0.74**	**0.45**	L747_08190	xsome
1374	hypothetical protein	-0.42	-0.27	0.30	0.56	L747_08195	xsome
1375	chloramphenicol acetyltransferase	**-0.97**	-0.09	0.30	**0.42**	L747_08200	xsome
1376	D-alanyl-D-alanine carboxypeptidase	-0.50	**3.86**	**5.40**	**1.53**	L747_08205	xsome
1377	D-alanyl-D-alanine carboxypeptidase	-0.28	**1.04**	**0.98**	-0.02	L747_08210	xsome
1378	ABC transporter permease	-0.56	**0.69**	**1.18**	**0.52**	L747_08215	xsome
1379	hypothetical protein	**-0.75**	0.27	**1.02**	**0.78**	L747_08220	xsome
1380	hypothetical protein	-0.59	0.20	**0.73**	**0.57**	L747_08225	xsome
1381	thiaminase	0.22	**0.69**	**1.14**	**0.49**	L747_08230	xsome
1382	hypothetical protein	0.01	0.12	-0.21	**-0.28**	L747_08235	xsome
1383	hypothetical protein	0.01	0.33	**1.08**	**0.77**	L747_08240	xsome
1384	hypothetical protein	0.51	**1.90**	**1.91**	0.04	L747_08245	xsome
1385	hypothetical protein	0.18	**1.34**	**0.47**	**-0.82**	L747_08250	xsome
1386	hypothetical protein	**0.66**	**1.34**	**1.42**	0.13	L747_08255	xsome
1387	productasparaginase	-0.36	-0.17	-0.22	-0.01	L747_08260	xsome
1388	hypothetical protein	-0.13	**-1.04**	**-1.03**	0.05	L747_08265	xsome

1389	xanthine permease	-0.05	**2.73**	**3.96**	**1.26**	L747_08270	xsome
1390	ABC transporter ATPase	**-0.97**	**-1.30**	**-1.04**	0.30	L747_08275	xsome
1391	ABC transporter permease	**-1.09**	**-1.00**	**-0.50**	**0.53**	L747_08280	xsome
1393	hypothetical protein	0.50	-0.74	-0.66	0.11	L747_08290	xsome
1394	membrane protein	-0.27	**-0.64**	**-0.48**	0.19	L747_08295	xsome
1395	hypothetical protein	0.44	**-0.91**	**-2.14**	**-1.15**	L747_08300	xsome
1396	glyoxal reductase	0.01	**2.07**	**1.00**	**-1.02**	L747_08305	xsome
1397	producthypothetical protein	-0.21	0.20	0.16	0.00	L747_08310	xsome
1398	pyrimidine dimer DNA glycosylase	-0.04	0.27	-0.17	-0.39	L747_08315	xsome
1399	glutamate decarboxylase	**0.50**	**1.72**	**2.05**	**0.37**	L747_08320	xsome
1400	productCrp/Fnr family transcriptional regulator	-0.14	**0.64**	**0.60**	0.00	L747_08325	xsome
1401	lactate racemization operon protein LarA	0.37	0.46	**0.82**	0.38	L747_08330	xsome
1402	1-(5-phosphoribosyl)-5-amino-4-imidazole-carboxylate carboxylase	0.01	0.23	0.57	0.33	L747_08335	xsome
1403	hypothetical protein	-0.30	0.45	0.63	0.19	L747_08340	xsome
1404	lactate racemization operon protein LarE	0.15	0.43	0.71	0.27	L747_08345	xsome
1405	glycerol uptake permease	-0.32	0.34	**1.21**	**0.89**	L747_08350	xsome
1406	nickel transporter NixA	**-0.86**	-0.59	0.45	**1.03**	L747_08355	xsome
1407	hypothetical protein	-0.30	**2.07**	**2.15**	0.10	L747_08360	xsome
1408	hypothetical protein	-0.25	-0.63	-1.78	-0.68	L747_08365	xsome
1409	spermidine/putrescine ABC transporter ATP-binding protein	**-1.01**	-0.24	0.02	**0.30**	L747_08370	xsome
1410	spermidine/putrescine ABC transporter permease	**-1.03**	**-0.58**	-0.25	**0.37**	L747_08375	xsome
1411	spermidine/purescine ABC transporter permease	-0.23	**-0.45**	-0.25	0.23	L747_08380	xsome
1412	spermidine/putrescine ABC transporter substrate-binding protein	0.08	0.20	0.15	-0.01	L747_08385	xsome
1413	phosphate ABC transporter ATP-binding protein; pstB	-0.16	-0.41	-0.26	0.18	L747_08390	xsome
1414	phosphate ABC transporter permease	0.35	0.30	-0.06	-0.30	L747_08395	xsome
1415	phosphate ABC transporter permease	-0.16	**-1.15**	**-1.90**	-0.63	L747_08400	xsome
1416	phosphate ABC transporter substrate-binding protein	-0.54	**-0.90**	**-1.71**	**-0.73**	L747_08405	xsome

1417	hypothetical protein	**0.94**	**2.51**	**2.56**	0.09	L747_08410	xsome
1418	hypothetical protein	-0.28	-1.07	-0.95	0.16	L747_08415	xsome
1419	transposase	-0.13	0.17	**0.57**	**0.43**	L747_08420	xsome
1420	transposase IS4	-0.06	-0.22	0.01	**0.26**	L747_08425	xsome
1421	hypothetical protein	**-0.89**	**-3.53**	**-3.90**	-0.25	L747_08430	xsome
1422	ATP-dependent helicase	**-0.69**	**-1.13**	0.17	**1.33**	L747_08435	xsome
1423	hypothetical protein	-0.62	**-0.95**	0.13	**1.09**	L747_08440	xsome
1424	hypothetical protein	-0.54	-0.35	0.46	**0.84**	L747_08445	xsome
1425	hypothetical protein	0.13	**0.56**	**0.33**	-0.19	L747_08450	xsome
1426	hypothetical protein	0.14	0.16	0.39	0.27	L747_08455	xsome
1427	amino acid:proton symporter	0.05	**-0.44**	**0.81**	**1.27**	L747_08460	xsome
1428	membrane protein	-0.61	**-3.09**	0.33	**3.30**	L747_08465	xsome
1429	membrane protein	**-0.90**	**-3.77**	**-0.99**	**2.68**	L747_08470	xsome
1430	Fur family transcriptional regulator	-0.40	**-0.71**	**2.28**	**2.99**	L747_08475	xsome
1431	N-acetylmuramoyl-L-alanine amidase	**-0.54**	**2.94**	**4.16**	**1.24**	L747_08480	xsome
1432	hypothetical protein	**0.70**	0.06	**-1.09**	**-1.10**	L747_08485	xsome
1433	membrane protein	**0.78**	**1.60**	**0.97**	**-0.59**	L747_08490	xsome
1434	membrane protein	0.47	**1.39**	**0.54**	**-0.80**	L747_08495	xsome
1435	hypothetical protein	0.70	**2.26**	**4.20**	**1.93**	L747_08500	xsome
1436	universal stress protein UspA	-0.44	**-0.84**	**-0.93**	-0.06	L747_08505	xsome
1437	hypothetical protein	**-0.71**	-0.09	0.27	**0.40**	L747_08510	xsome
1438	hypothetical protein	-0.25	-0.01	0.32	0.35	L747_08515	xsome
1439	MarR family transcriptional regulator	-0.01	-0.54	-0.70	-0.10	L747_08520	xsome
1440	peptidase M13	**0.43**	**2.05**	**2.37**	**0.36**	L747_08525	xsome
1441	ATP:cob(I)alamin adenosyltransferase	-0.35	**-1.32**	**-1.09**	0.27	L747_08530	xsome
1442	transposase ISL3	-0.19	-0.58	0.20	**0.80**	L747_08540	xsome
1443	hypothetical protein	**0.69**	**0.53**	**0.92**	**0.42**	L747_08545	xsome
1444	DEAD/DEAH box helicase	**0.47**	**1.94**	**3.05**	**1.15**	L747_08550	xsome
1445	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	0.26	**0.90**	**1.35**	**0.49**	L747_08555	xsome
1446	hypothetical protein	0.14	-0.20	-0.20	0.04	L747_08560	xsome

1447	heat shock protein HtpX	0.01	-0.11	-0.22	-0.07	L747_08565	xsome
1448	membrane protein	-0.01	-0.04	-0.12	-0.04	L747_08570	xsome
1449	protein tyrosine phosphatase	-0.20	0.02	**0.99**	**0.99**	L747_08575	xsome
1450	hypothetical protein	-0.35	-0.37	-0.29	0.11	L747_08580	xsome
1451	hypothetical protein	0.19	-0.63	**-0.92**	-0.24	L747_08585	xsome
1452	producthypothetical protein	**-0.90**	**-1.58**	**-1.64**	0.00	L747_08590	xsome
1453	hypothetical protein	-0.21	-0.03	-0.22	-0.15	L747_08595	xsome
1454	hypothetical protein	**0.70**	**-0.44**	0.06	**0.54**	L747_08600	xsome
1455	hypothetical protein	**0.75**	**-0.46**	-0.05	**0.44**	L747_08605	xsome
1456	integrase	0.08	**0.63**	**0.43**	-0.16	L747_08610	xsome
1457	hypothetical protein	**1.20**	**-1.79**	**-2.60**	**-0.73**	L747_08615	xsome
1458	hypothetical protein	-0.45	**-0.62**	**-0.98**	-0.31	L747_08620	xsome
1459	hypothetical protein	0.07	**-2.12**	**-2.78**	**-0.59**	L747_08625	xsome
1460	hypothetical protein	-0.12	**-2.60**	**-3.40**	**-0.72**	L747_08630	xsome
1461	hypothetical protein	0.27	**-1.73**	**-2.87**	**-1.00**	L747_08635	xsome
1462	sortase	0.42	**1.05**	**0.31**	**-0.69**	L747_08640	xsome
1463	50S ribosomal protein L31; rpmE2	-0.01	**0.23**	**1.23**	**1.02**	L747_08645	xsome
1464	productUDP-N-acetylglucosamine 1-carboxyvinyltransferase	0.14	**1.10**	**1.06**	0.00	L747_08650	xsome
1465	transposase IS30	-0.11	-0.37	-0.16	0.24	L747_08655	xsome
1466	alpha-acetolactate decarboxylase	**0.93**	**4.73**	**4.84**	0.14	L747_08660	xsome
1467	acetolactate synthase	**0.95**	**5.55**	**5.78**	**0.26**	L747_08665	xsome
1468	transcriptional regulator	0.22	**4.72**	**6.21**	**1.50**	L747_08670	xsome
1469	hypothetical protein	-0.49	0.14	**0.69**	**0.58**	L747_08675	xsome
1470	hypothetical protein	-0.07	0.18	0.04	-0.07	L747_08680	xsome
1471	hypothetical protein	-0.34	**-1.10**	-0.78	0.34	L747_08685	xsome
1472	CTP synthetase; pyrG	-0.29	-0.04	**-0.36**	**-0.28**	L747_08690	xsome
1473	DNA-directed RNA polymerase subunit delta	0.03	**0.35**	**0.43**	0.12	L747_08695	xsome
1474	hypothetical protein	-0.08	**0.38**	0.15	-0.19	L747_08700	xsome
1475	hypothetical protein	-0.10	0.13	0.35	0.17	L747_08705	xsome
1476	lipoate--protein ligase	0.01	0.38	**0.57**	0.23	L747_08710	xsome

1477	phosphohydrolase	-0.40	**-0.45**	-0.18	**0.31**	L747_08715	xsome
1478	sugar phosphate phosphatase	-0.36	-0.13	0.21	**0.37**	L747_08720	xsome
1479	transposase ISL3	0.09	-0.22	0.45	**0.69**	L747_08725	xsome
1480	phosphoketolase	-0.02	**0.42**	**0.60**	**0.21**	L747_08730	xsome
1481	GntR family transcriptional regulator	0.38	0.09	**-0.40**	**-0.44**	L747_08735	xsome
1482	hypothetical protein	0.28	**1.93**	**1.79**	-0.11	L747_08740	xsome
1483	hypothetical protein	-0.47	**-1.66**	**-1.59**	0.12	L747_08745	xsome
1484	ribose-phosphate pyrophosphokinase	-0.09	**0.34**	**0.75**	**0.44**	L747_08750	xsome
1485	N-acetylglucosamine-1-phosphate uridyltransferase; glmU	0.11	**0.36**	0.23	-0.09	L747_08755	xsome
1486	purine operon repressor	-0.10	**0.65**	**0.63**	0.02	L747_08760	xsome
1487	hypothetical protein	-0.61	**-1.16**	-1.09	0.11	L747_08765	xsome
1488	ABC transporter	0.44	**2.07**	**3.68**	**1.41**	L747_08770	xsome
1489	ABC transporter ATPase	0.38	**1.82**	**3.36**	**1.39**	L747_08775	xsome
1490	metal ABC transporter substrate-binding protein	**0.57**	**1.70**	**3.13**	**1.26**	L747_08780	xsome
1491	alpha/beta hydrolase	**0.59**	0.41	0.39	0.02	L747_08785	xsome
1492	hypothetical protein	-0.73	**-0.89**	**-0.66**	0.27	L747_08790	xsome
1493	hypothetical protein	-0.42	-0.47	0.01	**0.50**	L747_08795	xsome
1494	hypothetical protein	-0.14	0.17	0.79	**0.63**	L747_08800	xsome
1495	hypothetical protein	-0.11	**3.44**	-1.03	**-4.27**	L747_08805	xsome
1496	hypothetical protein	-0.59	**2.74**	**-1.78**	**-4.32**	L747_08810	xsome
1497	hypothetical protein	0.34	0.40	0.01	-0.34	L747_08815	xsome
1498	6-phospho-beta-glucosidase	0.25	-0.52	**-1.07**	-0.47	L747_08820	xsome
1499	transcriptional regulator	-0.14	0.01	-0.13	-0.10	L747_08825	xsome
1500	sodium:solute symporter	0.04	0.44	0.42	0.01	L747_08830	xsome
1501	alpha-glucosidase	-0.76	-0.09	0.70	**0.80**	L747_08835	xsome
1502	uracil-DNA glycosylase	0.02	**1.08**	**1.23**	0.19	L747_08840	xsome
1503	4-diphosphocytidyl-2-methyl-D-erythritol kinase ipk	0.20	**0.72**	**0.49**	-0.19	L747_08845	xsome
1504	VEG protein	**0.63**	0.20	**0.60**	**0.43**	L747_08850	xsome
1505	16S rRNA methyltransferase	0.14	**0.61**	0.22	**-0.35**	L747_08855	xsome

1506	ribonuclease M5	0.42	**0.95**	0.11	**-0.80**	L747_08860	xsome
1507	TatD family hydrolase	0.08	**0.66**	0.03	**-0.59**	L747_08865	xsome
1508	methionyl-tRNA synthetase	0.17	**0.31**	0.11	-0.15	L747_08870	xsome
1509	hypothetical protein	0.30	**0.97**	**0.70**	**-0.23**	L747_08875	xsome
1510	hypothetical protein	**0.63**	**-0.77**	**-1.84**	**-1.01**	L747_08880	xsome
1511	NAD-dependent deacetylase	**0.37**	-0.19	**-1.34**	**-1.10**	L747_08885	xsome
1512	IpaB/EvcA family protein	**0.42**	**0.27**	**-0.36**	**-0.58**	L747_08890	xsome
1513	3-hydroxy-3-methylglutaryl-CoA reductase	**0.54**	**0.61**	0.11	**-0.46**	L747_08895	xsome
1514	exopolyphosphatase	0.26	**0.43**	0.13	**-0.26**	L747_08900	xsome
1515	tryptophanyl-tRNA synthase	0.24	0.06	**-0.35**	**-0.38**	L747_08905	xsome
1516	transposase IS30	-0.29	0.03	-0.07	-0.06	L747_08910	xsome
1517	ATP-dependent DNA helicase	**-0.69**	**1.75**	**2.17**	**0.46**	L747_08915	xsome
1518	hypothetical protein	-0.23	**-1.33**	**-2.64**	**-1.24**	L747_08920	xsome
1519	hypothetical protein	0.32	-0.18	0.55	**0.74**	L747_08925	xsome
1520	transcriptional regulator	0.14	**-1.03**	**-1.01**	0.06	L747_08930	xsome
1521	6-phospho 3-hexuloisomerase	-0.16	**-1.48**	**-2.74**	**-1.15**	L747_08935	xsome
1522	3-hexulose-6-phosphate synthase	0.14	**-1.00**	**-2.25**	**-1.13**	L747_08940	xsome
1523	2-dehydro-3-deoxygluconokinase	0.06	**-1.69**	**-2.63**	**-0.83**	L747_08945	xsome
1524	gluconate:proton symporter	-0.82	**-1.84**	**-2.46**	-0.52	L747_08950	xsome
1525	sugar phosphate isomerase	-0.08	**-2.12**	**-3.11**	-0.76	L747_08955	xsome
1526	hypothetical protein	-0.10	**-3.13**	**-4.30**	-0.59	L747_08960	xsome
1527	sodium:proton antiporter	-0.41	**-0.71**	**-1.71**	**-0.93**	L747_08965	xsome
1528	hypothetical protein	-0.48	**-1.48**	**-1.72**	-0.17	L747_08970	xsome
1529	membrane protein	-0.18	1.12	1.01	-0.11	L747_08975	xsome
1530	glutathione reductase	**0.70**	**2.37**	**1.05**	**-1.27**	L747_08980	xsome
1531	hypothetical protein	0.32	0.34	-0.38	**-0.67**	L747_08985	xsome
1532	hypothetical protein	-0.25	-0.56	-0.11	0.47	L747_08990	xsome
1533	hypothetical protein	**-0.92**	**-0.65**	**-1.08**	-0.37	L747_08995	xsome
1534	membrane protein	0.09	0.23	-0.50	**-0.68**	L747_09000	xsome
1535	hypothetical protein	0.20	**0.70**	0.46	-0.20	L747_09005	xsome
1540	tyrosyl-tRNA synthase	0.19	**-0.90**	**-1.47**	**-0.53**	L747_09030	xsome

1541	hypothetical protein	0.39	-0.24	**-0.62**	**-0.33**	L747_09035	xsome
1542	manganese transporter	**1.47**	**2.79**	0.41	**-2.04**	L747_09040	xsome
1543	hypothetical protein	-0.32	**1.79**	**3.32**	**1.51**	L747_09045	xsome
1544	hypothetical protein	0.09	-0.35	-0.28	0.11	L747_09050	xsome
1545	hypothetical protein	-0.01	-0.14	**-0.36**	-0.18	L747_09055	xsome
1546	hypothetical protein	0.14	**1.32**	**0.41**	**-0.86**	L747_09060	xsome
1547	hypothetical protein	-0.02	**0.82**	-0.33	**-1.09**	L747_09065	xsome
1548	cytochrome C oxidase subunit III	**-0.51**	**-0.84**	**0.43**	**1.29**	L747_09070	xsome
1549	nucleoside 2-deoxyribosyltransferase	**-0.62**	**-0.92**	**0.40**	**1.34**	L747_09075	xsome
1550	membrane protein	-0.16	**-1.09**	-0.18	**0.90**	L747_09080	xsome
1551	hypothetical protein	-0.68	**-1.63**	-0.23	**1.39**	L747_09085	xsome
1552	hypothetical protein	-0.03	0.58	-0.03	-0.46	L747_09090	xsome
1553	hypothetical protein	-0.71	**-1.32**	**-1.70**	-0.31	L747_09095	xsome
1554	glycerate kinase	**0.95**	**-1.10**	**-1.58**	**-0.43**	L747_09100	xsome
1555	gluconate:proton symporter	**2.22**	**-1.84**	**-2.55**	**-0.63**	L747_09105	xsome
1556	hypothetical protein	-0.23	**-1.71**	**-0.69**	**1.02**	L747_09110	xsome
1557	hypothetical protein	0.06	**-1.05**	**-1.97**	**-0.86**	L747_09115	xsome
1558	acetoin ABC transporter ATP-binding protein; distrupted(psuedo)	-0.14	**-1.53**	**-1.99**	**-0.41**	L747_09120	xsome
1559	GntR family transcriptional regulator	-0.26	**-1.66**	**-2.21**	**-0.49**	L747_09125	xsome
1560	hypothetical protein	-0.12	**-1.30**	**-1.31**	0.03	L747_09130	xsome
1561	tRNA ligase	-0.08	**-2.19**	**-3.27**	**-1.02**	L747_09135	xsome
1562	arylsulfate sulfotransferase	-0.04	**0.86**	**1.15**	**0.32**	L747_09140	xsome
1563	hypothetical protein	-0.20	**2.08**	**2.73**	**0.65**	L747_09145	xsome
1564	inosine-uridine nucleoside N-ribohydrolase	0.20	0.20	0.12	-0.04	L747_09150	xsome
1565	membrane protein	-0.02	-0.03	0.13	0.20	L747_09155	xsome
1566	hypothetical protein	**0.35**	**-5.67**	**-6.54**	**-0.81**	L747_09160	xsome
1567	Apo-citrate lyase phosphoribosyl-CoA transferase	**0.33**	**-5.41**	**-5.99**	**-0.52**	L747_09165	xsome
1568	citrate lyase subunit alpha	**0.63**	**-5.24**	**-5.41**	-0.13	L747_09170	xsome
1569	citrate lyase B chain	**0.85**	**-5.22**	**-5.42**	-0.16	L747_09175	xsome
1570	citrate lyase gamma chain	**0.94**	**-5.14**	**-5.85**	**-0.65**	L747_09180	xsome

1571	citrate ligase	**0.79**	**-5.37**	**-5.54**	-0.12	L747_09185	xsome
1572	malate permease	**0.90**	**-5.90**	**-5.56**	**0.38**	L747_09190	xsome
1573	oxaloacetate decarboxylase	**0.84**	**-4.70**	**-4.32**	**0.42**	L747_09195	xsome
1574	citrate lyase transcriptional regulator, CitI	-0.48	-0.32	0.38	**0.73**	L747_09200	xsome
1575	hypothetical protein	0.01	0.39	**1.16**	**0.79**	L747_09205	xsome
1576	heme ABC transporter ATP-binding protein	0.07	**-1.41**	**-0.35**	**1.10**	L747_09210	xsome
1577	hypothetical protein	0.24	**-0.81**	-0.54	0.30	L747_09215	xsome
1578	MFS transporter	-0.04	**0.57**	**1.08**	**0.54**	L747_09220	xsome
1579	transcriptional regulator	-0.03	**-1.62**	**-1.03**	**0.62**	L747_09225	xsome
1580	membrane protein	-0.60	-0.99	**2.65**	**3.48**	L747_09230	xsome
1581	permease; distrupted(permease)	-0.41	**-1.88**	**-0.90**	**0.93**	L747_09235	xsome
1582	productArsR family transcriptional regulator	-0.13	-0.12	-0.15	0.01	L747_09240	xsome
1583	multicopper oxidase; distrupted(psuedo)	**0.46**	0.25	**-0.35**	**-0.55**	L747_09245	xsome
1584	hypothetical protein	0.43	-0.33	**0.51**	**0.86**	L747_09250	xsome
1585	cobalt ABC transporter ATP-binding protein	-0.44	**-2.47**	**-2.88**	-0.36	L747_09255	xsome
1586	hypothetical protein	**-0.66**	**-0.94**	**-1.88**	**-0.87**	L747_09260	xsome
1587	hypothetical protein	**0.93**	**3.22**	**2.69**	**-0.48**	L747_09265	xsome
1588	transposase IS204	-0.49	-0.26	-0.13	0.17	L747_09270	xsome
1589	GntR family transcriptional regulator	-0.20	**-0.89**	-0.25	**0.66**	L747_09275	xsome
1590	hypothetical protein	-0.27	**-2.42**	**-3.11**	**-0.64**	L747_09280	xsome
1591	bacitracin ABC transporter ATP-binding protein	0.15	**-2.60**	**-3.75**	**-1.07**	L747_09285	xsome
1592	aldo/keto reductase	0.21	**2.61**	**2.45**	-0.12	L747_09290	xsome
1593	sugar MFS transporter	-0.31	0.18	0.39	0.24	L747_09295	xsome
1594	MarR family transcriptional regulator	**-0.99**	0.09	-0.41	**-0.45**	L747_09300	xsome
1595	hypothetical protein	-0.68	0.52	**0.55**	0.06	L747_09305	xsome
1596	glycosyl transferase	-0.40	**0.81**	**0.39**	**-0.38**	L747_09310	xsome
1597	Rrf2 family transcriptional regulator	-0.19	**0.62**	**-0.72**	**-1.28**	L747_09315	xsome
1598	3-ketoacyl-ACP reductase	0.25	**0.57**	-0.17	**-0.69**	L747_09320	xsome
1599	hypothetical protein	0.28	-0.06	**-0.97**	**-0.86**	L747_09325	xsome
1600	beta-xylosidase	0.32	**1.14**	**1.27**	0.15	L747_09330	xsome
1601	MFS transporter	-0.15	0.02	0.40	0.40	L747_09335	xsome

1602	short-chain dehydrogenase	-0.05	**0.76**	**0.63**	-0.10	L747_09340	xsome
1603	permease	-0.19	**0.98**	**1.50**	**0.54**	L747_09345	xsome
1604	hypothetical protein	-0.01	-0.30	-0.14	0.19	L747_09350	xsome
1605	hypothetical protein	-0.33	**0.55**	0.24	-0.27	L747_09355	xsome
1606	hypothetical protein	-0.57	-1.04	-0.23	**0.77**	L747_09360	xsome
1607	D-alanyl-D-alanine carboxypeptidase	**-2.61**	**0.44**	**2.71**	**2.28**	L747_09365	xsome
1608	ArsR family transcriptional regulator	0.04	**1.55**	**1.93**	**0.41**	L747_09370	xsome
1609	hypothetical protein	-0.11	**1.30**	**0.75**	**-0.51**	L747_09375	xsome
1610	transposase	0.03	**0.62**	**0.39**	-0.19	L747_09380	xsome
1611	transposase IS30	-0.09	0.36	0.31	-0.02	L747_09385	xsome
1612	hypothetical protein	-0.10	**-0.65**	**-0.95**	**-0.25**	L747_09390	xsome
1613	hypothetical protein	**0.36**	**0.55**	**0.45**	-0.06	L747_09395	xsome
1614	hypothetical protein	**0.51**	0.07	0.05	0.03	L747_09400	xsome
1615	hypothetical protein	**0.42**	**-0.63**	**-0.96**	**-0.28**	L747_09405	xsome
1616	aldose 1-epimerase	0.38	**2.26**	**2.91**	**0.68**	L747_09410	xsome
1617	30S ribosomal protein S14	0.06	**4.33**	**5.17**	**0.78**	L747_09415	xsome
1618	hypothetical protein	0.22	**1.36**	**2.12**	**0.79**	L747_09420	xsome
1619	maltose phosphorylase	**-1.34**	**-3.08**	**-3.69**	**-0.57**	L747_09425	xsome
1620	major facilitator transporter	**-1.64**	**-3.50**	**-3.95**	**-0.39**	L747_09430	xsome
1621	LacI family transcriptional regulator	0.29	0.20	-0.05	-0.21	L747_09435	xsome
1622	hypothetical protein	0.53	**2.83**	**2.57**	**-0.22**	L747_09440	xsome
1623	transcriptional regulator	0.38	**2.26**	**1.38**	**-0.83**	L747_09445	xsome
1624	hypothetical protein	**-0.78**	**-0.72**	**0.37**	**1.12**	L747_09450	xsome
1625	hypothetical protein	-0.24	**-2.18**	**-3.78**	**-1.49**	L747_09455	xsome
1626	sodium:proton antiporter	-0.37	**-0.79**	**-1.69**	**-0.85**	L747_09460	xsome
1627	membrane protein	**0.79**	**0.89**	**2.00**	**1.12**	L747_09465	xsome
1628	GTP pyrophosphokinase	-0.03	-0.24	-0.10	0.18	L747_09470	xsome
1629	multidrug transporter	0.57	0.30	**0.54**	**0.27**	L747_09475	xsome
1630	3-beta-hydroxysteroid dehydrogenase	-0.12	**-5.78**	**-5.11**	**0.69**	L747_09480	xsome
1631	productmembrane protein	0.46	**1.42**	**1.41**	0.01	L747_09485	xsome
1632	permease	0.00	**1.09**	0.45	**-0.60**	L747_09490	xsome

1633	hypothetical protein	-0.22	**-0.45**	**-0.70**	-0.21	L747_09495	xsome
1634	acyltransferase	-0.30	**-0.68**	**-1.00**	**-0.28**	L747_09500	xsome
1635	hypothetical protein	**-0.69**	-0.31	**-1.11**	**-0.75**	L747_09505	xsome
1636	hypothetical protein	0.31	0.42	-0.06	**-0.44**	L747_09510	xsome
1637	S-ribosylhomocysteinase	**0.69**	**-0.25**	**-0.62**	**-0.33**	L747_09515	xsome
1638	alpha/beta hydrolase	0.21	**-0.94**	**-1.34**	**-0.36**	L747_09520	xsome
1639	hypothetical protein	0.23	**0.67**	**0.97**	**0.34**	L747_09525	xsome
1640	hypothetical protein	-0.50	-0.13	**-0.80**	**-0.62**	L747_09530	xsome
1641	hypothetical protein	-0.57	-0.34	**-1.12**	**-0.72**	L747_09535	xsome
1642	hypothetical protein	-0.50	-0.13	**-0.66**	**-0.48**	L747_09540	xsome
1643	thiol-disulfide isomerase	0.21	**1.07**	0.45	**-0.57**	L747_09545	xsome
1644	NADH oxidase	-0.18	**0.27**	0.10	-0.13	L747_09550	xsome
1645	hypothetical protein	**-1.03**	**1.32**	0.05	**-1.22**	L747_09555	xsome
1646	integrase	0.24	0.14	**0.86**	**0.75**	L747_09560	xsome
1647	hypothetical protein	0.53	**2.27**	0.73	**-1.47**	L747_09565	xsome
1648	manganese transporter	**0.51**	**2.81**	**2.03**	**-0.73**	L747_09570	xsome
1649	3-beta-hydroxysteroid dehydrogenase	**0.58**	**-0.54**	**-1.62**	**-1.04**	L747_09575	xsome
1650	ketohydroxyglutarate aldolase	-0.21	-0.73	0.07	**0.75**	L747_09580	xsome
1651	2-dehydro-3-deoxygluconokinase	-0.75	**-1.46**	**-1.18**	0.30	L747_09585	xsome
1652	2-keto-3-deoxygluconate permease	-0.40	**-1.52**	-1.00	0.49	L747_09590	xsome
1653	2-deoxy-D-gluconate 3-dehydrogenase	-0.46	0.04	0.18	0.19	L747_09595	xsome
1654	product5-keto-4-deoxyuronate isomerase	-0.40	-0.19	-0.23	0.00	L747_09600	xsome
1655	hypothetical protein	-0.07	**3.70**	**4.09**	**0.40**	L747_09605	xsome
1656	sugar phosphate isomerase	-0.68	**1.46**	**1.01**	**-0.41**	L747_09610	xsome
1657	transcriptional regulator	0.29	**0.90**	**0.80**	-0.07	L747_09615	xsome
1658	succinate-semialdehyde dehdyrogenase	**1.33**	**7.31**	**7.07**	**-0.20**	L747_09620	xsome
1659	hypothetical protein	0.02	-0.01	**-0.26**	**-0.20**	L747_09625	xsome
1660	methylated-DNA--protein-cysteine methyltransferase	-0.08	-0.27	**-4.38**	**-3.95**	L747_09630	xsome
1661	NADH dehydrogenase	-0.10	**0.34**	**-1.03**	**-1.32**	L747_09635	xsome
1662	hypothetical protein	-0.54	0.48	**0.77**	0.31	L747_09640	xsome

1663	integrase	0.00	**-0.36**	**0.75**	**1.15**	L747_09650	xsome
1664	hypothetical protein	0.31	**2.15**	**3.21**	**1.02**	L747_09655	xsome
1665	hypothetical protein	-0.19	**1.81**	**2.69**	**0.90**	L747_09660	xsome
1666	hypothetical protein	0.08	**1.62**	**2.53**	**0.93**	L747_09665	xsome
1667	hypothetical protein	0.13	**4.26**	**4.07**	-0.16	L747_09670	xsome
1668	pyruvate oxidase	0.11	**3.03**	**2.84**	-0.14	L747_09675	xsome
1669	transposase IS204; distrupted(psuedo)	-0.18	0.02	0.19	0.20	L747_13705	xsome
1670	hypothetical protein	**-0.48**	**0.56**	**1.45**	**0.92**	L747_09680	xsome
1671	membrane protein	**-1.67**	**-0.48**	**0.85**	**1.36**	L747_09685	xsome
1672	hypothetical protein	0.18	**-0.81**	**-1.46**	**-0.59**	L747_09690	xsome
1673	oligo-1,6-glucosidase	0.25	**-0.92**	**-1.04**	-0.08	L747_09695	xsome
1674	multidrug ABC transporter ATP-binding protein	-0.15	**3.99**	**3.79**	-0.20	L747_09700	xsome
1675	ABC transporter permease	**-0.90**	**3.22**	**2.82**	**-0.36**	L747_09705	xsome
1676	membrane protein	-0.38	**1.15**	**2.05**	**0.92**	L747_09710	xsome
1677	hypothetical protein	0.16	**3.98**	**4.93**	**0.96**	L747_09715	xsome
1678	glycosyl hydrolase family 25	-0.39	**3.16**	**3.55**	**0.41**	L747_09720	xsome
1679	glycosyltransferase	0.22	**1.95**	**2.41**	**0.50**	L747_09725	xsome
1680	hypothetical protein	0.65	**0.58**	**1.45**	**0.89**	L747_09730	xsome
1681	plastocyanin	**0.90**	**1.44**	**2.22**	**0.81**	L747_09735	xsome
1682	peroxidase	-0.30	**0.58**	**1.10**	**0.55**	L747_09740	xsome
1683	multidrug transporter	-0.25	**2.93**	**3.81**	**0.91**	L747_09745	xsome
1684	hypothetical protein	-0.79	**-1.26**	-0.46	**0.77**	L747_09750	xsome
1685	hypothetical protein	NA	1.36	2.14	0.39	L747_09755	xsome
1686	glycine/betaine ABC transporter ATP-binding protein	**-0.39**	**-0.33**	**0.31**	**0.67**	L747_09760	xsome
1687	choline ABC transporter permease	-0.14	**0.50**	**1.09**	**0.62**	L747_09765	xsome
1688	glycine/betaine ABC transporter substrate-binding protein	-0.18	-0.23	-0.10	0.18	L747_09770	xsome
1689	amino acid ABC transporter permease	**0.52**	**0.82**	**1.25**	**0.47**	L747_09775	xsome
1690	membrane protein	-0.49	-0.38	0.06	**0.47**	L747_09780	xsome
1691	hypothetical protein	0.18	**0.43**	**1.25**	**0.85**	L747_09785	xsome

1692	tautomerase	0.34	**1.64**	**1.05**	**-0.55**	L747_09790	xsome
1693	daunorubicin resistance protein DrrC	0.39	**1.32**	0.04	**-1.23**	L747_09795	xsome
1694	membrane protein	-0.13	**2.58**	**2.08**	**-0.46**	L747_09800	xsome
1695	sodium:solute symporter	0.12	**2.21**	**1.49**	**-0.67**	L747_09805	xsome
1696	hypothetical protein	0.18	**-1.01**	**-2.40**	**-1.34**	L747_09810	xsome
1697	hypothetical protein	-0.26	**0.55**	0.22	**-0.29**	L747_09815	xsome
1698	cytidine deaminase	0.53	0.13	0.41	0.31	L747_09820	xsome
1699	signal peptidase	**0.68**	**2.09**	**2.78**	**0.72**	L747_09825	xsome
1700	isomerase	**0.77**	**1.58**	**2.10**	**0.56**	L747_09830	xsome
1701	hypothetical protein	-0.05	**0.47**	0.20	**-0.23**	L747_09835	xsome
1702	acyltransferase	0.02	0.14	0.23	0.13	L747_09840	xsome
1703	hypothetical protein	-0.59	**-1.12**	**-0.54**	**0.61**	L747_09845	xsome
1704	hypothetical protein	**-0.86**	**-1.01**	-0.03	**0.98**	L747_09850	xsome
1705	hypothetical protein	-0.35	**-0.53**	0.06	**0.61**	L747_09855	xsome
1706	hypothetical protein	-0.77	**-3.60**	**-4.70**	-0.38	L747_09860	xsome
1707	copper-binding protein	-0.17	0.25	**1.29**	**1.00**	L747_09865	xsome
1708	cation-transporting ATPase	-0.76	0.38	**1.31**	**0.96**	L747_09870	xsome
1709	2%2C5-diketo-D-gluconic acid reductase	0.27	**1.01**	**1.58**	**0.60**	L747_09875	xsome
1710	D-alanyl-D-alanine carboxypeptidase	0.14	**2.25**	**2.59**	**0.37**	L747_09880	xsome
1711	uracil phosphoribosyltransferase	**-1.06**	-0.42	-0.17	0.29	L747_09885	xsome
1712	ATPase	**-0.93**	**0.51**	**1.06**	**0.58**	L747_09890	xsome
1713	multidrug transporter	-0.20	0.25	**1.19**	**0.98**	L747_09895	xsome
1714	ferrous iron transporter A	0.24	**2.94**	**2.82**	-0.08	L747_09900	xsome
1715	maltose O-acetyltransferase	-0.04	**3.93**	**4.43**	**0.51**	L747_09905	xsome
1716	hypothetical protein	0.19	0.09	-0.05	-0.10	L747_09910	xsome
1717	peptidase C69	**0.42**	**-0.48**	**-0.81**	**-0.28**	L747_09915	xsome
1718	cation-transporting ATPase	0.08	0.21	**0.42**	**0.25**	L747_09920	xsome
1719	hypothetical protein	-0.31	**-0.73**	**-0.83**	-0.05	L747_09925	xsome
1720	hypothetical protein	**0.56**	**0.83**	**1.34**	**0.55**	L747_09930	xsome
1721	NADPH-dependent FMN reductase	-0.14	-0.12	**0.36**	**0.52**	L747_09935	xsome
1722	hypothetical protein	**-0.87**	**2.13**	**1.53**	-0.50	L747_09940	xsome

1723	glycine/betaine ABC transporter permease	-0.11	**1.10**	**0.59**	**-0.47**	L747_09945	xsome
1724	ABC transporter ATPase	-0.03	**1.07**	0.14	**-0.88**	L747_09950	xsome
1725	transcriptional regulator	-0.04	0.41	**1.05**	**0.65**	L747_09955	xsome
1726	alcohol dehydrogenase	-0.38	**-1.45**	-0.61	**0.78**	L747_09960	xsome
1727	succinyl-diaminopimelate desuccinylase	-0.20	0.01	**-0.42**	**-0.39**	L747_09965	xsome
1728	hypothetical protein	**0.90**	0.53	0.32	-0.17	L747_09970	xsome
1729	hypothetical protein	-0.07	**-2.22**	**-1.73**	0.42	L747_09975	xsome
1730	hypothetical protein	-0.18	0.26	**0.80**	**0.58**	L747_09980	xsome
1731	alanine glycine permease	**-0.40**	**-0.88**	-0.19	**0.73**	L747_09985	xsome
1732	GNAT family acetyltransferase	-0.16	**0.74**	**0.79**	0.09	L747_09990	xsome
1733	flavodoxin	-0.36	**1.37**	**0.97**	**-0.36**	L747_09995	xsome
1734	trans-2-enoyl-CoA reductase	-0.17	**1.16**	**0.75**	**-0.37**	L747_10000	xsome
1735	tRNA-dihydrouridine synthase	0.11	**2.08**	**1.34**	**-0.69**	L747_10005	xsome
1736	hypothetical protein	-0.38	**-1.53**	**-3.00**	**-1.34**	L747_10010	xsome
1737	hypothetical protein	-0.57	**-2.68**	**-4.47**	**-1.40**	L747_10015	xsome
1738	PadR family transcriptional regulator	-0.64	**-2.90**	**-4.08**	**-0.89**	L747_10020	xsome
1739	isochorismatase	-0.18	**2.26**	**2.39**	0.16	L747_10025	xsome
1740	major facilitator transporter	0.35	**0.69**	0.09	**-0.54**	L747_10030	xsome
1741	hypothetical protein	0.39	**1.30**	0.26	**-0.99**	L747_10035	xsome
1742	hypothetical protein	-0.31	-0.02	-0.47	**-0.39**	L747_10040	xsome
1743	productglycosyl hydrolase family 3	-0.31	0.15	0.11	0.00	L747_10045	xsome
1744	transposase ISL3	-0.02	-0.44	0.11	**0.57**	L747_10050	xsome
1745	hypothetical protein	-0.14	-0.17	**-0.45**	-0.24	L747_10055	xsome
1746	peptidase	-0.18	-0.07	0.05	0.15	L747_10060	xsome
1747	glycerol kinase; glpK	**0.81**	**1.13**	**-0.66**	**-1.73**	L747_10065	xsome
1748	endonuclease III	-0.13	**-1.17**	**-2.34**	**-1.08**	L747_10070	xsome
1749	hypothetical protein	**0.66**	0.19	0.04	-0.11	L747_10075	xsome
1750	glutamine amidotransferase	-0.29	**0.69**	**1.27**	**0.61**	L747_10080	xsome
1751	membrane protein	-0.08	0.30	**1.39**	**1.12**	L747_10085	xsome
1752	hypothetical protein	**-0.86**	**-0.89**	**-1.55**	**-0.61**	L747_10090	xsome
1753	hypothetical protein	**0.72**	0.02	**-0.46**	**-0.44**	L747_10095	xsome

1754	hypothetical protein	0.13	**0.90**	**0.75**	-0.11	L747_10100	xsome
1755	hydrolase	-0.31	0.03	0.44	**0.45**	L747_10105	xsome
1756	TetR family transcriptional regulator	0.35	-0.62	0.09	**0.72**	L747_10110	xsome
1757	hypothetical protein	0.28	**1.39**	**2.03**	**0.66**	L747_10115	xsome
1758	succinate-semialdehyde dehdyrogenase	**0.73**	**1.18**	**1.77**	**0.62**	L747_10120	xsome
1759	hypothetical protein	0.47	**2.14**	**2.91**	**0.79**	L747_10125	xsome
1760	adenylosuccinate lyase	-0.61	0.11	**0.61**	**0.53**	L747_10130	xsome
1761	adenylosuccinate synthetase	-0.16	**0.45**	**0.44**	0.03	L747_10135	xsome
1762	guanosine 5'-monophosphate oxidoreductase	-0.08	**0.99**	**1.13**	0.17	L747_10140	xsome
1763	hypothetical protein	**-1.74**	**-4.11**	**-5.30**	**-1.08**	L747_10145	xsome
1764	manganese transporter; distrupted(psuedo)	**3.43**	**4.89**	**3.75**	**-1.09**	L747_13605	xsome
1765	universal stress protein UspA	**2.12**	**2.43**	**2.37**	-0.03	L747_10150	xsome
1766	hypothetical protein	0.51	0.19	-0.25	-0.38	L747_10155	xsome
1767	membrane protein	-0.44	**-2.06**	**-1.51**	0.44	L747_10160	xsome
1768	hypothetical protein	-0.21	**-2.19**	**-2.06**	0.17	L747_10165	xsome
1769	hypothetical protein	0.05	**-2.73**	**-3.06**	-0.19	L747_10170	xsome
1770	hypothetical protein	-0.16	**-1.45**	**-1.88**	-0.24	L747_10175	xsome
1771	MarR family transcriptional regulator	**2.98**	**2.09**	**1.78**	-0.27	L747_10180	xsome
1772	GCN5 family N-acetyltransferase	**3.50**	**1.07**	-0.20	**-1.21**	L747_10185	xsome
1773	CoA reductase	-0.25	-0.49	**-1.14**	-0.55	L747_10190	xsome
1774	TetR family transcriptional regulator	-0.31	**0.84**	**0.75**	-0.05	L747_10195	xsome
1775	PadR family transcriptional regulator	0.36	0.11	0.25	0.18	L747_10200	xsome
1776	phenolic acid decarboxylase padC	-0.15	**1.89**	**2.06**	0.19	L747_10205	xsome
1777	hypothetical protein	0.28	0.02	-0.10	-0.08	L747_10210	xsome
1778	ADP-ribose pyrophosphatase	-0.09	0.44	0.37	-0.03	L747_10215	xsome
1779	hypothetical protein	0.38	-0.15	-0.53	-0.27	L747_10220	xsome
1780	hypothetical protein	-0.26	-0.39	-0.10	0.32	L747_10225	xsome
1781	hypothetical protein	0.01	-0.29	-0.18	0.15	L747_10230	xsome
1782	N-acetyltransferase GCN5	-0.30	**-0.86**	**-0.67**	0.23	L747_10235	xsome
1783	transposase	-0.28	**0.59**	**0.98**	**0.42**	L747_10240	xsome
1784	transposase IS4	0.04	-0.11	0.30	**0.45**	L747_10245	xsome

1785	diguanylate cyclase	**-1.52**	**0.72**	**1.53**	**0.84**	L747_10250	xsome
1786	hypothetical protein	-0.48	**-0.79**	**-1.75**	**-0.87**	L747_10255	xsome
1787	hypothetical protein	0.02	0.11	0.03	-0.04	L747_10260	xsome
1788	hypothetical protein	0.30	**1.44**	0.46	**-0.93**	L747_10265	xsome
1789	hypothetical protein	0.41	**1.13**	0.22	**-0.85**	L747_10270	xsome
1790	D-lactate dehydrogenase	0.29	-0.50	-0.22	0.32	L747_10275	xsome
1791	malate permease	-0.20	-0.59	-0.04	**0.58**	L747_10280	xsome
1792	sodium:proton antiporter	0.37	**0.52**	**4.09**	**3.57**	L747_10285	xsome
1793	hypothetical protein	0.10	**2.48**	**2.53**	0.08	L747_10290	xsome
1794	hypothetical protein	0.29	**1.04**	**2.13**	**1.11**	L747_10295	xsome
1795	glyoxal reductase	-0.25	**-0.33**	**-0.32**	0.05	L747_10300	xsome
1796	hypothetical protein	0.15	**0.70**	**0.68**	0.01	L747_10305	xsome
1797	potassium transporter Kef	0.40	**1.00**	**1.74**	**0.78**	L747_10310	xsome
1798	succinyl-diaminopimelate desuccinylase	0.31	**-0.72**	-0.20	**0.54**	L747_10315	xsome
1799	hypothetical protein	**-0.84**	**-0.91**	**-0.70**	0.25	L747_10320	xsome
1800	universal stress protein UspA	**-0.82**	**-0.62**	-0.14	**0.51**	L747_10325	xsome
1801	producthypothetical protein	-0.17	**-0.60**	**-0.65**	-0.01	L747_10330	xsome
1802	hypothetical protein	0.40	**-1.90**	**-3.67**	**-1.44**	L747_10335	xsome
1803	acetoin reductase	**0.54**	**1.45**	**1.71**	**0.30**	L747_10340	xsome
1804	major facilitator transporter	-0.65	0.26	-0.32	**-0.52**	L747_10345	xsome
1805	D-xylulose kinase	-0.17	0.09	-0.34	**-0.39**	L747_10350	xsome
1806	xylose isomerase	-0.23	**1.27**	**1.21**	-0.02	L747_10355	xsome
1807	producthypothetical protein	-0.26	-1.64	-1.77	0.02	L747_10360	xsome
1808	hypothetical protein	-0.05	0.44	0.24	-0.15	L747_10365	xsome
1809	hypothetical protein	0.16	**0.39**	**0.48**	0.13	L747_10370	xsome
1810	hypothetical protein	**0.64**	0.19	**-0.61**	**-0.76**	L747_10375	xsome
1811	lipoate-protein ligase A	-0.04	**-1.12**	**-2.28**	**-1.12**	L747_10380	xsome
1812	Rrf2 family transcriptional regulator	**0.54**	**-0.33**	0.07	**0.43**	L747_10385	xsome
1813	peptide ABC transporter ATPase	-0.50	**4.34**	**4.27**	0.05	L747_10390	xsome
1814	peptide ABC transporter substrate-binding protein	-0.46	**4.76**	**4.57**	-0.15	L747_10395	xsome
1815	peptide ABC transporter permease	-0.50	**4.71**	**4.59**	-0.09	L747_10400	xsome

1816	peptide ABC transporter permease	-0.37	**4.70**	**4.20**	**-0.45**	L747_10405	xsome
1817	peptide ABC transporter substrate-binding protein	-0.58	**5.79**	**4.98**	**-0.76**	L747_10410	xsome
1818	hypothetical protein	-0.17	**-1.34**	**-1.86**	-0.43	L747_10415	xsome
1819	hypothetical protein	-0.61	-0.89	-0.29	**0.60**	L747_10420	xsome
1820	cobalt-zinc-cadmium resistance protein	0.04	0.02	0.78	**0.75**	L747_10425	xsome
1821	universal stress protein UspA	-0.19	**-1.33**	**-0.48**	**0.88**	L747_10430	xsome
1822	pseudouridine synthase	0.12	-0.43	0.29	**0.74**	L747_10435	xsome
1823	hypothetical protein	-0.02	-0.54	-0.52	0.07	L747_10440	xsome
1824	3-oxoacyl-ACP reductase	**-0.98**	**-2.95**	**-3.35**	-0.33	L747_10445	xsome
1825	hypothetical protein	-0.01	0.77	0.33	-0.40	L747_10450	xsome
1826	hypothetical protein	0.20	0.29	-0.42	**-0.62**	L747_10455	xsome
1827	hypothetical protein	0.02	0.22	-0.61	**-0.72**	L747_10460	xsome
1828	hypothetical protein	-0.26	0.75	-0.48	**-1.06**	L747_10465	xsome
1829	hypothetical protein	-0.11	**0.99**	**0.93**	-0.03	L747_10470	xsome
1830	transcription elongation factor GreA	-0.16	0.22	0.49	0.30	L747_10475	xsome
1831	hypothetical protein	-0.48	**0.66**	**0.59**	-0.03	L747_10480	xsome
1832	GntR family transcriptional regulator	0.25	**0.84**	**0.81**	0.01	L747_10485	xsome
1833	aminoacyl-tRNA deacylase	-0.02	**1.17**	**1.27**	0.14	L747_10490	xsome
1834	hypothetical protein	-0.37	**1.16**	**1.35**	**0.22**	L747_10495	xsome
1835	hypothetical protein	**0.41**	**-4.29**	**-5.05**	**-0.69**	L747_10500	xsome
1836	DNA-binding protein	0.04	-0.04	-0.33	-0.23	L747_10505	xsome
1837	hypothetical protein	0.24	0.35	0.39	0.07	L747_10510	xsome
1838	MFS transporter	-0.09	0.02	**0.75**	**0.75**	L747_10515	xsome
1839	hypothetical protein	-0.05	**2.31**	**2.46**	0.13	L747_10520	xsome
1840	hypothetical protein	0.09	0.14	**0.67**	**0.57**	L747_10525	xsome
1841	glucuronate isomerase	-0.14	-0.43	-0.01	0.45	L747_10530	xsome
1842	hypothetical protein	-0.43	-0.60	-0.58	0.05	L747_10535	xsome
1843	major facilitator transporter	-0.08	-1.09	-0.43	0.61	L747_10540	xsome
1844	LacI family transcription regulator	0.28	**-0.41**	**-0.84**	**-0.39**	L747_10545	xsome
1845	mannonate dehydratase	0.24	**-0.53**	**-1.06**	**-0.49**	L747_10550	xsome
1846	transcriptional regulator	-0.21	**-0.63**	**-1.13**	**-0.45**	L747_10555	xsome

1847	6-phosphogluconate dehydrogenase	**-0.37**	**-3.02**	**-1.85**	**1.20**	L747_10560	xsome
1848	gluconokinase	-0.20	-0.02	**-0.44**	**-0.38**	L747_10565	xsome
1849	2-hydroxyacid dehydrogenase	-0.20	**-0.78**	**-0.96**	-0.14	L747_10570	xsome
1850	fructose-2%2C6-bisphosphatase	-0.60	**-0.92**	**-0.66**	0.29	L747_10575	xsome
1851	galactose mutarotase	-0.26	**-1.43**	**-1.29**	0.18	L747_10580	xsome
1852	transposase IS4	0.11	-0.18	0.14	**0.36**	L747_10585	xsome
1853	transposase	0.09	**0.55**	**0.85**	**0.34**	L747_10590	xsome
1854	dioxygenase	**-0.72**	**-2.15**	**-2.33**	-0.13	L747_10595	xsome
1855	mannonate dehydratase	0.09	0.04	-0.39	-0.37	L747_10600	xsome
1856	transcriptional regulator	**-0.86**	**-1.69**	**-0.87**	**0.85**	L747_10605	xsome
1857	MFS transporter	-0.51	-0.78	**-0.97**	-0.13	L747_10610	xsome
1858	hypothetical protein	-0.09	-0.14	0.18	0.25	L747_10615	xsome
1859	glucuronate isomerase	-0.38	-0.77	-0.47	0.32	L747_10620	xsome
1860	2-dehydro-3-deoxygluconokinase	-0.31	-0.77	-0.33	0.41	L747_10625	xsome
1861	ketohydroxyglutarate aldolase	-0.49	-0.97	-0.49	0.46	L747_10630	xsome
1862	Gnt-II system L-idonate transporter IdnT	**-1.07**	-0.98	**-1.13**	-0.09	L747_10635	xsome
1863	beta-D-glucuronidase	**-0.76**	**-0.90**	**-1.07**	-0.12	L747_10640	xsome
1864	productalpha-glucosidase	-0.19	-0.33	**-0.38**	-0.01	L747_10645	xsome
1865	melibiose carrier protein	-0.58	**-1.26**	**-0.84**	0.44	L747_10650	xsome
1866	glucuronate isomerase	-0.25	**-1.01**	**-0.67**	0.36	L747_10655	xsome
1867	multidrug transporter	-0.25	0.12	**0.68**	**0.58**	L747_10660	xsome
1868	phosphohydrolase	0.46	**3.53**	**4.40**	**0.88**	L747_10665	xsome
1869	ABC transporter ATP-binding protein	0.60	**4.19**	**5.52**	**1.33**	L747_10670	xsome
1870	hypothetical protein	**0.60**	**5.19**	**6.55**	**1.37**	L747_10675	xsome
1871	hypothetical protein	-0.37	**5.39**	**6.35**	**0.98**	L747_10680	xsome
1872	acetate kinase	**-0.51**	**-3.00**	**-3.48**	-0.42	L747_10685	xsome
1873	2-dehydro-3-deoxygluconokinase	0.37	**1.14**	**1.16**	0.05	L747_10690	xsome
1874	5-keto-4-deoxyuronate isomerase	0.16	0.33	0.18	-0.11	L747_10695	xsome
1875	hypothetical protein	0.03	**1.08**	0.71	-0.32	L747_10700	xsome
1876	producthypothetical protein	0.66	1.04	1.03	0.00	L747_10705	xsome
1877	aggregation promoting factor surface protein;	**-0.92**	0.12	**1.71**	**1.60**	L747_10710	xsome

	distrupted(psuedo)						
1878	peptidase M23B	**-0.48**	**4.76**	**6.01**	**1.26**	L747_10715	xsome
1879	hypothetical protein	-0.17	-0.52	0.16	0.58	L747_10720	xsome
1880	peptidase M23B	**-1.17**	**-0.44**	**0.57**	**1.04**	L747_10725	xsome
1881	producthypothetical protein	-0.14	**-3.75**	**-4.46**	NA	L747_10730	xsome
1882	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	**-0.75**	**-7.36**	**-7.33**	0.08	L747_10735	xsome
1883	glycerophosphoryl diester phosphodiesterase	**-0.95**	**-0.44**	-0.10	**0.37**	L747_10740	xsome
1884	hypothetical protein	-0.12	**2.15**	**1.68**	**-0.43**	L747_10745	xsome
1885	cation-transporting ATPase	**1.03**	**1.84**	**1.73**	-0.07	L747_10750	xsome
1886	hypothetical protein	-0.31	0.58	**1.04**	0.48	L747_10755	xsome
1887	glyoxal reductase	-0.14	**-1.02**	**-0.95**	0.12	L747_10760	xsome
1888	hypothetical protein	-0.05	-0.62	0.32	0.56	L747_10765	xsome
1889	productamino acid ABC transporter permease	0.26	**-4.00**	**-4.06**	-0.01	L747_10770	xsome
1890	molecular chaperone	-0.48	**1.43**	**2.00**	**0.59**	L747_10775	xsome
1891	hypothetical protein	**1.43**	**2.52**	**1.47**	**-1.00**	L747_10780	xsome
1892	hemolysin	0.06	**1.18**	**1.44**	**0.29**	L747_10785	xsome
1893	short-chain dehydrogenase	**0.62**	**0.57**	**-0.44**	**-0.96**	L747_10790	xsome
1894	hypothetical protein	**0.69**	0.18	-0.51	**-0.64**	L747_10795	xsome
1895	transcriptional regulator	0.45	0.33	**-0.65**	**-0.92**	L747_10800	xsome
1896	2-deoxy-D-gluconate 3-dehydrogenase	0.05	**1.35**	**-1.94**	**-3.18**	L747_10805	xsome
1897	hypothetical protein	0.21	-0.07	0.09	0.19	L747_10810	xsome
1898	DSBA oxidoreductase	0.36	**0.84**	**1.10**	**0.29**	L747_10815	xsome
1899	ABC transporter ATPase	0.39	**1.09**	**1.04**	-0.01	L747_10820	xsome
1900	ribonucleotide reductase	-0.30	**1.35**	**2.11**	**0.79**	L747_10825	xsome
1901	nitroreductase	-0.17	**1.08**	**1.71**	**0.66**	L747_10830	xsome
1902	hypothetical protein	**-0.75**	**-1.12**	**-0.81**	**0.35**	L747_10835	xsome
1903	multidrug MFS transporter	0.39	**1.25**	**1.31**	0.09	L747_10840	xsome
1904	hypothetical protein	0.19	**1.39**	**1.56**	0.19	L747_10845	xsome
1905	glycerol-3-phosphatase transporter	**-1.52**	**-3.23**	**-3.26**	0.01	L747_10850	xsome
1906	MFS transporter	-0.41	**-0.38**	**-0.74**	**-0.32**	L747_10855	xsome

1907	hypothetical protein	0.28	**2.55**	**2.56**	0.05	L747_10860	xsome
1908	aldo/keto reductase	**0.91**	**2.35**	**1.88**	**-0.43**	L747_10865	xsome
1909	productconiferyl aldehyde dehydrogenase	-0.02	-0.47	-0.51	0.01	L747_10870	xsome
1910	hypothetical protein	0.59	**-0.63**	-0.23	**0.43**	L747_10875	xsome
1911	hypothetical protein	0.05	0.25	0.49	0.28	L747_10880	xsome
1912	glucose-6-phosphate 1-dehydrogenase	0.14	**-1.53**	**-2.12**	**-0.54**	L747_10885	xsome
1913	integrase	0.10	0.34	**1.24**	**0.93**	L747_10890	xsome
1914	hypothetical protein	0.45	**-1.20**	**-0.80**	**0.44**	L747_10895	xsome
1915	hypothetical protein	**1.92**	**-2.41**	**-2.67**	-0.14	L747_10900	xsome
1916	hypothetical protein	0.72	**-1.36**	**-1.23**	0.16	L747_10905	xsome
1917	6-phosphogluconate dehydrogenase	**0.31**	**0.25**	**0.25**	0.04	L747_10910	xsome
1918	hypothetical protein	0.42	0.18	0.06	-0.08	L747_10915	xsome
1919	hypothetical protein	**0.88**	**0.81**	**0.55**	**-0.22**	L747_10920	xsome
1920	short-chain dehydrogenase	-0.50	**-0.71**	-0.43	0.31	L747_10925	xsome
1921	hypothetical protein	-0.01	**0.64**	**0.56**	-0.04	L747_10930	xsome
1922	peroxiredoxin	-0.23	**-2.45**	**0.42**	**2.87**	L747_10935	xsome
1923	proton glutamate symport protein	-0.33	**-0.92**	**-0.37**	**0.59**	L747_10940	xsome
1927	tRNA-dihydrouridine synthase	0.32	**1.00**	**0.75**	**-0.21**	L747_10960	xsome
1928	heat shock protein Hsp33	0.15	**0.74**	**0.60**	-0.10	L747_10965	xsome
1929	cell division protein FtsH	**0.49**	**0.68**	**0.28**	**-0.35**	L747_10970	xsome
1930	hypoxanthine phosphoribosyltransferase	0.21	**-0.85**	**-1.37**	**-0.48**	L747_10975	xsome
1931	hypothetical protein	0.33	**-0.50**	**-1.33**	**-0.78**	L747_10980	xsome
1932	RNA-binding protein	0.14	**0.41**	-0.08	**-0.45**	L747_10985	xsome
1933	hypothetical protein	0.05	-0.02	**0.89**	**0.94**	L747_10990	xsome
1934	hypothetical protein	0.29	**-2.16**	**-2.58**	**-0.37**	L747_10995	xsome
1935	hypothetical protein	0.06	-0.21	-0.16	0.10	L747_11000	xsome
1936	transcription-repair coupling factor	-0.09	**-0.49**	**-0.39**	0.14	L747_11005	xsome
1937	peptidyl-tRNA hydrolase	-0.46	**-1.10**	**-0.79**	**0.34**	L747_11010	xsome
1938	L-lactate dehydrogenase; ldh	-0.11	**-0.66**	-0.05	**0.65**	L747_11015	xsome
1939	hypothetical protein	**0.83**	**2.51**	**2.55**	0.07	L747_11020	xsome
1940	hypothetical protein	-0.18	-0.12	0.55	0.60	L747_11025	xsome

1941	hypothetical protein	0.38	**3.05**	**3.42**	**0.40**	L747_11030	xsome
1942	PemK family transcriptional regulator	0.48	0.26	-0.03	-0.24	L747_11035	xsome
1943	hypothetical protein	**0.56**	**-1.12**	**-1.83**	**-0.66**	L747_11040	xsome
1944	alanine racemase	0.18	**-0.62**	**-0.57**	0.08	L747_11045	xsome
1945	4'-phosphopantetheinyl transferase	**0.47**	**-1.13**	**-2.39**	**-1.20**	L747_11050	xsome
1946	hypothetical protein	-0.05	0.04	0.65	0.58	L747_11055	xsome
1947	hypothetical protein	0.03	-0.60	0.10	**0.72**	L747_11060	xsome
1948	hypothetical protein	-0.21	**-0.89**	**-1.74**	**-0.75**	L747_11065	xsome
1949	hypothetical protein	-0.27	**-1.37**	**-1.79**	-0.35	L747_11070	xsome
1950	hypothetical protein	-0.47	**-1.66**	**-1.34**	0.34	L747_11075	xsome
1951	ribonucleoside-triphosphate reductase	**-1.16**	-0.07	**1.07**	**1.16**	L747_11080	xsome
1952	hypothetical protein	-0.13	-0.53	-1.96	-0.58	L747_11085	xsome
1953	hypothetical protein	-0.06	-0.52	-0.49	0.07	L747_11090	xsome
1954	LacI family transcriptional regulator	0.43	**1.75**	**1.44**	**-0.27**	L747_11100	xsome
1955	peptidase S24	0.22	**0.94**	**0.45**	**-0.45**	L747_11105	xsome
1956	UDP-glucose 4-epimerase	0.34	**0.81**	**0.58**	-0.19	L747_11110	xsome
1957	productgalactokinase	0.42	**0.41**	0.37	-0.01	L747_11115	xsome
1958	sodium:solute symporter	0.58	**0.85**	0.07	**-0.74**	L747_11120	xsome
1959	hypothetical protein	0.34	**0.98**	-0.66	**-1.54**	L747_11125	xsome
1960	phosphoglucomutase	**0.54**	**0.39**	0.03	**-0.32**	L747_11130	xsome
1961	aldose 1-epimerase	**0.54**	**0.39**	-0.25	**-0.60**	L747_11135	xsome
1962	acyl-CoA dehydrogenase	-0.01	**-1.59**	**-2.17**	-0.45	L747_11140	xsome
1963	electron transfer flavoprotein subunit beta	-0.37	**-1.98**	**-1.48**	0.45	L747_11145	xsome
1964	electron transfer flavoprotein subunit alpha	-0.41	**-1.78**	**-1.36**	0.43	L747_11150	xsome
1965	NADPH:quinone reductase; distrupted(psuedo)	0.53	**0.98**	0.49	**-0.45**	L747_13660	xsome
1966	ArsR family transcriptional regulator	-0.38	-0.09	0.02	0.15	L747_11155	xsome
1967	peptidase C56	0.13	0.15	0.53	0.41	L747_11160	xsome
1968	phosphoenolpyruvate carboxykinase	0.23	0.07	**-0.36**	**-0.38**	L747_11165	xsome
1969	hypothetical protein	-0.40	**-1.53**	**-1.73**	-0.12	L747_11170	xsome
1970	hypothetical protein	-0.27	**-1.40**	**-1.67**	-0.19	L747_11175	xsome
1971	hypothetical protein	-0.51	-0.21	0.01	0.23	L747_11180	xsome

1972	hypothetical protein	-0.59	**-0.96**	-0.58	0.40	L747_11185	xsome
1973	RNA-binding protein	-0.05	0.47	**0.95**	**0.51**	L747_11190	xsome
1974	integrase	0.41	**0.50**	**1.25**	**0.78**	L747_11195	xsome
1975	MFS transporter	-0.22	**-0.82**	**1.24**	**2.07**	L747_11200	xsome
1976	hypothetical protein	0.64	**0.56**	**0.68**	0.16	L747_11205	xsome
1977	methionine aminopeptidase	0.09	**0.81**	**1.29**	**0.51**	L747_11210	xsome
1978	ubiquinone/menaquinone biosynthesis methyltransferase	0.28	**1.40**	**1.88**	**0.51**	L747_11215	xsome
1979	hypothetical protein	-0.06	-0.14	-0.22	-0.03	L747_11220	xsome
1980	3-methyladenine DNA glycosylase	-0.08	**-1.29**	**-1.79**	**-0.44**	L747_11225	xsome
1981	hypothetical protein	0.43	0.32	0.38	0.10	L747_11230	xsome
1982	beta-phosphoglucomutase	0.22	0.18	0.22	0.08	L747_11235	xsome
1983	universal stress protein UspA	0.44	**0.70**	**1.23**	**0.57**	L747_11240	xsome
1984	glycine/betaine ABC transporter	**0.67**	**1.70**	**2.58**	**0.90**	L747_11245	xsome
1985	galactoside O-acetyltransferase	0.05	**-1.16**	**-1.04**	0.16	L747_11250	xsome
1986	3-demethylubiquinone-9 3-methyltransferase	0.04	**0.97**	**0.40**	**-0.53**	L747_11255	xsome
1987	ribonuclease	-0.24	0.29	**-0.52**	**-0.76**	L747_11260	xsome
1988	hypothetical protein	**0.48**	**1.62**	**1.91**	**0.32**	L747_11265	xsome
1989	cytosine deaminase	0.17	**0.26**	0.20	-0.02	L747_11270	xsome
1990	hypothetical protein	0.36	0.08	-0.44	**-0.47**	L747_11275	xsome
1991	phosphomethylpyrimidine kinase	-0.41	**1.05**	**1.03**	0.02	L747_11280	xsome
1992	hypothetical protein	-0.16	0.37	0.28	-0.07	L747_11285	xsome
1993	NADPH-quinone reductase	-0.02	**0.64**	0.13	**-0.46**	L747_11290	xsome
1995	hypothetical protein	-0.06	**-0.53**	**-0.63**	-0.06	L747_11300	xsome
1996	hypothetical protein	-0.01	**-1.53**	**-1.53**	0.04	L747_11305	xsome
1997	transposase IS204	-0.20	0.00	0.18	0.21	L747_11310	xsome
1998	cytochrome B5	0.61	0.15	**-1.40**	**-1.40**	L747_11315	xsome
1999	cold-shock protein	**1.26**	**1.77**	**3.33**	**1.46**	L747_11320	xsome
2000	hypothetical protein	0.30	**0.97**	**1.01**	0.07	L747_11325	xsome
2001	hypothetical protein	0.28	0.23	-0.21	**-0.39**	L747_11330	xsome
2002	DNA glycosylase	0.07	**-0.97**	**-0.86**	0.15	L747_11335	xsome

2003	hypothetical protein	-0.19	**0.60**	**0.91**	**0.35**	L747_11340	xsome
2004	hypothetical protein	**-0.80**	**-1.32**	**-1.54**	-0.18	L747_11345	xsome
2005	transposase IS204	-0.26	-0.28	-0.27	0.06	L747_11350	xsome
2006	hypothetical protein	0.35	0.18	-0.33	**-0.46**	L747_11355	xsome
2007	hypothetical protein	0.02	**-0.77**	**-1.71**	**-0.88**	L747_11360	xsome
2008	glycine cleavage system protein H	0.35	-0.33	-0.20	0.17	L747_11365	xsome
2009	productaldo/keto reductase	0.20	-0.01	-0.06	-0.01	L747_11370	xsome
2010	hypothetical protein	0.31	0.16	**1.33**	**1.19**	L747_11375	xsome
2011	membrane protein	0.29	**-0.78**	-0.09	**0.72**	L747_11380	xsome
2012	ABC transporter permease	-0.09	**2.73**	**2.85**	0.14	L747_11385	xsome
2013	methionine ABC transporter ATP-binding protein	-0.37	**1.22**	**0.70**	-0.42	L747_11390	xsome
2014	ABC transporter substrate-binding protein	-0.47	**0.50**	0.29	-0.17	L747_11395	xsome
2015	methyladenine glycosylase	-0.14	**-1.57**	**-1.42**	0.20	L747_11400	xsome
2016	peptide ABC transporter substrate-binding protein	**-1.54**	**-2.90**	**-2.67**	0.27	L747_11405	xsome
2017	hypothetical protein	**-0.89**	**-1.16**	-0.04	**1.10**	L747_11410	xsome
2018	penicillin V acylase	-0.09	**1.37**	**1.13**	-0.21	L747_11415	xsome
2019	hypothetical protein	0.14	**-0.41**	-0.19	**0.26**	L747_11420	xsome
2020	exonuclease SbcD subunit D	-0.12	-0.03	**0.45**	**0.52**	L747_11425	xsome
2021	3-methyladenine DNA glycosylase	0.17	**-0.58**	-0.36	0.26	L747_11430	xsome
2022	hypothetical protein	**-0.57**	**-0.87**	**-1.19**	**-0.27**	L747_11435	xsome
2023	1,4-beta-N-acetylmuramidase	**-0.55**	**2.63**	**4.07**	**1.45**	L747_11440	xsome
2024	endoglucanase	-0.09	**-0.50**	**-0.57**	-0.03	L747_11445	xsome
2025	4-carboxymuconolactone decarboxylase	0.53	0.55	-0.05	**-0.54**	L747_11450	xsome
2026	LytTR family transcriptional regulator	**0.84**	0.43	-0.56	**-0.91**	L747_11455	xsome
2027	MerR family transcriptional regulator	0.69	0.25	**-0.96**	**-1.14**	L747_11460	xsome
2028	TetR family transcriptional regulator	-0.02	**-0.72**	**-1.61**	**-0.84**	L747_11465	xsome
2029	hypothetical protein	0.47	0.37	-0.20	-0.50	L747_11470	xsome
2030	hypothetical protein	**1.06**	**-1.00**	**-1.75**	**-0.69**	L747_11475	xsome
2031	integrase	0.08	**0.69**	**0.39**	**-0.25**	L747_11480	xsome
2032	2,5-diketo-D-gluconic acid reductase	**1.35**	**3.12**	**1.86**	**-1.22**	L747_11485	xsome
2033	MFS transporter	0.13	0.11	-0.22	-0.28	L747_11490	xsome

2034	MFS transporter	-0.05	-0.16	-0.07	0.12	L747_11495	xsome
2035	hypothetical protein	-0.36	-0.72	-0.10	**0.62**	L747_11500	xsome
2036	hypothetical protein	-0.20	-0.14	0.93	**0.97**	L747_11505	xsome
2037	hypothetical protein	0.02	-0.09	**-0.83**	**-0.65**	L747_11510	xsome
2038	NADH:flavin oxidoreductase	0.17	-0.21	**-0.65**	-0.40	L747_11515	xsome
2039	ethanolamine utilization protein EutN	0.13	**-0.84**	**-2.47**	**-1.40**	L747_11520	xsome
2040	hypothetical protein	-0.39	**-1.03**	0.21	**1.27**	L747_11525	xsome
2041	hypothetical protein	0.09	**-0.90**	**-0.63**	**0.30**	L747_11530	xsome
2042	Fucose-binding lectin II	-0.07	**-0.90**	**-0.86**	0.08	L747_11535	xsome
2043	membrane protein	-0.09	**-1.34**	**-2.18**	**-0.79**	L747_11540	xsome
2044	cobalt ABC transporter ATP-binding protein	**0.78**	**0.57**	**0.53**	-0.01	L747_11545	xsome
2045	hypothetical protein	**0.63**	**0.82**	**0.40**	**-0.37**	L747_11550	xsome
2046	transposase ISL3	-0.04	-0.19	0.41	**0.62**	L747_11555	xsome
2047	amino acid decarboxylase	-0.25	0.76	0.33	-0.39	L747_11560	xsome
2048	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	0.00	0.94	0.35	-0.53	L747_11565	xsome
2049	3,4-dihydroxybenzoate decarboxylase	0.25	**0.80**	0.41	-0.34	L747_11570	xsome
2050	transcriptional regulator	-0.65	**-1.20**	-0.48	**0.70**	L747_11575	xsome
2051	peptide deformylase	0.46	-0.34	**0.55**	**0.90**	L747_11580	xsome
2052	phospholipid phosphatase	-0.53	**-0.82**	-0.42	0.41	L747_11585	xsome
2053	integrase	0.26	**0.84**	**0.59**	**-0.22**	L747_11590	xsome
2054	hypothetical protein	-0.07	0.92	-0.08	**-0.92**	L747_11595	xsome
2055	aquaporin	-0.07	**3.04**	**1.69**	**-1.31**	L747_11600	xsome
2056	pyrrolidone-carboxylate peptidase	-0.13	0.50	-0.14	**-0.59**	L747_11605	xsome
2057	ABC transporter permease; distrupted(psuedo)	-0.43	-0.30	-0.32	0.02	L747_11610	xsome
2058	MarR family transcriptional regulator	-0.12	**-0.95**	**-1.46**	-0.43	L747_11615	xsome
2059	sulfite export protein	0.00	-0.71	0.01	0.67	L747_11620	xsome
2060	hypothetical protein	0.00	0.03	0.33	0.32	L747_11625	xsome
2061	hypothetical protein	**-0.75**	**-2.07**	**-2.42**	-0.30	L747_11630	xsome
2062	ABC transporter ATP-binding protein	**-0.52**	**-2.37**	**-3.42**	**-0.97**	L747_11635	xsome
2063	GntR family transcriptional regulator	**-0.52**	**-3.42**	**-5.97**	**-2.29**	L747_11640	xsome
2064	beta-lactamase	-0.22	-1.03	**-1.90**	-0.67	L747_11645	xsome

2065	glutathione peroxidase	**0.77**	**1.13**	**1.13**	0.04	L747_11650	xsome
2066	hypothetical protein	**0.77**	**0.52**	**0.61**	0.13	L747_11655	xsome
2067	bicyclomycin resistance protein	0.52	**0.50**	**0.86**	**0.40**	L747_11660	xsome
2068	haloacid dehalogenase	-0.05	**-0.64**	**-0.55**	0.13	L747_11665	xsome
2069	methionine aminopeptidase	**-0.49**	**-1.52**	**-1.74**	-0.17	L747_11670	xsome
2070	hypothetical protein	-0.06	**-3.70**	**-6.03**	**-2.07**	L747_11675	xsome
2071	oxidoreductase	**1.59**	**2.96**	-0.20	**-3.08**	L747_11680	xsome
2072	regulatory protein	0.11	**0.62**	0.26	-0.31	L747_11685	xsome
2073	alkaline-shock protein	**1.46**	**2.95**	**1.36**	**-1.54**	L747_11690	xsome
2074	alkaline-shock protein	**1.45**	**2.37**	**0.37**	**-1.94**	L747_11695	xsome
2075	hypothetical protein	**1.46**	**2.28**	-0.22	**-2.45**	L747_11700	xsome
2076	hypothetical protein	**1.65**	**2.62**	**0.83**	**-1.74**	L747_11705	xsome
2077	membrane protein	**1.28**	**2.93**	**1.51**	**-1.36**	L747_11710	xsome
2078	membrane protein	-0.37	0.40	**2.08**	**1.71**	L747_11715	xsome
2079	asparaginyl-tRNA synthetase	-0.08	**0.32**	**0.53**	**0.25**	L747_11720	xsome
2080	hypothetical protein	0.12	**2.67**	**3.50**	**0.86**	L747_11725	xsome
2081	hypothetical protein	-0.02	**2.99**	**3.38**	**0.41**	L747_11730	xsome
2082	transglycosylase	**-2.46**	**1.10**	**2.35**	**1.26**	L747_11735	xsome
2083	tRNA synthetase subunit beta	**1.90**	0.06	**-0.70**	**-0.71**	L747_11740	xsome
2084	arginine deiminase	-0.05	0.06	**-0.60**	**-0.61**	L747_11745	xsome
2085	carbamate kinase	**1.38**	**-0.44**	**-1.84**	**-1.34**	L747_11750	xsome
2086	aminotransferase	**1.08**	**-1.03**	**-2.80**	**-1.71**	L747_11755	xsome
2087	amino acid APC transporter	**0.77**	**-1.19**	**-2.43**	**-1.17**	L747_11760	xsome
2088	ornithine carbamoyltransferase	**1.07**	**-1.25**	**-1.90**	**-0.60**	L747_11765	xsome
2089	arginine deiminase; distrupted(psuedo)	**0.48**	**-2.34**	**-3.12**	**-0.73**	L747_13730	xsome
2090	hypothetical protein	**-0.84**	**-2.95**	**-2.91**	0.08	L747_11770	xsome
2091	histidine kinase	-0.36	**-2.11**	**-1.81**	0.33	L747_11775	xsome
2092	transcriptional regulator	-0.22	**-1.85**	**-1.96**	-0.05	L747_11780	xsome
2093	hypothetical protein	-0.02	**-1.83**	**-1.21**	0.61	L747_11785	xsome
2094	transposase	0.43	-0.45	0.15	**0.63**	L747_11790	xsome
2095	transposase	-0.16	**-1.33**	**-0.89**	**0.47**	L747_11795	xsome

2096	arginine:ornithine antiporter	**1.18**	**0.65**	0.37	-0.25	L747_11800	xsome
2097	hypothetical protein	**0.62**	**0.97**	**0.92**	-0.01	L747_11805	xsome
2098	hypothetical protein	0.34	**-0.76**	**-2.57**	**-1.59**	L747_11810	xsome
2099	hypothetical protein	-0.36	-0.19	0.31	**0.54**	L747_11815	xsome
2100	transposase ISL3	-0.16	-0.57	-0.12	**0.48**	L747_11820	xsome
2101	hypothetical protein	0.23	**0.81**	**-0.74**	**-1.48**	L747_11825	xsome
2102	hypothetical protein	-0.61	**-1.71**	-0.43	**1.18**	L747_11830	xsome
2103	hypothetical protein	0.02	**-1.78**	**-2.56**	**-0.72**	L747_11835	xsome
2104	DSBA oxidoreductase	-0.16	**-0.52**	**0.50**	**1.04**	L747_11840	xsome
2105	LacI family transcriptional regulator	-0.48	**-0.79**	**-0.38**	**0.44**	L747_11845	xsome
2106	beta-D-glucuronidase	0.47	-0.19	**-0.88**	**-0.65**	L747_11850	xsome
2107	hypothetical protein	0.54	0.27	-0.06	**-0.29**	L747_11855	xsome
2108	integrase	0.11	**0.74**	**0.47**	**-0.23**	L747_11860	xsome
2109	transporter	**-0.92**	**-2.86**	**-1.93**	0.80	L747_11865	xsome
2110	hypothetical protein	0.22	**-0.59**	-0.19	**0.44**	L747_11870	xsome
2111	hypothetical protein	0.32	0.55	-0.77	**-1.20**	L747_11875	xsome
2112	hypothetical protein	**0.87**	-0.48	**-1.37**	**-0.80**	L747_11880	xsome
2113	hypothetical protein	0.32	-0.15	**-0.71**	**-0.51**	L747_11885	xsome
2114	aldo/keto reductase; distrupted(psuedo)	-0.21	**-0.65**	-0.48	0.20	L747_11890	xsome
2115	integrase	0.10	**0.66**	**0.44**	-0.18	L747_11895	xsome
2116	hypothetical protein	0.18	2.19	**2.89**	0.35	L747_11900	xsome
2117	hypothetical protein	-0.08	-0.32	-0.33	0.03	L747_11905	xsome
2118	MerR family transcriptional regulator	0.15	0.45	0.32	-0.09	L747_11910	xsome
2119	hypothetical protein	-0.57	**-1.28**	**-1.64**	-0.31	L747_11915	xsome
2120	hypothetical protein	0.14	-0.55	**-1.19**	**-0.56**	L747_11920	xsome
2121	hypothetical protein	-0.70	-0.42	**-0.70**	-0.23	L747_11925	xsome
2122	phosphoglyceromutase; gpmA	0.00	**1.51**	**2.12**	**0.65**	L747_11930	xsome
2123	hypothetical protein	-0.16	-0.20	-0.47	-0.23	L747_11935	xsome
2124	membrane protein	0.32	-0.02	**-0.44**	**-0.37**	L747_11940	xsome
2125	ROK family transcriptional regulator	**-0.98**	**-0.71**	**-0.64**	0.11	L747_11945	xsome
2126	hypothetical protein	**-0.38**	-0.22	**-0.83**	**-0.57**	L747_11950	xsome

2127	hypothetical protein	-0.24	**0.53**	**-1.49**	**-1.95**	L747_11955	xsome
2128	cyclopropane-fatty-acyl-phospholipid synthase	0.26	**2.04**	**2.04**	0.03	L747_11960	xsome
2129	branched-chain amino acid transporter	-0.44	**1.74**	**2.13**	**0.42**	L747_11965	xsome
2130	branched-chain amino acid ABC transporter	0.02	**1.07**	**0.73**	**-0.30**	L747_11970	xsome
2131	N-acetyltransferase	0.01	0.25	**0.54**	0.32	L747_11975	xsome
2132	glutathione reductase	**0.91**	**3.60**	**2.50**	**-1.04**	L747_11980	xsome
2133	hypothetical protein	-0.12	0.56	0.66	0.14	L747_11985	xsome
2134	integrase	0.22	0.37	**1.09**	**0.75**	L747_11990	xsome
2135	hypothetical protein	-0.10	-0.02	**-0.44**	**-0.38**	L747_11995	xsome
2136	hypothetical protein	-0.23	**-1.79**	-0.35	**1.25**	L747_12000	xsome
2137	hypothetical protein	**-1.15**	**-1.46**	0.06	**1.55**	L747_12005	xsome
2138	D-alanyl-D-alanine carboxypeptidase	0.17	**1.69**	**1.86**	**0.20**	L747_12010	xsome
2139	sensor histidine kinase	**0.67**	**2.21**	**1.50**	**-0.66**	L747_12015	xsome
2140	PhoB family transcriptional regulator	**0.57**	**1.64**	**0.61**	**-0.99**	L747_12020	xsome
2141	hypothetical protein	-0.35	**-1.75**	**-1.65**	0.15	L747_12025	xsome
2142	hypothetical protein	0.30	**0.41**	**0.58**	**0.22**	L747_12030	xsome
2143	GTP-binding protein YchF	0.28	**0.61**	0.05	**-0.51**	L747_12035	xsome
2144	hypothetical protein	0.66	-0.04	-0.43	-0.34	L747_12040	xsome
2145	Lb464_xsome partitioning protein ParB	0.06	-0.02	**-0.71**	**-0.64**	L747_12045	xsome
2146	sporulation initiation inhibitor Soj	0.17	**0.59**	-0.16	**-0.70**	L747_12050	xsome
2147	Lb464_xsome partitioning protein ParB	0.09	**0.40**	**-0.45**	**-0.79**	L747_12055	xsome
2148	16S rRNA methyltransferase	0.04	0.26	-0.19	**-0.41**	L747_12060	xsome
2149	producthypothetical protein	-0.72	-0.37	-0.43	-0.02	L747_12065	xsome
2150	hypothetical protein	-0.31	-1.41	-1.27	0.16	L747_12070	xsome
2151	ribonucleoside hydrolase	**-0.58**	**-1.36**	**-1.06**	**0.33**	L747_12075	xsome
2152	permease	**-0.50**	**-2.04**	**-1.74**	**0.34**	L747_12080	xsome
2153	hypothetical protein	0.06	**-1.56**	**-1.85**	**-0.24**	L747_12085	xsome
2154	NAD-dependent dehydratase	0.03	**0.51**	**-0.49**	**-0.95**	L747_12090	xsome
2155	hypothetical protein	**0.48**	**1.24**	**2.23**	**1.02**	L747_12095	xsome
2156	hypothetical protein	-0.26	0.30	**0.89**	**0.61**	L747_12100	xsome
2157	hypothetical protein	0.37	**1.43**	**2.49**	**1.08**	L747_12105	xsome

2158	transposase IS204	-0.61	-0.33	-0.25	0.12	L747_12110	xsome
2159	hypothetical protein	-0.50	-0.03	-0.21	-0.12	L747_12115	xsome
2160	producthypothetical protein	-0.57	**-1.83**	**-1.89**	0.03	L747_12120	xsome
2161	hypothetical protein	-0.17	**-2.64**	**-2.96**	-0.17	L747_12125	xsome
2162	hypothetical protein	-0.49	**-0.78**	**-1.26**	-0.41	L747_12130	xsome
2163	hypothetical protein	-0.19	-0.36	-1.36	-0.78	L747_12135	xsome
2164	transposase	-0.29	**-1.47**	**-1.26**	0.25	L747_12140	xsome
2165	hypothetical protein	-0.09	-0.72	-0.62	0.14	L747_12145	xsome
2166	hypothetical protein	**-0.34**	**-1.37**	**-0.81**	**0.60**	L747_12150	xsome
2167	hypothetical protein	-0.22	**-0.58**	**-0.73**	-0.11	L747_12155	xsome
2168	hypothetical protein	0.11	**1.17**	**1.36**	**0.23**	L747_12160	xsome
2169	hypothetical protein	0.16	**1.57**	**1.77**	**0.24**	L747_12165	xsome
2170	aspartate aminotransferase	-0.22	**0.65**	**1.32**	**0.69**	L747_12170	xsome
2171	LysR family transcriptional regulator	**-0.88**	**-1.28**	0.29	**1.58**	L747_12175	xsome
2172	hypothetical protein	0.00	0.07	**1.29**	**1.14**	L747_12180	xsome
2173	hypothetical protein	0.34	0.29	0.61	0.31	L747_12185	xsome
2174	hypothetical protein	0.43	**1.38**	**1.86**	**0.49**	L747_12190	xsome
2175	hypothetical protein	0.24	0.85	**1.38**	**0.54**	L747_12195	xsome
2176	hypothetical protein	**1.47**	**3.42**	**4.15**	**0.74**	L747_12200	xsome
2177	hypothetical protein	**1.87**	**3.45**	**4.12**	**0.67**	L747_12205	xsome
2178	hypothetical protein	**2.26**	**2.45**	**2.85**	**0.42**	L747_12210	xsome
2179	ABC transporter ATP-binding protein	**-0.66**	**-1.30**	**-2.17**	**-0.81**	L747_12215	xsome
2180	thiamine-phosphate pyrophosphorylase	-0.19	**0.92**	0.09	**-0.77**	L747_12220	xsome
2181	phosphomethylpyrimidine kinase	-0.38	-0.10	**-1.69**	**-1.53**	L747_12225	xsome
2182	hydroxyethylthiazole kinase; distrupted(psuedo)	**-0.81**	-0.03	**-1.22**	**-1.13**	L747_12230	xsome
2183	short-chain dehydrogenase	0.15	**1.67**	0.69	**-0.93**	L747_12235	xsome
2184	membrane protein	-0.37	0.68	-0.38	**-0.99**	L747_12240	xsome
2185	putrescine/spermidine ABC transporter ATP-binding protein	-0.10	**1.52**	0.86	**-0.62**	L747_12245	xsome
2186	RNA methyltransferase	-0.12	**1.09**	**1.29**	**0.23**	L747_12250	xsome
2187	hypothetical protein	**1.29**	**2.25**	**3.26**	**1.03**	L747_12255	xsome

2188	hypothetical protein	**0.72**	**-2.19**	**-1.56**	**0.63**	L747_12260	xsome
2189	hypothetical protein	0.18	**-1.73**	**-1.61**	0.16	L747_12265	xsome
2190	integrase	**0.70**	**-0.82**	-0.09	**0.76**	L747_12270	xsome
2191	hypothetical protein	**0.70**	**-3.88**	**-5.17**	**-1.04**	L747_12275	xsome
2192	hypothetical protein	**0.91**	**-3.77**	**-3.43**	0.37	L747_12280	xsome
2193	hypothetical protein	0.52	**-1.87**	**-1.30**	0.56	L747_12285	xsome
2194	hypothetical protein	0.07	**-1.87**	**-1.64**	0.25	L747_12290	xsome
2195	MerR family transcriptional regulator	0.50	**1.36**	**1.58**	0.25	L747_12295	xsome
2196	polyketide cyclase	0.33	**1.07**	**1.14**	0.09	L747_12300	xsome
2197	hypothetical protein	-0.67	-0.16	**0.59**	**0.76**	L747_12305	xsome
2198	hypothetical protein	0.14	0.65	0.81	0.19	L747_12310	xsome
2199	xylose repressor	0.21	**-0.67**	**-0.62**	0.09	L747_12315	xsome
2200	2,5-diketo-D-gluconic acid reductase	0.21	**1.05**	**0.75**	-0.26	L747_12320	xsome
2201	recombinase RarA	**0.61**	**0.98**	0.31	**-0.62**	L747_12325	xsome
2202	ribonuclease Y 2	**0.56**	**1.33**	**0.94**	**-0.35**	L747_12330	xsome
2203	hypothetical protein	-0.05	0.03	-0.35	-0.33	L747_12335	xsome
2204	alpha/beta hydrolase	-0.70	-0.25	**-0.83**	**-0.53**	L747_12340	xsome
2205	oligoendopeptidase	**-0.66**	**1.35**	**1.46**	0.15	L747_12345	xsome
2206	Cro/Cl family transcriptional regulator	0.11	**1.08**	**1.20**	0.16	L747_12350	xsome
2207	SAM-dependent methyltransferase	**-0.81**	**0.52**	**0.67**	0.19	L747_12355	xsome
2208	membrane protein	0.31	**1.09**	**0.48**	**-0.57**	L747_12360	xsome
2209	branched-chain amino acid transporter II carrier protein	-0.45	**1.65**	**1.13**	**-0.47**	L747_12365	xsome
2210	hypothetical protein	0.54	**1.72**	**1.77**	0.09	L747_12370	xsome
2211	amino acid permease	0.17	**0.54**	**0.72**	0.22	L747_12375	xsome
2212	hypothetical protein	**-0.83**	-0.52	-0.09	**0.47**	L747_12380	xsome
2213	hypothetical protein	**-0.70**	**-0.60**	**-0.47**	0.17	L747_12385	xsome
2214	hypothetical protein	-0.29	-0.19	0.31	**0.51**	L747_12390	xsome
2216	hypothetical protein	-0.56	0.31	-0.43	**-0.69**	L747_12405	xsome
2217	hypothetical protein	-0.05	0.33	-0.02	**-0.31**	L747_12410	xsome
2218	carbohydrate kinase	0.49	**1.85**	**1.74**	-0.07	L747_12415	xsome

2219	hypothetical protein	-0.38	**-0.89**	-0.52	0.40	L747_12420	xsome
2220	hypothetical protein	-0.52	**-1.81**	**-1.52**	0.31	L747_12425	xsome
2221	D-alanyl-D-alanine dipeptidase	-0.44	**1.14**	**0.60**	**-0.50**	L747_12430	xsome
2222	hypothetical protein	-0.11	0.01	0.12	0.14	L747_12435	xsome
2223	penicillin V acylase	0.06	**-1.23**	**-0.89**	0.37	L747_12440	xsome
2224	hydrolase	**-0.92**	**1.99**	**4.03**	**2.03**	L747_12445	xsome
2225	sugar transporter	0.38	**1.17**	**0.98**	-0.15	L747_12450	xsome
2226	hypothetical protein	0.18	-0.06	0.11	0.19	L747_12455	xsome
2227	hypothetical protein	0.05	**2.97**	**3.09**	0.15	L747_12460	xsome
2228	hypothetical protein	-0.44	0.04	**0.76**	**0.75**	L747_12465	xsome
2229	hypothetical protein	-0.32	**0.36**	0.15	-0.16	L747_12470	xsome
2230	regulatory protein	0.07	0.03	**1.08**	**1.08**	L747_12475	xsome
2231	hypothetical protein	**-0.60**	-0.33	**0.52**	**0.88**	L747_12480	xsome
2232	3'-cyclic nucleotide 2'-phosphodiesterase	**-0.48**	-0.17	0.26	**0.47**	L747_12485	xsome
2233	arylsulfate sulfotransferase	0.39	**5.40**	**5.83**	**0.45**	L747_12490	xsome
2234	hypothetical protein	0.48	**2.04**	**3.10**	**1.08**	L747_12495	xsome
2235	ribonucleoside hydrolase; rihB	**-0.90**	**-1.61**	**-1.80**	-0.14	L747_12500	xsome
2237	hypothetical protein	0.13	-0.25	0.40	**0.69**	L747_12510	xsome
2238	hypothetical protein	-0.02	**-1.21**	**-0.79**	**0.45**	L747_12515	xsome
2239	producthypothetical protein	0.11	**-1.45**	**-1.49**	0.01	L747_12520	xsome
2240	membrane protein	0.10	**-2.18**	**-3.71**	**-1.29**	L747_12525	xsome
2241	hydrogenase expression protein	-0.20	**-1.85**	**-2.61**	-0.61	L747_12530	xsome
2242	hypothetical protein	**1.39**	-0.25	-0.10	0.18	L747_12535	xsome
2243	LytR family transcriptional regulator	**2.48**	-0.10	-0.22	-0.07	L747_12540	xsome
2244	taurine ABC transporter ATP-binding protein	**1.97**	-0.68	**-0.89**	-0.14	L747_12545	xsome
2245	hypothetical protein	**1.65**	0.12	-0.04	-0.11	L747_12550	xsome
2246	cell division protein FtsW	**1.66**	**1.97**	**1.02**	**-0.91**	L747_12555	xsome
2247	hypothetical protein	0.52	**1.77**	0.41	**-1.29**	L747_12560	xsome
2248	MarR family transcriptional regulator	-0.05	-0.88	**-0.88**	0.04	L747_12565	xsome
2249	hypothetical protein	-0.33	**-0.83**	**-1.09**	-0.20	L747_12570	xsome
2250	glyoxalase	**0.44**	**-5.37**	**-5.60**	-0.16	L747_12575	xsome

2251	fructose permease	**0.52**	**-5.34**	**-5.92**	**-0.51**	L747_12580	xsome
2252	alcohol dehydrogenase	**0.64**	**-6.15**	**-6.42**	-0.22	L747_12585	xsome
2253	fructokinase	**0.70**	**-7.02**	**-6.90**	0.17	L747_12590	xsome
2254	hypothetical protein	0.12	**2.27**	**2.92**	**0.67**	L747_12595	xsome
2255	transposase ISL3	-0.67	**-0.73**	-0.08	**0.67**	L747_12600	xsome
2256	glycerol dehydrogenase; gldA	0.33	-0.21	**-0.35**	-0.10	L747_12605	xsome
2257	multidrug resistance protein SMR	0.01	0.07	-0.24	-0.26	L747_12610	xsome
2258	multidrug resistance protein SMR	0.28	-0.15	-0.31	-0.11	L747_12615	xsome
2259	GNAT family acetyltransferase	0.13	0.11	0.18	0.10	L747_12620	xsome
2260	phosphoglycerate kinase	0.06	**-3.80**	**-4.47**	**-0.61**	L747_12625	xsome
2261	hypothetical protein	-0.12	0.15	0.04	-0.05	L747_12630	xsome
2262	hypothetical protein	0.30	**-1.85**	**-2.48**	**-0.57**	L747_12635	xsome
2263	hypothetical protein	0.38	**-2.73**	**-3.15**	-0.35	L747_12640	xsome
2264	sugar:proton symporter	-0.25	**-2.92**	**-3.19**	-0.20	L747_12645	xsome
2265	integrase	0.18	-0.14	**1.25**	**1.41**	L747_12650	xsome
2266	hypothetical protein	**0.68**	**1.57**	**1.39**	-0.14	L747_12655	xsome
2267	hypothetical protein	-0.31	**-1.42**	**-1.55**	-0.05	L747_12660	xsome
2268	serine acetyltransferase	-0.28	-0.95	-0.81	0.18	L747_12665	xsome
2269	NADH-dependent flavin oxidoreductase	**1.60**	**1.70**	**1.06**	**-0.60**	L747_12670	xsome
2270	hypothetical protein	**1.34**	**2.04**	0.79	**-1.16**	L747_12675	xsome
2271	MarR family transcriptional regulator	0.66	0.09	-0.46	**-0.49**	L747_12680	xsome
2272	peptidase S66	-0.28	-0.26	0.30	**0.59**	L747_12685	xsome
2273	rihB	0.06	-0.34	0.01	0.38	L747_12690	xsome
2274	transposase IS204	-0.42	-0.33	-0.12	0.24	L747_12695	xsome
2275	hypothetical protein	-0.33	-0.06	-0.16	-0.05	L747_12700	xsome
2276	transcriptional regulator	-0.36	-0.62	**-0.98**	-0.30	L747_12705	xsome
2277	cobalt-zinc-cadmium resistance protein	0.29	**0.71**	0.02	**-0.63**	L747_12710	xsome
2278	1,3-propanediol dehydrogenase	**-0.94**	**-3.22**	**-3.46**	-0.20	L747_12715	xsome
2279	hypothetical protein	-0.31	-0.01	**0.66**	**0.69**	L747_12720	xsome
2280	producthypothetical protein	-0.12	0.07	0.03	0.00	L747_12725	xsome
2281	envelope protein	0.04	0.79	**1.64**	**0.84**	L747_12730	xsome

2282	hypothetical protein	0.12	**1.42**	**1.76**	**0.38**	L747_12735	xsome
2283	arabinose ABC transporter permease	**0.87**	**0.44**	0.04	**-0.35**	L747_12740	xsome
2284	peptide ABC transporter ATP-binding protein	0.04	**1.58**	**0.38**	**-1.15**	L747_12745	xsome
2285	ABC transporter permease	0.02	**0.91**	-0.15	**-1.01**	L747_12750	xsome
2286	hypothetical protein	-0.11	**-0.61**	**-0.94**	-0.28	L747_12755	xsome
2287	MFS permease	0.53	-0.43	-0.42	0.05	L747_12760	xsome
2288	hypothetical protein	0.31	0.32	-0.01	-0.26	L747_12765	xsome
2289	MFS transporter	0.25	0.53	0.56	0.05	L747_12770	xsome
2290	beta-D-galactosidase	0.36	0.18	0.11	-0.03	L747_12775	xsome
2291	L-ribulose-5-phosphate 4-epimerase; sgbE	0.67	0.23	-0.02	-0.21	L747_12780	xsome
2292	L-xylulose 5-phosphate 3-epimerase	-0.12	-0.70	-0.92	-0.14	L747_12785	xsome
2293	hypothetical protein	-0.26	**-1.40**	**-1.31**	0.13	L747_12790	xsome
2294	hypothetical protein	0.21	-0.41	-1.90	-0.58	L747_12795	xsome
2295	hypothetical protein	0.06	-0.93	**-1.97**	-0.69	L747_12800	xsome
2296	2-hydroxyacid dehydrogenase	0.19	0.14	-0.07	-0.16	L747_12805	xsome
2297	hypothetical protein	**-0.94**	-0.09	0.24	**0.36**	L747_12810	xsome
2298	thiW protein	0.51	**1.84**	0.44	**-1.34**	L747_12815	xsome
2299	malate permease	**0.96**	**5.48**	**6.36**	**0.91**	L747_12820	xsome
2300	Malolactic enzyme	**1.30**	**5.82**	**6.72**	**0.92**	L747_12825	xsome
2301	LysR family transcriptional regulator	-0.30	0.15	**0.63**	**0.50**	L747_12830	xsome
2302	phosphosugar-binding protein	0.46	**1.30**	**1.10**	-0.16	L747_12835	xsome
2303	agmatine deiminase	0.41	**1.00**	0.12	**-0.83**	L747_12840	xsome
2304	carbamate kinase	0.24	**1.35**	**1.10**	-0.21	L747_12845	xsome
2305	agmatine deiminase	0.20	**4.08**	**4.53**	**0.48**	L747_12850	xsome
2306	amino acid:proton antiporter	0.39	**4.81**	**5.39**	**0.61**	L747_12855	xsome
2307	putrescine carbamoyltransferase	-0.24	**4.46**	**4.83**	**0.40**	L747_12860	xsome
2308	sodium:proton antiporter	**-1.11**	**-1.65**	**-2.35**	**-0.63**	L747_12865	xsome
2309	amino acid permease	**-1.82**	**-3.17**	**-3.65**	-0.40	L747_12870	xsome
2310	decarboxylase	**-2.29**	**-3.44**	**-3.85**	-0.34	L747_12875	xsome
2311	integrase	0.30	**0.45**	**1.21**	**0.79**	L747_12880	xsome
2312	tyrosyl-tRNA synthase	0.29	**-1.51**	**-2.73**	**-1.16**	L747_12885	xsome

2313	hypothetical protein	-0.59	**-2.62**	**-1.02**	**1.46**	L747_12890	xsome
2314	hypothetical protein	**1.09**	**-1.50**	**1.55**	**3.02**	L747_12895	xsome
2315	hypothetical protein	**0.82**	**-1.71**	**1.09**	**2.79**	L747_12900	xsome
2316	3-ketoacyl-CoA thiolase	0.27	**2.21**	**3.09**	**0.91**	L747_12905	xsome
2317	hypothetical protein	0.53	**2.66**	**3.22**	**0.58**	L747_12910	xsome
2318	permease	-0.06	0.04	0.38	**0.37**	L747_12915	xsome
2319	alpha-L-arabinofuranosidase	0.23	0.07	-0.50	**-0.52**	L747_12920	xsome
2320	hypothetical protein	**0.46**	**1.08**	**-0.37**	**-1.40**	L747_12925	xsome
2321	membrane protein	**0.55**	0.26	**-1.58**	**-1.79**	L747_12930	xsome
2322	hypothetical protein	0.27	**1.13**	**1.22**	0.12	L747_12935	xsome
2323	hypothetical protein	0.27	-0.48	-0.12	**0.38**	L747_12940	xsome
2324	glycosyl hydrolase family 30	-0.48	**-0.58**	0.20	**0.80**	L747_12945	xsome
2325	hypothetical protein	-0.35	-0.19	0.27	0.44	L747_12950	xsome
2326	hypothetical protein	-0.17	**-0.63**	**-1.35**	**-0.67**	L747_12955	xsome
2327	hypothetical protein	-0.37	-0.17	0.69	**0.85**	L747_12960	xsome
2328	phosphoenolpyruvate-protein phosphotransferase	0.39	0.11	-0.05	-0.12	L747_12965	xsome
2329	integrase	0.33	**0.74**	**0.57**	-0.13	L747_12970	xsome
2330	hypothetical protein	-0.10	**-1.22**	-0.83	0.39	L747_12975	xsome
2331	PTS ascorbate transporter subunit IIC	-0.49	**-1.47**	-0.85	0.57	L747_12980	xsome
2332	hypothetical protein	-0.17	-1.20	-0.47	0.60	L747_12985	xsome
2333	PTS mannose transporter subunit IID	-0.36	**-1.53**	-1.06	0.43	L747_12990	xsome
2334	PTS sorbose transporter subunit IIC	0.11	-0.36	0.14	0.47	L747_12995	xsome
2335	fructose-2%2C6-bisphosphatase	0.18	**-1.26**	**-1.48**	-0.10	L747_13000	xsome
2336	hypothetical protein	0.43	**1.15**	**1.37**	**0.26**	L747_13005	xsome
2337	integrase	0.21	**0.47**	**1.17**	**0.73**	L747_13010	xsome
2338	orotate phosphoribosyltransferase	-0.32	**-1.05**	-0.52	**0.55**	L747_13015	xsome
2339	orotidine 5'-phosphate decarboxylase	-0.51	**-0.97**	-0.15	**0.82**	L747_13020	xsome
2340	MFS transporter	0.01	**1.06**	**2.78**	**1.74**	L747_13025	xsome
2341	hypothetical protein	0.67	0.40	-0.49	**-0.82**	L747_13030	xsome
2342	proline iminopeptidase	0.52	**-0.64**	**-1.43**	**-0.71**	L747_13035	xsome
2343	ribokinase	0.47	**-1.93**	**-2.29**	-0.26	L747_13040	xsome

2344	allantoin permease	0.11	**-2.23**	**-2.80**	-0.46	L747_13045	xsome
2345	crystallin	-0.18	**-2.12**	**-2.72**	-0.48	L747_13050	xsome
2346	GntR family transcriptional regulator	-0.43	**-2.70**	**-2.69**	0.07	L747_13055	xsome
2347	hypothetical protein	-0.13	**-0.93**	**-1.39**	-0.37	L747_13060	xsome
2348	GMP synthase	0.31	**1.07**	**0.98**	-0.05	L747_13065	xsome
2349	elongation factor G	0.11	0.15	**0.44**	**0.33**	L747_13070	xsome
2350	hypothetical protein	-0.61	0.19	**0.76**	**0.60**	L747_13075	xsome
2351	integrase	-0.12	**-0.41**	**0.74**	**1.18**	L747_13080	xsome
2352	polygalacturonase	-0.14	-0.72	-0.59	0.16	L747_13085	xsome
2353	MFS transporter	-0.08	**-1.06**	-0.71	0.36	L747_13090	xsome
2354	aspartate aminotransferase	-0.18	-0.19	**1.50**	**1.70**	L747_13095	xsome
2355	cyclopentanol dehydrogenase	-0.32	**-1.95**	-0.68	**1.23**	L747_13100	xsome
2356	hypothetical protein	-0.53	**-1.34**	**-1.65**	-0.26	L747_13105	xsome
2357	hypothetical protein	-0.10	**1.41**	**2.43**	**1.04**	L747_13110	xsome
2358	MFS transporter	0.00	**-0.87**	-0.11	**0.77**	L747_13115	xsome
2359	transposase	-0.11	0.03	**0.37**	**0.38**	L747_13120	xsome
2360	hypothetical protein	-0.14	-0.57	0.13	0.67	L747_13125	xsome
2361	galactoside O-acetyltransferase; lacA	0.15	**-1.11**	**-1.49**	-0.33	L747_13130	xsome
2362	hypothetical protein	**0.65**	0.33	**-1.42**	**-1.68**	L747_13135	xsome
2363	producthypothetical protein	-0.62	**-1.81**	**-1.88**	-0.01	L747_13140	xsome
2364	carbonic anhydrase	-0.23	**-0.41**	**-0.43**	0.02	L747_13145	xsome
2365	thymidine kinase	**-0.74**	-0.05	**0.75**	**0.83**	L747_13150	xsome
2366	DNA-binding protein	-0.40	**-1.58**	**-2.62**	**-0.94**	L747_13155	xsome
2367	hypothetical protein	-0.50	-0.56	**-0.62**	-0.02	L747_13160	xsome
2368	hypothetical protein	0.21	**-0.97**	**-0.89**	0.11	L747_13165	xsome
2369	hypothetical protein	-0.09	**-2.01**	**-2.30**	-0.17	L747_13170	xsome
2370	hypothetical protein	-0.53	**-1.08**	**-0.86**	0.25	L747_13175	xsome
2371	hypothetical protein	-0.16	**-0.91**	**-0.80**	0.14	L747_13180	xsome
2372	hypothetical protein	-0.72	**-1.35**	**-1.44**	-0.03	L747_13185	xsome
2373	hypothetical protein	-0.15	**1.43**	**2.33**	**0.90**	L747_13190	xsome
2374	multidrug MFS transporter	-0.23	**1.47**	**2.48**	**1.02**	L747_13195	xsome

2375	hypothetical protein	0.53	**1.36**	**2.28**	**0.94**	L747_13200	xsome
2376	hypothetical protein	0.46	**0.83**	**1.47**	**0.68**	L747_13205	xsome
2377	hypothetical protein	0.54	**-0.54**	0.14	**0.70**	L747_13210	xsome
2378	molecular chaperone GroES	0.49	**1.25**	0.36	**-0.85**	L747_13215	xsome
2379	hypothetical protein	0.37	**0.66**	**-1.17**	**-1.73**	L747_13220	xsome
2380	hypothetical protein	0.44	0.41	**-1.82**	**-2.10**	L747_13225	xsome
2381	hypothetical protein	**0.80**	0.33	**-1.35**	**-1.58**	L747_13230	xsome
2382	hypothetical protein	0.54	0.19	**-1.13**	**-1.21**	L747_13235	xsome
2383	hypothetical protein	**-0.89**	**-1.63**	0.04	**1.68**	L747_13240	xsome
2384	hypothetical protein	0.23	0.60	0.87	0.25	L747_13245	xsome
2385	hypothetical protein	0.23	0.03	0.21	0.21	L747_13250	xsome
2386	CoA reductase	-0.29	0.42	**0.69**	0.30	L747_13255	xsome
2387	beta-galactosidase	**0.39**	**0.46**	-0.08	**-0.49**	L747_13260	xsome
2388	beta-galactosidase	-0.05	0.21	-0.09	**-0.26**	L747_13265	xsome
2389	Rrf2 family transcriptional regulator	0.40	**2.01**	**2.58**	**0.59**	L747_13270	xsome
2390	hypothetical protein	**0.64**	**1.46**	**1.90**	**0.47**	L747_13275	xsome
2391	AraC family transcriptional regulator	0.24	**-0.56**	**-1.35**	**-0.75**	L747_13280	xsome
2392	hypothetical protein	**-0.63**	**-1.06**	**-0.52**	**0.57**	L747_13285	xsome
2393	ATP-dependent helicase	0.34	0.01	0.16	**0.20**	L747_13290	xsome
2394	ATP-dependent helicase	-0.05	-0.08	**0.39**	**0.51**	L747_13295	xsome
2395	succinyl-diaminopimelate desuccinylase	0.40	**2.87**	**3.35**	**0.51**	L747_13300	xsome
2396	xylanase	-0.10	**1.17**	**-0.56**	**-1.68**	L747_13305	xsome
2397	hypothetical protein	**0.60**	**0.55**	**1.16**	**0.64**	L747_13310	xsome
2398	hypothetical protein	-0.03	0.40	-0.32	**-0.67**	L747_13315	xsome
2399	oxidoreductase ion channel protein IolS	**-0.75**	**0.78**	**1.13**	**0.39**	L747_13320	xsome
2400	guanine permease	**-0.90**	0.13	**0.62**	**0.52**	L747_13325	xsome
2401	hypothetical protein	0.10	-0.10	**-0.62**	**-0.47**	L747_13330	xsome
2402	ABC transporter ATP-binding protein	0.38	**2.06**	**1.53**	**-0.49**	L747_13335	xsome
2403	GntR family transcriptional regulator	0.27	**2.09**	**1.62**	**-0.43**	L747_13340	xsome
2404	1,4-dihydroxy-2-naphthoate prenyltransferase	0.05	**0.57**	0.34	-0.18	L747_13345	xsome
2405	hypothetical protein	0.31	0.16	**0.35**	**0.22**	L747_13350	xsome

2406	hypothetical protein	0.07	**-1.76**	**-0.68**	**1.10**	L747_13355	xsome
2407	hypothetical protein	-0.03	**-1.41**	-1.29	0.15	L747_13360	xsome
2408	transposase ISL3; distrupted(psuedo)	-0.13	-0.55	-0.13	0.44	L747_13720	xsome
2409	hypothetical protein	-0.12	-0.49	-0.29	0.24	L747_13365	xsome
2410	hypothetical protein	-0.38	**-0.93**	-0.50	0.45	L747_13370	xsome
2411	hypothetical protein	-0.57	**-1.47**	**-1.20**	0.30	L747_13375	xsome
2412	hypothetical protein	-0.16	**-2.61**	**-3.24**	**-0.57**	L747_13380	xsome
2413	hypothetical protein	0.02	-1.16	-0.28	0.73	L747_13385	xsome
2414	hypothetical protein	NA	0.24	NA	-0.11	L747_13390	xsome
2415	hypothetical protein	0.36	-0.24	0.00	0.24	L747_13395	xsome
2416	hypothetical protein	-0.03	**-0.95**	**-1.21**	-0.21	L747_13400	xsome
2417	hypothetical protein	-0.21	-0.82	0.01	**0.77**	L747_13405	xsome
2418	hypothetical protein	-0.27	**-1.20**	**-2.40**	**-1.15**	L747_13410	xsome
2419	hypothetical protein	0.22	-0.38	**-1.05**	**-0.61**	L747_13415	xsome
2420	integrase	0.04	**0.75**	**0.49**	**-0.21**	L747_13420	xsome
2421	hypothetical protein	0.01	-0.53	-0.26	0.30	L747_13425	xsome
2422	beta-xylosidase	-0.12	**2.06**	**1.47**	**-0.55**	L747_13430	xsome
2423	xyloside transporter	0.38	**1.05**	0.47	**-0.54**	L747_13435	xsome
2424	AraC family transcriptional regulator	0.01	**-1.28**	**-1.91**	**-0.58**	L747_13440	xsome
2425	cardiolipin synthase	-0.03	**-0.29**	-0.18	0.15	L747_13445	xsome
2426	hypothetical protein	0.12	0.51	1.01	0.49	L747_13450	xsome
2427	DNA-entry nuclease	-0.34	**-1.23**	**-1.18**	0.10	L747_13455	xsome
2428	hypothetical protein	0.12	**1.78**	**2.35**	**0.61**	L747_13460	xsome
2429	hypothetical protein	0.04	**1.65**	**2.34**	**0.71**	L747_13465	xsome
2430	transposase IS30	-0.18	**0.51**	0.20	**-0.27**	L747_13470	xsome
2431	hypothetical protein	-0.37	-0.52	-0.34	0.21	L747_13475	xsome
2432	hypothetical protein	**-1.02**	**-0.97**	**-1.33**	-0.29	L747_13480	xsome
2433	hypothetical protein	0.40	**1.78**	**1.10**	**-0.64**	L747_13485	xsome
2434	hypothetical protein	0.28	0.16	-0.32	**-0.44**	L747_13490	xsome
2435	aminopeptidase	0.28	**3.18**	**1.93**	**-1.20**	L747_13495	xsome
2436	hypothetical protein	0.45	**1.78**	**1.37**	**-0.37**	L747_13500	xsome

2437	signal peptidase	0.01	**0.76**	**0.49**	-0.23	L747_13505	xsome
2438	MFS transporter permease	0.24	**1.11**	**1.75**	**0.67**	L747_13510	xsome
2439	hypothetical protein	**0.78**	**2.67**	**2.71**	0.08	L747_13515	xsome
2440	acyl-CoA hydrolase	0.08	**1.52**	**2.13**	**0.64**	L747_13520	xsome
2441	hypothetical protein	-0.03	0.24	**0.78**	**0.57**	L747_13525	xsome
2442	NADH-flavin reductase	**0.53**	**2.74**	**2.31**	**-0.39**	L747_13530	xsome
2443	branched-chain amino acid transporter II carrier protein	-0.02	**0.70**	**1.16**	**0.49**	L747_13535	xsome
2444	hypothetical protein	0.31	**1.04**	**1.90**	**0.87**	L747_13540	xsome
2445	haloacid dehalogenase	-0.29	**-0.99**	**-0.74**	0.28	L747_13545	xsome
2446	hypothetical protein	0.15	-0.23	0.26	0.48	L747_13550	xsome
2447	hypothetical protein	-0.15	**-1.49**	**-1.72**	-0.18	L747_13555	xsome
2448	tRNA uridine 5-carboxymethylaminomethyl modification protein	0.14	**2.21**	**2.15**	-0.02	L747_13560	xsome
2449	tRNA modification GTPase	0.35	**2.14**	**1.68**	**-0.42**	L747_13565	xsome
2450	RNA-binding protein	0.48	**1.06**	**1.44**	**0.42**	L747_13570	xsome
2451	membrane protein	**0.39**	0.21	**0.74**	**0.56**	L747_13575	xsome
2452	ribonuclease P; rnpA	**0.49**	-0.20	0.07	**0.31**	L747_13580	xsome
2453	50S ribosomal protein L34	**0.67**	**0.31**	**1.48**	**1.19**	L747_13585	xsome
2454	producthypothetical protein; distruped(psuedo)	-0.54	-1.30	**-1.37**	0.01	L747_00005	pLb464-1
2455	initiator RepB protein	**-0.47**	**-1.27**	**-1.22**	0.09	L747_00010	pLb464-1
2456	hypothetical protein	0.39	**-1.49**	**-0.62**	**0.87**	L747_00015	pLb464-1
2457	glycosyl transferase	0.21	**0.26**	**0.85**	**0.63**	L747_00020	pLb464-1
2458	HorA	**0.49**	**0.77**	**1.19**	**0.46**	L747_00025	pLb464-1
2459	1-acyl-sn-glycerol-3-phosphate acyltransferase	-0.18	**-0.61**	**-0.45**	**0.20**	L747_00030	pLb464-1
2460	glycosyl transferase	**-0.33**	**-0.31**	**-0.44**	-0.09	L747_00035	pLb464-1
2461	glycerol acyltransferase	-0.17	**-0.24**	-0.08	**0.20**	L747_00040	pLb464-1
2462	hypothetical protein	-0.07	-0.12	-0.09	0.06	L747_00045	pLb464-1
2463	hypothetical protein	0.01	**-1.06**	**-1.06**	0.04	L747_00050	pLb464-1
2464	transposase	**0.54**	**0.64**	**1.65**	**1.04**	L747_00055	pLb464-1
2465	transposase IS605	**0.89**	**0.89**	**1.84**	**0.98**	L747_00060	pLb464-1

2466	hypothetical protein	-0.39	0.39	**0.63**	0.27	L747_00065	pLb464-1
2467	hypothetical protein	-0.25	0.09	**0.56**	**0.50**	L747_00070	pLb464-1
2468	growth inhibitor PemK	-0.05	-0.14	-0.30	-0.12	L747_00075	pLb464-1
2469	PbsX family transcriptional regulator	0.06	**-1.08**	**-1.95**	**-0.82**	L747_00080	pLb464-1
2470	integrase	-0.30	0.22	-0.02	-0.20	L747_00085	pLb464-1
2471	hypothetical protein	-0.15	**0.83**	**0.95**	0.15	L747_00090	pLb464-1
2472	cro regulatory protein	-0.37	**0.48**	**0.86**	**0.42**	L747_00095	pLb464-2
2473	toxin of toxin-antitoxin stability system	**-0.62**	**-0.97**	**-0.59**	**0.42**	L747_00100	pLb464-2
2474	antitoxin	-0.23	**-1.24**	-0.13	**1.11**	L747_00105	pLb464-2
2475	integrase	-0.20	-0.05	0.03	0.12	L747_00110	pLb464-2
2476	hypothetical protein	-0.55	**0.78**	**0.72**	-0.02	L747_00115	pLb464-2
2477	hypothetical protein	-0.58	**1.32**	**1.26**	-0.02	L747_00120	pLb464-2
2478	hypothetical protein	-0.49	**2.11**	**1.65**	**-0.41**	L747_00125	pLb464-2
2479	ATPase	**-0.72**	**-0.84**	**-1.18**	**-0.29**	L747_00130	pLb464-2
2480	hypothetical protein	-0.38	-0.66	-0.76	-0.06	L747_00135	pLb464-2
2481	DNA-damage-inducible protein J	**-0.92**	**-0.78**	**-1.56**	**-0.73**	L747_00140	pLb464-2
2482	plasmid replication initiation protein	**-1.40**	**-2.12**	**-2.06**	0.10	L747_00145	pLb464-2
2483	dihydrodipicolinate reductase	0.31	0.19	0.00	-0.15	L747_00150	pLb464-2
2484	PRTRC system protein D	**0.52**	-0.17	**-0.29**	-0.08	L747_00155	pLb464-2
2485	transposase	0.14	**2.15**	**2.81**	**0.69**	L747_00160	pLb464-2
2486	transposase	-0.04	**2.15**	**2.68**	**0.57**	L747_00165	pLb464-2
2487	hypothetical protein	-0.35	**0.93**	**2.29**	**1.38**	L747_00170	pLb464-2
2488	transposase ISL3	-0.11	-0.13	0.43	**0.59**	L747_00175	pLb464-2
2489	enolase; eno	**0.79**	**0.93**	**1.42**	**0.53**	L747_00180	pLb464-2
2490	hypothetical protein	-0.04	-0.10	0.26	**0.39**	L747_00185	pLb464-2
2491	transposase	-0.06	0.13	**1.03**	**0.93**	L747_00190	pLb464-2
2492	transposase	-0.07	**-0.76**	**-0.37**	**0.43**	L747_00195	pLb464-2
2493	hypothetical protein	0.05	**1.82**	**2.81**	**0.93**	L747_00200	pLb464-2
2494	hypothetical protein_HorB	**-2.09**	**-1.58**	**-1.35**	0.26	L747_00210	pLb464-2
2495	hypothetical protein_HorC	**4.07**	**4.64**	**5.45**	**0.83**	L747_00215	pLb464-2
2496	transposase IS30	**2.60**	**1.88**	**2.39**	**0.54**	L747_00220	pLb464-2

2497	hypothetical protein	**0.68**	**1.13**	**2.16**	**1.06**	L747_00225	pLb464-2
2498	glycosyltransferase	**0.68**	**1.29**	**2.10**	**0.84**	L747_00230	pLb464-2
2499	membrane protein	**0.81**	**1.59**	**2.67**	**1.11**	L747_00235	pLb464-2
2500	membrane protein	**0.96**	**2.22**	**3.90**	**1.70**	L747_00240	pLb464-2
2501	hypothetical protein	0.00	**1.62**	**2.93**	**1.27**	L747_00245	pLb464-2
2502	nickase	-0.08	-0.18	**0.84**	**1.04**	L747_00250	pLb464-2
2503	hypothetical protein; distruped(psuedo)	-0.47	-0.54	0.08	**0.65**	L747_00255	pLb464-2
2504	initiator RepB protein	-0.22	**0.45**	0.15	**-0.25**	L747_00260	pLb464-2
2505	replication protein RepB	**-0.52**	**2.49**	**2.20**	**-0.24**	L747_00265	pLb464-2
2506	ferritin	**-0.31**	**2.14**	**1.78**	**-0.32**	L747_00270	pLb464-2
2507	hypothetical protein	-0.18	**-1.04**	-0.33	**0.74**	L747_00275	pLb464-2
2508	hypothetical protein	-0.40	**-1.38**	**-0.82**	**0.59**	L747_00290	pLb464-3
2509	Mg2+ and Co2+ transporter	-0.11	**-1.14**	**-0.47**	**0.71**	L747_00295	pLb464-3
2510	manganese transporter	**1.28**	**0.48**	**0.26**	-0.18	L747_00300	pLb464-3
2511	transcriptional regulator	**1.40**	**1.17**	**0.69**	**-0.44**	L747_00305	pLb464-3
2512	transposase	-0.09	**-0.67**	-0.53	0.18	L747_00310	pLb464-3
2513	plasmid replication protein	**-1.15**	**-1.54**	**-0.99**	**0.58**	L747_00315	pLb464-3
2514	hypothetical protein	**-0.61**	**-1.45**	**-0.70**	**0.78**	L747_00320	pLb464-3
2515	hypothetical protein	-0.33	**-0.78**	**0.64**	**1.42**	L747_00325	pLb464-3
2516	transposase IS30	-0.34	-0.24	-0.10	0.17	L747_00330	pLb464-3
2517	hypothetical protein	-0.31	-0.57	0.25	**0.82**	L747_00335	pLb464-3
2518	hypothetical protein	**-1.14**	**-1.65**	**-1.81**	-0.12	L747_00340	pLb464-3
2519	LtrC	0.34	**-0.35**	-0.26	0.13	L747_00345	pLb464-3
2520	transposase	0.21	0.49	**0.96**	**0.49**	L747_00350	pLb464-3
2521	transposase IS4	-0.03	-0.27	-0.03	**0.27**	L747_00355	pLb464-3
2522	hypothetical protein	0.02	**-1.13**	**-1.56**	**-0.39**	L747_00365	pLb464-3
2523	DNA topoisomerase	-0.18	**-0.26**	**-0.68**	**-0.38**	L747_00370	pLb464-3
2524	nickase	**-0.30**	-0.18	**-1.34**	**-1.11**	L747_00375	pLb464-3
2525	hypothetical protein	**-1.10**	**-0.30**	-0.23	0.12	L747_00380	pLb464-3
2526	hypothetical protein	**-0.77**	0.19	-0.19	**-0.34**	L747_00385	pLb464-3
2527	hypothetical protein	-0.36	**-0.99**	-0.31	**0.71**	L747_00390	pLb464-3

2528	transposase; distrupted(psuedo)	-0.37	**-1.60**	**-1.24**	**0.39**	L747_00395	pLb464-3
2529	DeoR family transcriptional regulator	**-0.53**	**-1.12**	**-1.35**	-0.18	L747_00405	pLb464-4
2530	membrane protein	**0.67**	**0.63**	**-0.88**	**-1.46**	L747_00410	pLb464-4
2531	hypothetical protein	0.26	**0.61**	**-1.13**	**-1.68**	L747_00415	pLb464-4
2532	membrane protein	0.29	**2.05**	**1.42**	**-0.59**	L747_00420	pLb464-4
2533	transposase IS30; distrupted(psuedo)	0.37	**0.94**	0.19	**-0.71**	L747_01000	pLb464-4
2534	multidrug ABC transporter permease	**0.46**	**0.43**	-0.24	**-0.62**	L747_00425	pLb464-4
2535	ABC transporter ATP-binding protein	0.23	**0.38**	**0.47**	0.13	L747_00430	pLb464-4
2536	resolvase	0.27	**0.45**	**0.47**	0.06	L747_00435	pLb464-4
2537	hypothetical protein	-0.46	**-1.23**	**-0.69**	**0.58**	L747_00440	pLb464-4
2538	hypothetical protein	-0.27	**-1.71**	**-1.65**	0.11	L747_00445	pLb464-4
2539	hypothetical protein	**-0.76**	**-0.91**	**-1.00**	-0.05	L747_00450	pLb464-4
2540	hypothetical protein	**-0.96**	0.09	0.14	0.08	L747_00455	pLb464-4
2541	LtrC	**-0.64**	**-0.37**	-0.26	0.15	L747_00460	pLb464-4
2542	hypothetical protein	0.14	0.74	**1.73**	**0.96**	L747_00465	pLb464-4
2543	DNA topoisomerase	**-0.57**	**-0.36**	**-0.36**	0.04	L747_00470	pLb464-4
2544	nickase	**-0.83**	**-0.73**	**-1.28**	**-0.50**	L747_00475	pLb464-4
2545	hypothetical protein	**-1.34**	0.05	0.33	**0.32**	L747_00480	pLb464-4
2546	hypothetical protein	**-1.33**	**-0.69**	**-1.07**	**-0.33**	L747_00485	pLb464-4
2547	hypothetical protein	-0.17	**1.05**	**1.46**	**0.44**	L747_00490	pLb464-4
2548	hypothetical protein	**-1.04**	-0.74	0.19	**0.92**	L747_00495	pLb464-4
2549	hypothetical protein	-0.50	0.08	**1.17**	**1.12**	L747_00500	pLb464-4
2550	hypothetical protein	**-1.00**	**-0.49**	0.11	**0.63**	L747_00505	pLb464-4
2551	hypothetical protein	NA	**4.02**	1.55	**-2.57**	L747_00510	pLb464-4
2552	DNA-damage-inducible protein J	**-0.93**	**-0.33**	0.17	**0.54**	L747_00515	pLb464-4
2553	replication protein	**-1.35**	**-1.03**	**-1.05**	0.03	L747_00520	pLb464-4
2554	transposase	NA	0.24	NA	-0.11	L747_00525	pLb464-4
2555	hypothetical protein	NA	1.68	0.48	-0.90	L747_00530	pLb464-4
2556	hypothetical protein	**-0.43**	**0.75**	**0.49**	**-0.21**	L747_00535	pLb464-4
2557	hypothetical protein	NA	**4.06**	0.89	**-3.09**	L747_00540	pLb464-4
2558	hypothetical protein; distruped(psuedo)	NA	1.71	NA	**-1.28**	L747_00545	pLb464-4

2559	hypothetical protein	NA	1.68	0.48	**-0.94**	L747_00550	pLb464-4
2560	hypothetical protein	NA	**2.34**	NA	**-1.86**	L747_00555	pLb464-4
2561	hypothetical protein	NA	**4.34**	NA	**-4.06**	L747_00560	pLb464-4
2562	hypothetical protein	NA	**3.28**	0.81	**-2.20**	L747_00565	pLb464-4
2563	hypothetical protein	NA	**2.73**	0.26	**-2.11**	L747_00570	pLb464-4
2564	hypothetical protein	NA	**3.48**	NA	**-3.10**	L747_00575	pLb464-4
2565	hypothetical protein	NA	**5.12**	1.19	**-4.06**	L747_00580	pLb464-4
2566	hypothetical protein	NA	1.55	NA	**-1.13**	L747_00585	pLb464-4
2567	hypothetical protein	NA	**4.48**	0.25	**-4.05**	L747_00590	pLb464-4
2568	hypothetical protein	-0.06	**5.06**	0.16	**-4.74**	L747_00595	pLb464-4
2569	hypothetical protein	NA	**5.14**	0.89	**-4.37**	L747_00600	pLb464-4
2570	hypothetical protein	NA	**6.57**	2.49	**-4.72**	L747_00605	pLb464-4
2571	hypothetical protein	NA	**2.44**	NA	**-1.95**	L747_00610	pLb464-4
2572	hypothetical protein	NA	**3.57**	0.26	**-3.02**	L747_00615	pLb464-4
2573	tail protein	NA	**5.99**	1.32	**-5.03**	L747_00620	pLb464-4
2574	hypothetical protein	NA	**5.19**	0.48	**-4.61**	L747_00625	pLb464-4
2575	hypothetical protein	NA	**5.02**	0.71	**-4.32**	L747_00630	pLb464-4
2576	hypothetical protein	NA	**4.50**	0.71	**-3.76**	L747_00635	pLb464-4
2577	DNA packaging protein	NA	**4.78**	0.25	**-4.25**	L747_00640	pLb464-4
2578	capsid protein	NA	**6.44**	2.32	**-4.70**	L747_00645	pLb464-4
2579	peptidase	NA	**6.05**	1.44	**-4.97**	L747_00650	pLb464-4
2580	portal protein	NA	**6.39**	1.92	**-4.94**	L747_00655	pLb464-4
2581	hypothetical protein	NA	1.09	NA	-0.73	L747_00660	pLb464-4
2582	terminase; distrupted(psuedo)	NA	**6.64**	1.91	**-5.28**	L747_00665	pLb464-4
2583	terminase	NA	**5.39**	1.19	**-4.42**	L747_00670	pLb464-4
2584	hypothetical protein	NA	**4.23**	0.48	**-3.57**	L747_00675	pLb464-4
2585	HNH endonuclease	NA	**4.67**	1.05	**-3.70**	L747_00680	pLb464-4
2586	hypothetical protein	NA	1.36	0.25	-0.79	L747_00685	pLb464-4
2587	hypothetical protein	NA	**2.59**	0.26	**-1.93**	L747_00690	pLb464-4
2589	hypothetical protein	0.09	**4.17**	NA	**-3.76**	L747_00700	pLb464-4
2590	hypothetical protein	NA	1.96	NA	**-1.51**	L747_00705	pLb464-4

2591	hypothetical protein	NA	1.36	NA	**-0.95**	L747_00710	pLb464-4
2592	producthypothetical protein	NA	NA	NA	NA	L747_00715	pLb464-4
2593	hypothetical protein	NA	**3.00**	0.48	**-2.20**	L747_00720	pLb464-4
2594	hypothetical protein	NA	**2.81**	NA	**-2.38**	L747_00725	pLb464-4
2595	hypothetical protein	NA	**2.65**	NA	**-2.21**	L747_00730	pLb464-4
2596	hypothetical protein	NA	**2.60**	NA	**-2.15**	L747_00735	pLb464-4
2597	hypothetical protein	NA	1.75	NA	**-1.31**	L747_00740	pLb464-4
2598	hypothetical protein	NA	**2.71**	NA	**-2.26**	L747_00745	pLb464-4
2599	hypothetical protein	NA	**3.38**	NA	**-3.00**	L747_00750	pLb464-4
2600	hypothetical protein	NA	**3.87**	NA	**-3.54**	L747_00755	pLb464-4
2601	hypothetical protein	NA	**2.94**	NA	**-2.53**	L747_00760	pLb464-4
2602	hypothetical protein	NA	1.46	NA	**-1.04**	L747_00765	pLb464-4
2603	hypothetical protein	NA	**3.13**	0.25	**-2.54**	L747_00770	pLb464-4
2604	single-stranded DNA-binding protein	NA	**2.25**	NA	**-1.77**	L747_00775	pLb464-4
2605	hypothetical protein	NA	**3.29**	0.25	**-2.69**	L747_00780	pLb464-4
2606	recombinase	NA	**3.61**	0.48	**-2.84**	L747_00785	pLb464-4
2607	hypothetical protein	NA	**3.26**	NA	**-2.79**	L747_00790	pLb464-4
2608	hypothetical protein	NA	1.55	NA	**-1.13**	L747_00795	pLb464-4
2609	producthypothetical protein	NA	NA	NA	NA	L747_00800	pLb464-4
2610	hypothetical protein	NA	0.51	NA	-0.32	L747_00805	pLb464-4
2611	hypothetical protein	NA	1.86	NA	**-1.40**	L747_00810	pLb464-4
2612	hypothetical protein	NA	**4.24**	1.19	**-3.08**	L747_00815	pLb464-4
2613	hypothetical protein	NA	0.81	NA	-0.48	L747_00820	pLb464-4
2614	hypothetical protein	NA	1.53	NA	**-1.04**	L747_00825	pLb464-4
2615	hypothetical protein	NA	1.59	0.26	**-0.98**	L747_00830	pLb464-4
2616	hypothetical protein	NA	**2.35**	NA	**-1.90**	L747_00835	pLb464-4
2617	hypothetical protein	NA	1.46	NA	**-1.04**	L747_00840	pLb464-4
2618	hypothetical protein	NA	**2.40**	0.25	**-1.76**	L747_00845	pLb464-4
2619	producthypothetical protein	NA	NA	NA	NA	L747_00850	pLb464-4
2620	hypothetical protein	NA	**4.66**	0.88	**-3.76**	L747_00855	pLb464-4
2621	hypothetical protein	NA	2.14	0.25	**-1.51**	L747_00860	pLb464-4

2622	hypothetical protein	NA	1.90	0.25	**-1.28**	L747_00865	pLb464-4
2623	hypothetical protein	NA	**2.25**	0.48	**-1.46**	L747_00870	pLb464-4
2624	hypothetical protein	NA	**2.30**	NA	**-1.72**	L747_00875	pLb464-4
2625	hypothetical protein	-0.11	**8.51**	**5.35**	**-3.45**	L747_00880	pLb464-4
2626	hypothetical protein	NA	**4.19**	1.19	**-3.06**	L747_00885	pLb464-4
2627	Lb464_xsome partitioning ATPase	NA	**3.51**	0.48	**-2.81**	L747_00890	pLb464-4
2628	hypothetical protein	-0.18	0.63	**1.20**	**0.56**	L747_00895	pLb464-4
2629	DNA integrase	NA	**3.44**	0.88	**-2.44**	L747_00900	pLb464-4
2630	metal ABC transporter substrate-binding protein	NA	**5.36**	2.07	**-3.60**	L747_00905	pLb464-4
2631	N-acetylmuramoyl-L-alanine amidase; distrupted(psuedo)	NA	**5.79**	1.32	**-4.54**	L747_00910	pLb464-4
2632	hypothetical protein	NA	**2.61**	NA	**-2.16**	L747_00915	pLb464-4
2633	30S ribosomal protein S14	NA	**3.39**	0.89	**-2.36**	L747_00920	pLb464-4
2634	50S ribosomal protein L33	NA	**4.07**	0.64	**-3.05**	L747_00925	pLb464-4
2635	transposase IS30; distrupted(psuedo)	-0.05	**2.28**	0.80	**-1.38**	L747_01005	pLb464-4
2636	glycosyl hydrolase family 25	NA	**4.28**	1.55	**-2.84**	L747_00930	pLb464-4
2637	transposase	0.27	**0.83**	**1.23**	**0.43**	L747_00935	pLb464-4
2638	transposase IS4	0.47	0.46	**0.77**	**0.35**	L747_00940	pLb464-4
2639	hypothetical protein	-0.71	**0.86**	**1.40**	**0.57**	L747_00945	pLb464-4
2640	plasmid replication initiation protein	-0.40	-0.43	**-1.59**	**-1.07**	L747_00950	pLb464-4
2641	integrase	0.31	**0.78**	**0.63**	-0.11	L747_00955	pLb464-4
2642	hypothetical protein	NA	1.36	0.25	-0.79	L747_00960	pLb464-4
2643	hypothetical protein	NA	1.53	NA	**-1.04**	L747_00965	pLb464-4
2644	hypothetical protein	NA	1.88	0.25	**-1.24**	L747_00970	pLb464-4
2645	peptide transporter	NA	**3.55**	0.71	**-2.63**	L747_00975	pLb464-4
2646	MFS transporter	NA	**4.53**	1.44	**-3.20**	L747_00980	pLb464-4
2647	pyrimidine dimer DNA glycosylase; distrupted(psuedo)	NA	**6.79**	**2.89**	**-4.54**	L747_00985	pLb464-4
2648	integrase	-0.05	**6.70**	**3.23**	**-3.78**	L747_00990	pLb464-4
2649	universal stress protein UspA	0.29	**2.23**	**1.37**	**-0.80**	L747_00995	pLb464-4
2650	integrase	**-1.03**	**-0.73**	-0.34	**0.43**	L747_01010	pLb464-5

2651	RelB	**-0.78**	**-2.36**	**-2.60**	-0.19	L747_01015	pLb464-5
2652	translation repressor RelE	**-0.69**	**-2.91**	**-3.16**	-0.03	L747_01020	pLb464-5
2653	hypothetical protein	-0.40	**-0.59**	**0.44**	**1.06**	L747_01025	pLb464-5
2654	type I restriction-modification protein subunit M	-0.12	-0.29	0.27	**0.59**	L747_01030	pLb464-5
2655	type I restriction enzyme R protein	**-0.47**	**-0.91**	**-0.58**	**0.37**	L747_01035	pLb464-5
2656	hypothetical protein	**-1.02**	**-1.01**	-0.04	**0.92**	L747_01040	pLb464-5
2657	RepB family protein	-0.66	**-0.49**	**-0.55**	-0.03	L747_01045	pLb464-5
2658	initiator RepB protein	**-0.95**	**-1.01**	**-0.37**	**0.68**	L747_01050	pLb464-5
2659	hypothetical protein	**-0.75**	-0.19	-0.32	-0.09	L747_01060	pLb464-6
2660	relaxase	**-0.90**	**-0.62**	**-0.76**	-0.10	L747_01065	pLb464-6
2661	initiator RepB protein	-0.34	**-0.93**	**-1.30**	**-0.33**	L747_01070	pLb464-6
2662	hypothetical protein	-0.59	-0.55	-0.46	0.12	L747_01075	pLb464-6
2663	cadmium transporter	**-1.04**	**-1.18**	**-1.12**	0.10	L747_01080	pLb464-6
2664	ArsR family transcriptional regulator	**-1.79**	**-1.29**	**-1.29**	0.04	L747_01085	pLb464-6
2665	protein rep	**-1.16**	**-1.90**	**-2.36**	**-0.41**	L747_01090	pLb464-7
2666	ATP-dependent Clp protease ATP-binding protein	**0.26**	**2.17**	**0.28**	**-1.84**	L747_01095	pLb464-8
2667	transposase	-0.22	0.54	**0.77**	0.26	L747_01100	pLb464-8
2668	hypothetical protein	-0.25	0.57	**0.79**	0.24	L747_01105	pLb464-8
2669	hypothetical protein	**1.03**	-0.11	0.26	**0.41**	L747_01110	pLb464-8
2670	DeoR family transcriptional regulator	**0.36**	0.09	**0.45**	**0.40**	L747_01115	pLb464-8
2671	transposase IS1216	0.04	-0.04	**0.83**	**0.91**	L747_01120	pLb464-8
2672	hypothetical protein	-0.44	-0.42	0.46	**0.88**	L747_01125	pLb464-8
2673	transposase	0.14	-0.47	0.14	**0.64**	L747_01130	pLb464-8
2674	FMN-binding protein	-0.26	-0.46	0.47	**0.94**	L747_01135	pLb464-8
2675	NAD(FAD)-dependent dehydrogenase	**0.51**	**1.01**	**1.40**	**0.43**	L747_01140	pLb464-8
2676	transposase	-0.29	-0.03	**0.44**	**0.51**	L747_01145	pLb464-8
2677	hypothetical protein	0.02	**0.36**	**1.39**	**1.06**	L747_01150	pLb464-8
2678	hypothetical protein	-0.17	-0.12	**0.45**	**0.60**	L747_01155	pLb464-8
2679	transposase	-0.32	-0.08	**0.33**	**0.45**	L747_01165	pLb464-8
2680	hypothetical protein	0.09	0.16	**0.60**	**0.46**	L747_01170	pLb464-8
2681	copy number control protein	-0.16	-0.17	**-0.70**	**-0.49**	L747_01175	pLb464-8

2682	hypothetical protein	0.18	**-1.03**	**0.49**	**1.52**	L747_01180	pLb464-8
2683	plasmid replication initiation protein	**-0.86**	**-0.73**	**-0.53**	**0.24**	L747_01185	pLb464-8
2684	hypothetical protein	-0.19	0.01	**0.41**	**0.43**	L747_01190	pLb464-8
2685	hypothetical protein	-0.61	**-0.84**	-0.49	**0.39**	L747_01195	pLb464-8
2686	nicakse; distrupted(psuedo)	**-0.36**	**-0.83**	**-0.66**	**0.21**	L747_01200	pLb464-8
2687	transposase	0.12	**0.73**	**0.53**	-0.17	L747_01205	pLb464-8
2688	LtrC	-0.31	**-1.03**	**-0.75**	**0.33**	L747_01210	pLb464-8
2689	2-hydroxyacid dehydrogenase	-0.03	**-1.38**	**-0.72**	**0.69**	L747_01215	pLb464-8
2690	hypothetical protein	0.10	0.46	**1.11**	**0.68**	L747_01220	pLb464-8
2691	acetyl-CoA carboxylase	-0.13	0.06	0.31	0.28	L747_01225	pLb464-8
2692	UV-damage repair protein uvrX	0.09	0.07	**0.63**	**0.59**	L747_01230	pLb464-8
2693	hypothetical protein	-0.08	**0.76**	**3.23**	**2.09**	L747_01235	pLb464-8
2694	hypothetical protein	**-0.63**	**-0.84**	-0.08	**0.80**	L747_01240	pLb464-8
2695	producttransposase	0.15	**0.43**	**0.39**	0.00	L747_01245	pLb464-8
2696	hypothetical protein	-0.43	-0.18	**0.62**	**0.83**	L747_01250	pLb464-8
2697	restriction endonuclease subunit M	**-0.65**	**-0.69**	-0.12	**0.60**	L747_01255	pLb464-8
2698	type I restriction endonuclease	**-0.94**	**-1.40**	**-0.70**	**0.73**	L747_01260	pLb464-8
2699	holin	-0.47	**1.33**	**2.89**	**1.55**	L747_01265	pLb464-8
2700	hypothetical protein	**-0.57**	0.07	0.05	0.02	L747_01270	pLb464-8
2701	hypothetical protein	**-0.88**	**-0.58**	**-1.03**	**-0.41**	L747_01275	pLb464-8
2702	resolvase	**-1.33**	**-0.64**	-0.16	**0.52**	L747_01280	pLb464-8
2703	integrase	0.00	**-0.31**	**0.77**	**1.11**	L747_01285	pLb464-8
2704	FAD-dependent pyridine nucleotide-disulfide oxidoreductase	**0.76**	**1.36**	**1.94**	**0.62**	L747_01290	pLb464-8
2705	hypothetical protein	**0.54**	**1.03**	**2.09**	**1.09**	L747_01295	pLb464-8
2706	integrase	0.27	**0.59**	**1.49**	**0.93**	L747_01300	pLb464-8
2707	hypothetical protein	-0.16	0.12	0.33	0.24	L747_01305	pLb464-8

Appendix 2: Table S7.2 – DESeq2 analysis of Pc344 gene expression grown in hops.

Black indicates Log_2 Fold Change (FC) of expression in first listed condition. **BOLD** indicates significant FC (< 0.1 FDR).
Blue (-) indicates Log_2 Fold Change (FC) of expression in second listed condition. **BOLD** indicates significant FC (< 0.1 FDR).

Gene	*Product*	*30 BU Hops vs. mMRS*	*Locus_tag*	*Location*
0	50S ribosomal protein L34	**0.33**	PECL_1	xsome
1	chromosomal replication initiator protein DnaA	0.21	PECL_2	xsome
2	DNA polymerase III subunit beta	-0.24	PECL_3	xsome
3	S4 domain-containing protein YaaA	0.10	PECL_4	xsome
4	DNA replication and repair RecF family protein	**-0.33**	PECL_5	xsome
5	DNA gyrase subunit B	0.02	PECL_6	xsome
6	DNA gyrase subunit A	-0.10	PECL_7	xsome
7	30S ribosomal protein S6	**-0.70**	PECL_8	xsome
8	single-stranded DNA-binding protein ssb	**-0.39**	PECL_9	xsome
9	30S ribosomal protein S18	-0.09	PECL_10	xsome
10	DHH family protein	-0.08	PECL_11	xsome
11	50S ribosomal protein L9	-0.18	PECL_12	xsome
12	replicative DNA helicase	0.00	PECL_13	xsome
13	major facilitator superfamily protein	**0.29**	PECL_14	xsome
14	nlpC, FP60 family protein	**1.09**	PECL_15	xsome
15	hypothetical protein	0.41	PECL_16	xsome
16	uracil DNA glycosylase superfamily protein	**1.00**	PECL_17	xsome
17	hypothetical protein	**1.75**	PECL_18	xsome
18	ABC transporter family protein	**0.97**	PECL_19	xsome
19	EAL domain-signal transduction regulation protein	**-0.67**	PECL_20	xsome
20	NADH oxidase	**-0.51**	PECL_21	xsome
21	diguanylate cyclase domain protein	-0.23	PECL_22	xsome
22	hypothetical protein	**1.61**	PECL_23	xsome
23	ABC transporter family protein	-0.02	PECL_24	xsome

24	ABC-2 transporter family protein	**0.63**	PECL_25	xsome
25	FAD linked oxidase%2C C-terminal domain-containing protein	**0.54**	PECL_26	xsome
26	hypothetical protein	0.21	PECL_27	xsome
27	hypothetical protein	0.54	PECL_28	xsome
28	hypothetical protein	-0.19	PECL_29	xsome
29	type III restriction enzyme, res subunit	-0.25	PECL_30	xsome
30	metallo-beta-lactamase superfamily protein	**0.46**	PECL_31	xsome
31	PTS system fructose IIA component family protein	**0.28**	PECL_32	xsome
32	hypothetical protein	**0.43**	PECL_33	xsome
33	PTS system sorbose subIIB component family protein	-0.18	PECL_34	xsome
34	PTS system sorbose-specific IIC component family	**0.44**	PECL_35	xsome
35	PTS system mannose/fructose/sorbose IID component family protein	**0.44**	PECL_36	xsome
36	predicted transcriptional regulator of N-Acetylglucosamine utilization/GntR family; psuedo	0.17	PECL_2019	xsome
37	sugar isomerase	**0.28**	PECL_39	xsome
38	N-acetylglucosamine-6-phosphate deacetylase	0.22	PECL_40	xsome
39	HAD hydrolase family protein	**0.47**	PECL_41	xsome
40	tagatose 1,6-diphosphate aldolase	**0.34**	PECL_42	xsome
42	prolyl oligopeptidase family protein	**-0.53**	PECL_44	xsome
43	diacylglycerol kinase family protein	**-1.05**	PECL_45	xsome
44	hypothetical protein	**1.53**	PECL_46	xsome
45	hypothetical protein	-0.26	PECL_47	xsome
46	alpha/beta hydrolase superfamily protein	**-1.15**	PECL_48	xsome
47	thioredoxin family protein	**1.10**	PECL_49	xsome
48	NAD-dependent epimerase/dehydratase family protein	**0.53**	PECL_50	xsome
49	NAD dependent epimerase/dehydratase family protein	**1.26**	PECL_51	xsome
50	short chain dehydrogenase family protein	**1.31**	PECL_52	xsome
51	dipeptidase family protein	**-0.73**	PECL_53	xsome
52	MarR family transcriptional regulator	**-0.72**	PECL_54	xsome
53	NADPH-dependent FMN reductase family protein	**-0.37**	PECL_55	xsome
54	tyrosine--tRNA ligase	**-0.75**	PECL_56	xsome

55	major facilitator superfamily protein	**2.24**	PECL_57	xsome
60	acetyltransferase family protein	-0.26	PECL_65	xsome
61	permease for cytosine/purines,uracil,thiamine, allantoin family protein	**-0.74**	PECL_66	xsome
62	cytosine deaminase	-0.28	PECL_67	xsome
63	zinc-binding dehydrogenase family protein	0.40	PECL_68	xsome
64	hypothetical protein	**2.04**	PECL_69	xsome
65	iron dependent repressor, marR family	**1.15**	PECL_70	xsome
66	substrate binding domain of ABC-type glycine betaine transport system family protein	-0.17	PECL_71	xsome
67	ABC transporter family protein	**-0.37**	PECL_72	xsome
68	pyridine nucleotide-disulfide oxidoreductase family protein	**-0.28**	PECL_73	xsome
69	proton antiporter-2 family protein	-0.06	PECL_74	xsome
70	zeta toxin family protein	**-0.62**	PECL_75	xsome
71	pyruvate carboxylase	**-0.64**	PECL_76	xsome
72	beta-eliminating lyase family protein	-0.24	PECL_77	xsome
73	glycosyl hydrolase family 3, N terminal domain containing protein	0.05	PECL_78	xsome
74	uvrD-FREP helicase family protein	**1.31**	PECL_79	xsome
75	tryptophan--tRNA ligase	**-0.61**	PECL_80	xsome
76	alpha/beta hydrolase fold family protein	**2.70**	PECL_81	xsome
77	hypothetical protein	**1.15**	PECL_82	xsome
78	NAD dependent epimerase/dehydratase family protein	**2.13**	PECL_83	xsome
79	Ppx/GppA phosphatase family protein	**-2.10**	PECL_84	xsome
80	hydroxymethylglutaryl-CoA reductase, degradative	**-1.86**	PECL_85	xsome
81	hypothetical protein	**-0.91**	PECL_86	xsome
82	extracellular solute-binding, 5 Middle family protein	**-2.01**	PECL_87	xsome
83	Putative stress-responsive transcriptional regulator	**1.12**	PECL_88	xsome
84	hypothetical protein	**-0.72**	PECL_89	xsome
85	hypothetical protein	**-0.37**	PECL_90	xsome
86	methionine-tRNA ligase	**-0.48**	PECL_2021	xsome
87	TatD family hydrolase	**-0.52**	PECL_92	xsome

88	ribonuclease M5	**-1.16**	PECL_93	xsome
89	dimethyladenosine transferase	**-1.19**	PECL_94	xsome
90	hypothetical protein	**0.50**	PECL_95	xsome
91	4-cytidine 5-diphospho-methyl-D-erythritol kinase	**-0.36**	PECL_96	xsome
92	TetR family transcriptional regulator	**-0.72**	PECL_97	xsome
93	universal stress family protein	**-0.78**	PECL_98	xsome
94	universal stress family protein	-0.26	PECL_99	xsome
95	cytochrome b5-like Heme/Steroid binding domain protein	**1.95**	PECL_100	xsome
96	hypothetical protein	**-0.78**	PECL_101	xsome
97	hypothetical protein	**2.67**	PECL_102	xsome
98	RelB/DinJ family addiction module antitoxin	**2.20**	PECL_103	xsome
99	beta-hydroxyacyl-acyl-carrier-proteindehydratase FabZ	**-0.77**	PECL_104	xsome
100	3-oxoacyl-Bacyl-carrier-synthase III family protein	**-1.14**	PECL_105	xsome
101	acyl carrier protein	**-0.94**	PECL_106	xsome
102	malonyl CoA-acyl carrier protein transacylase	**-1.45**	PECL_107	xsome
103	3-oxoacyl-ACP reductase	**-1.97**	PECL_108	xsome
104	beta-ketoacyl-acyl-carrier-protein synthase II	**-1.51**	PECL_109	xsome
105	acetyl-CoA carboxylase%2C biotin carboxyl carrier protein	**-1.67**	PECL_110	xsome
106	hydroxymyristoyl-ACP dehydratase	**-1.42**	PECL_111	xsome
107	acetyl-CoA carboxylase%2C biotin carboxylase subunit	**-1.74**	PECL_112	xsome
108	acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	**-1.81**	PECL_113	xsome
109	acetyl co-enzyme A carboxylase carboxyltransferase alpha subunit	**-1.72**	PECL_114	xsome
110	enoyl-Bacyl-carrier-protein-D reductase-NADH; FabI	**-1.83**	PECL_115	xsome
111	4-phosphopantetheinyl transferase	**-1.88**	PECL_116	xsome
112	bioY family protein	**-1.65**	PECL_117	xsome
113	glycosyl transferase 2 family protein; psuedo	**-1.84**	PECL_2020	xsome
114	hypothetical protein	**-1.22**	PECL_120	xsome
115	beta-lactamase	**-0.71**	PECL_121	xsome
116	hypothetical protein	**-0.83**	PECL_122	xsome
117	hypothetical protein	**-0.93**	PECL_123	xsome
118	D-serine ammonia-lyase	**-1.79**	PECL_124	xsome

119	ABC transporter family protein	**0.32**	PECL_125	xsome
120	branched-chain amino acid transport system permease component family protein	**0.50**	PECL_126	xsome
121	ABC transporter substrate binding family protein	0.18	PECL_127	xsome
122	hypothetical protein	0.14	PECL_128	xsome
123	putative phosphinothricin acetyltransferase YwnH	0.20	PECL_129	xsome
124	putative membrane protein; psuedo	**-0.78**	PECL_2023	xsome
125	hypothetical protein	**-1.12**	PECL_132	xsome
126	glycosyl transferase 2 family protein	**-1.07**	PECL_133	xsome
127	cellulose synthase subunit	**-0.83**	PECL_134	xsome
128	hypothetical protein	**-0.34**	PECL_135	xsome
129	hypothetical protein	**-0.43**	PECL_136	xsome
130	hypothetical protein	**3.36**	PECL_137	xsome
131	GntR family transcriptional regulator	**0.99**	PECL_138	xsome
132	PTS system Lactose/Cellobiose specific IIB subunit	**1.50**	PECL_139	xsome
133	PTS system Lactose/Cellobiose specific IIA subunit	**1.36**	PECL_140	xsome
134	6-phospho-beta-glucosidase	**1.27**	PECL_141	xsome
135	helix-turn-helix domain/ rpiR family protein	**1.24**	PECL_142	xsome
136	hypothetical protein	**0.71**	PECL_143	xsome
137	sortase	**1.03**	PECL_144	xsome
138	aspartate-semialdehyde dehydrogenase	**0.57**	PECL_145	xsome
139	aminotransferase class-V family protein	0.26	PECL_146	xsome
140	dihydrodipicolinate reductase	**0.52**	PECL_147	xsome
141	dihydrodipicolinate synthase	**0.68**	PECL_148	xsome
142	amidohydrolase family protein	**0.74**	PECL_149	xsome
143	5-tetrahydropyridine-6-carboxylate N-succinyltransferase	**1.15**	PECL_150	xsome
144	diaminopimelate decarboxylase	**1.31**	PECL_151	xsome
145	hypothetical protein	0.26	PECL_152	xsome
146	aspartate kinase domain protein	**0.77**	PECL_153	xsome
147	diaminopimelate epimerase	**1.02**	PECL_154	xsome
148	glycosyl transferase 2 family protein	**1.54**	PECL_155	xsome

149	hypothetical protein	1.01	PECL_156	xsome
150	putative outer membrane protein	**1.08**	PECL_157	xsome
151	mga helix-turn-helix domain protein	**0.93**	PECL_158	xsome
152	acetyltransferase	0.09	PECL_159	xsome
153	ABC-type cation transporter periplasmic component	0.10	PECL_160	xsome
154	ABC-type cation transporter ATPase component	0.21	PECL_161	xsome
155	ABC-type cation transporter permease component	**0.40**	PECL_162	xsome
156	pur operon repressor PurR	**-0.31**	PECL_163	xsome
157	UDP-N-acetylglucosamine diphosphorylase/glucosamine-1-phosphate N-acetyltransferase	**-0.30**	PECL_164	xsome
158	ribose-phosphate pyrophosphokinase	**-0.56**	PECL_165	xsome
159	polysaccharide biosynthesis family protein	**1.74**	PECL_166	xsome
160	MerR family transcriptional regulator	**0.83**	PECL_167	xsome
161	hypothetical protein	**2.73**	PECL_168	xsome
162	MIP channel s family protein	**-1.70**	PECL_169	xsome
163	hypothetical protein	**-0.64**	PECL_170	xsome
164	hypothetical protein	0.01	PECL_171	xsome
165	D-alanyl-D-alanine carboxypeptidase	**-0.80**	PECL_172	xsome
166	hypothetical protein	**-0.95**	PECL_173	xsome
167	HAD hydrolase	**-0.40**	PECL_174	xsome
168	putative malate permease	-0.11	PECL_175	xsome
170	carbonic anhydrase	**-0.58**	PECL_177	xsome
171	TetR family transcriptional regulator	**-0.62**	PECL_178	xsome
172	nitroreductase family protein	**-0.77**	PECL_179	xsome
173	dihydrodipicolinate synthetase family protein	**-0.58**	PECL_180	xsome
174	acetyltransferase family protein	**0.67**	PECL_181	xsome
175	TetR family transcriptional regulator	**0.82**	PECL_182	xsome
176	H-antiporter-2 family protein	**1.16**	PECL_183	xsome
177	H-antiporter-2 family protein	**1.56**	PECL_184	xsome
178	methyladenine glycosylase family protein	**1.02**	PECL_185	xsome
179	hypothetical protein	**0.95**	PECL_186	xsome

180	guanosine monophosphate reductase	-0.09	PECL_187	xsome
181	hypothetical protein	**-1.21**	PECL_188	xsome
182	H- antiporter-2 family protein	**-1.08**	PECL_189	xsome
183	putative transcriptional regulator	**-0.75**	PECL_190	xsome
184	riboflavin transporter RibU	**-0.90**	PECL_191	xsome
185	acetyltransferase family protein	**-0.73**	PECL_192	xsome
186	D-alanine—poly-phosphoribitol ligase subunit 1	-0.08	PECL_193	xsome
187	D-alanyl-lipoteichoic acid biosynthesis protein DltB	-0.19	PECL_194	xsome
188	D-alanine—poly phosphoribitol ligase subunit 2	**0.55**	PECL_195	xsome
189	D-alanyl-lipoteichoic acid biosynthesis protein DltD	0.06	PECL_196	xsome
190	hypothetical protein	-0.14	PECL_197	xsome
191	hypothetical protein	0.10	PECL_198	xsome
192	hypothetical protein	**-0.34**	PECL_199	xsome
193	TetR family transcriptional regulator	0.35	PECL_200	xsome
194	hypothetical protein	0.11	PECL_201	xsome
195	FAD-NAD-dependent oxidoreductase	-0.06	PECL_202	xsome
196	HAD hydrolase family protein	**0.36**	PECL_204	xsome
197	hypothetical protein	0.41	PECL_203	xsome
198	CAAX amino terminal protease self- immunity family protein	-0.23	PECL_205	xsome
199	MATE efflux family protein	**0.52**	PECL_206	xsome
200	hypothetical protein	**1.06**	PECL_207	xsome
201	hypothetical protein	**0.71**	PECL_208	xsome
202	alternate 30S ribosomal protein S14	**2.58**	PECL_209	xsome
203	hypothetical protein	**1.94**	PECL_210	xsome
204	iron dependent repressor N-terminal DNA binding domain protein	**0.74**	PECL_211	xsome
205	hypothetical protein	0.20	PECL_212	xsome
206	hypothetical protein	0.07	PECL_213	xsome
207	HD domain-containing protein	-0.16	PECL_214	xsome
208	hypothetical protein	-0.22	PECL_215	xsome
209	putative DNA-directed RNA polymerase%2C delta subunit	**-0.28**	PECL_216	xsome
210	CTP synthase	**-0.36**	PECL_217	xsome

211	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	**-1.03**	PECL_218	xsome
212	transcription termination factor Rho	**-1.11**	PECL_219	xsome
213	50S ribosomal protein L31	**-0.35**	PECL_220	xsome
214	major facilitator superfamily protein	-0.22	PECL_221	xsome
215	sortase family protein	**1.63**	PECL_222	xsome
216	lemA family protein	**0.47**	PECL_223	xsome
217	peptidase M48 family protein	-0.03	PECL_224	xsome
218	hypothetical protein	-0.01	PECL_225	xsome
219	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	**-0.96**	PECL_226	xsome
220	DEAD/DEAH box helicase	**-1.23**	PECL_227	xsome
221	holo-acyl-carrier-protein synthase	**-2.43**	PECL_228	xsome
222	alanine racemase	**-1.95**	PECL_229	xsome
223	hypothetical protein	0.25	PECL_230	xsome
224	mRNA interferase EndoA	0.27	PECL_231	xsome
225	queT transporter family protein	-0.04	PECL_232	xsome
226	hypothetical protein	**0.37**	PECL_233	xsome
227	D-transpeptidase catalytic domain-containing protein	0.23	PECL_234	xsome
228	L-lactate dehydrogenase	0.04	PECL_235	xsome
229	formate acetyltransferase	0.31	PECL_236	xsome
230	pyruvate formate-lyase activating enzyme	0.31	PECL_237	xsome
231	VIT family protein	-0.35	PECL_238	xsome
232	feS assembly ATPase SufC	**-0.39**	PECL_239	xsome
233	FeS assembly protein SufD	-0.19	PECL_240	xsome
234	cysteine desulfurase SufS	-0.07	PECL_241	xsome
235	NifU family SUF system FeS assembly protein	-0.38	PECL_242	xsome
236	feS assembly protein SufB	-0.27	PECL_243	xsome
237	hypothetical protein	0.24	PECL_244	xsome
238	alpha/beta hydrolase fold family protein	0.15	PECL_246	xsome
239	putative sugar-binding domain protein	**0.41**	PECL_245	xsome
240	hypothetical protein	-0.40	PECL_247	xsome
241	hypothetical protein	-0.49	PECL_248	xsome

242	hypothetical protein	-0.02	PECL_249	xsome
243	hypothetical protein	0.17	PECL_250	xsome
244	Malate dehydrogenase Oxaloacetate-decarboxylase	**1.63**	PECL_251	xsome
245	citrate malate transporter	**1.64**	PECL_252	xsome
246	citrate pro-3S-lyase ligase	**1.68**	PECL_253	xsome
247	citrate lyase acyl carrier protein	**1.59**	PECL_254	xsome
248	citrate pro-lyase subunit beta	**1.84**	PECL_255	xsome
249	citrate lyase subunit alpha	**1.76**	PECL_256	xsome
250	holo-ACP synthase CitX	**1.74**	PECL_257	xsome
251	triphosphoribosyl-dephospho-CoA synthase CitG	**1.73**	PECL_258	xsome
252	1,4-alpha-glucan-branching protein	-0.25	PECL_259	xsome
253	glucose-1-phosphate adenylyltransferase	0.37	PECL_260	xsome
254	glucose-1-phosphate adenylyltransferase GlgD subunit	-0.50	PECL_261	xsome
255	glycogen starch synthase	**-0.93**	PECL_262	xsome
256	glycogen starch/alpha-glucan phosphorylases family protein	**-0.58**	PECL_263	xsome
257	alpha amylase	**-0.57**	PECL_264	xsome
258	peptidyl-tRNA hydrolase	**-0.90**	PECL_265	xsome
259	transcription-repair coupling factor	**-0.59**	PECL_266	xsome
260	polysaccharide biosynthesis protein	**-0.56**	PECL_267	xsome
261	S4 domain-containing protein	-0.04	PECL_268	xsome
262	septum formation initiator family protein	-0.15	PECL_269	xsome
263	S1 RNA binding domain-containing protein	**-0.86**	PECL_270	xsome
264	tRNA Ile-lysidine synthetase	**-0.76**	PECL_271	xsome
265	hypoxanthine phosphoribosyltransferase	-0.13	PECL_272	xsome
266	ATP-dependent metallopeptidase HflB family protein	-0.20	PECL_273	xsome
267	33 kDa chaperonin	0.01	PECL_274	xsome
268	TIM-barrel nifR3 family protein	**-0.49**	PECL_275	xsome
269	lysine-tRNA ligase	**-0.33**	PECL_276	xsome
277	H:symporter family protein	**-1.45**	PECL_287	xsome
278	phosphoesterase family protein	**0.55**	PECL_288	xsome
281	multicopper oxidase mco	**2.35**	PECL_291	xsome

282	hypothetical protein	**1.51**	PECL_2022	xsome
283	sodium:hydrogen exchanger family protein	**1.11**	PECL_292	xsome
284	NADH peroxidase	**3.61**	PECL_293	xsome
285	phosphotransferase enzyme family protein	**3.28**	PECL_294	xsome
286	hypothetical protein	1.05	PECL_295	xsome
287	H antiporter-2 family protein	0.56	PECL_296	xsome
288	MarR family transcriptional regulator	**-0.49**	PECL_297	xsome
289	lysM domain protein	**-0.46**	PECL_298	xsome
290	hypothetical protein	**-0.61**	PECL_299	xsome
291	putative potassium transport system protein kup	**0.96**	PECL_300	xsome
292	acetyltransferase family protein	-0.14	PECL_301	xsome
293	cadmium resistance transporter family protein	**-1.29**	PECL_302	xsome
294	ArsR family transcriptional regulator	**-0.98**	PECL_303	xsome
295	peptide-methionine-S-oxide reductase	0.08	PECL_304	xsome
296	hypothetical protein	**0.55**	PECL_305	xsome
297	5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase	**-0.51**	PECL_306	xsome
298	10-methylenetetrahydrofolate reductase	**-0.81**	PECL_307	xsome
299	major facilitator superfamily protein	**0.94**	PECL_308	xsome
300	pyrroline-5-carboxylate reductase	**-0.64**	PECL_309	xsome
301	N-acetylglucosamine-6-phosphate deacetylase	**-0.72**	PECL_310	xsome
302	GntR family transcriptional regulator	**-0.67**	PECL_311	xsome
303	hypothetical protein	**1.88**	PECL_312	xsome
304	metal ion transporter metal ion family protein	**2.32**	PECL_313	xsome
305	hypothetical protein	**-4.32**	PECL_314	xsome
306	accessory gene regulator C	**-2.80**	PECL_315	xsome
307	accessory gene regulator protein A	**-2.22**	PECL_316	xsome
308	WecB/TagA/CpsF family glycosyl transferase	**-1.60**	PECL_317	xsome
309	nicotinate phosphoribosyltransferase	**-1.69**	PECL_318	xsome
310	NH-dependent NAD synthetase	**-1.81**	PECL_319	xsome
311	calcium-translocating P-type ATPase	**-1.48**	PECL_320	xsome

312	Cation transporting ATPase	0.26	PECL_321	xsome
313	pyridine nucleotide-disulfide oxidoreductase family protein	**-0.63**	PECL_322	xsome
314	ubiA prenyltransferase family protein	**-0.38**	PECL_323	xsome
315	geranylgeranyl pyrophosphate synthase	**-0.96**	PECL_324	xsome
316	thiol reductant ABC exporter CydC subunit	-0.05	PECL_325	xsome
317	thiol reductant ABC exporter CydD subunit	0.25	PECL_326	xsome
318	cytochrome d ubiquinol oxidase subunit II	**0.41**	PECL_327	xsome
319	cytochrome d ubiquinol oxidase subunit I	**0.40**	PECL_328	xsome
320	S1 RNA binding domain-containing protein	**-0.75**	PECL_329	xsome
321	sprT-like family protein	**-0.67**	PECL_330	xsome
323	hypothetical protein	**1.27**	PECL_332	xsome
324	H%2B antiporter-2 family protein	**1.58**	PECL_333	xsome
325	TetR family transcriptional regulator	**0.59**	PECL_334	xsome
326	major facilitator superfamily protein	-0.10	PECL_335	xsome
327	transcriptional regulator	0.06	PECL_336	xsome
328	hypothetical protein	0.01	PECL_337	xsome
329	nicotinamide mononucleotide transporter PnuC family protein	0.18	PECL_338	xsome
330	hypothetical protein	0.29	PECL_339	xsome
331	PTS system mannose/fructose/sorbose family IIA component domain protein	**-0.62**	PECL_340	xsome
332	PTS system sorbose-specific IIc component	**-0.94**	PECL_341	xsome
333	PTS system mannose/fructose/sorbose IID component family protein	**-0.33**	PECL_342	xsome
334	hypothetical protein	-0.18	PECL_343	xsome
335	transcriptional regulator	**0.37**	PECL_344	xsome
336	PTS system mannose/fructose/sorbose family IIA component protein	0.06	PECL_345	xsome
337	PTS system sorbose subIIB component family protein	-0.06	PECL_346	xsome
338	hypothetical protein	**1.93**	PECL_347	xsome
339	hypothetical protein	0.66	PECL_348	xsome
340	enterocin A Immunity family protein	**-0.96**	PECL_349	xsome
341	hypothetical protein	**-1.05**	PECL_350	xsome
342	hypothetical protein	-0.68	PECL_351	xsome

343	hypothetical protein	-0.61	PECL_352	xsome
344	hypothetical protein	1.10	PECL_353	xsome
345	hypothetical protein	**1.98**	PECL_354	xsome
346	hypothetical protein	**1.51**	PECL_355	xsome
347	ABC-type bacteriocin transporter family protein	**0.82**	PECL_356	xsome
348	bacteriocin secretion accessory family protein	**0.56**	PECL_358	xsome
349	Response regulator of the LytTR family	**-1.00**	PECL_359	xsome
350	accessory gene regulator C	-0.33	PECL_361	xsome
351	bacteriocin-type signal sequence domain protein	1.09	PECL_360	xsome
352	hypothetical protein	0.60	PECL_362	xsome
353	CAAX amino terminal protease	**0.67**	PECL_363	xsome
354	3-oxoacyl-acyl-carrier-synthase III family protein	0.02	PECL_364	xsome
355	acetyl-CoA carboxylase biotin carboxyl carrier protein	**-0.58**	PECL_365	xsome
356	acetyl-CoA carboxylase biotin carboxylase subunit	**-1.09**	PECL_366	xsome
357	acetyl-coA carboxylase carboxyl transferase beta subunit	**-0.80**	PECL_367	xsome
358	acetyl-coA carboxylase carboxyl transferase alpha subunit	**-0.97**	PECL_368	xsome
359	phosphoglycerate mutase	**-0.80**	PECL_369	xsome
360	hypothetical protein	-0.02	PECL_370	xsome
361	peptidase C1-like family protein	**0.74**	PECL_371	xsome
362	ribose 5-phosphate isomerase A	**0.67**	PECL_372	xsome
363	putative acetyltransferase	**0.37**	PECL_2024	xsome
364	putative dUTPase	**-0.75**	PECL_375	xsome
365	DNA repair protein RadA	**-0.70**	PECL_376	xsome
366	PIN domain-containing protein	-0.18	PECL_377	xsome
367	glutamyl-tRNA synthetase	**-0.57**	PECL_378	xsome
368	hypothetical protein	**2.55**	PECL_379	xsome
369	peroxide-responsive repressor perR	**1.49**	PECL_380	xsome
370	major facilitator superfamily protein	-0.16	PECL_381	xsome
371	Ribonuclease BN-like family protein	0.23	PECL_382	xsome
372	hypothetical protein	**0.86**	PECL_383	xsome
373	major facilitator superfamily protein	**0.45**	PECL_384	xsome

374	hypothetical protein	**1.05**	PECL_385	xsome
375	hypothetical protein	**0.40**	PECL_386	xsome
376	protein-tyrosine phosphatase	**0.71**	PECL_387	xsome
377	HAD hydrolase	**0.85**	PECL_388	xsome
378	putative ribonucleotide reduction protein NrdI	**1.07**	PECL_389	xsome
379	protein-tyrosine phosphatase	**1.11**	PECL_390	xsome
380	prolyl oligopeptidase family protein	**1.19**	PECL_391	xsome
381	hemolysin-like protein containing CBS domains	**0.66**	PECL_392	xsome
382	bacterial low temperature requirement A family protein; psuedo	-0.21	PECL_393	xsome
383	aldo-keto reductase family protein	**0.81**	PECL_395	xsome
384	hypothetical protein	0.16	PECL_396	xsome
385	hypothetical protein	0.66	PECL_397	xsome
386	H antiporter-2 family protein	**0.57**	PECL_398	xsome
387	PadR family transcriptional regulator	0.15	PECL_399	xsome
388	glutamine ABC transporter permease substrate-binding protein	**-1.50**	PECL_400	xsome
389	Glutamine ABC transporter ATP-binding protein	**-1.54**	PECL_401	xsome
390	eamA-like transporter family protein	**-1.60**	PECL_402	xsome
391	hypothetical protein	**-0.81**	PECL_403	xsome
393	oligo-1,6-glucosidase	**-0.96**	PECL_405	xsome
394	galactose mutarotase related enzyme	0.31	PECL_406	xsome
395	dihydrodipicolinate reductase family protein	**1.60**	PECL_407	xsome
396	phosphomethylpyrimidine kinase	**1.19**	PECL_408	xsome
397	PadR family transcriptional regulator	0.12	PECL_409	xsome
398	hypothetical protein	**-0.41**	PECL_410	xsome
399	hypothetical protein	-0.23	PECL_411	xsome
400	hypothetical protein	0.28	PECL_412	xsome
401	low temperature requirement A family protein	**0.89**	PECL_413	xsome
402	nitroreductase family protein	0.14	PECL_414	xsome
403	DNA-repair protein-SOS response UmuC-like protein	**0.83**	PECL_416	xsome
404	hypothetical protein	0.44	PECL_417	xsome
405	hypothetical protein	**0.73**	PECL_418	xsome

406	phosphatidylethanolamine-binding family protein	**0.91**	PECL_419	xsome
407	hypothetical protein	0.19	PECL_420	xsome
408	ABC transporter ATPase	0.39	PECL_421	xsome
409	ABC transporter permease	**0.55**	PECL_422	xsome
410	TetR family transcriptional regulator	0.01	PECL_424	xsome
411	exodeoxyribonuclease III	**-0.83**	PECL_425	xsome
412	NAD-dependent DNA ligase	**-0.43**	PECL_426	xsome
413	GtcA family membrane protein	-0.07	PECL_427	xsome
414	glycosyl transferase 2 family protein	**-0.59**	PECL_428	xsome
415	Teichoic acid translocation permease protein TagG	0.22	PECL_429	xsome
416	teichoic acids export%2C ATP-binding protein TagH	0.18	PECL_430	xsome
417	hypothetical protein	0.04	PECL_431	xsome
418	hypothetical protein	**-0.45**	PECL_432	xsome
419	two-component response regulator	**-0.48**	PECL_433	xsome
420	Signal transduction histidine kinase	**-0.52**	PECL_434	xsome
421	PAP2 superfamily protein	**-0.65**	PECL_435	xsome
422	glycosyl transferase 2 family protein	-0.21	PECL_436	xsome
423	hypothetical protein	**-0.92**	PECL_437	xsome
424	ion channel family protein	**1.00**	PECL_438	xsome
425	asparaginase family protein	**-0.78**	PECL_439	xsome
426	neutral endopeptidase	**-0.65**	PECL_440	xsome
427	lipoprotein	**1.15**	PECL_441	xsome
428	permease family protein	-0.29	PECL_442	xsome
429	RNase H family protein	-0.28	PECL_444	xsome
430	acetyltransferase family protein	0.33	PECL_443	xsome
431	hypothetical protein	**1.76**	PECL_445	xsome
432	major facilitator superfamily protein	**1.35**	PECL_446	xsome
433	hypothetical protein	**2.84**	PECL_447	xsome
434	hypothetical protein	**0.48**	PECL_448	xsome
435	hypothetical protein	**1.09**	PECL_449	xsome
436	hypothetical protein	**-0.37**	PECL_450	xsome

437	aldehyde dehydrogenase family protein	**1.66**	PECL_451	xsome
438	glycosyl transferase 2 family protein	**0.96**	PECL_452	xsome
439	glucosamine-6-phosphate deaminase	**-0.47**	PECL_453	xsome
440	lytTr DNA-binding domain protein	0.11	PECL_454	xsome
441	hypothetical protein	-0.28	PECL_455	xsome
442	hypothetical protein	**3.53**	PECL_456	xsome
443	PTS system mannitol-specific IIC component family protein	**0.92**	PECL_457	xsome
444	PRD domain-containing protein	0.34	PECL_458	xsome
445	mannitol-specific phosphotransferase enzyme IIA component	0.36	PECL_459	xsome
446	mannitol-1-phosphate 5-dehydrogenase	**0.66**	PECL_460	xsome
447	acetate kinase	**-0.46**	PECL_461	xsome
448	phosphate acetyltransferase	**-0.78**	PECL_462	xsome
449	truncated resolvase N terminal domain; psuedo	**0.74**	PECL_463	xsome
450	hypothetical protein	**-0.34**	PECL_464	xsome
451	alpha-beta hydrolase family protein	0.32	PECL_465	xsome
452	cysteinyl-tRNA synthetase	**-1.04**	PECL_466	xsome
453	RNase3 domain-containing protein	**-1.29**	PECL_467	xsome
454	RNA-O ribose methyltransferase substrate binding family protein	**-0.85**	PECL_468	xsome
455	igma-70 family RNA polymerase sigma factor	**-0.99**	PECL_469	xsome
456	Preprotein translocase subunit SecE	**1.76**	PECL_470	xsome
457	transcription termination antitermination factor NusG	**-0.36**	PECL_471	xsome
458	50S ribosomal protein L11	**-0.88**	PECL_472	xsome
459	50S ribosomal protein L1	**-0.51**	PECL_473	xsome
460	50S ribosomal protein L10	**-0.42**	PECL_474	xsome
461	50S ribosomal protein L7%2FL12	**-0.36**	PECL_475	xsome
462	hypothetical protein	0.30	PECL_476	xsome
463	orotidine-phosphate decarboxylase	0.43	PECL_477	xsome
464	orotate phosphoribosyltransferase	0.05	PECL_478	xsome
465	aspartate carbamoyltransferase	**-0.96**	PECL_479	xsome
466	dihydroorotase	**-0.60**	PECL_480	xsome
467	carbamoyl-phosphate synthase small subunit	-0.49	PECL_481	xsome

468	carbamoyl-phosphate synthase large subunit	-0.32	PECL_482	xsome
469	dihydroorotate dehydrogenase NAD catalytic subunit	-0.08	PECL_483	xsome
470	flavodoxin family protein	**1.54**	PECL_484	xsome
471	hypothetical protein	**-0.77**	PECL_485	xsome
472	H antiporter-2 family protein	-0.16	PECL_486	xsome
473	TetR family transcriptional regulator	**-0.66**	PECL_487	xsome
474	hypothetical protein	**-0.32**	PECL_488	xsome
475	ribonucleoside-diphosphate reductase subunit beta	**0.34**	PECL_489	xsome
476	ribonucleoside-diphosphate reductase subunit alpha	0.27	PECL_490	xsome
477	glutaredoxin-like protein nrdH	**1.09**	PECL_491	xsome
478	transposase family protein	**1.26**	PECL_492	xsome
479	transposase	**0.91**	PECL_493	xsome
480	methyltransferase small domain family protein	-0.50	PECL_494	xsome
481	cytidine and deoxycytidylate deaminase zinc-binding region family protein	**-0.73**	PECL_495	xsome
482	DNA polymerase IIIC subunit gamma and tau	-0.13	PECL_496	xsome
483	hypothetical protein	**-0.76**	PECL_497	xsome
484	recombination protein RecR	0.18	PECL_498	xsome
485	thymidylate kinase	-0.08	PECL_499	xsome
486	hypothetical protein	**-0.45**	PECL_500	xsome
487	DNA polymerase III subunit delta	-0.24	PECL_501	xsome
488	hypothetical protein	-0.04	PECL_502	xsome
489	ribosomal RNA small subunit methyltransferase I	-0.10	PECL_503	xsome
490	oleoyl-Bacyl-carrier protein thioesterase	-0.17	PECL_504	xsome
491	UDP-glucose 4-epimerase	0.27	PECL_505	xsome
492	glycoprotease family protein	-0.27	PECL_506	xsome
493	ribosomal-protein-alanine acetyltransferase	**-0.37**	PECL_507	xsome
494	metallohydrolase glycoprotease Kae1 family protein	-0.24	PECL_508	xsome
495	beta-lactamase family protein	-0.10	PECL_509	xsome
496	CAAX amino terminal protease self- immunity family protein	**0.86**	PECL_510	xsome
497	10 kDa chaperonin	**-0.89**	PECL_511	xsome

498	chaperonin GroL	**-0.40**	PECL_512	xsome
499	amino acid polyamine organocation transporter	-0.06	PECL_513	xsome
500	glycosyl transferase 4 family protein	**-0.61**	PECL_515	xsome
501	hypothetical protein	0.25	PECL_516	xsome
502	ComF operon protein A	0.53	PECL_517	xsome
503	ComF operon protein C	0.31	PECL_518	xsome
504	Sigma 54 modulation protein S30EA ribosomal protein	**0.77**	PECL_519	xsome
505	Preprotein translocase subunit SecA	**-0.39**	PECL_520	xsome
506	peptide chain release factor 2	**-0.61**	PECL_521	xsome
507	alkaline phosphatase synthesis transcriptional regulatory protein phoP	-0.27	PECL_522	xsome
508	Phosphate regulon sensor histidine kinase protein PhoR	**-0.76**	PECL_523	xsome
509	phosphate binding family protein	-0.31	PECL_524	xsome
510	phosphate ABC transporter permease	-0.10	PECL_525	xsome
511	phosphate ABC transporter permease	-0.20	PECL_526	xsome
512	phosphate ABC transporter ATP-binding protein	0.26	PECL_527	xsome
513	phosphate ABC transporter ATP-binding protein	0.28	PECL_528	xsome
514	phosphate transport system regulatory proteinPhoU	-0.16	PECL_529	xsome
515	pspC domain protein	0.24	PECL_530	xsome
516	hypothetical protein	-0.01	PECL_531	xsome
517	Serine kinase phosphatase	0.16	PECL_532	xsome
518	prolipoprotein diacylglyceryl transferase	-0.09	PECL_533	xsome
519	glycerol-3-phosphate dehydrogenase	**-0.82**	PECL_534	xsome
520	UTP-glucose-1-phosphate uridylyltransferase	**-0.75**	PECL_535	xsome
521	thioredoxin-disulfide reductase	**0.39**	PECL_536	xsome
522	amino acid permease family protein	0.26	PECL_537	xsome
523	phosphoglucomutase phosphomannomutase	-0.24	PECL_538	xsome
524	excinuclease ABC subunit B	**1.52**	PECL_539	xsome
525	excinuclease ABC subunit A	**0.84**	PECL_540	xsome
526	P-loop ATPase protein	**-0.32**	PECL_541	xsome
527	hypothetical protein	**-0.61**	PECL_542	xsome
528	Putative sporulation transcription regulator whiA	0.01	PECL_543	xsome

529	ATP-dependent Clp protease proteolytic subunit ClpP	-0.19	PECL_544	xsome
531	RNA polymerase sigma-54 factor	0.03	PECL_546	xsome
532	central glycolytic gene regulator	-0.16	PECL_547	xsome
533	glyceraldehyde-3-phosphate dehydrogenase type I	0.27	PECL_548	xsome
534	phosphoglycerate kinase	0.06	PECL_549	xsome
535	triose-phosphate isomerase	**-0.38**	PECL_550	xsome
536	enolase	0.13	PECL_551	xsome
537	hypothetical protein	**1.11**	PECL_553	xsome
538	preprotein translocase subunit SecG	-0.05	PECL_554	xsome
539	ribonuclease R	-0.12	PECL_555	xsome
540	ssrA-binding protein	**-0.61**	PECL_556	xsome
541	high-affinity gluconate transporter	-0.32	PECL_557	xsome
542	FGGY family carbohydrate kinase	**-0.44**	PECL_558	xsome
543	6-phosphogluconate dehydrogenase	**-0.86**	PECL_559	xsome
544	helix-turn-helix domain, rpiR family protein	**-1.15**	PECL_560	xsome
545	amino acid permease family protein	**-0.56**	PECL_561	xsome
546	acetyltransferase family protein	**0.57**	PECL_562	xsome
547	hypothetical protein	**0.45**	PECL_563	xsome
548	hydrolase haloacid dehalogenase-like protein	**0.35**	PECL_564	xsome
549	uracil-DNA glycosylase	-0.20	PECL_565	xsome
550	hypothetical protein	**-0.79**	PECL_566	xsome
551	GNAT family acetyltransferase	-0.11	PECL_568	xsome
552	exonuclease DNA polymerase III epsilon subunit family protein	0.13	PECL_567	xsome
553	oxidoreductase NAD-binding Rossmann fold family protein	**-0.80**	PECL_569	xsome
554	UDP-N-acetylenolpyruvoylglucosamine reductase	**-0.40**	PECL_570	xsome
555	disA bacterial checkpoint controller nucleotide-binding family protein	**-0.72**	PECL_571	xsome
556	hypothetical protein	**-0.38**	PECL_572	xsome
557	phosphoglucosamine mutase	**-0.31**	PECL_573	xsome
558	glutamine-fructose-6-phosphate transaminase	**-0.33**	PECL_574	xsome
559	hypothetical protein	**-1.08**	PECL_575	xsome

560	hypothetical protein	**-0.45**	PECL_576	xsome
561	ArsR family transcriptional regulator	-0.12	PECL_577	xsome
562	gbkey=CDS	0.13	PECL_578	xsome
563	polysaccharide deacetylase family protein	0.14	PECL_579	xsome
564	diguanylate cyclase domain protein	**-1.00**	PECL_580	xsome
565	Diguanylate cyclase phosphodiesterase	**-1.45**	PECL_581	xsome
566	D-isomer specific 2-hydroxyacid dehydrogenase NAD binding domain protein	0.21	PECL_582	xsome
567	licD family protein	0.18	PECL_583	xsome
568	hypothetical protein	**2.10**	PECL_584	xsome
569	phage integrase family protein	0.25	PECL_585	xsome
570	helix-turn-helix family protein	**-0.44**	PECL_586	xsome
571	hypothetical protein	0.74	PECL_587	xsome
572	Bifunctional DNA primase polymerase phage related protein	0.55	PECL_589	xsome
573	phage plasmid primase P4 family C-terminal domain	**0.77**	PECL_590	xsome
574	phage transcriptional regulator, ArpU family protein	0.23	PECL_591	xsome
575	hypothetical protein	**-1.04**	PECL_592	xsome
576	hypothetical protein	0.72	PECL_593	xsome
577	phage head-tail joining family protein	0.99	PECL_594	xsome
578	HNH endonuclease family protein phage-related protein	-0.07	PECL_595	xsome
579	phage terminase small subunit	-0.14	PECL_596	xsome
580	phage Terminase family protein	0.06	PECL_597	xsome
581	hypothetical protein	0.40	PECL_598	xsome
582	prophage Lp3 protein portal protein	**0.81**	PECL_599	xsome
583	phage prohead protease HK97 family	0.24	PECL_601	xsome
584	phage gp6-like head-tail connector family protein	0.44	PECL_602	xsome
585	hypothetical protein	**-0.81**	PECL_603	xsome
586	merR regulatory family protein truncated; psuedo	**1.11**	PECL_604	xsome
587	hypothetical protein	**0.83**	PECL_605	xsome
588	ubiA prenyltransferase family protein	**-0.65**	PECL_606	xsome
589	NADH-dependent butanol dehydrogenase A	**1.27**	PECL_607	xsome

590	ABC transporter ATP-binding protein	**0.71**	PECL_608	xsome
591	hypothetical protein	**1.28**	PECL_609	xsome
592	lysM domain protein	**2.25**	PECL_610	xsome
593	two-component response regulator	**-1.79**	PECL_611	xsome
594	Two-component system, sensor histidine kinase	**-1.32**	PECL_612	xsome
595	1,3-propanediol dehydrogenase	-0.16	PECL_613	xsome
596	hypothetical protein	0.49	PECL_614	xsome
597	fructose-1,6-bisphosphate aldolase	0.00	PECL_615	xsome
598	sugar transport family protein	-0.25	PECL_616	xsome
599	protein translocase subunit secA	0.00	PECL_617	xsome
600	Preprotein translocase subunit secY	**-0.40**	PECL_618	xsome
601	accessory Sec system protein Asp1	**-0.54**	PECL_619	xsome
602	accessory Sec system protein Asp2	-0.21	PECL_620	xsome
603	accessory Sec system protein Asp3	0.09	PECL_621	xsome
604	glycosyl transferases group 1 family protein	0.26	PECL_622	xsome
605	hypothetical protein	0.23	PECL_623	xsome
606	kxYKxGKxW signal peptide domain protein	**1.19**	PECL_624	xsome
607	putative cell-wall-anchored protein LPXTG motif	**1.47**	PECL_626	xsome
608	hypothetical protein	**1.83**	PECL_627	xsome
609	glucose-6-phosphate isomerase	0.18	PECL_628	xsome
610	anaerobic ribonucleoside-triphosphate reductase	**-0.60**	PECL_629	xsome
611	anaerobic ribonucleoside-triphosphate reductase activating protein	**-0.61**	PECL_630	xsome
612	cadmium-translocating P-type ATPase	**2.56**	PECL_631	xsome
615	LPXTG-motif cell wall anchor domain-containing protein	0.42	PECL_634	xsome
616	hypothetical protein	**-0.35**	PECL_635	xsome
617	hypothetical protein	0.17	PECL_636	xsome
618	aldehyde dehydrogenase family protein	**0.42**	PECL_637	xsome
619	metal ion transporter, metal ion family protein	**3.56**	PECL_638	xsome
620	hypothetical protein	**3.41**	PECL_639	xsome
621	transposase	**1.08**	PECL_640	xsome
622	hypothetical protein	**1.28**	PECL_641	xsome

623	hypothetical protein	**1.35**	PECL_642	xsome
624	transcription elongation factor, GreA/GreB C-term family protein	0.06	PECL_643	xsome
625	hypothetical protein	-0.12	PECL_644	xsome
626	TetR family transcriptional regulator	**0.94**	PECL_645	xsome
627	ABC transporter ATP-binding protein permease	**1.03**	PECL_646	xsome
628	multidrug ABC transporter ATPase permease	0.36	PECL_647	xsome
629	beta-lactamase family protein	0.22	PECL_649	xsome
630	mannose-6-phosphate isomerase	0.19	PECL_650	xsome
631	glycosyl transferase family protein	**-0.76**	PECL_651	xsome
632	glycosyl transferase family protein	-0.17	PECL_652	xsome
633	CobQ-like glutamine amidotransferase domain protein	**1.33**	PECL_653	xsome
634	mur ligase middle domain protein	**0.49**	PECL_654	xsome
635	thymidine kinase family protein	**-0.38**	PECL_655	xsome
636	peptide chain release factor 1	**-0.80**	PECL_656	xsome
637	protein-glutamine-methyltransferase	**-0.62**	PECL_657	xsome
638	hypothetical protein	**-0.55**	PECL_658	xsome
639	serine hydroxymethyltransferase family protein	**-0.44**	PECL_659	xsome
640	uracil phosphoribosyltransferase	**-0.59**	PECL_660	xsome
641	ATP synthase F0 subunit A	**1.04**	PECL_661	xsome
642	ATP synthase F0 subunit C	**1.35**	PECL_662	xsome
643	ATP synthase F0 subunit B	**0.50**	PECL_663	xsome
644	ATP synthase F1 subunit delta	**1.02**	PECL_664	xsome
645	ATP synthase F1 subunit alpha	**1.08**	PECL_665	xsome
646	ATP synthase F1 subunit gamma	**1.18**	PECL_666	xsome
647	ATP synthase F1 subunit beta	**1.07**	PECL_667	xsome
648	ATP synthase F1 subunit epsilon	**1.43**	PECL_668	xsome
649	hypothetical protein	-0.25	PECL_669	xsome
650	hypothetical protein	-0.01	PECL_670	xsome
651	hypothetical protein	**-0.64**	PECL_671	xsome
652	cell cycle family protein	-0.24	PECL_672	xsome
653	methionine import ATP-binding protein MetN 2	**-1.59**	PECL_673	xsome

654	methionine import system permease protein metI	**-1.12**	PECL_674	xsome
655	D-methionine ABC transporter substrate-binding protein	**-0.44**	PECL_675	xsome
656	endonuclease Exonuclease phosphatase family protein	**-1.50**	PECL_676	xsome
657	GntR family transcriptional regulator	**-1.55**	PECL_677	xsome
658	ABC transporter family protein	**-1.19**	PECL_678	xsome
659	hypothetical protein	**-1.31**	PECL_679	xsome
660	hypothetical protein	**-0.82**	PECL_680	xsome
661	amino acid permease family protein	**0.51**	PECL_681	xsome
662	hypothetical protein	**0.44**	PECL_682	xsome
663	zinc-binding alcohol dehydrogenase family protein	**0.49**	PECL_683	xsome
664	D-alanine--D-alanine ligase	**-0.62**	PECL_684	xsome
665	methyladenine glycosylase family protein	0.67	PECL_685	xsome
666	universal stress family protein	**1.10**	PECL_686	xsome
667	AAA ATPase	**0.40**	PECL_687	xsome
668	30S ribosomal protein S4	-0.12	PECL_688	xsome
669	GAF domain-containing protein	0.22	PECL_689	xsome
670	Septation ring formation regulator EzrA	-0.19	PECL_690	xsome
671	aminotransferase class-V family protein	**-0.38**	PECL_691	xsome
672	thiamine biosynthesis tRNA modification protein ThiI	-0.05	PECL_692	xsome
673	ahp, TSA family protein	0.13	PECL_693	xsome
674	valine--tRNA ligase	**-1.20**	PECL_694	xsome
675	bifunctional Dihydrofolate synthase tetrahydrofolylpolyglutamate synthase protein FolC	**-0.40**	PECL_695	xsome
676	DNA repair RadC family protein	**-0.75**	PECL_696	xsome
677	rod shape-determining protein MreC	-0.20	PECL_697	xsome
678	rod shape-determining protein MreD	-0.21	PECL_698	xsome
679	amino ABC transporter permease 3-TM region His-Glu-Gln-Arg-opine family domain protein	**-1.38**	PECL_699	xsome
680	ABC transporter ATPase	**-0.97**	PECL_700	xsome
681	polar amino acid ABC uptake transporter substrate binding protein	**-0.48**	PECL_701	xsome
682	cardiolipin synthase	**-0.47**	PECL_702	xsome

683	Zn-dependent peptidase	**-0.87**	PECL_703	xsome
684	peptidase M16 inactive domain protein	**-0.51**	PECL_705	xsome
685	short chain dehydrogenase family protein	**-0.58**	PECL_706	xsome
686	Putative transcriptional regulator	-0.10	PECL_707	xsome
687	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	0.15	PECL_708	xsome
688	Putative competence-damage inducible protein CinA	-0.34	PECL_709	xsome
689	protein RecA	**0.41**	PECL_710	xsome
690	ribonuclease Y	**-0.49**	PECL_711	xsome
691	DNA mismatch repair protein MutS	**-0.34**	PECL_712	xsome
692	DNA mismatch repair MutL family protein	-0.11	PECL_713	xsome
693	Holliday junction ATP-dependent DNA helicase RuvA	**-0.69**	PECL_714	xsome
694	Holliday junction ATP-dependent DNA helicase RuvB	**-0.42**	PECL_715	xsome
695	tRNA ribosyltransferase-isomerase	**-0.63**	PECL_716	xsome
696	queuine tRNA-ribosyltransferase	**-0.59**	PECL_717	xsome
697	preprotein translocase subunit YajC	**-0.45**	PECL_718	xsome
698	glucose-6-phosphate dehydrogenase	**0.43**	PECL_719	xsome
699	impB/mucB/samB family protein	**0.35**	PECL_720	xsome
700	DHH family protein	**-0.49**	PECL_721	xsome
701	DEAD/FDEAH box helicase	**-0.66**	PECL_722	xsome
702	alanine--tRNA ligase	**-1.18**	PECL_723	xsome
703	hypothetical protein	0.00	PECL_724	xsome
704	putative Holliday junction resolvase	**0.68**	PECL_725	xsome
705	hypothetical protein	**0.66**	PECL_726	xsome
706	mutS2 family protein	0.17	PECL_727	xsome
707	thioredoxin	**2.45**	PECL_728	xsome
708	glutamate racemase	**-0.49**	PECL_729	xsome
709	non-canonical purine NTP pyrophosphatase RdgB/HAM1 family	**-0.31**	PECL_730	xsome
710	mechanosensitive ion channel family protein	-0.12	PECL_731	xsome
711	hypothetical protein	**0.86**	PECL_732	xsome
712	hypothetical protein	**0.37**	PECL_733	xsome
713	pyruvate oxidase	**1.94**	PECL_734	xsome

714	xaa-Pro dipeptidase	0.04	PECL_735	xsome
715	catabolite control protein A	**-0.53**	PECL_736	xsome
716	glycerol facilitator-aquaporin gla	**-2.06**	PECL_737	xsome
717	hypothetical protein	**-0.72**	PECL_738	xsome
718	hypothetical protein	**-0.91**	PECL_739	xsome
719	hypothetical protein	**-0.62**	PECL_740	xsome
720	Hemolysin III	0.14	PECL_741	xsome
721	translation elongation factor P	**-0.31**	PECL_742	xsome
722	RDD family protein	**-0.75**	PECL_743	xsome
723	hypothetical protein	0.01	PECL_744	xsome
724	ribokinase	**-1.26**	PECL_745	xsome
725	type II FIV secretion system family protein	**-0.95**	PECL_746	xsome
726	Type II secretory pathway competence component	-0.52	PECL_747	xsome
727	competence protein GC	-0.24	PECL_748	xsome
728	prepilin-type N-terminal cleavage methylation domain-containing protein	-0.59	PECL_749	xsome
729	hypothetical protein	0.36	PECL_750	xsome
730	hypothetical protein	-0.33	PECL_751	xsome
731	hypothetical protein	0.29	PECL_752	xsome
732	N-6 DNA Methylase family protein	0.04	PECL_753	xsome
733	amino acid permease family protein	-0.02	PECL_754	xsome
734	phosphoesterase phosphohydrolase	0.25	PECL_755	xsome
735	hypothetical protein	**-1.73**	PECL_756	xsome
736	hydrolase haloacid dehalogenase family	**-1.19**	PECL_757	xsome
737	hypothetical protein	**-0.74**	PECL_759	xsome
738	protein dedA	0.02	PECL_758	xsome
739	RNA binding protein S1 domain	**-0.40**	PECL_760	xsome
753	hypothetical protein	-0.05	PECL_778	xsome
754	hypothetical protein	**0.63**	PECL_779	xsome
755	hypothetical protein	**1.22**	PECL_780	xsome
756	metallo-beta-lactamase family protein	**0.98**	PECL_781	xsome

757	regulatory protein spx	**1.43**	PECL_782	xsome
758	negative regulator of genetic competence family protein	**0.79**	PECL_783	xsome
759	competence CoiA-like family protein	0.11	PECL_784	xsome
760	hypothetical protein	0.52	PECL_785	xsome
761	dithiol-disulfide isomerase	**1.10**	PECL_786	xsome
762	putative GTP pyrophosphokinase	**-1.15**	PECL_787	xsome
763	ATP-NAD kinase family protein	**-0.54**	PECL_788	xsome
764	RluA family pseudouridine synthase	**-0.67**	PECL_789	xsome
765	magnesium transporter	**-0.31**	PECL_790	xsome
766	adenylosuccinate synthase	-0.61	PECL_791	xsome
767	adenylosuccinate lyase	**-0.58**	PECL_792	xsome
768	copper homeostasis protein CutC	**0.41**	PECL_793	xsome
769	hypothetical protein	0.09	PECL_794	xsome
770	PTS system glucitol/sorbitol-specific IIA component family protein	**-0.59**	PECL_795	xsome
771	hypothetical protein	-0.25	PECL_796	xsome
772	Putative tRNA cytidine-methyltransferase	**-0.47**	PECL_797	xsome
773	DNA translocase ftsK	**-0.45**	PECL_798	xsome
774	protein MraZ	**-0.80**	PECL_800	xsome
775	S-adenosyl-methyltransferase MraW	**-1.31**	PECL_801	xsome
776	cell division protein FtsL	**-1.58**	PECL_802	xsome
777	Cell division protein FtsI peptidoglycan synthetase	**-1.04**	PECL_803	xsome
778	phospho-N-acetylmuramoyl-pentapeptide-transferase	**-0.90**	PECL_804	xsome
779	UDP-N-acetylmuramoylalanine--D-glutamate ligase	**-0.59**	PECL_805	xsome
780	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	**-0.42**	PECL_806	xsome
781	Cell division protein FtsQ	-0.25	PECL_807	xsome
782	cell division protein FtsA	**-0.40**	PECL_808	xsome
783	cell division protein FtsZ	-0.14	PECL_809	xsome
784	hypothetical protein	-0.06	PECL_810	xsome
785	hypothetical protein	**-0.56**	PECL_811	xsome
786	divIVA domain protein	**-0.38**	PECL_812	xsome

787	isoleucine--tRNA ligase	**-1.06**	PECL_813	xsome
788	Cold-shock DNA-binding domain protein	**-0.97**	PECL_814	xsome
789	ADP-ribose pyrophosphatase	**-0.72**	PECL_815	xsome
790	hypothetical protein	**-0.88**	PECL_816	xsome
791	MTA/SAH nucleosidase	**-0.68**	PECL_817	xsome
792	aminotransferase class-V family protein	**-0.64**	PECL_818	xsome
793	hypothetical protein	**-0.44**	PECL_819	xsome
794	tRNA-methyl aminomethyl-2-thiouridylate-methyltransferase	-0.13	PECL_820	xsome
795	histidine phosphatase super family protein	-0.14	PECL_821	xsome
796	anaphase-promoting complex cyclosome subunit 3 family protein	0.05	PECL_822	xsome
797	viral Super1 RNA helicase family protein	-0.15	PECL_823	xsome
798	ribose-phosphate pyrophosphokinase	**-0.45**	PECL_824	xsome
799	hypothetical protein	-0.18	PECL_825	xsome
800	DeoR family transcriptional regulator	**0.78**	PECL_826	xsome
801	1-phosphofructokinase	**1.07**	PECL_827	xsome
802	PTS system fructose subfamily IIA component domain protein	**1.15**	PECL_828	xsome
803	hypothetical protein	0.54	PECL_829	xsome
804	lysM domain protein	**1.35**	PECL_830	xsome
805	hypothetical protein	**0.62**	PECL_831	xsome
806	hypothetical protein	0.05	PECL_832	xsome
807	hypothetical protein	**1.03**	PECL_833	xsome
808	peptide deformylase	**-0.59**	PECL_834	xsome
809	hypothetical protein	-0.15	PECL_835	xsome
810	inositol monophosphatase family protein	**-0.86**	PECL_836	xsome
811	GTP-binding protein TypA/BipA	-0.23	PECL_837	xsome
812	cell division protein	**0.33**	PECL_838	xsome
813	hypothetical protein	0.57	PECL_839	xsome
814	RsmD family RNA methyltransferase	-0.16	PECL_840	xsome
815	pantetheine-phosphate adenylyltransferase	-0.06	PECL_841	xsome
816	PDZ domain family protein	**-0.51**	PECL_842	xsome
817	competence ComEA helix-hairpin-helix repeat region domain protein	**-0.61**	PECL_843	xsome

818	comE operon protein 2	-0.19	PECL_844	xsome
819	DNA internalization-related competence protein ComEC/Rec2	-0.27	PECL_845	xsome
820	DNA polymerase III subunit delta	-0.08	PECL_846	xsome
821	30S ribosomal protein S20	-0.11	PECL_847	xsome
822	30S ribosomal protein S15	**-0.28**	PECL_848	xsome
823	RNA-metabolising metallo-beta-lactamase family protein	**-0.55**	PECL_849	xsome
824	hypothetical protein	**-0.63**	PECL_850	xsome
825	translation elongation factor Tu	**0.58**	PECL_851	xsome
826	trigger factor	**-0.63**	PECL_852	xsome
827	ATP-dependent Clp protease ATP-binding subunit ClpX	**-0.26**	PECL_853	xsome
828	ribosome biogenesis GTP-binding protein YsxC	**-0.74**	PECL_854	xsome
829	mazG nucleotide pyrophosphohydrolase domain protein	**-0.98**	PECL_855	xsome
830	hypothetical protein	**1.74**	PECL_856	xsome
831	Amino acid ABC transporter permease substrate-binding protein	-0.02	PECL_857	xsome
832	amino acid ABC transporter ATP-binding protein	**0.78**	PECL_858	xsome
833	excinuclease ABC subunit C	**0.72**	PECL_859	xsome
834	obg family GTPase CgtA	**-0.58**	PECL_860	xsome
835	acyltransferase family protein	**-0.28**	PECL_861	xsome
837	ribonuclease Z	**-0.76**	PECL_863	xsome
838	short chain dehydrogenase family protein	**-0.47**	PECL_864	xsome
839	single-stranded-DNA-specific exonuclease RecJ	**-0.68**	PECL_865	xsome
840	adenine phosphoribosyltransferase	-0.28	PECL_866	xsome
841	N-acetylmuramoyl-L-alanine amidase family protein	0.21	PECL_867	xsome
842	phage integrase truncated; psuedo	0.46	PECL_868	xsome
843	hypothetical protein	-0.18	PECL_869	xsome
844	hypothetical protein	0.01	PECL_870	xsome
845	hypothetical protein	**-0.55**	PECL_871	xsome
846	Prophage Lp1 protein	**-1.09**	PECL_872	xsome
847	hypothetical protein	**-1.37**	PECL_873	xsome
848	hypothetical protein	**-1.51**	PECL_874	xsome
849	hypothetical protein	**-1.49**	PECL_875	xsome

850	Prophage Lp1 protein 6	**-1.12**	PECL_876	xsome
851	Prophage Lp1 protein 5	**-1.06**	PECL_877	xsome
852	Prophage Lp1 protein 8	**-0.70**	PECL_878	xsome
853	hypothetical protein	-1.17	PECL_879	xsome
854	holin family protein truncated; psuedo	1.19	PECL_880	xsome
855	N-acetylmuramoyl-L-alanine amidase family protein	**0.98**	PECL_881	xsome
856	hypothetical protein	**-0.75**	PECL_882	xsome
857	hypothetical protein	-0.23	PECL_883	xsome
858	hypothetical protein	0.72	PECL_884	xsome
860	DNA primase	**0.84**	PECL_886	xsome
861	RNA polymerase sigma factor rpoD	**1.23**	PECL_887	xsome
862	tRNA adenin-methyltransferase TrmK	**-0.56**	PECL_888	xsome
863	NIF3 family protein	**-0.46**	PECL_889	xsome
864	hypothetical protein	**1.03**	PECL_890	xsome
865	DNA polymerase III subunit alpha	-0.21	PECL_891	xsome
866	6-phosphofructokinase	-0.23	PECL_893	xsome
867	pyruvate kinase	0.29	PECL_894	xsome
868	hypothetical protein	**-0.51**	PECL_895	xsome
869	hypothetical protein	**-0.46**	PECL_896	xsome
870	tyrosine recombinase XerD	**-0.56**	PECL_897	xsome
871	hypothetical protein	0.23	PECL_898	xsome
872	segregation and condensation protein A	-0.12	PECL_899	xsome
873	segregation and condensation protein B	**-0.69**	PECL_900	xsome
874	ribosomal large subunit pseudouridine synthase B	**-0.77**	PECL_901	xsome
875	hypothetical protein	-0.23	PECL_902	xsome
876	ATP-dependent DNA helicase RecQ family protein	**-0.41**	PECL_903	xsome
877	lysM domain protein	0.17	PECL_904	xsome
878	cytidylate kinase	-0.19	PECL_905	xsome
879	30S ribosomal protein S1	0.01	PECL_906	xsome
880	ribosome-associated GTPase EngA	-0.05	PECL_907	xsome
881	DNA-binding protein HU	**0.64**	PECL_908	xsome

882	anaphase-promoting complex cyclosome subunit 3 family protein	-0.01	PECL_909	xsome
883	hypothetical protein	0.19	PECL_910	xsome
884	nucleoside 2-deoxyribosyltransferase family protein	0.08	PECL_911	xsome
885	hypothetical protein	-0.14	PECL_912	xsome
886	poly A polymerase head domain protein	**-0.93**	PECL_913	xsome
887	heme ABC transporter ATP-binding protein CcmA	**-1.04**	PECL_914	xsome
888	thymidylate synthase	**-0.33**	PECL_915	xsome
889	dihydrofolate reductase	-0.16	PECL_916	xsome
890	EDD DegV family domain protein	-0.05	PECL_917	xsome
891	hypothetical protein	-0.41	PECL_918	xsome
892	C-terminal processing peptidase family protein	-0.08	PECL_919	xsome
893	GDSL-like Lipase cylhydrolase family protein	-0.10	PECL_920	xsome
894	hypothetical protein	0.17	PECL_921	xsome
895	ribosome biogenesis GTP-binding protein YlqF	0.28	PECL_922	xsome
896	ribonuclease HII family protein	**0.56**	PECL_923	xsome
897	DNA protecting protein DprA	0.22	PECL_924	xsome
898	DNA topoisomerase I	**0.45**	PECL_925	xsome
899	tyrosine recombinase XerC	**0.40**	PECL_926	xsome
900	ATP-dependent protease HslVU peptidase subunit	0.27	PECL_927	xsome
901	ATP-dependent carboxylate-amine ligase	**0.46**	PECL_928	xsome
902	galactose mutarotase related enzyme	**0.36**	PECL_929	xsome
903	acyl-phosphate glycerol 3-phosphate acyltransferase	**-0.33**	PECL_930	xsome
904	DNA topoisomerase IV subunit B	**-1.31**	PECL_931	xsome
905	DNA topoisomerase IV subunit A	**-1.18**	PECL_932	xsome
906	LysR family transcriptional regulator	-0.01	PECL_933	xsome
907	manganese-dependent inorganic pyrophosphatase	0.06	PECL_934	xsome
908	peptide methionine sulfoxide reductase MsrB	**3.63**	PECL_935	xsome
909	Peptide methionine sulfoxide reductase MsrA	**2.52**	PECL_936	xsome
910	inner membrane transporter yjeM	-0.24	PECL_937	xsome
911	hypothetical protein	**-1.11**	PECL_938	xsome
912	peptidase C26 family protein	**0.54**	PECL_939	xsome

913	amino acid permease family protein	0.18	PECL_940	xsome
914	crcB-like family protein	**-1.39**	PECL_941	xsome
915	crcB-like family protein	**-0.53**	PECL_942	xsome
916	protein degV	**-0.43**	PECL_943	xsome
917	hypothetical protein	-0.19	PECL_944	xsome
918	fibronectin-binding A family protein	-0.10	PECL_945	xsome
919	glycine--tRNA ligase subunit beta	**-0.57**	PECL_946	xsome
920	glycine--tRNA ligase alpha subunit	**-0.56**	PECL_947	xsome
921	DNA repair protein RecO	**-0.32**	PECL_948	xsome
922	GTP-binding protein Era	**-0.71**	PECL_949	xsome
923	undecaprenol kinase	-0.29	PECL_950	xsome
924	hypothetical protein	-0.15	PECL_951	xsome
925	istB-like ATP binding family protein	**-0.27**	PECL_952	xsome
926	Yqey-like protein	**-0.33**	PECL_953	xsome
927	30S ribosomal protein S21	**0.31**	PECL_954	xsome
928	hypothetical protein	0.13	PECL_955	xsome
929	putative endonuclease 4	-0.20	PECL_956	xsome
930	CDP-glycerol:poly-Glycerophosphate glycerophosphotransferase	-0.10	PECL_957	xsome
931	hypothetical protein	**-0.50**	PECL_958	xsome
932	aspartate--tRNA ligase	**-1.26**	PECL_959	xsome
933	histidine--tRNA ligase	**-1.35**	PECL_960	xsome
934	N-acetylmuramoyl-L-alanine amidase family protein	-0.27	PECL_962	xsome
935	HAD hydrolase family protein	**-1.10**	PECL_961	xsome
936	D-tyrosyl-tRNA-Tyr-deacylase	**-0.55**	PECL_963	xsome
937	GTP pyrophosphokinase	-0.13	PECL_964	xsome
938	RNA methyltransferase RsmE family protein	**-0.69**	PECL_965	xsome
939	50S ribosomal protein L11 methyltransferase	**-0.74**	PECL_966	xsome
940	uracil-xanthine permease	**-0.44**	PECL_967	xsome
941	hypothetical protein	**0.38**	PECL_968	xsome
942	hypothetical protein	-0.48	PECL_969	xsome
943	hypothetical protein	**0.70**	PECL_970	xsome

944	GTP-binding protein LepA	**-0.65**	PECL_971	xsome
945	chaperone protein DnaJ	**-1.52**	PECL_972	xsome
946	chaperone protein DnaK	**-1.09**	PECL_973	xsome
947	protein grpE	**-0.71**	PECL_974	xsome
948	heat-inducible transcription repressor HrcA	**-0.88**	PECL_975	xsome
949	riboflavin biosynthesis protein RibF	**-0.74**	PECL_977	xsome
950	tRNA pseudouridine synthase B	**-0.68**	PECL_978	xsome
951	ribosome-binding factor A	0.10	PECL_979	xsome
952	translation initiation factor IF-2	-0.15	PECL_980	xsome
953	ribosomal L7A/L30/S12=Gadd45 family protein	-0.13	PECL_981	xsome
954	hypothetical protein	0.21	PECL_982	xsome
955	transcription termination factor NusA	**-0.61**	PECL_983	xsome
956	Ribosome maturation factor rimP	**-0.81**	PECL_984	xsome
957	DNA polymerase III subunit alpha	**0.36**	PECL_985	xsome
958	proline--tRNA ligase	0.20	PECL_986	xsome
959	RIP metalloprotease RseP	-0.17	PECL_987	xsome
960	cytidylyltransferase family protein	-0.09	PECL_988	xsome
961	di-trans poly-cis-decaprenylcistransferase	**-0.32**	PECL_989	xsome
962	ribosome recycling factor	**0.42**	PECL_990	xsome
963	UMP kinase	-0.12	PECL_991	xsome
964	translation elongation factor Ts	**-0.89**	PECL_992	xsome
965	30S ribosomal protein S2	**-0.33**	PECL_993	xsome
966	D-lactate dehydrogenase	-0.19	PECL_994	xsome
967	hypothetical protein	-0.31	PECL_995	xsome
968	methyltransferase domain protein	**-0.34**	PECL_996	xsome
969	acyltransferase family protein	-0.11	PECL_997	xsome
970	hypothetical protein	**-0.84**	PECL_998	xsome
971	hypothetical protein	0.30	PECL_999	xsome
972	repressor LexA	**0.41**	PECL_1000	xsome
973	hypothetical protein	0.23	PECL_1001	xsome
974	hypothetical protein	**0.50**	PECL_1002	xsome

975	hydroxymethylglutaryl-CoA synthase	-0.24	PECL_1003	xsome
976	ABC transporter	**1.54**	PECL_1004	xsome
977	carbamoyl-phosphate synthase large subunit	-0.06	PECL_1005	xsome
978	carbamoyl-phosphate synthase small subunit	0.01	PECL_1006	xsome
979	bifunctional protein pyrR	-0.21	PECL_1007	xsome
980	RluA family pseudouridine synthase	0.20	PECL_1008	xsome
981	signal peptidase II	-0.19	PECL_1009	xsome
982	formate--tetrahydrofolate ligase 1	0.25	PECL_1010	xsome
983	pore-forming protein	-0.30	PECL_1011	xsome
984	FAD dependent oxidoreductase family protein	-0.13	PECL_1012	xsome
985	hypothetical protein	-0.09	PECL_1013	xsome
986	cell cycle protein gpsB	**0.75**	PECL_1014	xsome
987	hypothetical protein	**0.37**	PECL_1015	xsome
988	recombination protein U	-0.02	PECL_1016	xsome
989	transglycosylase	0.10	PECL_1017	xsome
990	DNA replication protein DnaD	**-0.33**	PECL_1018	xsome
991	Asparaginyl-tRNA synthetase	**-0.35**	PECL_1019	xsome
992	hypothetical protein	-0.18	PECL_1020	xsome
993	exonuclease%2C DNA polymerase III%2C epsilon subunit family domain protein	**-0.76**	PECL_1021	xsome
994	mevalonate kinase	0.17	PECL_1022	xsome
995	diphosphomevalonate decarboxylase	-0.25	PECL_1023	xsome
996	phosphomevalonate kinase	-0.12	PECL_1024	xsome
997	isopentenyl-diphosphate delta-isomerase	**-0.43**	PECL_1025	xsome
998	putative RNA methylase NOL1-NOP2-sun family	**-0.85**	PECL_1026	xsome
999	HAD hydrolase	-0.14	PECL_1027	xsome
1000	putative tautomerase ywhB	**0.58**	PECL_1028	xsome
1001	peptidase C69 family protein	-0.05	PECL_1029	xsome
1002	50S ribosomal protein L19	**-0.52**	PECL_1030	xsome
1003	tRNA guanine-N1-methyltransferase	**-1.26**	PECL_1031	xsome
1004	16S rRNA processing protein RimM	**-1.22**	PECL_1032	xsome

1005	30S ribosomal protein S16	**-0.59**	PECL_1033	xsome
1006	signal recognition particle protein	**-0.96**	PECL_1034	xsome
1007	hypothetical protein	**-1.85**	PECL_1035	xsome
1008	signal recognition particle-docking protein FtsY	**-1.09**	PECL_1036	xsome
1009	chromosome segregation protein SMC	**-1.18**	PECL_1037	xsome
1010	ribonuclease III	**-1.05**	PECL_1038	xsome
1011	acyl carrier protein	**-0.47**	PECL_1039	xsome
1012	fatty acid phospholipid synthesis protein PlsX	**-0.39**	PECL_1040	xsome
1013	ATP-dependent DNA helicase RecG	**-0.49**	PECL_1041	xsome
1014	hypothetical protein	-0.06	PECL_1042	xsome
1015	hypothetical protein	0.13	PECL_1043	xsome
1016	50S ribosomal protein L28	0.00	PECL_1044	xsome
1017	thiamine pyrophosphokinase	**-0.32**	PECL_1045	xsome
1018	ribulose-phosphate 3-epimerase	-0.26	PECL_1046	xsome
1019	ribosome small subunit-dependent GTPase A	**-0.38**	PECL_1047	xsome
1020	phosphotransferase enzyme family protein	-0.23	PECL_1048	xsome
1021	serine threonine phosphatase stp	**-0.49**	PECL_1049	xsome
1022	ribosomal RNA small subunit methyltransferase B	-0.22	PECL_1050	xsome
1023	methionyl-tRNA formyltransferase	-0.19	PECL_1051	xsome
1024	primosomal protein	0.02	PECL_1052	xsome
1025	phosphopantothenoylcysteine decarboxylase phosphopantothenate--cysteine ligase	0.06	PECL_1053	xsome
1026	DNA-directed RNA polymerase subunit omega	**-0.61**	PECL_1054	xsome
1027	guanylate kinase	**-1.44**	PECL_1055	xsome
1028	hypothetical protein	**1.91**	PECL_1056	xsome
1029	DNA repair protein RecN	**-0.54**	PECL_1057	xsome
1030	arginine repressor DNA binding domain protein	**-0.47**	PECL_1058	xsome
1031	hemolysin TlyA family protein	**-0.39**	PECL_1059	xsome
1032	farnesyl-diphosphate synthase	-0.32	PECL_1060	xsome
1033	exodeoxyribonuclease VII small subunit	-0.18	PECL_1061	xsome
1034	exodeoxyribonuclease VII large subunit	**-0.53**	PECL_1062	xsome

	Bifunctional methylenetetrahydrofolate dehydrogenase NADP			
1035	methenyltetrahydrofolate cyclohydrolase protein FolD	**-0.55**	PECL_1063	xsome
1036	transcription antitermination factor NusB	-0.31	PECL_1064	xsome
1037	hypothetical protein	-0.21	PECL_1065	xsome
1038	translation elongation factor P	**-0.92**	PECL_1066	xsome
1039	50S ribosomal protein L27	**0.37**	PECL_1067	xsome
1040	hypothetical protein	**0.27**	PECL_1068	xsome
1041	50S ribosomal protein L21	0.22	PECL_1069	xsome
1042	TetR family transcriptional regulator	0.06	PECL_1070	xsome
1043	membrane protein	**1.97**	PECL_1071	xsome
1044	hypothetical protein	**0.92**	PECL_1072	xsome
1045	putative cell-wall-anchored protein LPXTG motif	**1.73**	PECL_1073	xsome
1046	putative cell-wall-anchored protein LPXTG motif	**1.41**	PECL_1074	xsome
1047	hypothetical protein	**2.33**	PECL_1078	xsome
1048	hypothetical protein	**1.55**	PECL_1079	xsome
1049	hypothetical protein	0.30	PECL_1080	xsome
1051	hypothetical protein	**0.72**	PECL_1082	xsome
1052	dihydropteroate synthase	-0.24	PECL_1083	xsome
1053	Non-canonical purine NTP pyrophosphatase Rdg HAM1 family	**-0.37**	PECL_1084	xsome
1054	bifunctional Dihydrofolate synthase tetrahydrofolylpolyglutamate synthase protein FolC	-0.23	PECL_1085	xsome
1055	GTP cyclohydrolase I	**-0.56**	PECL_1086	xsome
1056	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	**-0.82**	PECL_1087	xsome
1057	dihydroneopterin aldolase	-0.32	PECL_1088	xsome
1058	dUTPase family protein	-0.10	PECL_1089	xsome
1059	hypothetical protein	**-0.31**	PECL_1090	xsome
1060	hypothetical protein	**-0.69**	PECL_1091	xsome
1061	glutamine synthetase type I	**-1.43**	PECL_1092	xsome
1062	HTH-type transcriptional regulator glnR	**-1.11**	PECL_1093	xsome
1063	aluminum resistance protein	**-0.39**	PECL_1094	xsome

1064	tRNA dimethylallyltransferase	-0.12	PECL_1096	xsome
1065	glycerophosphoryl diester phosphodiesterase	0.10	PECL_1097	xsome
1066	hypothetical protein	**0.64**	PECL_1098	xsome
1067	DNA topoisomerase III family protein	**-1.05**	PECL_1099	xsome
1068	rhodanese-like domain-containing protein	**-1.06**	PECL_1100	xsome
1069	glucokinase	**-1.40**	PECL_1101	xsome
1070	hypothetical protein	**-1.26**	PECL_1102	xsome
1071	rhomboid family protein	-0.22	PECL_1103	xsome
1072	5-formyltetrahydrofolate cyclo-ligase	**-0.39**	PECL_1104	xsome
1073	penicillin binding transpeptidase domain protein	0.12	PECL_1105	xsome
1074	hypothetical protein	0.17	PECL_1106	xsome
1075	transcription elongation factor greA	-0.25	PECL_1107	xsome
1076	uridine kinase	**-0.59**	PECL_1108	xsome
1077	aminodeoxychorismate lyase	**-0.58**	PECL_1110	xsome
1078	phenylalanine--tRNA ligase beta subunit	**-1.50**	PECL_1111	xsome
1079	phenylalanine--tRNA ligase alpha subunit	**-1.61**	PECL_1112	xsome
1080	HxlR family transcriptional regulator	**1.91**	PECL_1113	xsome
1081	HD domain-containing protein	**2.36**	PECL_1114	xsome
1082	RNA-O ribose methyltransferase substrate binding family protein	**0.32**	PECL_1115	xsome
1083	acylphosphatase family protein	**0.56**	PECL_1116	xsome
1084	membrane insertase, YidC/Oxa1 family domain protein	**0.72**	PECL_1117	xsome
1085	Two-component system sensor histidine kinase	**-0.41**	PECL_1118	xsome
1086	two-component response regulator	-0.11	PECL_1120	xsome
1087	6-phosphogluconate dehydrogenase	**0.67**	PECL_1121	xsome
1088	50S ribosomal protein L32	0.08	PECL_2025	xsome
1089	hypothetical protein	-0.23	PECL_1122	xsome
1090	hypothetical protein	**-0.82**	PECL_1123	xsome
1091	methyltransferase domain protein	**-0.97**	PECL_1124	xsome
1092	iojap-like ribosome-associated protein	**-0.50**	PECL_1125	xsome
1093	hypothetical protein	**-0.91**	PECL_1126	xsome
1094	nicotinate/nicotinamide nucleotide adenylyltransferase	**-0.69**	PECL_1127	xsome

1095	putative RNA-binding protein	**-1.23**	PECL_1128	xsome
1096	ribosome biogenesis GTPase YqeH	**-1.00**	PECL_1129	xsome
1097	HAD hydrolase family protein	**-0.56**	PECL_1130	xsome
1098	alcohol dehydrogenase GroES-like domain protein	0.27	PECL_1131	xsome
1099	pyridoxamine-phosphate oxidase family protein	**1.70**	PECL_1132	xsome
1100	50S ribosomal protein L20	**0.33**	PECL_1133	xsome
1101	50S ribosomal protein L35	**-0.40**	PECL_1134	xsome
1102	translation initiation factor IF-3	**0.29**	PECL_1135	xsome
1103	threonine--tRNA ligase	**-0.74**	PECL_1136	xsome
1104	Primosomal protein DnaI	-0.22	PECL_1137	xsome
1105	replication initiation and membrane attachment protein	-0.21	PECL_1138	xsome
1106	Transcriptional repressor NrdR	-0.24	PECL_1139	xsome
1107	dephospho-CoA kinase	**-0.69**	PECL_1140	xsome
1108	formamidopyrimidine-DNA glycosylase	**-0.87**	PECL_1141	xsome
1109	DNA polymerase I	**-0.66**	PECL_1142	xsome
1110	glutathione peroxidase	-0.24	PECL_1143	xsome
1111	inhibitor of apoptosis-promoting Bax1 family protein	0.12	PECL_1144	xsome
1112	UDP-N-acetylmuramate--L-alanine ligase	**-0.86**	PECL_1145	xsome
1113	DNA translocase sftA	**-0.55**	PECL_1146	xsome
1114	putative tRNA binding domain protein	**-0.31**	PECL_1147	xsome
1115	hypothetical protein	-0.08	PECL_1148	xsome
1116	tRNA-guanine-N-methyltransferase	-0.22	PECL_1149	xsome
1117	ABC transporter EcsB family protein	**-0.35**	PECL_1150	xsome
1118	ABC transporter ATP-binding protein	-0.06	PECL_1151	xsome
1119	Histidine triad HIT-domain protein	0.27	PECL_1152	xsome
1120	hypothetical protein	**0.63**	PECL_1153	xsome
1121	foldase protein prsA	**-0.31**	PECL_1154	xsome
1122	hypothetical protein	-0.23	PECL_1155	xsome
1123	hypothetical protein	-0.20	PECL_1156	xsome
1124	calcineurin-like phosphoesterase family protein	0.15	PECL_1157	xsome
1125	hypothetical protein	0.17	PECL_1158	xsome

1126	penicillin-binding 1A family protein	**-0.48**	PECL_1159	xsome
1127	arginine repressor DNA binding domain protein	**-1.46**	PECL_1160	xsome
1128	cyclic nucleotide-binding domain-containing protein	**-1.38**	PECL_1161	xsome
1129	arginine--tRNA ligase	**-1.68**	PECL_1162	xsome
1131	hypothetical protein	**2.27**	PECL_2026	xsome
1132	hypothetical protein	-0.11	PECL_1164	xsome
1133	glycosyl transferases group 1 family protein	**2.41**	PECL_1165	xsome
1134	aspartate racemase	**-0.45**	PECL_1166	xsome
1135	ATP-grasp enzyme	**-0.34**	PECL_1167	xsome
1136	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase	**-0.54**	PECL_1168	xsome
1137	asparagine synthase	-0.18	PECL_1169	xsome
1138	hypothetical protein	-0.61	PECL_1170	xsome
1139	TetR family transcriptional regulator	**-0.66**	PECL_1171	xsome
1140	glutamate gamma-aminobutyrate antiporter	**0.37**	PECL_1172	xsome
1141	serine O-acetyltransferase	**1.91**	PECL_1173	xsome
1142	cysteine synthase A	**1.88**	PECL_1174	xsome
1143	DNA RNA non-specific endonuclease family protein	**-1.02**	PECL_1175	xsome
1145	hypothetical protein	0.03	PECL_1177	xsome
1146	hypothetical protein	**-0.48**	PECL_1178	xsome
1147	hypothetical protein	-0.44	PECL_1179	xsome
1148	HAD hydrolase	**-1.03**	PECL_1180	xsome
1149	deoxyguanosine kinase	**-0.40**	PECL_1181	xsome
1150	universal stress family protein	**0.49**	PECL_1182	xsome
1151	pseudouridine synthase	**-1.75**	PECL_1183	xsome
1152	polysaccharide biosynthesis family protein	**-1.56**	PECL_1184	xsome
1153	leucyl-tRNA synthetase	**-1.59**	PECL_1185	xsome
1154	PAP2 superfamily protein	**-0.46**	PECL_1186	xsome
1155	putative rRNA methylase	**-0.45**	PECL_1187	xsome
1156	H antiporter-2 family protein	**-0.75**	PECL_1188	xsome
1157	S-adenosylmethionine synthetase	-0.06	PECL_1189	xsome
1158	hypothetical protein	**1.00**	PECL_1191	xsome

1159	L-lysine permease, lysE type	0.28	PECL_1192	xsome
1184	hypothetical protein	**-0.68**	PECL_1220	xsome
1185	glycosyl transferases group 1 family protein	**-0.71**	PECL_1221	xsome
1186	glycosyl transferases group 1 family protein	**-0.78**	PECL_1222	xsome
1187	alpha-beta hydrolase fold family protein	**-0.89**	PECL_1223	xsome
1188	phosphoenolpyruvate-protein phosphotransferase	**0.50**	PECL_1224	xsome
1189	phosphocarrier protein HPr	**0.69**	PECL_1225	xsome
1190	hypothetical protein	**0.49**	PECL_1226	xsome
1191	ATP-dependent Clp protease ATP-binding subunit ClpE	0.00	PECL_1227	xsome
1192	hypothetical protein	**-0.41**	PECL_1228	xsome
1193	sulfite exporter TauE/SafE family protein	**-0.62**	PECL_1229	xsome
1194	type I phosphodiesterase nucleotide pyrophosphatase family protein	**-0.35**	PECL_1230	xsome
1195	peptide chain release factor 3	0.03	PECL_1231	xsome
1196	glycerol-3-phosphate cytidylyltransferase	**0.63**	PECL_1232	xsome
1197	hypothetical protein	**-0.60**	PECL_1233	xsome
1198	hypothetical protein	**-0.58**	PECL_1234	xsome
1199	beta-lactamase family protein	-0.08	PECL_1235	xsome
1200	regulatory protein recX	-0.28	PECL_1236	xsome
1201	flavodoxin	**0.64**	PECL_1237	xsome
1202	UDP-N-acetylglucosamine 2-epimerase	-0.06	PECL_1238	xsome
1203	aminopeptidase pepS	**1.14**	PECL_1239	xsome
1205	hypothetical protein	-0.02	PECL_1241	xsome
1206	hypothetical protein	**-0.37**	PECL_1242	xsome
1207	Cell envelope-associated transcriptional attenuator LytR-Cps	**-0.67**	PECL_1243	xsome
1208	DEAD/DEAH box helicase	**-1.29**	PECL_1244	xsome
1209	oxidoreductase NAD-binding Rossmann fold family protein	**-0.76**	PECL_1245	xsome
1210	hypothetical protein	**0.45**	PECL_1247	xsome
1211	glycosyl hydrolases 25 family protein	-0.08	PECL_1246	xsome
1212	serine threonine exchanger, SteT	**0.48**	PECL_1248	xsome
1213	vanZ like family protein	**1.17**	PECL_1249	xsome
1216	polysaccharide biosynthesis family protein	**-0.31**	PECL_1252	xsome

1217	UDP-galactopyranose mutase	-0.21	PECL_1253	xsome
1218	CDP-glycerol:poly-Glycerophosphate glycerophosphotransferase	-0.18	PECL_1254	xsome
1219	hypothetical protein	-0.29	PECL_1255	xsome
1220	glycosyl transferase 2 family protein	**-0.58**	PECL_1256	xsome
1221	sugar transferase	**-0.38**	PECL_1257	xsome
1222	Tyrosine-protein phosphatase ywqE	-0.10	PECL_1258	xsome
1223	tyrosine-protein kinase YwqD	**-0.50**	PECL_1259	xsome
1224	Tyrosine-protein kinase transmembrane modulator	-0.26	PECL_1260	xsome
1225	beta-lactamase family protein	-0.07	PECL_1261	xsome
1226	23S rRNA uracil-5-methyltransferase RumA	**-0.56**	PECL_1262	xsome
1227	Diacylglycerol kinase	**-0.35**	PECL_1263	xsome
1228	aspartyl glutamyl-tRNA-Asn-Gln amidotransferase subunit B	-0.25	PECL_1264	xsome
1229	aspartyl glutamyl-tRNA amidotransferase subunit A	-0.21	PECL_1265	xsome
1230	aspartyl glutamyl-tRNA-Asn-Gln amidotransferase subunit C	**-0.39**	PECL_1266	xsome
1231	lipoprotein pheromone	-0.24	PECL_1267	xsome
1232	ATP-dependent DNA helicase PcrA	**-1.22**	PECL_1268	xsome
1233	phosphoribosylaminoimidazole carboxylase ATPase subunit	**-0.61**	PECL_1269	xsome
1234	xanthine phosphoribosyltransferase	**-0.61**	PECL_1270	xsome
1235	mannosyl-glycoendo-beta-N-acetylglucosaminidase family protein	**-0.65**	PECL_1271	xsome
1236	hypothetical protein	-0.28	PECL_1272	xsome
1237	D-isomer specific 2-hydroxyacid dehydrogenase NAD binding domain protein	**0.82**	PECL_1273	xsome
1238	30S ribosomal protein S9	-0.02	PECL_1274	xsome
1239	50S ribosomal protein L13	**-0.46**	PECL_1275	xsome
1240	tRNA pseudouridine synthase A	**-0.35**	PECL_1276	xsome
1241	energy-coupling factor transporter transmembrane protein EcfT	-0.23	PECL_1277	xsome
1242	energy-coupling factor transporter ATP-binding protein EcfA2	-0.24	PECL_1278	xsome
1243	Energy-coupling factor transporter ATP-binding protein EcfA1	**-0.48**	PECL_1279	xsome
1244	50S ribosomal protein L17	**-0.47**	PECL_1280	xsome
1245	DNA-directed RNA polymerase subunit alpha	**-0.81**	PECL_1281	xsome
1246	30S ribosomal protein S11	**-1.25**	PECL_1282	xsome

1247	30S ribosomal protein S13	**-0.97**	PECL_1283	xsome
1248	translation initiation factor IF-1	**-0.94**	PECL_1284	xsome
1249	adenylate kinase	**-1.07**	PECL_1285	xsome
1250	preprotein translocase subunit SecY	**-0.93**	PECL_1286	xsome
1251	50S ribosomal protein L15	**-0.80**	PECL_1287	xsome
1252	50S ribosomal protein L30	**-1.09**	PECL_1288	xsome
1253	30S ribosomal protein S5	**-1.09**	PECL_1289	xsome
1254	50S ribosomal protein L18	**-1.46**	PECL_1290	xsome
1255	50S ribosomal protein L6	**-0.88**	PECL_1291	xsome
1256	30S ribosomal protein S8	**-0.90**	PECL_1292	xsome
1257	30S ribosomal protein S14	**-1.09**	PECL_2027	xsome
1258	50S ribosomal protein L5	**-0.95**	PECL_1293	xsome
1259	50S ribosomal protein L24	**-1.45**	PECL_1294	xsome
1260	50S ribosomal protein L14	**-1.15**	PECL_1295	xsome
1261	30S ribosomal protein S17	**-1.01**	PECL_1296	xsome
1262	50S ribosomal protein L29	**-1.45**	PECL_1297	xsome
1263	50S ribosomal protein L16	**-0.60**	PECL_1298	xsome
1264	30S ribosomal protein S3	**-1.16**	PECL_1299	xsome
1265	50S ribosomal protein L22	**-1.37**	PECL_1300	xsome
1266	30S ribosomal protein S19	**-0.68**	PECL_1301	xsome
1267	50S ribosomal protein L2	**-0.40**	PECL_1302	xsome
1268	50S ribosomal protein L23	**-0.77**	PECL_1303	xsome
1269	50S ribosomal protein L4	-0.16	PECL_1304	xsome
1270	50S ribosomal protein L3	**-0.56**	PECL_1305	xsome
1271	30S ribosomal protein S10	-0.05	PECL_1306	xsome
1272	major facilitator superfamily protein	-0.27	PECL_1307	xsome
1273	translation elongation factor G	**-0.71**	PECL_1308	xsome
1274	30S ribosomal protein S7	-0.17	PECL_1309	xsome
1275	30S ribosomal protein S12	**-0.31**	PECL_1310	xsome
1276	Peptidase A24 N-terminal domain protein	-0.41	PECL_1311	xsome
1277	DNA-directed RNA polymerase subunit beta%27	-0.05	PECL_1313	xsome

1278	DNA-directed RNA polymerase subunit beta	0.03	PECL_1314	xsome
1279	ATP-dependent Clp protease ATP-binding subunit ClpC	**0.62**	PECL_1315	xsome
1280	CtsR family transcriptional regulator	**0.31**	PECL_1316	xsome
1287	serine--tRNA ligase	**-0.53**	PECL_1323	xsome
1288	deoxyguanosine kinase	**-0.42**	PECL_1324	xsome
1289	amino acid permease family protein	**-0.35**	PECL_1325	xsome
1290	hypothetical protein	0.08	PECL_1326	xsome
1291	lipase%2Festerase	**0.92**	PECL_1327	xsome
1292	cell envelope-related function transcriptional attenuator common domain-containing protein	**0.91**	PECL_1328	xsome
1293	hypothetical protein	0.10	PECL_1329	xsome
1294	hypothetical protein	0.32	PECL_1330	xsome
1295	cold shock protein CspC	**0.37**	PECL_1331	xsome
1296	metalloregulator ScaR	**0.64**	PECL_1332	xsome
1297	Cobalt%2Fzinc%2Fcadmium cation efflux pump protein	0.10	PECL_1333	xsome
1298	PAP2 superfamily protein	**-0.67**	PECL_1334	xsome
1299	hypothetical protein	**-1.12**	PECL_1335	xsome
1300	hypothetical protein	**-1.21**	PECL_1336	xsome
1301	hypothetical protein	**-1.01**	PECL_1337	xsome
1302	GNAT family acetyltransferase	**-1.03**	PECL_1338	xsome
1303	RluA family pseudouridine synthase	**-0.76**	PECL_1339	xsome
1304	hypothetical protein	**-0.57**	PECL_1340	xsome
1305	hypothetical protein	0.32	PECL_1341	xsome
1306	ATP-dependent DNA helicase RecQ	0.27	PECL_1342	xsome
1307	protein containing glycosyl hydrolase family 25 domain and kxYKxGKxW signal peptide	**0.67**	PECL_1343	xsome
1308	major facilitator superfamily protein	-0.25	PECL_1344	xsome
1309	hypothetical protein	-0.11	PECL_1345	xsome
1310	hypothetical protein	**1.28**	PECL_1346	xsome
1311	proline dipeptidase	**1.55**	PECL_1347	xsome
1312	dipeptidase A	-0.05	PECL_1348	xsome

1313	csbD-like family protein	**1.73**	PECL_1349	xsome
1314	branched-chain amino acid aminotransferase	**-0.66**	PECL_1350	xsome
1315	hypothetical protein	0.17	PECL_1351	xsome
1316	malonate semialdehyde decarboxylase	**0.44**	PECL_1352	xsome
1317	hypothetical protein	0.70	PECL_1353	xsome
1318	lytTr DNA-binding domain protein	-0.15	PECL_1354	xsome
1319	GMP synthase	**-0.84**	PECL_1355	xsome
1320	hypothetical protein	**-0.54**	PECL_1356	xsome
1321	pantothenate kinase	**-0.71**	PECL_1357	xsome
1322	alpha-acetolactate decarboxylase	**-0.57**	PECL_1358	xsome
1323	acetolactate synthase	-0.18	PECL_1359	xsome
1324	DNA helicase	**0.70**	PECL_1360	xsome
1325	histidine phosphatase super family protein	**-1.82**	PECL_1361	xsome
1326	amino acid transporter	**-1.33**	PECL_1362	xsome
1327	trans-2-enoyl-CoA reductase catalytic region family protein	**0.68**	PECL_1363	xsome
1328	glycerol kinase	**0.82**	PECL_1364	xsome
1329	protein tyrosine serine phosphatase	0.14	PECL_1365	xsome
1330	flavin oxidoreductase NADH oxidase family protein	-0.11	PECL_1366	xsome
1331	propanediol utilization protein PduU	0.01	PECL_1367	xsome
1332	putative propionate kinase Facetate kinase	-0.27	PECL_1368	xsome
1333	propanol dehydrogenase	0.07	PECL_1369	xsome
1334	CoA-dependent propionaldehyde dehydrogenase	0.16	PECL_1370	xsome
1335	Propanediol utilization protein PduObis	0.02	PECL_1371	xsome
1336	Propanediol utilization protein PduO	-0.21	PECL_1372	xsome
1337	propanediol utilization protein PduN	-0.71	PECL_1373	xsome
1338	Propanediol utilization protein PduM	0.01	PECL_1374	xsome
1339	phosphate propanoyltransferase	0.47	PECL_1375	xsome
1340	propanediol utilization protein PduJ	0.83	PECL_1376	xsome
1341	propanediol utilization protein PduK	1.03	PECL_1377	xsome
1342	diol dehydratase-reactivating factor small subunit	-0.27	PECL_1378	xsome
1343	diol dehydratase-reactivating factor large subunit	**0.67**	PECL_1379	xsome

1344	propanediol dehydratase small subunit	1.07	PECL_1380	xsome
1345	propanediol dehydratase medium subunit	0.75	PECL_1381	xsome
1346	propanediol dehydratase large subunit	**1.01**	PECL_1382	xsome
1347	Propanediol utilization protein PduB	1.03	PECL_1383	xsome
1348	propanediol utilization protein PduA	0.99	PECL_1384	xsome
1349	ethanolamine utilization protein EutP	-0.39	PECL_1385	xsome
1350	nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase	-0.26	PECL_1386	xsome
1351	hypothetical protein	**-0.79**	PECL_1387	xsome
1352	sirohydrochlorin cobaltochelatase 2	0.26	PECL_1389	xsome
1353	precorrin-methyltransferase	0.16	PECL_1390	xsome
1354	sirohydrochlorin cobaltochelatase	-0.22	PECL_1391	xsome
1355	uroporphyrin-III C-methyltransferase uroporphyrinogen-III synthase	0.17	PECL_1392	xsome
1356	precorrin-6x reductase	0.00	PECL_1393	xsome
1357	precorrin-methyltransferase	0.33	PECL_1394	xsome
1358	cobalamin vitamin B12 biosynthesis protein CbiG	-0.03	PECL_1395	xsome
1359	precorrin-methyltransferase	-0.17	PECL_1396	xsome
1360	precorrin-methyltransferase subunit CbiT	**0.99**	PECL_1397	xsome
1361	precorrin-methyltransferase decarboxylating, CbiE subunit	0.39	PECL_1398	xsome
1362	cobalt-precorrin-6A synthase	0.53	PECL_1399	xsome
1363	cobalt-precorrin-8X methylmutase	0.55	PECL_2015	xsome
1364	adenosylcobinamide-phosphate synthase	0.52	PECL_1402	xsome
1365	cobyrinic acid-diamide synthase	0.27	PECL_1403	xsome
1366	threonine-phosphate decarboxylase	0.13	PECL_1404	xsome
1367	hypothetical protein	0.82	PECL_1405	xsome
1368	cobyrinic acid-diamide adenosyltransferase	0.57	PECL_1406	xsome
1369	flavodoxin	-0.24	PECL_1407	xsome
1370	putative uroporphyrinogen-III synthase HemD	-0.04	PECL_1408	xsome
1371	alpha-ribazole-phosphate phosphatase	0.09	PECL_1409	xsome
1372	cobalamin-phosphate synthase	0.42	PECL_1410	xsome
1373	bifunctional protein CobU adenosylcobinamide kinase	0.94	PECL_1411	xsome

	adenosylcobinamide-phosphate guanylyltransferase			
1374	hypothetical protein	**1.39**	PECL_1412	xsome
1375	glutamate-1-semialdehyde-2-aminomutase	0.59	PECL_1413	xsome
1376	delta-aminolevulinic acid dehydratase	0.63	PECL_1414	xsome
1377	porphobilinogen deaminase	0.34	PECL_1415	xsome
1378	glutamyl-tRNA reductase	**1.69**	PECL_1416	xsome
1379	Precorrin-2 dehydrogenase	0.13	PECL_1417	xsome
1380	cobyric acid synthase CbiP	**0.83**	PECL_1418	xsome
1381	cobalt ABC transporter ATP-binding protein CbiO	-0.23	PECL_1419	xsome
1382	cobalt ABC transporter permease CbiQ	0.36	PECL_1420	xsome
1383	cobalt transport protein CbiN	0.06	PECL_1421	xsome
1384	Cobalt transport protein CbiM	-0.17	PECL_1422	xsome
1385	AraC family transcriptional regulator	**1.03**	PECL_1423	xsome
1386	high-affinity nickel-transport protein nixA	**0.75**	PECL_1424	xsome
1387	oligopeptide dipeptide ABC transporter ATP-binding protein OppF	**-0.37**	PECL_1425	xsome
1388	oligopeptide dipeptide ABC transporter ATP-binding protein OppD	**-0.39**	PECL_1426	xsome
1389	oligopeptide dipeptide ABC transporter permease protein OppC	**-0.48**	PECL_1427	xsome
1390	oligopeptide dipeptide ABC transporter permease protein OppB	**-0.77**	PECL_1428	xsome
1391	oligopeptide dipeptide ABC transporter periplasmic binding protein OppA	**0.90**	PECL_1429	xsome
1392	GTP-binding protein HflX	-0.01	PECL_1430	xsome
1393	oxidoreductase FAD/NAD-binding domain protein	**1.62**	PECL_1431	xsome
1394	apbE family protein	**1.69**	PECL_1432	xsome
1395	FMN-binding domain-containing protein	**1.65**	PECL_1433	xsome
1396	short chain dehydrogenase family protein	**0.90**	PECL_1434	xsome
1397	MerR family transcriptional regulator	**1.03**	PECL_1435	xsome
1398	mgtC family protein	**2.12**	PECL_1436	xsome
1399	PTS system lactose/cellobiose IIC component family protein	**0.94**	PECL_1437	xsome
1400	cellulase family protein	**1.06**	PECL_1438	xsome
1401	GntR family transcriptional regulator	0.16	PECL_1439	xsome
1402	lytTr DNA-binding domain protein	-0.11	PECL_1440	xsome

1403	SH3 domain-containing protein	-0.09	PECL_1441	xsome
1404	L-fucose:H symporter permease	**1.78**	PECL_1442	xsome
1405	D-ribose pyranase	**2.11**	PECL_1443	xsome
1406	ribokinase	**1.44**	PECL_1444	xsome
1407	Ribose operon transcriptional regulator LacI family	**-0.93**	PECL_1445	xsome
1408	amino acid ABC transporter permease protein–His-Glu-Gln-Arg-opine family	**-0.68**	PECL_1446	xsome
1409	amino acid ABC transporter permease protein His-Glu-Gln-Arg-opine family	**-0.88**	PECL_1447	xsome
1410	amino acid ABC transporter amino acid-binding periplasmic protein His-Glu-Gln-Arg-opine family	**-0.66**	PECL_1448	xsome
1411	amino acid ABC transporter ATP-binding protein His-Glu-Gln-Arg-opine family	**-0.71**	PECL_1449	xsome
1412	hypothetical protein	**-1.18**	PECL_1450	xsome
1413	hypothetical protein	**-1.86**	PECL_1451	xsome
1414	zinc-specific metalloregulatory protein	**-1.45**	PECL_1452	xsome
1415	guanylate kinase family protein	**-1.64**	PECL_1453	xsome
1416	hypothetical protein	**2.06**	PECL_1454	xsome
1417	NUDIX domain-containing protein	-0.14	PECL_1455	xsome
1418	amino acid permease family protein	0.27	PECL_1456	xsome
1419	xaa-Pro dipeptidyl-peptidase	**0.30**	PECL_1458	xsome
1420	biotin-acetyl-CoA-carboxylase ligase	0.22	PECL_1457	xsome
1421	proline-specific peptidases family protein	**0.37**	PECL_1459	xsome
1422	aldo-keto reductase family protein	**0.59**	PECL_1460	xsome
1423	flavin oxidoreductase NADH oxidase family protein	**1.76**	PECL_1461	xsome
1424	heme ABC transporter ATP-binding protein CcmA	-0.12	PECL_1462	xsome
1425	bleomycin resistance protein	-0.06	PECL_1463	xsome
1426	signal peptidase I	0.18	PECL_1464	xsome
1427	glutamate-5-semialdehyde dehydrogenase	**-0.31**	PECL_1465	xsome
1428	glutamate 5-kinase	-0.23	PECL_1466	xsome
1429	methionine aminopeptidase	0.27	PECL_1467	xsome

1430	EDD DegV family domain protein	**-0.31**	PECL_1468	xsome
1431	thioredoxin	0.09	PECL_1469	xsome
1432	hypothetical protein	**-0.50**	PECL_1470	xsome
1433	corA-like Mg2transporter family protein	-0.14	PECL_1471	xsome
1434	yibE-like family protein	-0.29	PECL_1472	xsome
1435	hypothetical protein	**-0.35**	PECL_1473	xsome
1436	acetyltransferase family protein	-0.22	PECL_1474	xsome
1437	sulfatase family protein	0.20	PECL_1475	xsome
1438	ferredoxin-NADP reductase	**0.73**	PECL_1476	xsome
1439	Purine nucleosidase	-0.04	PECL_1477	xsome
1440	sodium:hydrogen antiporter	**-0.90**	PECL_1478	xsome
1441	hypothetical protein	**-0.99**	PECL_1479	xsome
1442	prolyl oligopeptidase family protein	**-1.65**	PECL_1480	xsome
1443	helix-turn-helix family protein	-0.35	PECL_1481	xsome
1444	putative SAM-dependent methyltransferase	**-0.57**	PECL_1482	xsome
1445	hypothetical protein	0.26	PECL_1483	xsome
1446	hypothetical protein	**0.65**	PECL_1484	xsome
1447	LD-transpeptidase catalytic domain-containing protein	**0.58**	PECL_1485	xsome
1448	hypothetical protein	**0.71**	PECL_1486	xsome
1449	major facilitator superfamily protein	**-0.79**	PECL_1487	xsome
1450	universal stress family protein	**1.29**	PECL_1488	xsome
1451	TetR family transcriptional regulator	**2.01**	PECL_1489	xsome
1452	ABC transporter permease component; psuedo	**1.53**	PECL_2016	xsome
1453	ABC transporter ATPase	**0.99**	PECL_1492	xsome
1454	RNA pseudouridylate synthase family protein	**1.28**	PECL_1493	xsome
1455	xylulose-5-phosphate phosphoketolase	**0.57**	PECL_1494	xsome
1456	GntR family transcriptional regulator	**-0.36**	PECL_1495	xsome
1457	Ybak-EbsC family protein	0.03	PECL_1496	xsome
1458	hypothetical protein	**0.38**	PECL_1497	xsome
1459	hypothetical protein	**2.69**	PECL_1498	xsome
1460	hypothetical protein	0.07	PECL_1499	xsome

1461	hypothetical protein	**0.51**	PECL_1500	xsome
1462	eamA-like transporter family protein	**-0.85**	PECL_1501	xsome
1463	aldose 1-epimerase family protein	**0.96**	PECL_1502	xsome
1464	HAD hydrolase	**1.48**	PECL_1504	xsome
1465	Maltose o-acetyltransferase; psuedo	**1.45**	PECL_1503	xsome
1466	Malate permease	**2.28**	PECL_1505	xsome
1467	malolactic protein	**2.31**	PECL_1506	xsome
1468	malolactic regulator, LysR family	0.36	PECL_1507	xsome
1469	hypothetical protein	0.93	PECL_1508	xsome
1470	peptidyl-prolyl cis-trans isomerase	-0.23	PECL_1509	xsome
1471	HAD hydrolase family protein	0.04	PECL_1510	xsome
1472	voltage gated chloride channel family protein	**0.41**	PECL_1512	xsome
1473	glyoxalase family protein	0.11	PECL_1511	xsome
1474	oligoendopeptidase F	**0.92**	PECL_1513	xsome
1475	hypothetical protein	-0.11	PECL_1514	xsome
1476	quaternary ammonium compound-resistance protein sugE	-0.16	PECL_1515	xsome
1477	enolase phosphopyruvate hydratase	**1.28**	PECL_1516	xsome
1478	hypothetical protein	0.25	PECL_1517	xsome
1479	major facilitator superfamily protein	**-0.48**	PECL_1518	xsome
1480	hypothetical protein	**-0.54**	PECL_1519	xsome
1481	aryl-phospho-beta-D-glucosidase BglH	0.35	PECL_1521	xsome
1482	fructokinase	**1.05**	PECL_1522	xsome
1483	Methionine synthase II Cobalamin-independent	**1.23**	PECL_1523	xsome
1484	D-methionine ABC transporter substrate-binding protein	**1.41**	PECL_1524	xsome
1485	D-methionine ABC transporter permease	**1.14**	PECL_1525	xsome
1486	D-methionine ABC transporter ATP-binding protein	**0.87**	PECL_1526	xsome
1487	hypothetical protein	**0.60**	PECL_1527	xsome
1488	TetR family transcriptional regulator	**0.58**	PECL_1528	xsome
1489	metal-dependent hydrolase	**1.22**	PECL_1529	xsome
1490	firmicute fructose-1,6-bisphosphatase family protein; psuedo	0.16	PECL_2017	xsome
1491	S-ribosylhomocysteinase	**0.96**	PECL_1532	xsome

1492	aminotransferase class I and II family protein	-0.16	PECL_1533	xsome
1493	isochorismatase family protein	-0.18	PECL_1534	xsome
1494	EDD DegV family domain protein	**-0.49**	PECL_1535	xsome
1495	pemK-like family protein	0.02	PECL_1536	xsome
1496	hypothetical protein	**-0.78**	PECL_1537	xsome
1497	hypothetical protein	**1.78**	PECL_1538	xsome
1498	TetR family transcriptional regulator	0.24	PECL_1539	xsome
1499	hypothetical protein	0.88	PECL_1540	xsome
1500	acetyltransferase	-0.02	PECL_1541	xsome
1501	hypothetical protein	-0.73	PECL_1542	xsome
1502	PTS system lactose/cellobiose IIC component family protein	0.08	PECL_1543	xsome
1503	AAA ATPase	**4.19**	PECL_1544	xsome
1504	hypothetical protein	**3.25**	PECL_1545	xsome
1505	nucleoside diphosphate kinase	**0.41**	PECL_1546	xsome
1506	hypothetical protein	-0.18	PECL_1547	xsome
1507	polypeptide deformylase family protein	**-0.48**	PECL_1548	xsome
1508	putative NADPH-quinone reductase	-0.02	PECL_1549	xsome
1509	hypothetical protein	0.00	PECL_1550	xsome
1510	HAD hydrolase	-0.15	PECL_1551	xsome
1511	hypothetical protein	**1.44**	PECL_1552	xsome
1512	hypothetical protein	1.07	PECL_1554	xsome
1513	hypothetical protein	0.83	PECL_1553	xsome
1514	TENA-THI-PQQC family protein	**-0.60**	PECL_1555	xsome
1515	cobalt transport family protein	**-0.45**	PECL_1556	xsome
1516	heme ABC transporter ATP-binding protein CcmA	**-0.57**	PECL_1557	xsome
1517	ABC-type cobalt transport system permease component family protein	**-0.58**	PECL_1558	xsome
1518	thiamine-phosphate pyrophosphorylase	**-0.66**	PECL_1559	xsome
1519	phosphomethylpyrimidine kinase	-0.24	PECL_1560	xsome
1520	hydroxyethylthiazole kinase family protein	-0.29	PECL_1561	xsome
1521	GNAT family acetyltransferase	**-2.29**	PECL_1562	xsome

1522	hypothetical protein	**-1.22**	PECL_1563	xsome
1523	TetR family transcriptional regulator	**-1.38**	PECL_1564	xsome
1524	gbkey=CDS	**1.36**	PECL_1565	xsome
1525	CAAX amino terminal protease self- immunity family protein	0.17	PECL_1566	xsome
1526	igma-70 family RNA polymerase sigma factor	-0.03	PECL_1567	xsome
1527	hypothetical protein	0.20	PECL_1568	xsome
1528	hypothetical protein	NA	PECL_1569	xsome
1529	transglycosylase associated family protein	**1.80**	PECL_1570	xsome
1530	hypothetical protein	**1.79**	PECL_1571	xsome
1531	hypothetical protein	**1.91**	PECL_1572	xsome
1532	hypothetical protein	**1.19**	PECL_1573	xsome
1533	hypothetical protein	**1.71**	PECL_1574	xsome
1534	hypothetical protein	**1.56**	PECL_1575	xsome
1535	hypothetical protein	**0.46**	PECL_1576	xsome
1536	GDSL-like Lipase Acylhydrolase family protein	0.05	PECL_1577	xsome
1537	MarR family transcriptional regulator	0.11	PECL_1578	xsome
1538	copper-translocating P-type ATPase	**0.45**	PECL_1579	xsome
1539	hypothetical protein	**0.44**	PECL_1580	xsome
1540	hypothetical protein	0.57	PECL_1581	xsome
1541	LysR family transcriptional regulator	-0.32	PECL_1582	xsome
1542	multiple substrate aminotransferase	0.41	PECL_1583	xsome
1543	glycosyl transferase 2 family protein	**2.70**	PECL_1584	xsome
1544	hypothetical protein	**2.67**	PECL_1585	xsome
1545	L-2-hydroxyisocaproate dehydrogenase	-0.02	PECL_1586	xsome
1546	hypothetical protein	**-0.49**	PECL_1587	xsome
1547	LysR family transcriptional regulator	0.55	PECL_1588	xsome
1548	NAD dependent epimerase dehydratase family protein	**1.17**	PECL_1589	xsome
1549	glycerate kinase	**-1.23**	PECL_1590	xsome
1550	monooxygenase	**3.75**	PECL_1591	xsome
1551	NADP oxidoreductase coenzyme F420-dependent family protein	**3.62**	PECL_1592	xsome
1552	pyruvate oxidase	**2.62**	PECL_1593	xsome

1553	ABC transporter family protein	**0.89**	PECL_1594	xsome
1554	ftsX-like permease family protein	**0.98**	PECL_1595	xsome
1555	two-component response regulator	**1.01**	PECL_1596	xsome
1556	histidine kinase-DNA gyrase and HSP90-like ATPase family protein	**0.99**	PECL_1597	xsome
1557	hypothetical protein	-0.13	PECL_1598	xsome
1558	hypothetical protein	**-0.95**	PECL_1599	xsome
1559	PadR family transcriptional regulator	**-1.91**	PECL_1600	xsome
1560	polysaccharide deacetylase family protein	**0.51**	PECL_1601	xsome
1561	Poly-beta-1,6-N-acetyl-D-glucosamine synthase	**0.51**	PECL_1602	xsome
1562	beta-N-acetylhexosaminidase	**0.99**	PECL_1603	xsome
1563	hypothetical protein	**1.11**	PECL_1604	xsome
1564	prolyl oligopeptidase family protein	**3.99**	PECL_1605	xsome
1565	acetyl-CoA acetyltransferase	**0.79**	PECL_1606	xsome
1566	hypothetical protein	**0.63**	PECL_1607	xsome
1567	astacin family protein	**2.31**	PECL_1608	xsome
1568	hypothetical protein	0.71	PECL_1609	xsome
1569	hypothetical protein	-0.47	PECL_1610	xsome
1570	carboxymuconolactone decarboxylase family protein	0.26	PECL_1611	xsome
1571	hypothetical protein	0.03	PECL_1612	xsome
1572	LysR family transcriptional regulator	0.23	PECL_1613	xsome
1573	Ribonuclease BN-like family protein	**-0.57**	PECL_1614	xsome
1574	cyclic nucleotide-binding domain-containing protein	**-0.72**	PECL_1615	xsome
1575	NADPH-dependent FMN reductase family protein	**-0.64**	PECL_1616	xsome
1576	methyltransferase domain protein	**-0.36**	PECL_1617	xsome
1577	TetR family transcriptional regulator	**-1.08**	PECL_1618	xsome
1578	H antiporter-2 family protein	**0.99**	PECL_1619	xsome
1579	alpha-beta hydrolase family protein	**0.49**	PECL_1620	xsome
1580	TetR family transcriptional regulator	0.07	PECL_1621	xsome
1581	sodium:hydrogen exchanger family protein	0.33	PECL_1622	xsome
1582	alpha amylase catalytic domain protein truncated; psuedo	0.02	PECL_1623	xsome
1583	NAD dependent epimerase dehydratase family protein	0.23	PECL_1624	xsome

1584	DJ-PfpI family protein	0.37	PECL_1625	xsome
1585	ArsR family transcriptional regulator	0.44	PECL_1626	xsome
1586	helix-turn-helix family protein	**1.31**	PECL_1627	xsome
1587	major facilitator superfamily protein	**2.60**	PECL_1628	xsome
1588	TetR family transcriptional regulator	**5.29**	PECL_1629	xsome
1589	ABC transporter family protein	**5.54**	PECL_1630	xsome
1590	hypothetical protein	**0.89**	PECL_1631	xsome
1591	hypothetical protein	**1.16**	PECL_1632	xsome
1592	pfkB carbohydrate kinase family protein	**1.47**	PECL_1633	xsome
1593	prolyl oligopeptidase family protein	**1.49**	PECL_1634	xsome
1594	TetR family transcriptional regulator	0.41	PECL_1635	xsome
1595	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	-0.03	PECL_1636	xsome
1596	lipoprotein	**0.36**	PECL_1637	xsome
1597	methyltransferase domain protein	**0.45**	PECL_1638	xsome
1598	short chain dehydrogenase family protein	**1.28**	PECL_1639	xsome
1599	short chain dehydrogenase family protein	**1.65**	PECL_1640	xsome
1600	MerR family transcriptional regulator	**1.14**	PECL_1641	xsome
1601	hypothetical protein	**1.42**	PECL_1642	xsome
1602	pyridine nucleotide-disulfide oxidoreductase family protein	**1.02**	PECL_1643	xsome
1603	branched-chain amino acid transporter AzlD family	**-0.99**	PECL_1644	xsome
1604	branched-chain amino acid transport protein AzlC family	-0.22	PECL_1645	xsome
1605	beta-lactamase family protein	**-1.32**	PECL_1646	xsome
1606	histidine kinase-DNA gyrase and HSP90-like ATPase family protein	-0.23	PECL_1647	xsome
1607	two-component response regulator	**-0.30**	PECL_1648	xsome
1608	methylated-DNA--protein-cysteine methyltransferase inducible	**0.44**	PECL_1649	xsome
1609	inosine-monophosphate dehydrogenase	**-0.34**	PECL_1650	xsome
1610	hypothetical protein	**-0.57**	PECL_1651	xsome
1611	hypothetical protein	**-1.19**	PECL_1652	xsome
1612	GTPase	**-1.02**	PECL_1653	xsome
1613	CAAX amino terminal protease self- immunity family protein	**-1.16**	PECL_1654	xsome
1614	hypothetical protein	**-0.66**	PECL_1655	xsome

1615	hypothetical protein	0.30	PECL_1656	xsome
1616	parB-like partition s domain protein	**-0.81**	PECL_1657	xsome
1617	16S rRNA guanine-N-methyltransferase GidB	**-0.70**	PECL_1658	xsome
1618	galactose-1-phosphate uridyl transferase%2C N-terminal domain protein	**-1.09**	PECL_1659	xsome
1619	galactokinase	**-0.92**	PECL_1660	xsome
1620	Putative esterase	0.21	PECL_1661	xsome
1621	alpha-beta fold family hydrolase	**0.67**	PECL_1662	xsome
1622	glycosyl hydrolases 31 family protein	0.02	PECL_1663	xsome
1623	LacI family transcriptional regulator	**-0.82**	PECL_1664	xsome
1624	GIY-YIG catalytic domain-containing protein	**-1.03**	PECL_1665	xsome
1625	helix-turn-helix domain rpiR family protein	**-0.85**	PECL_1666	xsome
1626	PTS system glucose subfamily IIABC protein	0.36	PECL_1667	xsome
1627	N-acetylmuramic acid 6-phosphate etherase	0.53	PECL_1668	xsome
1628	hypothetical protein	0.07	PECL_1669	xsome
1629	MerR family transcriptional regulator	**0.85**	PECL_1670	xsome
1630	PTS system trehalose-specific transporter subunit IIABC	0.32	PECL_1671	xsome
1631	PTS system trehalose-specific transporter subunit IIABC	**1.69**	PECL_1672	xsome
1632	trehalose operon repressor	**0.57**	PECL_1673	xsome
1633	trehalose-6-phosphate hydrolase	**0.84**	PECL_1674	xsome
1634	hypothetical protein	0.49	PECL_1675	xsome
1635	major facilitator superfamily protein	0.46	PECL_1676	xsome
1636	hypothetical protein	0.73	PECL_1677	xsome
1637	hypothetical protein	-0.78	PECL_1678	xsome
1638	hypothetical protein	0.66	PECL_1679	xsome
1639	non-specific ribonucleoside hydrolase rihC	**-0.89**	PECL_1680	xsome
1640	purine nucleoside transport protein nupG	**-1.39**	PECL_1681	xsome
1641	DEAD/DEAH box helicase	**-1.39**	PECL_1682	xsome
1642	hypothetical protein	**-1.48**	PECL_1683	xsome
1643	drug resistance transporter CflA subfamily protein	**-1.40**	PECL_1684	xsome
1644	23S rRNA uracil-methyltransferase RumA	0.19	PECL_1685	xsome

1645	dipeptidase	0.28	PECL_1686	xsome
1646	MerR family transcriptional regulator	**-0.49**	PECL_1687	xsome
1647	sir2 family protein	0.27	PECL_1688	xsome
1648	NAD dependent epimerase/dehydratase family protein	-0.02	PECL_1689	xsome
1649	putative lipoprotein	**-0.49**	PECL_1690	xsome
1650	TetR family transcriptional regulator	**0.73**	PECL_1691	xsome
1651	peroxidase family protein	**1.62**	PECL_1692	xsome
1652	prolyl oligopeptidase family protein	**1.33**	PECL_1693	xsome
1653	MarR family transcriptional regulator	-0.10	PECL_1694	xsome
1654	beta-phosphoglucomutase	0.33	PECL_1695	xsome
1655	hydrolase	0.09	PECL_1696	xsome
1656	ABC transporter family protein	**0.67**	PECL_1697	xsome
1657	hypothetical protein	-0.13	PECL_1698	xsome
1658	hypothetical protein	0.94	PECL_1699	xsome
1659	multiple sugar ABC transporter permease protein 1	-0.17	PECL_1700	xsome
1660	multiple sugar ABC transporter permease protein 2	0.48	PECL_1701	xsome
1661	multiple sugar ABC transporter substrate-binding protein	**0.66**	PECL_1702	xsome
1662	helix-turn-helix domain, rpiR family protein	**0.71**	PECL_1703	xsome
1663	agmatine deiminase	**0.57**	PECL_1704	xsome
1664	carbamate kinase	**1.15**	PECL_1705	xsome
1665	agmatine deiminase	**2.22**	PECL_1706	xsome
1666	agmatine-putrescine antiporter	**3.57**	PECL_1707	xsome
1667	putrescine carbamoyltransferase	**3.90**	PECL_1708	xsome
1668	alpha-amylase	**-0.94**	PECL_1709	xsome
1669	alpha amylase	**-2.17**	PECL_1710	xsome
1670	LacI family transcriptional regulator	**-2.11**	PECL_1711	xsome
1671	hypothetical protein	**-1.49**	PECL_1712	xsome
1672	PTS system cellobiose-specific transporter subunit IIC	0.26	PECL_1713	xsome
1673	hypothetical protein	-0.15	PECL_1714	xsome
1674	peptide ABC transporter peptide-binding protein	**1.07**	PECL_1715	xsome
1675	hypothetical protein	0.22	PECL_1716	xsome

1676	hypothetical protein	0.17	PECL_1717	xsome
1677	hypothetical protein	0.12	PECL_1718	xsome
1678	hypothetical protein	0.30	PECL_1719	xsome
1679	putative lysine decarboxylase family protein	-0.21	PECL_1720	xsome
1680	tnp;psuedo	**-0.52**	PECL_1721	xsome
1681	Type IIS restriction enzyme FokI	**0.35**	PECL_1722	xsome
1682	modification methylase FokI	**1.09**	PECL_1723	xsome
1683	integrase; psuedo	**0.84**	PECL_2018	xsome
1684	CAAX amino terminal protease self- immunity family protein	0.49	PECL_1726	xsome
1685	mRNA interferase ChpB	**0.87**	PECL_1727	xsome
1686	hypothetical protein	0.39	PECL_1728	xsome
1687	hypothetical protein	**0.68**	PECL_1729	xsome
1688	ABC transporter ATPase	-0.06	PECL_1730	xsome
1689	ABC transporter permease	-0.16	PECL_1731	xsome
1690	hypothetical protein	0.06	PECL_1732	xsome
1691	PAP2 superfamily protein	-0.19	PECL_1733	xsome
1692	MIP channel s family protein	**0.81**	PECL_1734	xsome
1693	PTS-dependent dihydroxyacetone kinase phosphotransferase subunit dhaM	**1.38**	PECL_1735	xsome
1694	PTS-dependent dihydroxyacetone kinase ADP-binding subunit DhaL	**1.38**	PECL_1736	xsome
1695	PTS-dependent dihydroxyacetone kinase dihydroxyacetone-binding subunit DhaK	0.62	PECL_1737	xsome
1696	TetR family transcriptional regulator	**0.56**	PECL_1738	xsome
1697	NAD dependent epimerase dehydratase family protein	**0.98**	PECL_1739	xsome
1698	hypothetical protein	**0.60**	PECL_1740	xsome
1699	hypothetical protein	**0.72**	PECL_1741	xsome
1700	hypothetical protein	**0.72**	PECL_1742	xsome
1701	VIT family protein	**4.40**	PECL_1743	xsome
1702	hypothetical protein	**3.83**	PECL_1744	xsome
1703	hypothetical protein	-0.08	PECL_1745	xsome
1704	TetR family transcriptional regulator	-0.16	PECL_1746	xsome

1705	hypothetical protein	0.25	PECL_1747	xsome
1706	D-isomer specific 2-hydroxyacid dehydrogenase NAD binding domain protein	0.19	PECL_1748	xsome
1707	hypothetical protein	0.16	PECL_1749	xsome
1708	orotidine-phosphate decarboxylase HUMPS family protein	**0.44**	PECL_1750	xsome
1709	pfkB carbohydrate kinase family protein	0.34	PECL_1751	xsome
1710	hypothetical protein	**0.97**	PECL_1752	xsome
1711	hypothetical protein	**1.50**	PECL_1753	xsome
1712	xylose isomerase-like TIM barrel family protein	**2.44**	PECL_1754	xsome
1713	LacI family transcriptional regulator	-0.27	PECL_1755	xsome
1714	glycosyl transferases group 1 family protein	-0.05	PECL_1756	xsome
1715	glycosyl transferases group 1 family protein	**-0.48**	PECL_1757	xsome
1716	LPXTG-motif cell wall anchor domain-containing protein	**-0.44**	PECL_1758	xsome
1717	MarR family transcriptional regulator	**-1.11**	PECL_1759	xsome
1718	cell surface hydrolase	**0.40**	PECL_1761	xsome
1719	phosphotransferase system EIIC family protein	**0.45**	PECL_1762	xsome
1720	ABC transporter family protein	0.12	PECL_1763	xsome
1721	serine threonine exchanger SteT	**1.57**	PECL_1764	xsome
1722	ftsX-like permease family protein	**-0.34**	PECL_1765	xsome
1723	HAD ATPase P-type IC family protein	**-1.42**	PECL_1766	xsome
1724	short chain dehydrogenase family protein	**0.86**	PECL_1767	xsome
1725	TetR family transcriptional regulator	**1.11**	PECL_1768	xsome
1726	Glycine betaine-carnitine-choline ABC transporter permease protein OpuCD	**-0.75**	PECL_1769	xsome
1727	Glycine betaine-carnitine-choline ABC transporter substrate-binding protein OpuCC	**-1.16**	PECL_1770	xsome
1728	Glycine betaine-carnitine-choline ABC transporter permease protein OpuCB	**-0.56**	PECL_1771	xsome
1729	Glycine betaine-carnitine-choline ABC transporter ATP-binding protein OpuCA	**-0.73**	PECL_1772	xsome
1730	short chain dehydrogenase family protein	0.32	PECL_1773	xsome
1731	MerR family transcriptional regulator	**0.69**	PECL_1775	xsome

1732	short chain dehydrogenase family protein	**1.25**	PECL_1774	xsome
1733	peptidase T	0.03	PECL_1776	xsome
1734	PTS system N-acetylglucosamine-specific transporter subunit IIBC	0.16	PECL_1777	xsome
1735	hypothetical protein	0.65	PECL_1778	xsome
1736	PP-loop family protein	0.29	PECL_1779	xsome
1737	major intrinsic family protein	0.51	PECL_1780	xsome
1738	hypothetical protein	0.38	PECL_1781	xsome
1739	AIR carboxylase family protein	0.16	PECL_1782	xsome
1740	hypothetical protein	0.84	PECL_1783	xsome
1741	Transcription regulator Crp family	0.09	PECL_1784	xsome
1742	high-affinity nickel-transport protein nixA	**0.93**	PECL_1785	xsome
1743	Oxidoreductase family NAD-binding Rossmann fold protein	**1.62**	PECL_1786	xsome
1744	Glyoxalase bleomycin resistance protein dioxygenase superfamily protein	**1.41**	PECL_1787	xsome
1745	TetR family transcriptional regulator	**1.30**	PECL_1788	xsome
1746	efflux transporter hydrophobe amphiphile efflux-3 family protein	**1.66**	PECL_1789	xsome
1747	HAD ATPase P-type IC family protein	**1.28**	PECL_1790	xsome
1748	hhH-GPD superbase excision DNA repair family protein	-0.31	PECL_1791	xsome
1749	peptidase C69 family protein	**0.83**	PECL_1792	xsome
1750	short chain dehydrogenase family protein	-0.15	PECL_1794	xsome
1751	MarR family transcriptional regulator	**0.45**	PECL_1795	xsome
1752	helix-turn-helix family protein	**1.82**	PECL_1796	xsome
1753	LPXTG-motif cell wall anchor domain-containing protein	0.31	PECL_1797	xsome
1754	hypothetical protein	0.09	PECL_1798	xsome
1755	hypothetical protein	-0.34	PECL_1799	xsome
1756	hypothetical protein	**0.93**	PECL_1800	xsome
1757	short chain dehydrogenase family protein	**-0.52**	PECL_1801	xsome
1758	PHP domain-containing protein	0.27	PECL_1802	xsome
1759	beta-glucoside kinase	-0.06	PECL_1803	xsome
1760	putative phospho-beta-glucosidase	**1.29**	PECL_1804	xsome
1761	PTS system lactose/cellobiose IIC component family protein	**0.75**	PECL_1805	xsome

1762	hypothetical protein	0.15	PECL_1806	xsome
1763	hypothetical protein	**1.70**	PECL_1807	xsome
1764	hypothetical protein	**-0.70**	PECL_1808	xsome
1765	yhgE-Pip N-terminal domain protein	**0.85**	PECL_1809	xsome
1766	ArsR family transcriptional regulator	-0.23	PECL_1810	xsome
1767	EDD DegV family domain protein	-0.15	PECL_1811	xsome
1768	Histidine triad HIT domain protein	0.22	PECL_1812	xsome
1769	TetR family transcriptional regulator	**-0.47**	PECL_1813	xsome
1770	phosphatidylethanolamine-binding family protein	0.48	PECL_1814	xsome
1771	sulfite exporter TauE/SafE family protein	**0.93**	PECL_1815	xsome
1772	ketosteroid isomerase family protein	**1.35**	PECL_1816	xsome
1773	hypothetical protein	**0.74**	PECL_1818	xsome
1774	short chain dehydrogenase family protein	**1.48**	PECL_1817	xsome
1775	phosphoglycerate mutase	**1.10**	PECL_1819	xsome
1776	hypothetical protein	0.37	PECL_1820	xsome
1777	BCCT transporter family protein	**-1.55**	PECL_1821	xsome
1778	Maltose O-acetyltransferase	**-0.49**	PECL_1822	xsome
1779	chitosanase	**-0.47**	PECL_1823	xsome
1780	Protein with sigma-70 region 4 domain	**-0.54**	PECL_1824	xsome
1781	rRNA large subunit m3Psi methyltransferase RlmH	**-0.78**	PECL_1825	xsome
1782	trypsin family protein	0.12	PECL_1826	xsome
1783	metallo-beta-lactamase superfamily protein	-0.07	PECL_1827	xsome
1784	yycH family protein	**-0.62**	PECL_1828	xsome
1785	yycH family protein	**-0.82**	PECL_1829	xsome
1786	sensory box protein	**-0.64**	PECL_1830	xsome
1787	response regulator	**-0.40**	PECL_1831	xsome
1788	aminopeptidase N	0.28	PECL_1832	xsome
1790	fumarylacetoacetate FAA hydrolase family protein	**1.51**	PECL_1834	xsome
1791	hypothetical protein	**0.70**	PECL_1835	xsome
1792	hypothetical protein	**0.46**	PECL_1836	xsome
1793	Poly-glycerophosphate glycerophosphotransferase family protein	-0.19	PECL_1837	xsome

1794	Poly-glycerophosphate glycerophosphotransferase family protein	**-0.42**	PECL_1838	xsome
1795	zinc-binding dehydrogenase family protein	0.16	PECL_1839	xsome
1796	putative 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	**0.27**	PECL_1840	xsome
1797	isochorismatase family protein	**1.41**	PECL_1841	xsome
1798	acetyltransferase family protein	**1.18**	PECL_1842	xsome
1799	colicin V production family protein	**1.84**	PECL_1843	xsome
1800	hypothetical protein	**1.74**	PECL_1844	xsome
1801	hypothetical protein	**0.93**	PECL_2029	xsome
1802	glycerophosphoryl diester phosphodiesterase family protein	**0.77**	PECL_1845	xsome
1803	Alpha-beta hydrolase superfamily protein	**1.23**	PECL_1846	xsome
1804	acetyltransferase family protein	**0.67**	PECL_1847	xsome
1805	ATP-dependent helicase/nuclease subunit A	**-0.29**	PECL_1848	xsome
1806	ATP-dependent helicase/nuclease subunit B	0.05	PECL_1849	xsome
1807	hypothetical protein	0.22	PECL_1850	xsome
1808	xanthine permease family protein	**-1.14**	PECL_1851	xsome
1809	DNA-3-methyladenine glycosylase I	**-0.67**	PECL_1852	xsome
1810	GDSL-like Lipase Acylhydrolase family protein	0.15	PECL_1853	xsome
1811	hypothetical protein	**1.53**	PECL_1854	xsome
1812	Na:H antiporter NhaC	**-0.93**	PECL_1855	xsome
1813	phospholipase D	0.12	PECL_1856	xsome
1814	hypothetical protein	**0.52**	PECL_1857	xsome
1815	Histidine triad HIT domain protein	**0.43**	PECL_1858	xsome
1816	aminopeptidase E	0.26	PECL_1859	xsome
1817	signal peptidase I	**-0.31**	PECL_1860	xsome
1818	Transporter EamA family	-0.11	PECL_1861	xsome
1819	hypothetical protein	**1.03**	PECL_1862	xsome
1820	tRNA uridine 5-carboxymethylaminomethyl modification protein GidA	**-0.58**	PECL_1863	xsome
1821	tRNA modification GTPase TrmE	**-0.56**	PECL_1864	xsome
1822	membrane insertase YidC Oxa1 family domain protein	**-0.44**	PECL_1865	xsome
1823	ribonuclease P protein component	**-1.15**	PECL_1866	xsome

1824	plasmid replication protein	**0.58**	PECL_2013	pPECL-1
1825	copy-number control protein copG family	0.02	PECL_2014	pPECL-1
1826	plasmid replication protein	**-0.48**	PECL_2011	pPECL-2
1827	helix-turn-helix family protein	0.28	PECL_2012	pPECL-2
1828	replication initiation protein RepA	**2.03**	PECL_1972	pPECL-3
1829	hypothetical protein	**0.71**	PECL_1973	pPECL-3
1830	DNA protection during starvation protein	**2.83**	PECL_1974	pPECL-3
1831	hypothetical protein	**0.64**	PECL_1975	pPECL-3
1832	hypothetical protein	**0.67**	PECL_1976	pPECL-3
1833	putative peptidoglycan binding domain-containing protein	**1.98**	PECL_1977	pPECL-3
1834	pemK-like family protein	**2.59**	PECL_1978	pPECL-3
1835	AbrB family transcriptional regulator	**3.04**	PECL_1979	pPECL-3
1836	phage integrase family protein	**0.96**	PECL_1980	pPECL-3
1837	hypothetical protein	**1.16**	PECL_1981	pPECL-3
1838	plasmid replication protein	**2.10**	PECL_1982	pPECL-3
1839	hypothetical protein	**1.18**	PECL_1983	pPECL-3
1840	truncated Type I RM system specificity subunit hsdS; psuedo	**1.09**	PECL_1984	pPECL-3
1841	Transposase IS30 family	**1.08**	PECL_1985	pPECL-3
1842	truncated Type I RM system specificity subunit hsdS; psuedo	**0.55**	PECL_1986	pPECL-3
1843	pyridine nucleotide-disulfide oxidoreductase family protein	**1.08**	PECL_1987	pPECL-3
1844	putative bacteriocin immunity protein	**0.59**	PECL_1988	pPECL-3
1845	toxin of toxin-antitoxin stability system	**1.24**	PECL_1989	pPECL-3
1846	toxin-antitoxin TA system antitoxin	**1.32**	PECL_1990	pPECL-3
1847	phage integrase family protein	**1.35**	PECL_1991	pPECL-3
1848	hypothetical protein	**1.66**	PECL_1992	pPECL-3
1849	hypothetical protein	**1.35**	PECL_1993	pPECL-3
1850	plasmid replication protein	**0.52**	PECL_1908	pPECL-4
1851	transposase DDE domain family	0.30	PECL_1909	pPECL-4
1852	transposase IS66 family	**0.67**	PECL_1910	pPECL-4
1853	repA; psuedo	**0.53**	PECL_1911	pPECL-4
1854	antitoxin of toxin-antitoxin stability system RelB family	**1.83**	PECL_1912	pPECL-4

1855	hypothetical protein	**2.58**	PECL_1913	pPECL-4
1856	hypothetical protein	**0.98**	PECL_1914	pPECL-4
1857	hypothetical protein	**1.17**	PECL_1915	pPECL-4
1858	plasmid mobilization protein mobA, MobL family	**-0.74**	PECL_1916	pPECL-4
1859	transposase; psuedo	**-1.01**	PECL_1918	pPECL-4
1860	glycosyl hydrolase family 53 domain protein	0.15	PECL_1919	pPECL-4
1861	transposase IS66 family	**0.82**	PECL_1920	pPECL-4
1862	putative multicopper oxidase	**1.39**	PECL_1921	pPECL-4
1863	drug resistance MFS transporter drug:H antiporter-2	**1.55**	PECL_1922	pPECL-4
1864	site-specific recombinase%2C resolvase family	**0.41**	PECL_1923	pPECL-4
1865	site-specific recombinase%2C truncated;psuedo	**0.61**	PECL_1924	pPECL-4
1866	hypothetical protein	**0.84**	PECL_1925	pPECL-4
1867	hypothetical protein	**0.55**	PECL_1926	pPECL-4
1868	chromosome partitioning ATPase	**0.75**	PECL_1927	pPECL-4
1869	plasmid replication initiator protein A	**1.25**	PECL_1867	pPECL-5
1870	antitoxin of toxin-antitoxin stability system RelB family	**2.65**	PECL_1868	pPECL-5
1871	hypothetical protein	**2.65**	PECL_1871	pPECL-5
1872	hypothetical protein	**2.14**	PECL_1870	pPECL-5
1873	hypothetical protein	**1.99**	PECL_1872	pPECL-5
1874	plasmid mobilization protein mobA, MobL family	0.06	PECL_1873	pPECL-5
1875	hypothetical protein	-0.23	PECL_1874	pPECL-5
1876	hypothetical protein	-0.06	PECL_1875	pPECL-5
1877	transfer complex protein TrsB, TraB-like	**0.53**	PECL_1876	pPECL-5
1878	conjugation protein TrsC	0.30	PECL_1877	pPECL-5
1879	putative conjugation protein TrsD	0.13	PECL_1878	pPECL-5
1880	conjugation protein TrsE	**0.43**	PECL_1879	pPECL-5
1881	conjugation protein TrsF	0.11	PECL_1880	pPECL-5
1882	bacteriophage peptidoglycan hydrolase family protein	0.31	PECL_1881	pPECL-5
1883	hypothetical protein	**0.44**	PECL_1882	pPECL-5
1884	hypothetical protein	**0.66**	PECL_1883	pPECL-5
1885	conjugation protein TrsJ	**0.41**	PECL_2030	pPECL-5

1886	conjugation protein TrsK	**0.49**	PECL_1884	pPECL-5
1887	hypothetical protein	**0.92**	PECL_1885	pPECL-5
1888	conjugation protein TrsL	**1.17**	PECL_1886	pPECL-5
1889	hypothetical protein	**1.55**	PECL_1887	pPECL-5
1890	DNA topoisomerase III family protein	**0.69**	PECL_1888	pPECL-5
1891	hypothetical protein	**0.85**	PECL_1889	pPECL-5
1892	hypothetical protein	**0.81**	PECL_1890	pPECL-5
1893	hypothetical protein	**1.01**	PECL_1891	pPECL-5
1894	pyridine nucleotide-disulfide oxidoreductase class I	**0.47**	PECL_1892	pPECL-5
1895	arsenical resistance operon repressor	0.33	PECL_1893	pPECL-5
1896	arsenical pump membrane protein	**0.37**	PECL_1894	pPECL-5
1897	hypothetical protein	**1.04**	PECL_1895	pPECL-5
1898	site-specific recombinase resolvase family	**0.57**	PECL_1896	pPECL-5
1899	transposase truncated; psuedo	-0.07	PECL_1897	pPECL-5
1900	Cell surface protein with LPXTG-motif	**1.03**	PECL_1898	pPECL-5
1901	AraC family transcriptional regulator	**0.68**	PECL_1901	pPECL-5
1902	transposase;psuedo	**0.51**	PECL_1902	pPECL-5
1903	site-specific recombinase resolvase family	**1.51**	PECL_1905	pPECL-5
1904	putative plasmid partition protein	**2.79**	PECL_1906	pPECL-5
1905	hypothetical protein	**3.00**	PECL_1907	pPECL-5
1906	transposase; partial	0.64	PECL_2009	pPECL-6
1907	hypothetical protein	0.08	PECL_2008	pPECL-6
1908	MobA/MobL family mobilization protein	**0.52**	PECL_1934	pPECL-6
1909	hypothetical protein	**1.59**	PECL_1933	pPECL-6
1910	hypothetical protein	**1.63**	PECL_1931	pPECL-6
1911	hypothetical protein	**1.67**	PECL_1932	pPECL-6
1912	hypothetical protein	**2.24**	PECL_1930	pPECL-6
1913	antitoxin of toxin-antitoxin stability system, RelB family	**1.73**	PECL_1929	pPECL-6
1914	replication initiator protein A	**0.81**	PECL_1928	pPECL-6
1915	hypothetical protein	**0.69**	PECL_1948	pPECL-6
1916	hypothetical protein	-0.03	PECL_1947	pPECL-6

1917	putative plasmid partition protein	0.22	PECL_1946	pPECL-6
1918	resolvase family site-specific recombinase	**0.75**	PECL_1945	pPECL-6
1919	hypothetical protein	**1.19**	PECL_1944	pPECL-6
1920	type 2 lantibiotic biosynthesis protein LanM	**0.75**	PECL_1943	pPECL-6
1921	ABC-type bacteriocin transporter	-0.02	PECL_1942	pPECL-6
1922	ABC transporter ATP-binding protein	0.00	PECL_1941	pPECL-6
1923	ABC transporter permease	**0.41**	PECL_1940	pPECL-6
1924	ABC transporter permease	**0.57**	PECL_1939	pPECL-6
1925	transposase	**1.04**	PECL_1938	pPECL-6
1943	plasmid replication protein	**1.48**	PECL_2033	pPECL-8
1944	putative plasmid partition protein	**2.78**	PECL_2034	pPECL-8
1945	hypothetical protein	**2.48**	PECL_2035	pPECL-8
1946	hypothetical protein	**2.34**	PECL_2036	pPECL-8
1947	site-specific recombinase resolvase family	**2.69**	PECL_1949	pPECL-8
1948	Putative phospho-beta-glycosidase	**2.76**	PECL_1950	pPECL-8
1949	glycosyl transferase family 8	**2.78**	PECL_1951	pPECL-8
1950	phospholipid-glycerol acyltransferase	**2.67**	PECL_1952	pPECL-8
1951	Multidrug transporter HorA	**2.80**	PECL_1953	pPECL-8
1952	glycosyl transferase family 8	**2.28**	PECL_1954	pPECL-8
1953	codA	**2.25**	PECL_1955	pPECL-8
1954	truncated site-specific recombinase resolvase family; psuedo	**2.00**	PECL_1957	pPECL-8
1955	hypothetical protein	**3.18**	PECL_1958	pPECL-8
1956	hypothetical protein	**2.82**	PECL_1959	pPECL-8
1957	hypothetical protein	0.03	PECL_1960	pPECL-8
1958	DNA-repair protein-SOS response UmuC-like protein	**2.78**	PECL_1961	pPECL-8
1959	hypothetical protein	**2.23**	PECL_1962	pPECL-8
1960	hypothetical protein	**2.78**	PECL_1963	pPECL-8
1961	transcriptional regulator Xre family	**3.11**	PECL_1964	pPECL-8
1962	type I restriction and modification system restriction subunit R	**2.40**	PECL_1966	pPECL-8
1963	hsdM; psuedo	**2.60**	PECL_1967	pPECL-8
1964	type I restriction and modification system specificity subunit S	**2.59**	PECL_1969	pPECL-8

1965	hypothetical protein	**3.09**	PECL_2031	pPECL-8
1966	hypothetical protein	**2.64**	PECL_1970	pPECL-8
1967	hypothetical protein	**2.17**	PECL_1971	pPECL-8
1968	DNA topoisomerase	**2.66**	PECL_2037	pPECL-8
1969	putative nickase	**1.55**	PECL_2038	pPECL-8
1970	hypothetical protein	**2.45**	PECL_2039	pPECL-8
1971	hypothetical protein	**3.40**	PECL_2040	pPECL-8
1972	hypothetical protein	**2.63**	PECL_2041	pPECL-8
1973	hypothetical protein	**2.41**	PECL_2042	pPECL-8
1974	transposase	**1.10**	PECL_2043	pPECL-8
1975	transposase	**0.92**	PECL_2044	pPECL-8
1976	putative ncRNA between PECL_171 & PECL_172	**5.35**	PECL_2059	xsome
1977	bacterial small signal recognition particle RNA	-0.19	PECL_2057	xsome
1978	transfer messenger RNA ssrA	**2.39**	PECL_2056	xsome
1979	hypothetical protein	**0.58**	PECL_2046	xsome
1980	putative ncRNA between PECL_686 & PECL_687	**2.47**	PECL_2060	xsome
1981	hypothetical protein	**1.13**	PECL_2047	xsome
1982	hypothetical protein	0.16	PECL_2048	xsome
1983	Prophage Lp1 protein 7, nonsense mutations	**-0.66**	PECL_2049	xsome
1984	hypothetical protein	0.04	PECL_2050	xsome
1985	Ribonuclease P (RNase P) class B	**0.43**	PECL_2058	xsome
1986	50S ribosomal protein L33	-0.19	PECL_2051	xsome
1987	vanZ like family protein	**1.57**	PECL_2052	xsome
1988	putative ncRNA between PECL_1459 & PECL_1460	-0.23	PECL_2061	xsome
1989	putative ncRNA between PECL_1482 & PECL_1483	**1.01**	PECL_2062	xsome
1990	hypothetical protein	**1.61**	PECL_2053	xsome
1991	hypothetical protein	0.34	PECL_2054	xsome
1992	hypothetical protein	0.43	PECL_2055	xsome
1993	putative ncRNA between PECL_1907 & PECL_1867	**1.91**	PECL_2063	pPECL-5
1994	putative ncRNA between PECL_2033 & PECL_2044	NA	PECL_2064	pPECL-8
1995	putative ncRNA between PECL_2033 & PECL_2034	**1.67**	PECL_2065	pPECL-8