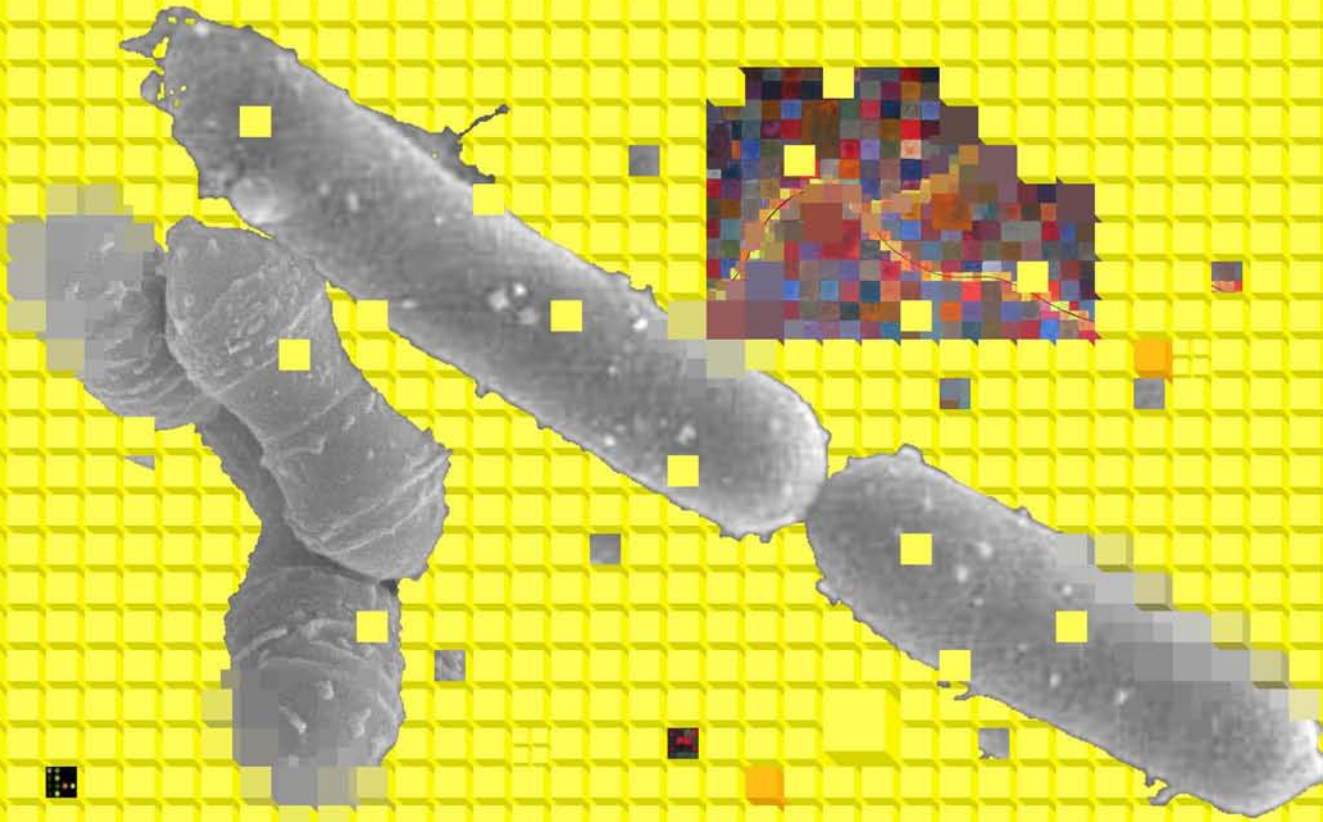
Analysis of Molecular Interactions between Yoghurt Bacteria by an Integrated Genomics Approach



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Thesis

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Abstract

The lactic acid bacteria (LAB) are a group of Gram-positive bacteria that ferment sugars such as lactose to produce mainly lactic acid. LAB are a group of industrially important microorganisms that are applied for the production of many fermented foods. These include foods produced with substrates from plant origin (e.g. sauerkraut and wine) and animal origin (e.g. fermented meats and dairy products such as yoghurt). The current market trends regarding sustainability and health-promoting foods demand more efficient and a more diverse range of fermentations. Most fermentations are carried out by multiple strains of different species. The interactions between consortium members are at the base of the performances of the individual microorganisms within a microbial ecosystem and therewith of the whole fermentation. These microbial interactions are often poorly understood. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are two LAB species that upon fermentation convert (bovine) milk into yoghurt. These two bacteria stimulate each other in growth and acid production. They produce exopolysaccharides (EPS), important for the texture of yoghurt, and characteristic flavor compounds such as acetaldehyde and diacetyl. However, the molecular basis of the mutualistic interactions between these two bacteria was poorly characterized.

In this thesis research, a combination was used of screening, mixed culture transcription profiling, whole-genome metabolic modeling, experimental evolution and next-generation sequencing. This was done to unravel the molecular basis of the interactions between *S. thermophilus* and *L. bulgaricus* in milk. The results showed that interactions were primarily based on the exchange of metabolites. Moreover, it was shown which genes or pathways were affected. Evidence was found that *S. thermophilus* provided *L. bulgaricus* with formic acid, folic acid (both involved in purine metabolism), long-chain fatty acids (by the action of lipolytic enzymes to break down milk fat) and CO_2. The proteolysis by the exoprotease of *L. bulgaricus*, in turn, provided both species peptides, which are taken up by the cell and broken down into amino acids (AA) by intracellular peptidases. However, this probably did not yield a sufficient supply of branched-chain and sulfur AA, leading to a higher expression of the genes for biosynthesis of these AA in both species when grown in mixed culture. Moreover, EPS biosynthesis genes were induced in the mixed culture, leading to increased EPS production and a higher viscosity of the yoghurt.

A mixed culture genome-scale metabolic model confirmed that cross-feeding interactions between the yoghurt bacteria were based on purine and AA

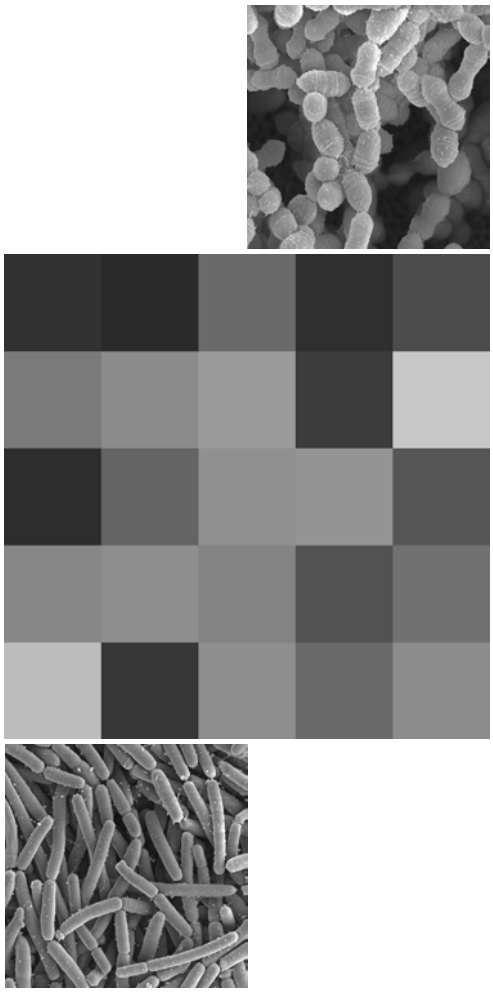
metabolism. Moreover, this model was used to show that the interactions provided a significant benefit to both bacteria, i.e. their biomass yield on lactose increased by around 50% in mixed culture.

Experimental evolution revealed that it is possible to co-adapt a novel combination of strains of *S. thermophilus* and *L. bulgaricus.* It was shown that their mutual stimulation increased by optimizing their interactions by fine-tuning expression of pathways involved in the interactions. Furthermore, as little as ~1000 generations of co-culture was sufficient to transform the relatively slow growing mixed culture into one that showed similar performance as commercial starters with respect to key characteristics such as acidification rate and viscosity.

Improved understanding of the described interactions that are at the base of the yoghurt fermentation provides us targets for the rational optimization of existing mixed culture fermentations and the rational development of industrially relevant mixed cultures, such as those containing probiotics. Moreover, the results are in particular interesting for the field of microbial ecology as they show how mutual nutritional dependencies evolve and structure the microbial composition of this ecosystem.

Chapter 1

General introduction



Historically, many food products were preserved by fermentation by lactic acid bacteria (LAB). For a large part this can be attributed to the desired functionalities of LAB, such as fast growth and the production of acids, volatile compounds and exopolysaccharides. This makes fermentation by LAB the method of choice for improving the taste and structure of food products. With the industrialization of the fermentation processes during the last century, LAB have even gained importance. For example, the cheese market has a worldwide turnover of more than 75 billion US $ and the yoghurt market is worth over 35 billion US $ (59). Most fermentations are carried out by a mixture of species or strains and the last decades it has become clear that the molecular interactions between these different LAB are of key importance for the success of fermentations. In order to control the fermentations and to develop new products, it is important to unravel these interactions. Yoghurt is a relatively well-studied mixed culture fermentation that involves *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, but the interactions between the two species are only poorly understood. The low complexity in terms of number of species involved and the high economical value make yoghurt a good model system to study the molecular interactions between the consortium members.

Here, we give an introduction to LAB and their application. In addition, their interactions in industrial fermentations are summarized with an emphasis on the yoghurt consortium. Moreover, new approaches used in modern biotechnology are discussed. Finally, an outline of this thesis is provided.

THE LACTIC ACID BACTERIA

Lactic acid bacteria or *Lactobacillales* are defined as a group of Gram-positive bacteria that ferment sugars such as lactose to produce primarily lactic acid (53). Within the *Lactobacillales* group, three main phylogenetic groups can be identified based on genomic similarity and GC content: *Lactobacillaceae, Leuconostocaceae* and *Streptococcaceae* (53). The LAB can be found ubiquitously on plants, in decomposing plant material, in dairy and in animal mucosa. The great variety in niches reflects that the LAB constitute a group of highly versatile organisms that are able to ferment many different substrates, ranging from simple disaccharides to complex carbohydrates like starch. Because the environments in which LAB occur are often relatively rich in carbon sources, amino acids or peptides and other nutrients, evolution of these organisms is mainly characterized by loss of gene function (see Figure 1) (53, 54), but also by horizontal gene transfer (HGT)

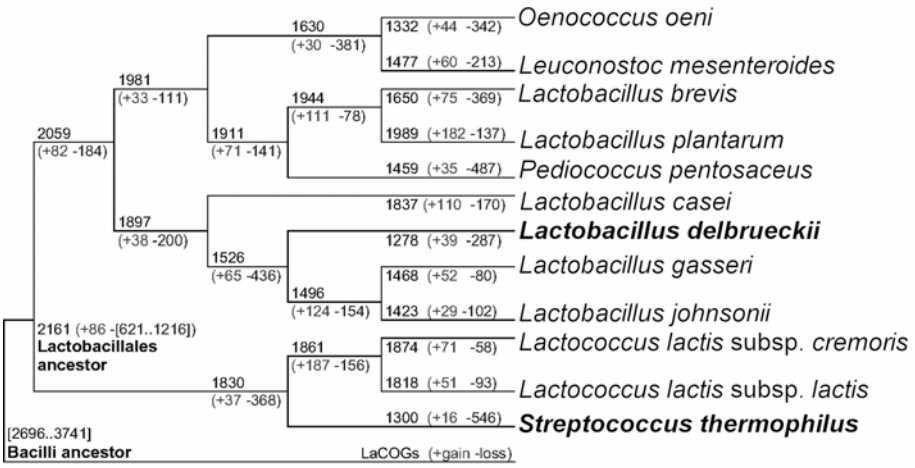


Figure 1. Reconstruction of gene content evolution in *Lactobacillales*. The tree is rooted by using *Bacillus subtilis* as the outgroup. For each species and each internal node of the tree, the inferred number of LaCOGs (*Lactobacillales*-specific clusters of orthologous genes – genes in two species that have a common ancestor gene) present, and the numbers of LaCOGs gained (+) and lost (-) along the branch leading to the given node (species) are indicated. Especially note the low amounts of LaCOGs in *Lactobacillus delbrueckii* (1278) and *Streptococcus thermophilus* (1300) that are caused by reductive evolution in these species (indicated in bold). Figure adapted from Makarova *et al*. (53).

between different LAB species. It was suggested that the last common ancestor of *Lactobacillales* (estimated genome size 2100 to 2200 genes) has lost 600 to 1200 genes (25 to 30%) and gained less than 100 genes after diverging from the *Bacilli* ancestor (genome size 2700 to 3700 genes), an event that has taken place billions rather than millions of years ago (54). Only since the rise of mammalian life, i.e. the only natural source of lactose, LAB have become the specialized lactose-utilizing bacteria (19). The adaptation to the relatively nutrient-rich and constant dairy environment was the cause of the reductive evolution (70). An illustrative example of reductive evolution is the case of *Lactococcus lactis*. This species includes strains that are found on plants while other strains are specifically adapted to the dairy environment and are used for the industrial production of cheese. Genomic and physiological studies have shown that the dairy *Lc. lactis* strains have lost the ability to ferment many carbohydrates while retaining the capacity to utilize efficiently the milk sugar lactose; conversely, the plant-derived *Lc. lactis* strains are not capable of fast growth on lactose but have a large capacity to degrade a variety of sugars (58, 81). Similarly, adaptation to milk was shown to often lead to auxotrophy for histidine and branched chain amino acids (BCAA) as a result of the accumulation of mutations mainly in the promoter regions of *his* genes (21), and nonsense mutations and small deletions in the *ile* and *val* genes (34).

Expression of genes has both costs and benefits, and therefore optimizing the gene repertoire and its expression for a given niche will determine the evolutionary success of a strain (3, 20). In nutritionally-rich environments, it may be advantageous to harvest compounds required for biosynthesis from the medium rather than expressing biochemical pathways needed for *de novo* biosynthesis. As a result these pathways are often not functional in many LAB and one can observe genome decay through the accumulation of mutations that result in loss of function, usually by introducing stop codons by frame shifts. These non-functional genes are annotated as pseudogenes. In addition, gene loss may occur, a process in which genes or major parts of these are completely deleted. Moreover, microorganisms that have a long history of growing together may have optimized their genetic repertoire to support associated growth and develop nutritional interactions. This also occurs frequently among LAB, leading to complex cross-feeding relationships and cooperative behavior as for instance in different types of cheese (73), plant-based food products (29, 82) and beverages (55, 77). Similarly, the close proximities in space and time of different species have facilitated horizontal gene transfer (HGT) leading to genomes containing regions with a deviating GC content originating from other species in the same habitat (4, 5, 53).

LAB ferment sugars in a homofermentative (leading to lactic acid) or heterofermentative (leading to a mixture of lactic acid, carbon dioxide, acetic acid and ethanol) way (40). Most *Lactobacillales* are microaerophilic, meaning that they are oxygen tolerant, but do not perform well in an oxygen-rich (i.e. atmospheric) environment. Recently, it was found that several LAB may respire when cultured in the presence of specific cofactors or their precursors (9, 30). Respiration yields additional ATP per mol of substrate but LAB do not respire in a energy-rich environment such as milk as they have lost the ability to synthesize heme (9, 63). This indicates that the evolutionary strategy followed by most LAB is to optimize their growth and acid production rate rather than their yield (79). The resulting high acidification rate combined with the high acid tolerance makes LAB successful for the preservation of food products by fermentation (12).

INDUSTRIAL FERMENTATIONS WITH LACTIC ACID BACTERIA

LAB have a history of being safely applied in the preservation of food for thousands of years (56). Many LAB species are considered safe for application in fermented food and have acquired the “qualified presumption of safety” (QPS) status in the European Union and the “generally regarded as safe” (GRAS) status in the United states of America dependent on the food they are applied in (43). Since the start of human civilization fermentation has been applied to preserve milk, meat and vegetables and to improve the organoleptic characteristics of foods (12, 28). Initially, these fermentations occurred naturally, but later were started by inoculating the substrate with a small sample of the fermented product. The knowledge on the appropriate techniques was transferred from generation to generation. Only since the rapid scientific progress in the field of microbiology in the 19th century the processes executed by microorganisms that occur during fermentation are being understood (12). It was found that many fermentations relied on the action of mixed cultures of yeasts, filamentous fungi and different species of LAB. The industrialization in the late 19th century together with the isolation and identification of these species in the mid 20th century paved the way for large-scale food production by fermentation to support the increasing growth of the human population (see Table 1). This process continued in the 2nd half of the 20th century with the use of starter cultures to achieve a constant and reproducible quality of the fermented products. These starter cultures, for instance those used for the production of cheese and other fermented dairy products, consisted of either a single strain, multiple strains of one species, or multiple strains of multiple species. The fact that interactions between consortium members play a key role in the performance of a fermentation has only been recognized in the last decades. This notion implies that in the design of starter cultures for mixed fermentations not only single strain traits have to be addressed, but also the interaction potential needs to be included. Mixed strain fermentations have received additional attention with the emergence and rapid growth of the consumer interest in fermented foods containing LAB marketed as probiotics. These probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (27). Although some novel probiotic products contain only one – the probiotic – strain, there is much interest in novel strain combinations that typically consist of a traditional starter in combination with one or more probiotic strains. In addition, the probiotic activity may be modulated by the strain’s ability to grow in the desired substrate and its interactions with the starter culture. Similar

interactions are expected in the multiple strain probiotics that are currently marketed.

Table 1. Examples of fermented foods that are produced at industrial scale with lactic acid bacteria. Adapted from Caplice and Fitzgerald (12). *A.*, *Aspergillus*; *C.*, *Candida*; *L., Lactobacillus*; *Lc.*, *Lactococcus*; *Ln.*, *Leuconostoc*; *S.*, *Streptococcus*; *Sc.*, *Saccharomyces*; *St.*, *Staphylococcus*; LAB, lactic acid bacteria.

Product	Microorganism(s)	Substrate	Country
Bread	*Sc. cerevisiae*, other yeasts, LAB	Wheat, rye, other grains	International
Gari	*Corynebacterium manihot*, other yeasts, LAB (*L. plantarum*, *Streptococcus* spp.)	Cassava root	West Africa
Idli	LAB (*Ln. mesenteroides*, *Enterococcus faecalis*), *Torulopsis*, *Candida, Trichosporon pullulans*	Rice and black gram, dhal	India
Kimchi	LAB	Cabbage, vegetables, occasionally seafood, nuts	Korea
Mahewu	LAB	Maize	South Africa
Ogi	LAB, *Cephalosporium*, *Fusarium*, *Aspergillus*, *Penicillium* spp., *Sc. cerevisiae*, and *C. mycoderma*, *C. valida* or *C. vini*	Maize	Nigeria, West Africa
Soy sauce	*A. oryzae* or *A. soyae*, *Lactobacillus* spp, *Zygosaccharomyces rouxii*	Soybeans and wheat	Japan, China, Filippines
Nan	*S. cerevisiae*, LAB	White wheat flour	India
Cheese	LAB, (*Lc. lactis*, *S. thermophilus*, *L. shermanii*, *L. bulgaricus*, *Propionibacterium shermanii*, *Leuconostoc* spp), occasionally moulds (*Penicillium* spp.)	Milk	International
Yoghurt	*S. thermophilus*, *L. bulgaricus*	Milk, milk solids	International
Fermented sausages	LAB (lactobacilli, pediococci), catalase positive cocci (*St. carnosus*, *St. xylosus*, *Micrococcus varians*), occasionally yeasts and/or moulds	Mammalian meat, generally pork and / or beef, less often poultry	Southern and central Europe, U.S.A
Sauerkraut	LAB (*Ln. mesenteroides*, *L. brevis*, *L. plantarum*, *L. curvatus*, *L. sake*)	Cabbage	International
Pickles	*Pediococcus cerevisiae*, *L. plantarum*	Cucumber	International
Olives	*Ln. mesenteroides*, *L. plantarum*	Green olives	Mediterranian

INTERACTIONS BETWEEN MICROORGANISMS IN INDUSTRIAL FERMENTATIONS

Because of their ecological importance, there has been considerable interest in defining the nature of the interactions between microbes (25, 33). A total of six classes of interactions have been defined, which are distinguished based on differences in mutual effects on fitness that can occur between microorganisms: neutralism, competition, amensalism, commensalism, parasitism and mutualism (38, 72). Neutralism is the absence of interactions. Therefore, neutralism is not truly considered a class of interaction. In reality, this will not occur often in industrial fermentations because any action of a microorganism (e.g. the consumption of substrate or the production of metabolites) will inevitably have an effect on its neighboring microorganisms. Competition is perhaps the most obvious mode of interaction. Two microorganisms competing for a substrate will negatively affect each other in case the substrate becomes growth limiting. Amensalism is the type of interaction in which one organism adversely affects the other organism without being affected itself. This occurs frequently in mixed culture fermentations (49). Examples include the production of carboxylic acids such as lactic or acetic acid, alcohols (12, 49) and bacteriocins (1, 51). In contrast, when one species benefits from the other, while the second is not affected, it is called commensalism. Examples are fermentations where one microorganism consumes a product that is produced by another (14, 57). Such a trophic interaction can be very valuable in an applied context when a stable combination needs to be obtained or in the cases where a fermentation end metabolite needs to be removed because it is detrimental to the product characteristics. In surface-ripened cheeses, lactic acid is consumed by yeasts. This leads to de-acidification of the cheese surface enabling the outgrowth of aerobic bacteria necessary for the cheese its characteristic flavor (57). In parasitism, one species benefits at the expense of another. Perhaps the most frequently occurring examples are the bacteriophages. Phage attacks have been a longstanding problem. Still today, in an era of increased awareness of the importance of hygiene, it leads to major losses in the food industry. This resulted in the emergence of a complete field of research in mastering phages (76). Finally, mutualistic interactions are positive for both microorganism involved. Many industrial fermentations rely on the mutualistic interactions among LAB species or between LAB, other bacteria and eukaryotes (i.e. yeasts and filamentous fungi). One of the best known mutualistic interactions is that between *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* which was reclassified two decades ago into *L. delbrueckiii* subsp. *bulgaricus* (31, 83). Amensalism, commensalism,

parasitism and mutualism typically result from co-evolution as is exemplified by the co-evolution of *S. thermophilus* and bacteriophages (11). A more extensive overview of these interactions is presented in Chapter 2.

YOGHURT, A MILK PRODUCT FERMENTED BY TWO STRAINS

Yoghurt is a dairy product mostly made from bovine milk that is fermented by the lactic acid bacteria *S. thermophilus* and *L. bulgaricus*. Records of the application of yoghurt as a food product date back to as much as 8000 years ago (28, 77). Only during the last century the two species responsible for this fermentation were identified. In recent years, the genomes of several yoghurt strains have been characterized as is discussed below.

Streptococcus thermophilus

To date, there are three complete genome sequences available of *S. thermophilus*, namely strains CNRZ1066, LMG18311 (4) and LMD-9 (53). The *S. thermophilus* genome is approximately 1.8 Mb large and is predicted to contain 1915 protein-coding genes in strain CNRZ1066, the first strain to be characterized at the genome level (see Table 2 and Figure 2a).

The portion of pseudogenes in *S. thermophilus* CNRZ1066 (10%, see Table 2) is among the highest of all sequenced streptococcal genomes (4). The observation that many genes, mainly involved in transport and the carbohydrate metabolism (both ~30% of all sequences within the category, see Table 3), are nonfunctional (4), indicates that *S. thermophilus* has undergone a process of loss-of-function events. This is probably due to the presence of a single carbon source, lactose, in the dairy environment as was discussed for *Lc. lactis* above (4).

Table 2. Comparison of the genomes of *Streptococcus thermophilus* CNRZ1066 and *Lactobacillus bulgaricus* ATCC BAA-365.

	S. thermophilus CNRZ1066	*L. bulgaricus* ATCC BAA-365
Genome length (bp)	1796226	1856951
GC content %	39	49
Coding %	83	77
Genes	2000	2040
Protein coding	1915	1721
Pseudogenes	182	192

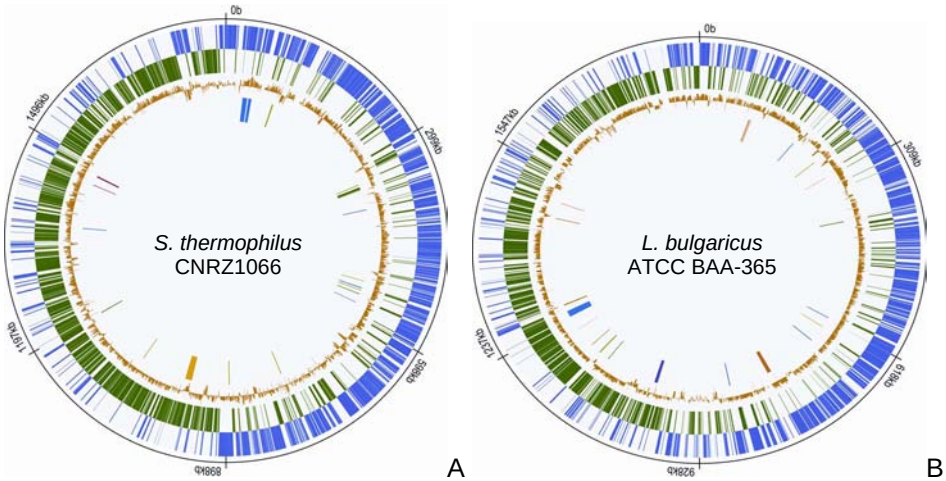


Figure 2. Genome-atlas view of the *S. thermophilus* CNRZ1066 chromosome (left) and the *L. bulgaricus* ATCC BAA-365 chromosome (right), with the predicted origin of replication at the top. In the left panel, the outer to inner circles show (i) positive strand ORFs, (ii) negative strand ORFs, (iii) GC-content and (iv) genes involved in purine biosynthesis and folate cycling (blue), sulfur AA biosynthesis (light green), BCAA biosynthesis and transport (dark green), EPS production (yellow) and the genes *pfl* and *pflA* (red). In the right panel, the outer to inner circles show (i) positive strand ORFs, (ii) negative strand ORFs, (iii) GC-content and (iv) genes involved in purine biosynthesis and folate cycling (blue), sulfur AA biosynthesis (light green), BCAA biosynthesis and transport (dark green), long-chain fatty acid biosynthesis (orange) and the gene *prtB* (purple). Figures were made with Microbial Genome Viewer (41) using the ORFs available at NCBI (accession no. NC_006449 for *S. thermophilus* and NC_008529 for *L. bulgaricus*).

Compared to *Lc. lactis* SK11, which has a genome of 2.4 Mb and contains only 6% of pseudogenes (53), the genome of *S. thermophilus* has undergone relatively much decay. Although milk is a protein-rich substrate, the *S. thermophilus* genome contains genes for functional biosynthetic pathways for most amino acids (AA) and this is confirmed by phenotypic analysis. Most *S. thermophilus* strains are auxotroph only for histidine and one of the sulfur AA (62). However, growth may be enhanced when methionine, proline, glutamic acid and valine are added to the growth medium (37, 46). Moreover, as most strains are only weakly proteolytic, *S. thermophilus* generally grows in milk in association with other protease-producing microorganisms and in particular with the PrtB-producing *L. bulgaricus*. It was shown that the exoprotease PrtS, which is present in a minority of *S. thermophilus* strains, does not have any effect on growth when a PrtB-producing *L. bulgaricus* is present (15). The presence of an extensive AA biosynthesis system and the fact that PrtS is not required in mixed cultures with proteolytic *L. bulgaricus* may also explain the absence of a functional exoprotease in most strains (15, 47).

Strikingly, in the *S. thermophilus* genome there are over 50 insertion sequences of which some display a GC content deviating from the rest of the genome, indicating that they were acquired by HGT. One fragment in the *S. thermophilus* genome likely originated form *L. bulgaricus* and contains a *metC* gene (95% identity to *metC* in *L. bulgaricus*) (4). This gene is involved in the biosynthesis of methionine, an amino acid that is present in low concentration in milk and casein. This indicates that the close proximities of *S. thermophilus* and *L. bulgaricus* in yoghurt may have led to HGT events (6, 50). Interestingly, a recent *in silico* study reported that the gene cluster *cbs-cblB/cglB-cysE*, involved in sulfur AA metabolism, is putatively transferred from *L. bulgaricus* to *S. thermophilus* (50). In addition, there are two clusters of genes involved in polysaccharide production in *S. thermophilus*: the exopolysaccharide (EPS) cluster and the rhamnose polysaccharide cluster. The first may have been acquired from *L. bulgaricus* (50). The EPS gene cluster codes for the complete pathway for EPS synthesis and secretion and this EPS imparts the viscous texture of yoghurt, one of the key desired properties (8). Although thus far a clear advantage of EPS production in terms of increasing fitness of *S. thermophilus* has not been identified, obviously, EPS production should confer a benefit that is stronger than the growth burden caused by expressing the genes and producing the EPS.

Lactobacillus bulgaricus

Two fully sequenced genomes of *L. bulgaricus* strains are publicly available, namely the strains LMG11842 (80) and ATCC BAA-365 (53). The genome of strain ATCC BAA-365 is approximately 1.9 Mb in size and contains 1721 protein coding sequences (see Table 2). Figure 2b gives a representation of the genome of this strain. In *L. bulgaricus* ATCC BAA-365, about 11% of all genes are pseudogenes and only 77% of the genome consists of (putative) coding sequences. Moreover, the number of rRNA and tRNA genes in *L. bulgaricus* is 50% higher than the average for a genome of its size (70). This means that the rRNA and tRNA content correspond to a genome of 3-4 Mb instead of its actual size of 1.9 Mb. These three observations indicate that *L. bulgaricus* is in a process of thorough genome reduction, which can be considered a result of a still ongoing extensive adaptation to the dairy environment (80). This is noteworthy *as L. bulgaricus* is considered to be a dairy species for already 5000 to 10000 years (31, 45). A relatively high number of transposases and insertion sequence elements (67, 68) may facilitate this process of reductive evolution (32). This reductive evolution likely caused that *L. bulgaricus* has lost most of its capacity to transport and metabolize sugars other than lactose or glucose. Similarly, the loss of function or even complete absence of

pathways involved in AA biosynthesis reflects the steady protein-rich environment *L. bulgaricus* resides in. The availability of the PrtB exoprotease in a protein-rich milk environment can provide a sufficient supply of most AA.

The *L. bulgaricus* genome encodes a complete pathway for the production of EPS and this is the cause of the slimy texture of yoghurt.

Table 3. Comparison of gene content involved in carbohydrate uptake among streptococcal genomes. [a], TC classification as described at http://tcdb.ucsd.edu/tcdb/background.php; [b], Genes which are either 3'-truncated, contain one or more frame shift(s), or a point mutation resulting in a stop codon. Adapted from Bolotin *et al*. (4).

Species/strain	Porters[a]	ABC transporters	PTS translocators	Accessory factors	Total
S. thermophilus CNRZ1066/LMG18311					
Complete genes	1	4	5	3	13
Pseudogenes[b]	0	2	4	0	6
S. pneumoniae TIGR4	0	15	50	3	68
S. pyogenes SF370 (M1)	0	15	28	3	46
S. agalactiae 2603 V/R	0	15	29	3	47
S. mutans UA159	0	13	20	3	36

Protocooperation between the yoghurt bacteria

S. thermophilus and *L. bulgaricus* were shown to stimulate each others' growth in a mixed culture in milk (64). This was expected to be a result of cross-feeding interactions, involving the exchange of nutrients and growth factors, a process referred to as protocooperation. Indeed, already in mid 20th century some compounds were shown to be produced by one species and to stimulate the other species. These include carbon dioxide (24), formic acid (16, 22) and folic acid (17) provided by *S. thermophilus* and peptides and amino acids released by the proteolytic action of the PrtB protease of *L. bulgaricus* (15, 69). The free AA content of milk is very low but the casein content is quite high (~2.5 %). As mentioned, *S. thermophilus* benefits from the proteolytic activity of *L. bulgaricus*. In turn, *L. bulgaricus* grows best in an anaerobic environment and thus the consumption of oxygen and concomitant production of carbon dioxide by the more oxygen tolerant *S. thermophilus* promotes *L. bulgaricus* growth. *L. bulgaricus* lacks pyruvate-formate lyase, which makes it dependent on an exogenous source of formic acid for an optimal purine synthesis. Similarly, a gene involved in the production of para-aminobenzoic acid, a precursor of folic acid, is missing which impedes purine metabolism for an optimal growth in milk (80). This is most likely the result of the long history of co-culture with *S. thermophilus*. It was shown that

folate is secreted by most *S. thermophilus* strains and consumed by *L. bulgaricus* (17). Therefore, exchange of folate is hypothesized as another mode of interaction. In addition, the production of EPS could confer advantages by facilitating exchange of these components by strained close proximities (13). A more detailed overview of the interactions between the yoghurt consortium members can be found in Chapter 2. Yet other modes of interaction exist and these are not all discovered or fully understood. With the classical microbiological methods that were available until the eighties of last century, it was quite difficult to deepen our understanding of interactions in the yoghurt consortium to the molecular level. This has changed with the emergence of genomics technologies that enable more holistic approaches to this well-defined ecosystem.

TRENDS IN MODERN BIOTECHNOLOGY: SCREENING, ~OMICS, MODELING AND EVOLUTION

The last decade the face of biotechnology changed rapidly due to developments that opened new avenues to so far unexplored research lines. One of the most powerful changes is the emergence of whole-genome sequencing and the ~omics technologies, i.e. transcriptomics, proteomics and metabolomics and more recently interactomics (84). The sequencing and annotation of complete genomes have provided insights in cellular regulation, the phylogeny of organisms and the molecular processes that lead to the evolution of species and strain diversity. The field of metagenomics studies the collective genomes of microbial communities, which sheds light on the molecular mechanisms responsible for structuring these communities (66, 84). In that sense, studying the regulation of genes specific for co-culture is essential. Transcription profiling with DNA microarrays is a powerful way of studying genome-wide responses (36). However, the application of microarrays has two major drawbacks in multi-species microbial communities: (i) it is limited to communities of which the genomes of their members is already known and (ii) the lack of variation between genes impedes proper profiling of the expression in closely related species or strains (7). Here, transcriptome profiling using next-generation sequencing (see below) may circumvent these drawbacks (2, 7, 75). Although transcription profiling is very informative for investigating the molecular basis behind responses on changing environmental factors, it does not necessarily reflect the cell's response at protein and metabolite level as these are also dependent on regulation at the posttranslational level, flux kinetics and thermodynamics. The field of proteomics studies the whole protein content of the

cell and can be used to determine the differences in protein content upon changing environmental factors and has been applied to *S. thermophilus* for physiological comparison of growth in a complex substrate (milk) with growth in a chemically defined laboratory medium (22). Metabolomics is defined as the study of all metabolites associated with the cell, i.e. substrates, products and intermediates. In order for any ~omics study to make sense, it should be combined with an overview of the reactions occurring in a cell. Taking together the cells' metabolites and protein-coding genes, it is possible to make a genome-scale metabolic model. By integrating ~omics data and, for instance, fermentation data, the link between molecules and physiology is made (10, 48). The field of research performing such analyses, constructing and applying these genome-scale models is systems biology. There are several genome-scale metabolic models available of LAB (60, 61, 78), but never before have these been used to describe co-culture growth or to predict molecular interactions between consortium members.

The sequencing of whole genomes has rapidly increased. With only two fully sequenced genomes in 1995, the amount has risen to an estimated amount of approximately 1500 of completed sequences and approximately 5000 incomplete sequences by the end of 2009 (GOLD database, http://genomesonline.org/). Similarly, the sequencing methods have developed from traditional Sanger sequencing to next-generation technologies such as Roche 454 sequencing and Illumina sequencing (52, 75). The latter is used in this thesis and will be discussed here in more detail (see Figure 3). In short, oligonucleotide adaptors are ligated to fragmented DNA and used for immobilizing the fragments to a substrate by binding. Subsequently, fold-back PCR results in isolated spots of amplified DNA. Fluorescently labeled terminator nucleotides and DNA polymerase are then used to create a complementary-strand DNA leading to fluorescent signals. Images are collected during each cycle. Stacking the images displays the sequence of the fragmented DNA. With Illumina sequencing typically reads of 36 bp are produced, although reads up to 100 bp are possible with optimized procedures (52). Moreover, pair-end sequencing can be applied and offers another option to assemble *de novo* sequences (44).

Because the method uses an amplification step by PCR, there is a bias in the distribution of sequence reads as amplification is never uniform for all sequences. This results in sequences with high and low coverage (52). Therefore, it is important to acquire a high average coverage, i.e. in the order of 10^2 in order to be able to assemble the short sequences properly. A high coverage (>20) and a high quality score (i.e. a high cut-off for the quality of the reads) are also necessary to reduce read errors (23, 74). Furthermore, the small sequences may prove hard to

assemble if a certain sequence occurs frequently in a genome, e.g. an IS element. However, if a reference genome is already available, the sequences can be aligned with it, facilitating assembly. This makes Illumina sequencing an excellent tool to detect mutations in strains derived from a species for which a genome sequence is available (52, 71).

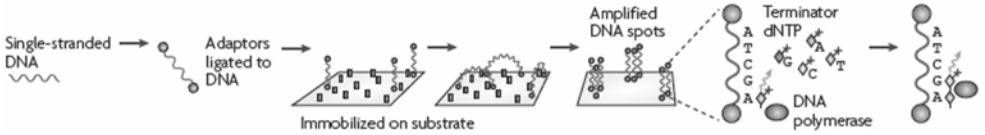


Figure 3. Schematic representation of the Illumina sequencing procedure. See text for explanation. Figure adapted from MacLean *et al.* (52).

Another field that has undergone major advances the last decade is that of high-throughput screening (HTS). With the availability of new fast screening methods and techniques such as fluorescent activated cell sorting (42), million-well growth chips (39) and rapid head space analysis using CG-MS (61) it becomes possible to screen thousands of strains for a specific trait in a relatively short time.

A field of research that is relatively old is one that deals with adaptation and (experimental) evolution. However, the face of this field changed tremendously the last decade with the application of mentioned ~omics techniques. Evolution studies are often combinations of a theoretical approach and a more experimental approach. The theoretical approach is to identify the underlying mechanisms behind evolution, such as the application of game-theory (65). With the true experimental approach the genetic adaptations in microorganisms in response to certain environmental characteristics are investigated (3, 26). It was shown in *Lc. lactis* that IS elements may cause a higher mutation frequency, especially when the microorganism is stressed (18). This may lead to a faster adaptation to unfavorable conditions. Also the evolution of interspecies interactions in biofilms has been investigated (35), but this has not been linked to mutations in the genomes of the species involved or any transcription profiling studies yet.

In the future, huge advances in mixed culture research can be expected by integrating information derived from HTS, next-generation sequencing, ~omics, modeling and evolution as each method complements what is lacking in the others.

Outline of this thesis

There is a continuous need to develop new fermented food products and to improve the efficiency of existing mixed culture fermentations. Therefore, it is of key importance to understand the microbial interactions that structure a microbial community and that lie at the basis of important product characteristics such as flavor and viscosity. The emergence of new tools and approaches in the genomics era opens up new avenues for research on classic topics in microbiology such as microbial ecology and microbial interactions. The research focus of this thesis is therefore the application of a combined approach of screening, next-generation sequencing, ~omics, modeling and evolution to map and explore the interactions between *S. thermophilus* and *L. bulgaricus*. The availability of genome sequences of these two species provided insight in the metabolic potential of these bacteria. Using some of the discussed ~omics technologies we revealed which genes or proteins are affected by co-culture growth. This provided insights in the molecular basis of the mutual stimulation of these two LAB species. Moreover, we applied experimental evolution and whole genome-based metabolic modeling to identify the optimization strategies of the two yoghurt bacteria leading to a mixed culture fermentation that performs better than the sum of the mono-cultures. The used combinations of approaches and technologies (see Figure 4) allow an improved characterization of the yoghurt consortium and the molecular basis behind (industrial) fermentations in general. The new information will facilitate the rational development of new mixed starter cultures or improvement of existing mixed culture fermentations.

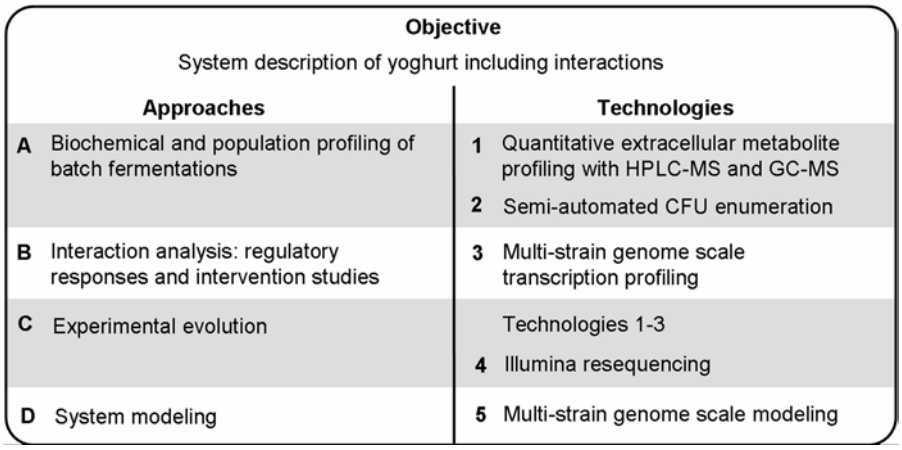


Figure 4. Schematic representation of the approaches and technologies used in this thesis research.

An overview of the current knowledge on microbial interactions in food fermentations is given in **Chapter 2**. Here, in more detail, is described what is the relevance of mixed cultures in industry and which considerations regarding the ecology should be made when designing or applying mixed cultures in food fermentations. Clearly, interactions between the consortium members are of key importance for the performance of the fermentation. Different types of interactions and their effects on mixed cultures are listed as well as what is known about the interactions affecting the yoghurt consortium. Finally the new developments in ~omics and genome-scale metabolic modeling and their possible uses in mixed culture research are further elaborated.

Although ~omics tools and genome scale metabolic modeling have become important and common tools the last decade, classical microbiology remains important and high-throughput screening has also undergone great developments in the past years. One of the methods that determine the amount of screening work that can be done in a fixed amount of time is determining viable counts by plating dilutions of cultures. Therefore, a relatively simple faster method for plating and plate counting was developed that can be incorporated in most laboratories without the need for special equipment. This method is described in **Chapter 3**.

Chapter 4 focuses on the interactions in the yoghurt consortium. Here, transcription profiles of mono and mixed cultures of *S. thermophilus* and *L. bulgaricus* in four different growth phases are compared to each other. These data are underpinned by transcription studies of cultures with additional formic acid and of yoghurt biofilms, and by studies of the effects of components suggested or proven to be involved in or influence the protocooperation.

A new topic in mixed culture research is the application of experimental evolution to unravel interactions between consortium members. **Chapter 5** explains the adaptation of two unfamiliar strains of *S. thermophilus* and *L. bulgaricus* to each other in the course of ~1000 generations under a very strict growth regime. A combination of phenotyping, *in silico* mutation analysis using Illumina sequencing and mixed culture transcription analysis sheds light on the optimization of interactions and shows that as little as 1000 generations can change a relatively inferior mixed culture (i.e. one with a low acidification rate and a low EPS production) into one that is comparable to a commercial starter.

Genome-scale metabolic models of *S. thermophilus* and *L. bulgaricus* are described in **Chapter 6**. Moreover, predictions in terms of growth and metabolite consumption/production (i.e. the carbon and nitrogen fluxes) from these models are compared to fermentation data. Notably the possibility is addressed to combine the

models of both bacteria in order to acquire a mixed culture “system” model that can be used for the prediction of molecular interactions.

Finally, in **Chapter 7** all the information on the interactions from chapters 2 to 6 is gathered and this (i) gives an improved understanding of known interactions between the yoghurt consortium members, (ii) sheds light on the nature of thus far unknown or only expected modes of interaction in this consortium and (iii) provides general concepts for the events that occur in mixed ecosystems, facilitating the rational improvement of existing or design of new industrial fermentations.

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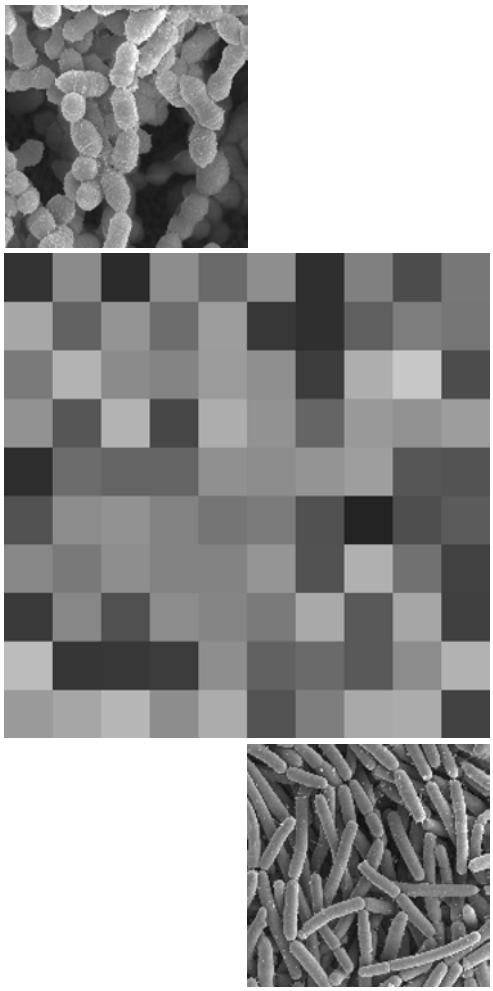
Chapter 2

Unraveling microbial interactions in food fermentations; from classical to genomics approaches



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Fermentation, the microbial degradation of organic compounds without net oxidation, is an important process in the global carbon cycle and is also exploited worldwide for the production and preservation of food. It is one of the oldest food processing technologies known with some records dating back to 6000 BC (50). The link between food and microbiology was laid by Pasteur who found that yeasts were responsible for alcoholic fermentation (106). Since that discovery the scientific and industrial interest in food microbiology started to grow and continues to increase until today. The number of food products that rely on fermentation in one or more steps of their production is tremendous. They form an important constituent of the daily diet and rank among the most innovative product categories in the food industry.

Most of the important microorganisms applied in the production of fermented foods have been studied for decades yielding a wealth of information on their physiology and genetics in relation to product functionalities such as the development of flavor, taste and texture. The recent emergence of genomics has opened new avenues for the systematic analysis of microbial metabolism and the responses of microorganisms to their environment. Additionally, genomics has boosted research on important food microbes (22, 90, 93). Much of this research focuses on the performance of a single strain including its interactions with the food matrix. However, food fermentations are typically carried out by mixed cultures consisting of multiple strains or species. Population dynamics plays a crucial role in the performance of mixed culture fermentations. For many years studies on mixed culture food fermentations have focused on analyzing population dynamics using classical and molecular methods. Many of these studies are mainly descriptive and relatively little is known about the mechanisms governing population dynamics in general and the molecular interactions that occur between the consortium members in particular. The availability of genome sequences for several species that are of industrial importance as well as technological advances in functional genomics enable new approaches to study food microbiology beyond the single species level and allow an integral analysis of interactions and metabolic activity in mixed cultures.

Here we review the current knowledge of important food fermentation processes focusing on the bacterial interactions. In addition, we illustrate how genomics approaches may contribute to the elucidation of the interaction networks between microbes including interactions with the food environment. This information may find application in industry through rational optimization and increased control over mixed culture fermentations.

MIXED CULTURE FOOD FERMENTATIONS – INDUSTRIAL PRACTICE AND CHALLENGES

Traditional fermentation processes relied on transfer of knowledge and methodologies associated with manufacturing from generation to generation. The industrialization of food production together with the blossoming of microbiology in the middle of the 19th century led to optimization and upscaling of many fermentation processes. Similarly, industrially produced starter cultures have emerged, leading to improved and reproducible product quality. Nowadays, the total economic value of fermented food products is huge and the worldwide turnover of fermented fresh products in the dairy segment alone represents a total economic value of 54.2 billion US $ annually whereas the cheese market is even larger (see Table 1) (111). In recent years there has been massive product diversification and many prebiotic and probiotic products with a high added-value emerged. Simultaneously, artisanal products have gained popularity due to their particular flavor and aroma characteristics (23).

Table 1. Overview of the total turnover in billion US $ worldwide in 2007 of three categories fermented fresh dairy products and cheese as estimated by Euromonitor International (111).

Category	Turnover (bln US $)
Yoghurt	34
Fermented dairy drinks	4.3
Fromage frais and quark	7.4
Cheese	74.4

At least two distinct product categories can be distinguished where control of mixed culture performance directly relates to key challenges of innovators in the food industry. The first relates to the dairy market, which includes important products such as cheese and fermented milks. This market is characterized by rapid growth of product varieties with distinct organoleptic properties. Examples include numerous applications in semihard cheeses where adjunct cultures are added to introduce additional flavor notes (49, 114). Additionally, there is an increasing number of products appearing in response to current health trends, such as low-fat and low-salt product varieties (46). Here, it is important to develop such products while maintaining good organoleptic properties. In low-fat cheese texture may be improved by the application of EPS-producing starter cultures (34). In fermented milks and yoghurts containing probiotic microbes off-flavor problems

may appear due to undesired metabolic activities (99, 152). Furthermore, the success of replacement or addition of the desired probiotic strain in mixed culture fermentation may largely depend on the interaction of this strain with the other strains in the starter culture (64). In general high numbers of viable probiotic bacteria are desired in these products at the moment of consumption. Typically between 5 and 8 logs CFU (Colony Forming Units) per gram of product is considered acceptable (99). Therefore, the growth, survival and activity of the probiotic strain in the product environment is of key importance and these traits are influenced both by specific environmental conditions (35, 152) and by interactions with the starter organisms (72, 99).

A second important product category is formed by food ingredients. Fermentation is widely applied to produce a broad range of ingredients such as amino acids and organic acids. Some of these fermentations are carried out with mixed cultures (54, 155). Challenges in this area include improvement of productivity and stability, and the elimination of unwanted by-products that interfere with down-stream processing. Moreover, such processes may become economically more attractive if cheaper raw substrates can be used with new (combinations of) strains. An example here is improved production of lactic acid from glucose by a mixed culture of *Lactobacillus delbrueckii* NRRL-B445 and *Lactobacillus helveticus* NRRL-B1937, of which the first is a good lactate producer and stimulated by the latter (86). Another example deals with a *S. cerevisiae* strain that was engineered with *L*-arabinose utilization genes from *Lactobacillus plantarum*, allowing it to utilize the *L*-arabinose moiety of lignocellulosic fractions of plant derived biomass (174).

Finally, we are seeing a rapid increase of industrialization of non-western fermented food products in Asia, Latin America and Africa (2, 144). As a result of demographic changes in Europe such products are also of increasing importance in the western market, especially since some are believed to bring specific health benefits (48, 113). Examples include fermented products produced from dairy, cassava, cereals, beans, meat, and fish (7, 167). Challenges here relate to the stability, reproducibility and productivity of fermentations.

MIXED CULTURES VERSUS PURE CULTURES - ECOLOGICAL CONSIDERATIONS

With few exceptions food fermentations rely on mixed cultures of microorganisms. There is a number of important considerations that are at the basis of the ecological success of mixed cultures and these will be discussed in this section. Microorganisms evolve to optimize their fitness and this is often achieved by specialization, e.g. optimization of their metabolism. This is exemplified in a number of elegant experimental evolution studies with *Escherichia coli* in well-defined and homogeneous laboratory systems. In one study in a continuous culture sequentially fed with glucose and acetate this organism differentiated into two ecotypes that displayed a large difference in lag phase when switching to growth on acetate after depletion of glucose (142). Another example with *E. coli* is that a single strain cultured for a prolonged period at glucose limitation diverged into two or three clonal variants in which one variant ferments the glucose and the fermentation products acetate and glycerol serve as growth substrates for the other strains (65, 132).

Most substrates for food fermentations have a highly heterogeneous physico-chemical composition which offers the possibility for simultaneous occupation of multiple niches by “specialized” strains, for instance through the utilization of different carbon sources. In these substrates coexisting strains often interact through trophic or nutritional relations via multiple mechanisms as will be discussed below.

Many food fermentations rely on spontaneous fermentation by the indigenous microbiota present in the food substrate. This implies that variations in the indigenous biota may affect the composition and activity of the fermenting community. This has a direct effect on product quality and the reproducibility of fermentations. A recent study showed large variations in the flavor and texture profiles of cheddar blocks produced at different factories (24). This is at least partially due to variations in proteolysis in the cheddar blocks. The application of starter cultures reduces the chance of unexpected population shifts and thereby ensures constant product characteristics and quality. Moreover, in combination with sterilization or pasteurization it allows the food to be fermented by species or strains that would be out competed otherwise.

CLASSIFYING INTERACTIONS ON THE BASIS OF MUTUALLY BENEFICIAL AND DETRIMENTAL EFFECTS ON FITNESS

Microbial interactions in mixed cultures occur via multiple mechanisms. Such interactions may be direct, as for instance through physical contact or via signaling molecules. Alternatively, indirect interactions may occur where changes in the physico-chemical properties of the environment induced by one strain trigger a response in another strain (21, 51). The effects of such interactions on the fitness of the strains involved may either be positive, neutral or negative. Mutual effects on fitness between interacting strains are an effective means of classifying interactions (68). These can be divided into five main classes – amensalism, competition, commensalism, parasitism and mutualism – all of which will be discussed below and illustrated with relevant examples from food fermentations (see Table 2).

Amensalism is an interspecies interaction in which one organism adversely affects the other organism without being affected itself. It frequently occurs in food fermentations since major end products of primary metabolism such as carboxylic acids and alcohols are effective growth inhibitors of the indigenous microbiota and spoilage organisms (23, 89). In fact, the lactic acid bacterial (LAB) metabolism is optimized for fast acid production rather than efficient growth (158). Another example is the production of antimicrobial compounds, such as bacteriocins, that are produced by many food-fermenting LAB and that play an important role in mixed culture population dynamics. Typically, bacteriocin-producing strains produce a dedicated immunity system that protects the host from detrimental effects. Lantibiotics, a special class of bacteriocins produced by LAB and other Gram-positives, have drawn specific attention. Nisin is a well-known lantibiotic produced by *Lactococcus lactis* and broadly applied as a food preservative. Its activity is based on the permeabilization of the cytoplasmic membrane leading to its depolarization (47, 70). Other potent bacteriocins include plantaricin and pediocin which are widely distributed among *L. plantarum* and Pediococci, respectively (41, 172). The broad activity spectrum of bacteriocins has been exploited for the inhibition of outgrowth of spoilage microbes and pathogens (4, 91).

The second class of interactions is competition. Microorganisms compete for energy sources and nutrients during fermentation. Carbon sources are often present in high concentrations in food substrates and competition therefore relates to rapid uptake of nutrients and conversion into biomass. In dairy fermentations, nitrogen is limiting and here organisms initially compete for the free amino acids and small peptides available in milk.

Table 2. Microbial (interspecies) interactions observed during food fermentations and in the fermented products.

Type of interaction	Product(s) or environment(s)	Organisms involved	References
Mutualism	Yoghurt	*S. thermophilus* *L. bulgaricus*	(1, 31-33, 43, 53, 66, 119, 140, 151, 154, 163, 179)
	Dairy	LAB	(108, 153)
	Cold milk	*Lactococcus lactis* subsp. *cremoris* *Pseudomonas fuorescens*	(80)
	Dairy, sourdough, laboratory medium	LAB Yeasts Fungi	(26, 108, 137)
	Milk	LAB Yeasts	(52, 108)
	Sourdough	*Saccharomyces exiguous* *Candida humilis* *Lactobacillus sanfranciscensis*	(37, 56, 57)
	Surface-ripened cheese	Different species of moulds, yeasts and bacteria	(30)
Amensalism	Dairy, vegetable broth	LAB *Listeria monocytogenes* Pseudomonads *Staphylococcus* *Yersinia* *Bacillus*	(9, 13, 14, 16, 71, 89, 94, 95, 102, 143)
	Broth culture, laboratory medium, vegetable broth	LAB *Escherichia coli* *Aspergilli* *Enterobacter* *Listeria monocytogenes* *Vibrio* *Salmonella*	(10, 11, 16, 25, 55, 63, 77, 92, 94, 95, 103, 115, 148, 159)
	Wine	*Lactobacillus hilgardii* *Pediococcus pentosaceus*	(94, 131)
	Yoghurt	*S. thermophilus* *L. bulgaricus*	(71, 117, 127, 179)
	Meat	LAB *Listeria monocytogenes*	(13)

Table 2. (continued)

Type of interaction	Product(s) or environment(s)	Organisms involved	References
	Wine	Malolactic bacteria Yeasts	(3)
	Surface-ripened cheese	*Lactobacillus plantarum* *Listeria monocytogenes*	(91)
	Lettuce	LAB *Listeria monocytogenes*	(4)
Commensalism	Dairy	LAB	(108, 153, 175)
	Yoghurt	*S. thermophilus* *L. bulgaricus* *Propionibacteria*	(175)
	Yoghurt, dairy	LAB *Propionibacteria*	(28, 175)
	Milk	LAB Yeasts	(52, 108)
	Wine	Malolactic bacteria Yeasts	(3)
	Surface-ripened cheese	LAB *Debaryomyces hansenii* *Geotrichum candidum* *Arthrobacter* sp. *Brevibacterium linens* *Corynebacterium ammoniagenes* Staphyolococci	(107)
	Laboratory medium	Yeasts *Bacterium linens*	(124)
	Fermented milks, yoghurt, cheeses	Yeasts Bacteria	(165)
Competition	Yoghurt	*S. thermophilus* *L. bulgaricus*	(105, 179)
	Dairy	LAB Yeasts	(52, 108)
Parasitism	Laboratory medium	Bacterium Phage	(29, 137)
	Milk	Bacterium Phage	(20, 145)
	Aquatic environments	Bacterium Phage	(169, 170)

In the later stages of fermentation they compete for the peptides released by the action of proteolytic enzymes. For this, they produce proteases, transport systems and peptidases. Growth rate and population dynamics in mixed dairy fermentations are largely determined by the ability to utilize amino acids efficiently (73, 75). Micronutrients such as iron have also been reported to be limiting for strains in the biota of smear cheeses. Strains compete for iron pools through the use of specialized molecular systems for harvesting iron including siderophores (109).

Commensalism is the third class of interactions. This is a situation in which one organism benefits from the interaction while the other strain is not affected. This also occurs in many food fermentations, for instance through trophic interactions. In Swiss-type cheeses propionic acid bacteria utilize the lactic acid produced by LAB starter bacteria (28). Similarly, in surface-ripened cheeses lactic acid is consumed by yeasts, in particular *Debaryomyces hansenii*, and by the filamentous fungus *Geotrichum candidum* (107). This leads to de-acidification of the cheese surface enabling the outgrowth of aerobic bacteria such as *Arthrobacter* species, *Brevibacterium linens, Corynebacterium ammoniagenes,* and staphylococci. One could argue that in this case the aerobic bacteria benefit while *D. hansenii* and *G. candidum* are unaffected. However, it may be difficult to prove that there is no effect if it can not be measured in terms of growth or survival. Another possible form of commensalism takes place in starters for Gouda cheese where PrtP- *Lc. lactis* strains benefit from the peptides that are released from milk protein through the action of extracellular proteases (PrtP) produced by PrtP+ strains (69, 74) whilst the PrtP+ strains do not directly seem affected. In milk, PrtP+ strains produce more biomass than their isogenic PrtP- variants lacking plasmids containing the protease gene, but growth is slower due to the cost of expressing this protease (173). In pure cultures of PrtP+ strains grown in milk, PrtP- variants rapidly occur. The outcome of long-term propagation of PrtP+ and PrtP- strains in a protein-containing medium like milk is that the strain that makes the least use of the resources in the medium, namely the PrtP- strain, will become dominant. In this case the immediate gain for the PrtP- strain is traded for the long-term community benefit. This particular example is also known as the “prisoner’s dilemma” in evolutionary game theory (6, 120). The population dynamics of PrtP+ and PrtP- isolates is highly dependent on the growth conditions that influence the costs and benefits of the proteolytic phenotype (69).

The fourth class of interactions is known as parasitism. Parasitism is the interaction in which one species benefits at the expense of another. A well-known example of parasitism in the microbial world is represented by bacteriophages. It is

well established that food fermentations, especially those repeatedly carried out in the same equipment, are highly vulnerable to phages. Phage attack may suddenly inactivate dominant strains in a fermenting culture leading to failure and product losses in industrial fermentations (145). In recent years our understanding of phage biology and the interactions with their hosts has increased significantly. The biology of bacteriophages has been studied extensively for LAB such as *Lc. lactis* and *Streptococcus thermophilus* (20, 145). This work has benefited much from genome sequencing efforts as for instance the genomes of at least seven phages specific for *S. thermophilus* have been sequenced (146). The diversity and fast evolution of phages typically results in the appearance of strains harboring different phage resistance phenotypes (170). Moreover, the recombination machinery of bacteriophages and their ability to transfer DNA from one bacterial cell to another may accelerate evolutionary processes in bacterial communities and contributes to the diversity in mixed culture fermentation processes, especially when back slopping, the sequential transfer of cultures to fresh medium, is applied (169, 170). Recent studies showed a thus far unknown system present in archaea and bacteria, amongst which *S. thermophilus*, that is involved in phage resistance (12, 40). In this CRISPR system, bacteria acquire resistance to phages by incorporating phage-specific short transcribed nucleotide sequences into regions of clustered regularly interspaced short palindromic repeats. It was shown that these regions evolve very rapidly, probably driven by the rapid evolution of phages (161).

Finally, during mutualism both participating microorganisms derive a benefit from the interaction. Many food fermentations rely on mutualistic interactions. Probably the best example is the yoghurt consortium, consisting of the LAB *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which will be discussed in detail in the next section. The interactions between the yoghurt bacteria are also often referred to as synergism or protocooperation, interactions in which enhanced growth rate is the main mutual benefit. Cultures consisting of yeasts, LAB and filamentous fungi are of key importance in a broad range of fermented foods in which mutualism is an important mode of interaction. For instance in kefir granules *S. cerevisiae* raises the pH by utilizing the lactic acid produced by *Lactobacillus kefiranofaciens* as carbon source enabling more growth of *L. kefiranofaciens* (26). In the sourdough fermentation there is a synergistic interaction between yeasts such as *Saccharomyces exiguous* or *Candida humilis* and LAB, especially *Lactobacillus sanfranciscensis* (56, 57). Yeast amylase releases maltose from starch which is fermented by *L. sanfranciscensis*. Part of the glucose derived from maltose is excreted by *L. sanfranciscensis* and is used as a carbon source by maltose-negative yeasts. In return, the yeasts stimulate growth of

L. sanfranciscensis by increasing the availability of amino acids and peptides, either through proteolysis or as a consequence of accelerated autolysis (37, 56). In wine interactions between yeasts and LAB also play a major role and these have been reviewed recently by Alexandre *et al.* (3)

YOGHURT CULTURES – THE MIXED CULTURE PARADIGM IN FOOD FERMENTATION

Yoghurt is the product of milk fermented by a defined mixed culture of two thermophilic LAB, *Streptococcus thermophilus* (15) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (93, 163). There are also many fermented milk products that contain only one or neither of these strains, but the name yoghurt is in several countries only allowed for those products that are produced with cultures containing both (82, 151, 179). Nowadays many yoghurts and yoghurt-based drinks are produced that contain probiotic strains, but these do not necessarily contribute to the fermentation. This mixed culture fermentation is of huge economic importance (see Table 1). It represents an attractive model system for research on interactions due to its relatively small complexicity. Some aspects of the yoghurt microbiology have already been reviewed elsewhere (23, 62, 151, 179). Here we present an updated review on comparative analysis with other mixed culture food fermentations.

Although *S. thermophilus* and *L. bulgaricus* are also able to ferment the milk individually, both species were found to be stimulated in growth and acid production in mixed cultures compared to single-strain cultures (119). Proteolysis plays an important role in yoghurt as is illustrated by growth of proteolytic *S. thermophilus* strains in milk (88). After inoculation the cells start growing exponentially using the amino acids, dipeptides, tripeptides and oligopeptides that are freely available. Subsequently, amino acids become limiting and the culture enters a non-exponential growth phase in which the synthesis of extracellular protease is initiated. Finally, in a second exponential phase, the proteolytic system is able to supply sufficient peptides for exponential growth but here the growth rate is lower than in the first exponential phase, probably due to a limited capacity of the peptide uptake system (88).

Most commonly, yoghurt cultures consist of proteolytic *L. bulgaricus* and non-proteolytic *S. thermophilus* (32, 119). During the first exponential phase of *S. thermophilus*, almost no growth of *L. bulgaricus* is observed. In the second phase, *S. thermophilus* growth decreases while *L. bulgaricus* starts to grow exponentially

and protease expression is initiated. The cell-wall anchored protease PrtB mainly catalyzes the hydrolysis of the hydrophobic caseins into small peptides, which are subsequently taken up using various peptide transport systems (76, 118). In the cytoplasm the peptides are further hydrolyzed into free amino acids by several endopeptidases and aminopeptidases (133, 135). Growth of *L. bulgaricus* continues in the third growth phase. At this stage the peptides released from milk casein also serve as a source of amino acids for *S. thermophilus* supporting a second exponential growth phase (31, 179).

Most *S. thermophilus* strains exhibit few amino acid auxotrophies and the fact that they have fewer nutritional requirements than *L. bulgaricus* may explain their preferential growth in milk. Only histidine is required by most strains and growth may be enhanced when methionine, proline, glutamic acid and valine are added to growth media (67, 87). With few exceptions, *S. thermophilus* strains do not possess extra-cellular proteases (31, 67). As a result growth of most *S. thermophilus* strains, except some highly proteolytic strains, is strongly stimulated in co-cultures with *L. bulgaricus* strains expressing *prtB* (31, 136).

The mutualistic coexistence of *S. thermophilus* and *L. bulgaricus* is also based on other interactions such as the exchange of several growth stimulating factors (see Figure 1). *S. thermophilus* provides *L. bulgaricus* with formic acid, pyruvic acid, folic acid (33) and carbon dioxide (43). The positive effects of formic acid and folic acid on growth of *L. bulgaricus* are related to the biosynthesis of purines (53). Formic acid is a precursor for purine synthesis and *L. bulgaricus* lacks a pyruvate-formate lyase, which may explain why it relies on other sources for formate (32). Pyruvate-formate lyase is a highly abundant protein in *S. thermophilus* grown in milk indicating that *S. thermophilus* may supply *L. bulgaricus* with formate during co-cultivation (39). Folic acid is involved as a cofactor in purine and amino acid biosynthesis (149, 168) and was shown to be excreted by *S. thermophilus* and consumed by *L. bulgaricus* (33). Genome sequence analysis of *L. bulgaricus* strain ATCC11842 has shown the absence of a biosynthetic pathway for *para*-aminobenzoic acid and therefore the biosynthetic pathway for folate in this strain is incomplete (163). *S. thermophilus* is capable of producing both *para*-aminobenzoic acid and folate and hence *L. bulgaricus* may benefit from elevated levels of either compound. A recent report on *Lc. lactis* shows the involvement of folate in the stimulation of a proteinase positive strain by a proteinase negative strain (121). Carbon dioxide is a precursor for the synthesis of aspartate (128, 166), glutamate (100), arginine and nucleotides (17). In heat-treated milk carbon dioxide levels may be too low for *L. bulgaricus* (43) and therefore it profits from the carbon dioxide released by *S. thermophilus* from the urea that is present in milk. In addition, the

urea catabolism plays a role in the synthesis of aspartate and glutamine, both essential amino acids (5, 104).

Yet other compounds may contribute to the mutualistic interaction between the yoghurt LAB. For instance, Partanen *et al.* (116) reported that several long chain fatty acids (LCFA) are stimulatory to *L. bulgaricus*. This is probably due to the fact that *L. bulgaricus* lacks part of the biosynthetic machinery required for *de novo* synthesis of long chain unsaturated fatty acids and one may speculate that *S. thermophilus* is also able to supply *L. bulgaricus* with LCFA. In the recent paper describing the genome sequence of *L. bulgaricus* ATCC11842*,* Van de Guchte *et al.* hypothesized that ornithine and putrescine may be produced by *S. thermophilus* and *L. bulgaricus*, respectively, and that the exchange of these metabolites mutually increases their resistance to oxidative stress (130, 163). Ornithine is involved in the metabolism of urea and putrescine turns *S*-adenosyl methionine into spermine via the intermediate spermidine. Spermine and spermidine are involved in the stabilization of DNA and DNA replication, respectively.

Growth-detrimental interactions have also been reported. Reddy and Shahani (127) reported that some strains of *L. bulgaricus* produce the bacteriocin bulgarican that inhibits growth of *S. thermophilus* (117). Moreover, some *S. thermophilus* strains were reported to produce peptide bacteriocins (71). Of seven strains of *L. bulgaricus* tested, one was inhibited by this peptide.

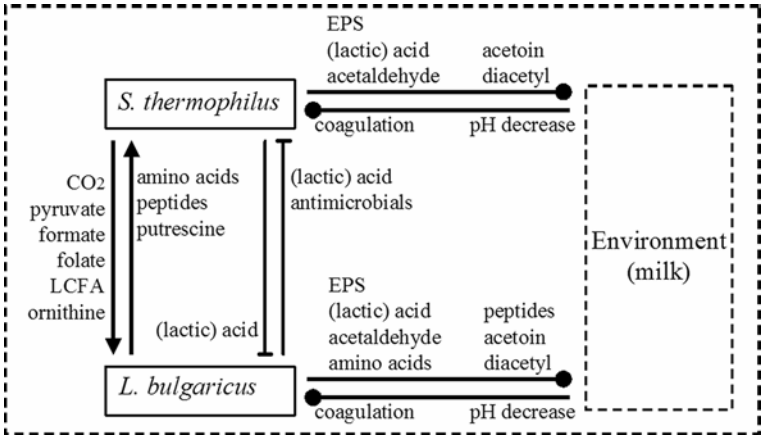

Figure 1. Schematic representation of the validated and hypothesized interactions that occur between *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and their environment, and compounds relevant for yoghurt characteristics. ▼, positive interactions; ⊥, negative interactions; ●, interactions that do not specifically promote or decrease growth of the other species. EPS, exopolysaccharides; LCFA, long chain fatty acids. See text for references.

A key sensory attribute that is introduced with fermentation during yoghurt production is its texture. Due to the acidification by the LAB, proteins coagulate and thereby change the viscosity of the milk. Furthermore, the bacteria, and mainly *S. thermophilus,* produce exopolysaccharides (EPS) that form a matrix with the milk proteins resulting in the final yoghurt structure. In kefir, kefiran production by LAB is stimulated by the presence of yeasts induced via direct physical contact (26). Similarly, interactions between yoghurt strains may influence EPS production as for instance by increasing the availability of nitrogen sources or through the interaction with non-EPS-producing *S. thermophilus* strains (38, 177). In general, associations of yeasts and lactic acid bacteria are of key importance for a broad range of fermented foods. However, surprisingly little information is available about molecular interaction mechanisms (165).

QUORUM SENSING AND PHYSICAL INTERACTIONS

Besides the interactions mentioned so far microorganisms may produce diffusible chemicals for the purpose of communication. This includes a process referred to as quorum sensing (QS) that is widely spread among Gram-negatives and Gram-positives and allows regulation at the population level of a wide range of traits, including competence, virulence, and stress responses (58, 78, 150). Two recent reviews link quorum sensing to motility, EPS production, biofilm formation, and toxin production, which are all important phenotypes in food fermentation (44, 59). As described previously, lantibiotics may play an important role in mixed culture population dynamics and their production is often regulated in a density-dependent way via quorum sensing (125, 129). Nisin production in *Lc. lactis* has been studied extensively and it has been shown that it acts as an autoinducer. It regulates its own production at the transcriptional level with the involvement of a two-component regulatory system (for a review see (83)). More recently, the involvement of QS in the regulation in EPS production and monospecies biofilms formation by *L. plantarum* and *Lactobacillus rhamnosus* was reported (85, 147).

Whereas intraspecies communication is quite common, fewer examples exist of interspecies communication and most examples are not related to food microbiology (78). An example of chemical communication has been observed in dental biofilms where *Veillonella atypica* and *Streptococcus gordonii* degrade complex carbohydrates in a way that benefits both species. *V. atypica* produces a yet to be identified signal molecule that induces amylase production in *S. gordonii* and thereby increases the degradation rate (45). A QS system that is present in a

major fraction of the bacterial population and allows interspecies signaling is the LuxS system. The LuxS enzyme is responsible for the production of a precursor of autoinducer-2, a signaling molecule involved the regulation of gene expression of for instance virulence factors, competence for genetic transformation, the production of antibiotics and secondary metabolites, and biofilm formation (36, 176). In co-cultures of the hyperthermophiles *Thermotoga maritima* and *Methanococcus jannaschii*, growth and EPS production of the former was stimulated by the presence of the latter. Additionally, a QS signaling peptide in *T. maritima* was upregulated. It was shown that EPS expression was enhanced in presence of this signaling peptide (72). Similar processes may also play an important role in biofilm formation in mixed culture food fermentations.

An example of interspecies communication via QS among lactic acid bacteria is represented by *L. plantarum* NC8, where not only plantaricin itself but also plantaricin-like peptides produced by other Gram-positive bacteria were shown to induce production of plantaricin by *L. plantarum* (96, 97).

Microbes may also interact and influence each others metabolism via physical contact and a few examples of such interactions have been described for mixed culture food fermentations. Cheirsilp *et al.* demonstrated that production of the capsular EPS kefiran was enhanced by physical contact between *Lactobacillus kefiranofaciens* and *S. cerevisiae* (26). The molecular basis of this effect remains to be established. It was postulated that the bacteria and yeasts may benefit from the enhanced kefiran production through interactions that occur in the kefir granules where interspecies contact or the exchange of growth factors is facilitated through physical contact.

GENOMICS APPROACHES FOR MIXED CULTURE RESEARCH

The genomic revolution has opened new avenues for research on mixed cultures. For an increasing number of relevant LAB genome sequences are available (90, 93). In several LAB genomes, amongst which *L. plantarum* (84), *S. thermophilus* (15) and *Lactobacillus salivarius* subsp. *salivarius* (27), there is evidence for horizontal gene transfer (HGT). Interestingly in many cases the acquired sequences appear to originate from species that frequently co-exists and interact. In the genome of *L. bulgaricus* ATCC11842 a region with a GC content of 38 % carrying an operon encoding an ABC transporter that could serve as an uptake transporter for putrescine and/or spermidine was found and proposed to be involved in the interactions between *S. thermophilus* and *L. bulgaricus* (163). This

GC content is significantly lower than the average value of 49.7% observed for the entire genome. Interestingly, this locus has only been found in a few bacterial genomes whereas it is not even present in the genome of another *L. bulgaricus* strain, ATCC BAA-365 (93) indicating that it may have been acquired recently via HGT (163).

Only a limited number of studies is available where genomics approaches are used to study the interactions in mixed culture food fermentations. Such studies pose at least two technical challenges that need to be addressed. The first relates to the complexity of most food fermentation substrates. The physicochemical composition and especially the high protein or fat content may interfere with experimental procedures for RNA and protein isolation that work well with laboratory media. A number of studies have appeared that describe successful transcriptome or proteome analyses on samples from fermented substrates and even from highly complex material such as fecal samples (81, 178). Most studies on dairy fermentations use skim milk where the precipitation of casein may be prevented by pretreatment with sodium citrate (126, 139). These studies have revealed several previously undescribed metabolic adaptations upon growth in milk such as the induction of pyruvate-formate lyase in *S. thermophilus* that may serve as a supply of formate required for the biosynthesis of purine bases or other anabolic processes (39) (see also Figure 1). A recent study deals with transcriptome analysis of *Lc. lactis* grown in milk in co-culture with *S. cerevisiae* (98). Although no difference in growth was observed compared to a *Lc. lactis* mono culture, a number of genes was differentially expressed, in particular genes involved in pyrimidine metabolism. Several other regulatory responses could be assigned to the ethanol produced by the yeast.

The potential of using functional genomics approaches for analyzing interactions is well illustrated by recent studies describing the genome-wide analysis of interactions of commensal or pathogenic microbes with their hosts. With the availability of microarray platforms for several plant species and soil bacteria it is possible to elucidate the response of both the host and the microorganism upon interactions as exemplified by the induction of defense proteins in *Arabidopsis thaliana* by *Agrobacterium tumefaciens* (42). Host-microbe interactions in the gastro-intestinal tract are of crucial importance for human health. The overwhelming complexity of this system with respect to composition and activity of the microbiota as well as its heterogeneity and poor accessibility to sampling requires inventive research approaches. The use of germ-free animal models has greatly facilitated the analysis of genome-wide responses of micro-organisms as well as the host upon colonization (18, 134). Other studies report the *in vivo* time

and spatial resolution of the expression of genes specifically expressed in the GI tract in the model probiotic *L. plantarum* (19). Recently, Sonnenburg *et al.* have extended these approaches in a study where they co-colonized germ-free mice with *Bacteroides thetaiotaomicron*, a prominent component of the adult gut microbiota, and *Bifidobacterium longum*, a frequently used probiotic microorganism. The results showed that co-colonization prompted *B. thetaiotaomicron* to increase expression of genes involved in the acquisition and metabolism of polysaccharides (141).

Most studies described above relate to the performance to the interactions of microbes in simplified model systems or defined systems composed of a limited microbial complexity. Recent advances in the field of metagenomics provide a radically new approach for very complex ecosystems as well as for ecosystems dominated by a moderate number of species and strains (162, 164). Random sequencing of environmental samples supplies information on the (amount of) species present in an environment, including uncultured microorganisms, as well as information on known and previously unknown genes that occur in that environment. By comparing habitat-specific fingerprints of genes present in various known environments, it is possible to interpret other environments. Tringe *et al.* (160) clustered similar environmental samples together and found only few genes specific for a certain environment. Based on relative abundance, it was clear that systems for transport of ions and inorganic compounds, energy production and (interspecies) communication were most discriminative between samples from the Sargasso sea, deep sea whale fall and farm soil. Currently, various groups are sequencing metagenomes of gut and oral microbiota (http://www.genomesonline.org/gold.cgi?want=Metagenomes), which will undoubtly boost research on dynamics and interactions within these complex microbial populations and support the development of prebiotics and probiotics.

DESCRIPTIVE AND PREDICTIVE MODELING IN THE GENOMICS ERA

Modeling has played an important role in food microbiology and an extensive review describing different modeling categories has been published recently (156). Historically, most of these studies aim at developing predictive models for the growth of desired or undesired microorganisms in the food matrix. As the demand for minimally processed foods increases, the risk of outgrowth of spoilage or pathogenic microbes rises. Accurate empirical models are of great value as they assist in the definition of processing conditions minimizing the risk of growth of

these bacteria such as *Bacillus cereus*, lactobacilli, or *E. coli* (95, 101, 122). Similarly, Sodini *et al.* used black box modeling for predicting the acidification of mixed cultures of *S. thermophilus* and *L. bulgaricus* and quantifying the interactions between the two species (140). In another example growth dynamics in a mixed yeast culture of killer and sensitive strains was reported (123). Here, the lethal action of the killer strain showed a lag phase probably due to the necessary accumulation of the toxin before it reached a lethal dose. The existing model was adapted for this effect.

Despite the value of such models in process optimization their predictive value is often limited to specific substrates and conditions and they do not provide additional mechanistic insight as for instance interaction effects. Other modeling strategies aim at predicting the performances of microbes in fermentations on the basis of their metabolic pathways and networks. Such “white box” or mechanistic models have been successfully applied for the optimization of industrial fermentations including food ingredients such as lactic acid and amino acids (8, 138). To our knowledge there are no examples of the integration of interaction effects in such models to the level where they can be used to predict the performance of mixed culture fermentations. However, Gregory *et al.* developed a computing system that allows the modelling of interactions and evolution in bacterial communities (60, 61). This model includes several aspects such as growth stimulatory interactions, antibiotic sensitivity, occurrence of antibiotic resistant mutants and growth on nutrients derived from killed cells in one model.

With the emergence of genomics a radically different modeling approach has drawn increasing attention (see (156) and references therein). Here, genome-scale metabolic models are constructed that allow a systematic exploration of the metabolic capacities and a number of such models have appeared in recent years for important microbes in mixed culture food fermentations like *L. plantarum* and *Lc. lactis* (112, 157). These may serve as references for future metabolic models thereby accelerating the process of model construction (110). A genome-scale metabolic model and associated constraint-based modeling techniques were used to analyze the physiology of growth of *L. plantarum* in a complex medium revealing the importance of amino acid catabolic pathways previously not associated with free-energy metabolism (158). With respect to mixed culture fermentations it will be interesting to see whether it is possible to connect genome-scale metabolic models of the individual components of mixed cultures through a limited number of interactions. Such “multi genome” scale models should be effective tools for the optimization of mixed culture performance with respect to growth and metabolite production.

CONCLUSIONS AND FUTURE PROSPECTS

Mixed culture food fermentations are of primary economic importance. The performance of such cultures, consisting of lactic acid bacteria, yeasts and/or filamentous fungi, is not the simple result of 'adding up' the individual single strain functionalities, but is largely determined by interactions at the level of substrates, exchange of metabolites and growth factors or inhibiting compounds.

Technological breakthroughs in the post-genomic era open up new avenues to study microbial communities and interaction networks beyond simple descriptive models. These are now mainly applied for ecological studies on highly complex systems such as the GI-tract or complex environmental ecosystems. On the other hand studies aiming at understanding more fundamental ecological principals underlying the success of evolutionary strategies typically make use of more artificial laboratory strains and ecosystems (79, 120). Food fermentations may provide a valuable alternative model with a high practical relevance. They typically have moderate microbial complexity and offer excellent possibilities for process control. Moreover, the availability of advanced genomics and genetic tools will allow the integration of mechanistic and evolutionary approaches (171).

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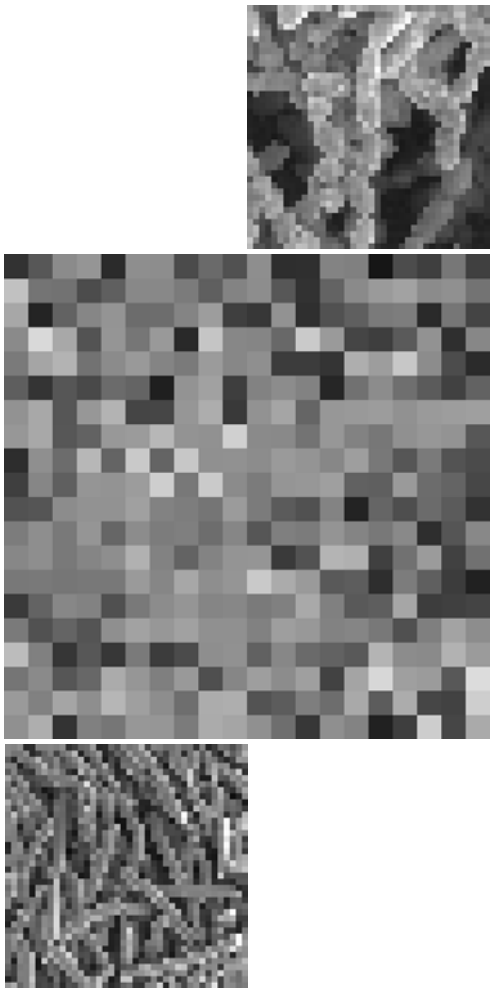
Chapter 3

A simple and fast method for determining colony forming units



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ABSTRACT

Aims: To develop a flexible and fast colony-forming unit (CFU) quantification method that can be operated in a standard microbiology laboratory.
Methods and results: A miniaturized plating method is reported where droplets of bacterial cultures are spotted on agar plates. Subsequently, minicolony spots are imaged with a digital camera and quantified using a dedicated plug-in developed for the freeware program ImageJ. A comparison between conventional and minicolony plating of industrial microorganisms including lactic acid bacteria, *Escherichia coli* and *Saccharomyces cerevisiae* showed that there was no significant difference in the results obtained with the methods.
Conclusion: The presented method allows downscaling of plating by 100-fold, is flexible, easy-to-use and is more labor-efficient and cost-efficient than conventional plating methods.
Significance and impact of the study: The method can be used for rapid assessment of viable counts of microorganisms similar to conventional plating using standard laboratory equipment. It is faster and cheaper than conventional plating methods.

KEYWORDS
Colony-forming units, plating, faster enumeration, lactic acid bacteria, *Saccharomyces cerevisiae*, *Escherichia coli*

The growth and maintenance of microbes on agar-containing media in Petri dishes has since long been common practice in microbiology. Traditionally, the preferred method for quantitative population analysis of pure and mixed cultures relies on plating of serial dilutions and subsequent counting of colony forming units (CFUs). In recent years a range of alternative, high-throughput (HT), methods relying on quantitative PCR (5, 14), fluorescent labeling (2, 8) or genome probing with micro arrays (1) have gained popularity (9). However, most of these methods measure different entities, i.e. all cells, including non-viable cells. Moreover, these methods may require the use of special equipment or extensive protocol development. In addition, some of these methods are poorly compatible with complex substrates and environmental samples. This largely explains why enumeration of microbes by colony counting is still a widely applied methodology. Presently, microbiology is increasingly moving towards HT analyses, which may require the use of large

quantities of plates. This results in serious drawbacks when using conventional plating and colony counting techniques. The preparation of media and plates as well as the counting of colonies is time-consuming and labor-intensive. Moreover, large volumes may lead to significant costs, in particular when expensive indicator or reporter substrates are used. Finally, many methods consume large amounts of materials including disposables, compromising their sustainability.

Several reports describe alternative plating technologies in which the required volumes are down-scaled (6, 7, 13, 16) or the colony counting process is automated (3, 12, 15). However, these examples require equipment that is not present in standard microbiology laboratories (4, 10), may result only in limited downscaling (4, 6, 7), or are poorly suited for automation (6, 13). Building upon this work, we report a simple and flexible method for determining cell counts capitalizing on microtiter plate formats. It integrates rapid miniaturized plating and (semi-) automated counting of minicolonies allowing 100-fold downscaling of the process compared to conventional CFU counting procedures. It can be fully operated with little more than a multichannel pipet, a digital camera and ImageJ, an image processing package that is available as freeware (http://rsb.info.nih.gov/ij/). A number of examples is presented to illustrate the power of the approach for rapid assessment of viable counts of important prokaryotic and eukaryotic industrial microorganisms.

For plating we used 10-fold serial dilutions that were prepared in 96-well micro plates using a Genex Alpha twelve channel pipet. For each dilution, samples of five µl were pipeted onto a plate containing agar medium using the same pipet. Typically, 60 to 120 droplets were applied per square 12 cm plate. The plates were air dried and then incubated until colonies were visible with an average size of 200 to 500 µm. Subsequently, these minicolonies were photographed with a Canon EOS 350D high resolution digital camera (Canon 0020X665, Canon Nederland NV) equipped with a Sigma 105 mm macro lens (AF 105MM F2.8 EX MACRO F, Sigma Corporation). Plates were put on a black surface and illuminated from the side in order to achieve a large contrast between colonies and background.

Digital images were further processed in ImageJ using a newly developed plug-in that can be downloaded as supplementary material. The counting process is schematically represented in Figure 1. Briefly, the color images were converted into eight-bit grayscale and inverted. By manual intervention using the ‘threshold’ function of ImageJ, colonies were selected from the background. Then all pictures were stacked. After inversion, the median was taken from each pixel with its neighboring pixels for noise reduction. The ’watershed’ function was used to separate merged colonies and the ‘analyze particles’ function was used for

counting. The output was saved as a text files and subsequently processed in Microsoft Excel.

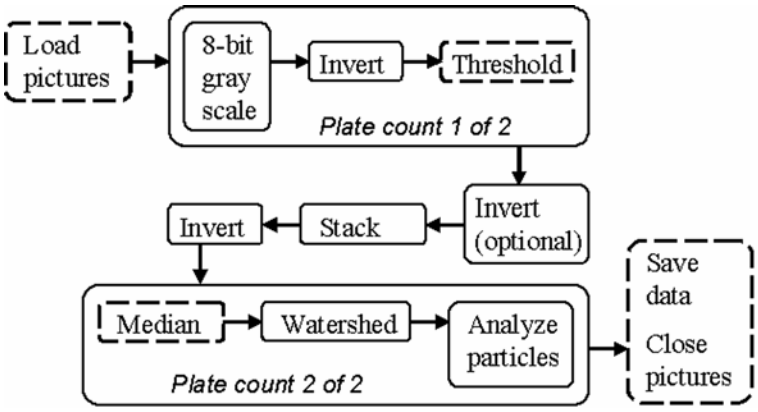

Figure 1. Flow chart of the process of image analysis using the ImageJ plug-in. Dashed boxes are steps that require human intervention. First, a number of pictures is loaded. Thereafter, the pictures are processed with macro 'Plate count 1 of 2' which transfers the pictures into an 8-bit gray scale image (necessary for the 'Threshold' function). Using 'Threshold' manually, the colonies can be selected from the background. This is done for each picture separately, because the images may vary in contrast and brightness. Subsequently, the pictures are stacked so that the following steps can be performed for all pictures simultaneously. For stacking it is necessary to have a black background and white colonies in all pictures, while for the next steps the pictures have to be inverted. In 'Plate count 2 of 2', the median of each pixel with its neighboring pixels is taken to remove noise. 'Watershed' is performed to separate merged colonies, and finally colonies are counted using the 'Analyze particles' function.

In our study we specifically aimed at developing fast CFU counting protocols for industrial microbes including lactic acid bacteria, *Escherichia coli* and *Saccharomyces cerevisiae.* When these strains are enumerated by conventional CFU counting, typically between 30 and 300 colonies per standard agar Petri dish of eight cm in diameter results in optimal counting. Larger numbers may easily result in underestimation as individual colonies can not be discriminated. Low numbers of colonies per plate result in large standard deviations, which is the squared root of the average in Poisson distribution. This implies that in case five colonies are counted the number of cells in the plated samples is between one and nine with 95% confidence whereas for 100 cells these values are 80 and 120. In the latter case the 95% confidence interval is 20% deviation of the average. We therefore aimed at developing a protocol that would allow the counting of at least 100 colonies. This implies that, in any process of downscaling of plate-counting, it is crucial to increase the number of colonies that can be counted per cm^2 of agar surface. In our experiments, this was achieved by counting minicolonies with a size

of 200 to 500 µm. Therefore, colonies were counted as soon as minicolonies were visible, which is earlier than normally is done with conventional counting. Moreover, growing many colonies on a relatively small surface area results in smaller colonies for instance due to limited substrate availability (10). This effectively increases the number of colonies that can be counted per cm^2 of agar surface.

Table 1. Culturing and plating conditions used in the comparison of the conventional plating method and minicolony plating method and resulting average CFU/ml counts with corresponding standard deviations within 12 replicates.

				Incubation of plates		Average CFU/ml (SD)	
Species	Liquid growth medium	Dilution medium	Plating medium	Temp.	Time (h)	Old method	Minicol. method
S. thermophilus	M17 broth + 1% glucose	M17 broth + 1% glucose	M17 agar + 1% glucose	37°C	28 (new method) 40 (Old method)	8.85E+07 (3.25E+07)	2.69E+08 (4.25E+07)
L. lactis	M17 broth + 1% glucose	M17 broth + 1% glucose	M17 agar + 1% glucose	30°C	20	1.88E+08 (3.17E+07)	1.04E+08 (2.30E+07)
L. plantarum	10% skim milk (Nilac) in co-culture with *S. thermophilus*	MRS broth	MRS agar + 1% galactose	30°C	24	2.86E+07 (9.21E+06)	1.37E+07 (1.21E+06)
E. coli	TY broth	TY broth	TY agar	30°C	18	2.04E+08 (1.58E+08)	3.12E+08 (8.29E+07)
S. cerevisiae	ME broth	ME broth	ME agar	30°C	18 (new method) 22 (old method)	8.80E+07 (7.29E+07)	4.87E+07 (2.19E+07)

We used the procedures described above to perform viable counting of various industrial microorganisms and bench-marked these data to conventional counting procedures. Therefore, *Streptococcus thermophilus* CNRZ1066, *Lactococcus lactis* MG1363, *Lactobacillus plantarum* WCFS1, *E. coli* DH5α and *S. cerevisiae* CBS57957 were cultured in appropriate media (see Table 1) for approximately 24 h at 37°C. Culture dilutions were prepared in 96-well plates in 12-fold and these were plated using both methods. For the conventional method 50 µl samples were pipetted onto a round agar plate of eight cm in diameter with a Gilson pipetman P100 and spread using a glass swab. The average CFU counts were comparable and the standard deviations comparable or lower with the minicolony method

confirming the suitability of the method for rapid CFU counting. From the standard deviations in Table 1, it can be concluded that the sensitivity of the fast method is comparable to the conventional method, although the variation between counts may differ with the type of pipets used, as argued previously by Jett *et al.* (7).

For example, *L. plantarum* spots containing minicolonies were around nine mm in diameter and therefore it is possible to count up to 200 colonies per cm^2 efficiently on MRS-galactose agar, which is typically one to two orders of magnitude higher than with conventional plating. We found that the sizes of spots and minicolonies may vary with the type of agar medium, drying time, sample matrice and species of interest (not shown). It may affect the number of colonies that can be enumerated per five µl spot, typically in the range between 10 and 150, but not on the number of colonies that can be counted per cm^2 for this certain species. The detection limit of the minicolony method is similar to that of the conventional method. The dynamic range of the new method was found equal to the conventional method (data not shown).

In recent years high-throughput alternatives and variants have been developed for many conventional microbiological techniques. The best examples are probably liquid batch cultivations for which various multi-well alternatives belong to standard laboratory equipment nowadays. Also several HT alternatives for counting of colony forming units are reported (3, 10, 15). Throughputs are increased by focusing either on automation of the counting process or on the miniaturization of the plating itself. The novelty of the method reported here relies on the integration of these aspects while it requires only standard laboratory equipment, a digital camera, imaging software that is available as freeware, and a dedicated plug-in that is available as supplemental material to this paper at the LAM website. The resulting fast plating and counting protocol is suitable for a quick determination of viable cell counts. The method is highly flexible, because it can easily be implemented for different microbial species and it is easy-to-use. Due to its miniaturization it reduces the amount of necessary materials by approximately 100-fold, which makes it a cost and labor efficient alternative for conventional methods. Because the counting is partially automated, the user can monitor critical steps in data acquisition and processing without the variability encountered from manual counting of CFUs (11). We anticipate that this protocol is a valuable tool for routine enumeration of industrial microbes in research and quality control laboratories.

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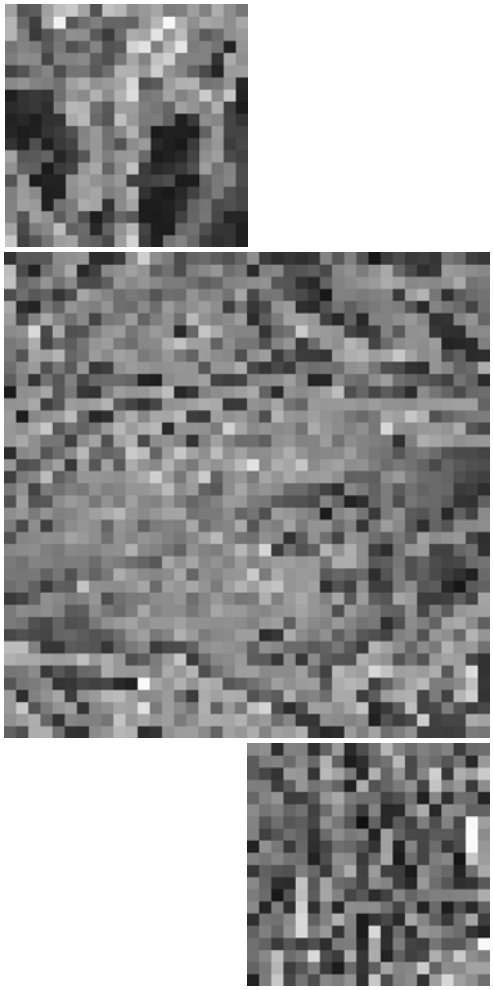
Chapter 4

Mixed culture transcriptome analysis reveals the molecular basis of co-culture growth and its consequences in *Streptococcus thermophilus* and *Lactobacillus bulgaricus*



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ABSTRACT

Many industrial food fermentations are carried out by mixed cultures of lactic acid bacteria. Interactions between different strains are of key importance for the performance of these fermentations. The yoghurt fermentation, in which bovine milk is fermented by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, is one of the best-described mixed culture fermentations. These species are known to stimulate each other's growth by the exchange of metabolites such as folic acid and carbon dioxide. Recently, postgenomic studies have been applied to reveal the global physiological response to mixed culture growth in *S. thermophilus* (19, 20) but an in-depth molecular analysis of mixed culture growth of both strains remains to be established. Here we report the application of mixed culture transcriptome profiling to unravel the molecular responses associated with co-culture growth *of S. thermophilus* and *L. bulgaricus* in milk. The results indicate that the interactions between these bacteria are primarily based on purine, amino acid and long-chain fatty acid metabolism. That is, formic acid and folic acid are provided by *S. thermophilus*, amino acids by proteolysis executed by *L. bulgaricus* and long-chain fatty acids possibly by lipolytic action of *S. thermophilus*. Moreover, we observed that genes involved in iron uptake in *S. thermophilus* are affected by co-culture growth, and that genes coding for exopolysaccharide production are higher expressed in both organisms in mixed culture.

INTRODUCTION

Many food products are fermented by mixed cultures consisting of bacteria, yeasts or filamentous fungi. Fermented dairy products are typically produced with lactic acid bacteria (LAB), a prominent group of Gram-positive bacteria. Yoghurt is bovine milk fermented by the LAB *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*). During fermentation, both species contribute to the texture and the flavor of the product by (i) acidifying the medium leading to coagulation of the milk proteins, (ii) producing exopolysaccharides (EPS) and (iii) generating characteristic flavor compounds, such as acetaldehyde and diacetyl (43). *S. thermophilus* and *L. bulgaricus* stimulate each others' growth and acid production in a mixed milk culture, a process also referred to as protocooperation (12). This mutual stimulation is based on the exchange of growth enhancing metabolites (for a recent review, see (35)). *S. thermophilus* provides *L. bulgaricus* with pyruvic acid, formic acid (14) and folic acid (13, 42) and carbon

dioxide (16), compounds that are all associated to purine biosynthesis either as precursors or cofactors. *L. bulgaricus* lacks pyruvate-formate lyase (PFL) and 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase, an essential gene in the biosynthetic pathway of folic acid (13, 45). Other metabolic interactions exist at the level of nitrogen metabolism. Milk contains low levels of free amino acids (AA) and small peptides but milk proteins provide a rich source of AA that can be liberated through the action of extracellular proteolytic enzymes. Typically, the non-proteolytic *S. thermophilus* profits from the proteolytic action of the membrane-resident protease prtB of *L. bulgaricus* (11, 34, 37). Similarly, *L. bulgaricus* was reported to be stimulated by long chain fatty acids (LCFA) such as oleic acid and lauric acid (29), but it remains to be established whether these are provided by *S. thermophilus* in mixed culture.

The metabolic interactions between the yoghurt bacteria have been elucidated mostly with classical microbiological approaches. More recently two postgenomic studies addressed the global response of *S. thermophilus* LMG18311 to growth in milk as a mono or mixed culture with *L. bulgaricus* ATCC11842 (19, 20). These studies revealed several additional metabolic responses to co-culture growth. The down-regulation of genes associated with purine metabolism and the upregulation of *stu0336*, a xanthine/uracil permease, suggested that purine (precursors) were provided by *L. bulgaricus* and consumed by *S. thermophilus*. Additionally the pathways for the biosynthesis of arginine and branched-chain AA (BCAA) were strongly upregulated in *S. thermophilus* in mixed culture. Finally there was a pronounced response in iron metabolism. The authors showed that in response to H_2O_2 produced by *L. bulgaricus*, *S. thermophilus* shows multiple responses that may lead to lower intracellular iron concentrations. (19). In this way *S. thermophilus* appears to minimize damage by reactive oxygen species (ROS) that are generated in the Fenton reaction.

The postgenomic analyses described above are only performed in *S thermophilus*. The global response to mixed culture growth in *L. bulgaricus* remains to be established. In this study we aimed at analyzing the global regulatory responses to co-cultivation in milk in both strains simultaneously. The correlation of the global responses with population dynamics in intervention studies and metabolite production confirmed the importance of purine and AA metabolism, but also showed that *L. bulgaricus* LCFA production genes are down-regulated in mixed culture despite its higher growth rate. This indicates that these LCFA were directly or indirectly provided by *S. thermophilus*. Similarly, the induction of genes responsible for EPS production in both species in co-culture shows a role for EPS in the protocooperation. These results provide further insight in the global

physiology of the yoghurt mixed culture fermentation and may facilitate rational improvement of other fermentations or the development of new (industrial) mixed cultures, for instance by providing targets for engineered nutritional dependencies.

MATERIALS AND METHODS

Strains and culture conditions.

Streptococcus thermophilus CNRZ1066 (3) and *Lactobacillus delbrueckii* subsp *bulgaricus* ATCC BAA-365 (25) were maintained as frozen stocks in M17 broth (Difco) and MRS broth (Difco), respectively with 22% glycerol (Scharlau) at -80 °C. Precultures were made as mono-cultures in UHT treated 10% reconstituted skim milk (Nilac, NIZO food research, the Netherlands) for 22 h at 42°C. Final liquid cultures were made by inoculating pre-warmed milk at the same conditions with, precultures of *S. thermophilus* and *L. bulgaricus* to density of approximately $1*10^5$ and $2*10^4$ CFU/mL, respectively.

For biofilm growth, *S. thermophilus* and *L. bulgaricus* mono-cultures in milk were incubated for 1-2 days at 42°C and subsequently pipeted together or separately onto an anopore slide (22) placed on top of a milk agar plate (8% skim milk and 1-1,5% agar). Plates were incubated anaerobically at 42°C for 2-4 days before staining procedures were carried out (see below) or at 45°C for 2 days for transcriptome analysis. Cell density of the inoculum was quantified by plating on MRS agar for *L. bulgaricus* and M17 agar supplemented with 1% (w/v) glucose for *S. thermophilus*.

In order to calculate the amount of lactic acid produced by the different cultures, uninoculated milk was acidified to various pH values with lactic acid. These recorded amounts of added lactic acid were used to calculate the lactic acid content of a culture at a certain pH.

Intervention studies.

Cultures of *S. thermophilus*, *L. bulgaricus* and the mixed culture were prepared in 0.8 volume reconstituted skim milk with 0.2 volume of a solution with compounds for which it has been established or hypothesized that they are involved in interaction (35): Na-pyruvate (1.82 mM), Na-formate (1.47 mM), folic acid (1 mM), nucleobases (10 mg/L each) (all representing purine and pyrimidine metabolism), Tween-20 (105.9 µM) (as a supply of lauric acid (15, 23)), Tween-80 (110 mg/L) (as a supply of oleic acid (23)), casitone (4 g/L), histidine (650 µM) (both representing proteolysis), L-ornithine monohydrochloride (590 µM) and putrescine

(1.13 mM) (both involved in arginine metabolism, urea cycle and tolerance to oxidative stress (7, 35, 45)). Here after these compounds are referred to as 'interaction compounds'. The LCFAs oleic acid and lauric acid are poorly soluble and therefore we used Tween-20 and Tween-80 (29). The effect of each of all interaction compounds on growth and acidification was tested in a single addition and a single omission strategy. Paired comparisons were made of a single compound versus nothing added (neg. control), and of all compounds minus one added versus all (pos. control). Acidification of quadruplicate cultures of 250 µL was measured at 37 °C for 19 h in hydroplates (PreSens - Precision Sensing GmbH, Germany) where after CFU counts were determined using a rapid miniplating method (36). Significant differences in acidification were determined by comparing the maximal acidification rate using a two-tailed Students t-test (p=0.05). Similarly, significant differences were calculated between the final pH values and between the colony-forming units.

Metabolite analyses.

Free amino acid content was determined as follows. Samples (100 µL) were diluted 10x by adding 100 µL 2.5 mM norvaline (Aldrich) as internal standard and 800 µL 0.1 M HCl (Fluka) / 0.2% (w/v) 3,3-thiodipropionic acid (Fluka). In order to remove proteins, this was filtered in a 3000 Da Microcon Ultracel YM-3 microcentrifuge filter (Sigma) by filtration at 3500 G for 30 min. 20 µL of this was mixed with 180 µL of a solution containing 0.15 M $NaHCO_3$ pH 8.6 to buffer pH. 200 µL 12.4 M dabsyl chloride (Fluka) in acetone (Sigma) was added. The samples were incubated for 15 min at 70°C and mixed with a vortex mixer after 0, 1 and 12 min to derivate the amino acids. This reaction was stopped by placing the samples on ice. The samples were centrifuged at 10000 G for 10 s, where after 400 µL of a solution was added consisting of 50% (v/v) acetonitrile (Sigma), 25% (v/v) ethanol (Sigma) and 25% (v/v) Eluens A (9 M NaH2PO4 pH 7.0 and 0.16% (v/v) triethylamine (Sigma)). The samples were mixed by vortexing and centrifuged at 15000 G for 5 min. Of the supernatants 10 µL was injected in the column for analysis with a Phenomenex ODS (C18), 3 µm SphereClone column and reversed-phase HPLC (detection at 436 nm with 738 A (VIS4205) (Applied Biosystems)). The concentrations were calculated by comparing the peak surface with that of a standard series.

For volatile compound analyses Solid Phase Dynamic Extraction (SPDE) head space analysis was performed similar to as described by Bachmann *et al*. (1). In short, triplicate cultures of 5 mL prepared in glass flasks were grown at 37°C for 24 h and subsequently frozen at -80°C. The flasks were incubated at 60°C and 1 mL

headspace was used for the SPDE (PDMS and 1% AC). The volatiles were fixed by cryofixation at -150°C for 0.5 min. Subsequently, the components were separated on a Factor Four V1ms column using a helium flow of 1.5 mL/min. The oven temperature was held at 40°C for 5 min, was increased to 50°C using a ramp rate of 10°C/min, brought to 60°C by 2°C/min, then brought to 150°C by 10°C/min and finally brought to 225°C for 4 min with a ramp rate of 50°C/min. Peak areas were determined and compounds were identified using the mainlib, replib and wiley7 (Nist) libraries in Xcalibur™ (Thermo Fisher Scientific Inc, Waltham, MA, USA). Quantification of components was performed by comparing peak areas with those of calibration series.

Exopolysaccharide isolation.

Different 275 mL yoghurt cultures were grown at 37°C for 24 h. Of this, 80 g was used for EPS isolation. The samples were incubated at 55°C to release all EPS from the cells, where after 5.3 mL of a 60% Na_3Citrate (Merck) solution was added. After stirring at RT for 1 h, the samples were centrifuged for 30 min at 6000 G and 4°C. The supernatant was applied to a flask and adjusted to pH 4.0 with 10 M NaOH (VWR). Dialysis tubes (Medicell International Ltd., England) were boiled in water with a spoon Na_2CO_3 for 5-10 minutes, rinsed with water and boiled in water for 5 min. The samples were applied to the tubes. Dialysis was performed in flowing tap water for 24 h and twice in MQ water for 3 h. Dialyzed samples were freeze dried in an ilShin freeze dryer (IlShin, South Korea) until all water had evaporated.

Microarray design.

Microarrays were spotted on the Agilent 8x15K platform (Agilent Technologies, Santa Clara, CA, USA) with a custom probe design (AMADID 015342) comprising the sequences of both *S. thermophilus* CNRZ1066 (released by NCBI, genbank accession no. NC_006449) and *L. bulgaricus* ATCC BAA-365 (released by JGI, genbank accession no. NC_008529). The probes were designed with the objective to minimize cross-hybridization: the probes were species-specific, i.e. all probes were designed as 60-mers with a target score of 100% to the target gene, allowing no binding of cDNA that is 1 base different (mismatch) if the correct hybridization temperature (65°C) and washing temperature (37°C) are used. In total there are 5438 probes representing 1899 genes of *S. thermophilus* and 4028 spots representing 1709 genes of *L. bulgaricus*. Most genes are represented by 3 probes or more. Only 55 genes in *S. thermophilus* and 77 in *L. bulgaricus* are represented by one probe and only 5 genes of *S. thermophilus* and 31 genes of *L. bulgaricus*

are lacking. The selectivity of strain specific gene detection was tested by a series of transcriptome profiling experiments of samples from MRS-grown mono-cultures of both strains. Comparative analysis of separate hybridizations and hybridization of a mixture of both samples showed that on average the probes showed 100-fold higher hybridization with RNA samples from the target strain. It was concluded that for a small number of genes strain specific gene expression analysis was not possible. These genes included rRNA genes (14 in *S. thermophilus*, 19 in *L. bulgaricus*), ribosomal proteins (4 and 12, respectively) and hypothetical proteins (8 and 2, respectively). They were excluded from further analysis.

RNA isolation from cultures grown in milk.

The high protein content of milk and the polysaccharide production by the grown microorganisms make cell harvesting problematic. Furthermore, sampling and quenching need to be carried out rapidly in order to prevent the introduction of technical errors in a transcriptomics experiment (10). Several procedures have been developed to “clear” the milk to enable cell harvest by centrifugation without the contamination with milk solids. However, milk cleaning procedures are time consuming and require drastic changes in pH and the addition of large quantities of sodium citrate (9). We considered that this procedure is prone to lead to changes in the transcriptome. Therefore, we developed an alternative method for cell harvesting and RNA extraction from yoghurt cultures suitable for transcriptomic profiling. Yoghurt cultures were quenched in 3 volumes 60% glycerol of -40°C leading to immediate arrest of cellular processes (32) and kept at -20°C for 0.5 h. Then pH was adjusted to 6.5 – 7.0 with 1 M NaOH and the medium was cleared with 4 mL 25% (w/v) Na_3Citrate per 100 mL at -20°C for 0.5 h with gently mixing each 5 min. Cells were spinned down at -20°C and 23000 G for 16 min and dissolved in a solution comprised off 50% (w/v) guanidinethiocyanate (Sigma), 0.5% (w/v) N-laurylsarcosine (Sigma) and 2.5% (v/v) of a 1 M sodium-citrate solution, adjusted to pH 7.0 with 0.1 M NaOH. After another centrifugation, the cells were resuspended in 500 µL 1xTE and applied to an RNA extraction tube containing 250 µL acidic phenol (Sigma), 250 µL chloroform (sigma), 30 µL NaAc (Merck) pH 5.2, 30 µL 10% SDS (Sigma) and 500 mg zirconium beads with 0.1 mm diameter (Biospec products Inc., OK, USA) which was immediately frozen in liquid nitrogen and kept at -80°C until RNA extraction.

For RNA isolation, a method was used that was already established for isolation from lactobacilli (39). Briefly, cells were disrupted 3 times 45 s in a Fastprep (Qbiogene Inc., France) at 5.5 m/s separated by 1 min on ice. After centrifugation for 1 min at 20800 G, 500 µL of the aqueous phase was purified with

400 µL chloroform and a second centrifugation step. The aqueous phase was used for RNA isolation with a High Pure kit (Roche Diagnostics, Mannheim, Germany), which included 1 h of treatment with DNase I. RNA was stored at -80°C. Quantity and quality were checked using a ND-1000 photospectrometer (Nanodrop Technologies, Wilmington, DE, USA) and capillary electrophoresis on a RNA 6000 Nano LabChip® kit (Agilent Technologies, Santa Clara, CA, USA) in a 2100 Bioanalyzer (Agilent).

cDNA synthesis, labeling and hybridization.

Five to seven µg of RNA was used for cDNA synthesis and labelling as described before (40). For each array, 0.3 µg of cDNA labeled with Cyanine 3 and Cyanine 5 was hybridized. Hybridizations were performed with solutions and following the protocol delivered by Agilent (version 5.5) for 8x15K slides. Arrays were hybridized at 65°C for 17 h. Hybridization schemes were designed that allowed duplicate comparisons between different stages within a fermentation experiment as well as and between mono and mixed cultures (see Figure 1). Here after, the microarray slides were washed according to the manufacturer's instructions (buffer 1: room temperature, buffer 2: 30-37°C) with the buffers supplied by Agilent. We found that washing at lower temperatures resulted in major cross-hybridization when hybridising *S. thermophilus* cDNA labelled with Cy5 and *L. bulgaricus* cDNA labelled with Cy3 simultaneously, but not when applying only one cDNA sample.

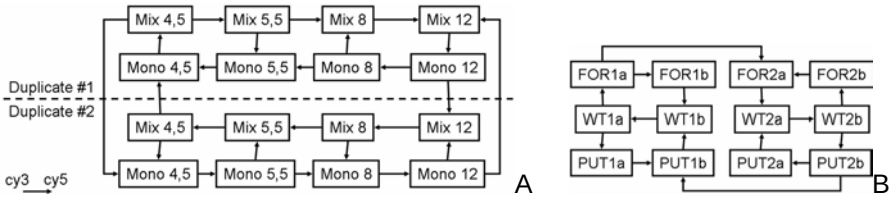


Figure 1. Hybridization schemes of the micro arrays performed in this study. Panel A, Mixed cultures were compared with mono-cultures in duplicate at four time-points in the fermentation: 4.5, 5.5, 8 and 12 h corresponding to the first exponential phase, transition phase, second exponential phase and stationary phase, respectively. Mono indicates a mixture of RNA from the mono-cultures of *S. thermophilus* and *L. bulgaricus*. Panel B, Mixed cultures without supplement (WT) were compared to mixed cultures supplemented with formic acid (FOR) and putrescine (PUT) at the transition phase (a) and the second exponential phase (b). The numbers 1 and 2 indicate biological duplicates.

Array analysis.

Slides were scanned using an Agilent microarray scanner (G2565BA), Laser lights of wavelengths at 532 and 635 nm were used to excite Cyanine3 and Cyanine5 dye, respectively. Fluorescent images were captured as multi-image-tagged image

file format and analyzed with Imagene software (Axon) (BioDiscovery, Marina del Rey, USA). The extent of hybridization was derived from a median value of pixel-by-pixel ratios. *S. thermophilus* and *L. bulgaricus* spots were normalised separately using Lowess (47). Differential regulation was determined by false-discovery rate (FDR) from the Cyber-T *p*-values by means of multiple testing connection (2, 48). Differential regulation was defined as a two-fold or higher differential expression with a FDR cut-off value of 0.05 or lower. Regulated genes were divided into functional classes as described by NCBI (*S. thermophilus*) and JGI (*L. bulgaricus*). Using Hierarchical clustering, principle component analysis and MicroPreP (46), the quality of the different hybridizations was verified. Finally, results were visualized by plotting onto KEGG maps, Simpheny (Genomatica Inc., San Diego, CA) metabolic maps (30, 44) and Minomics (6).

Staining, imaging and processing of biofilms.

For SybrGold staining, anopore strips containing minicolonies were transferred right-side up to a microscope slide covered with a 1-mm-thick film of 1% (w/v) solidified low-melting-point agarose (Sigma, The Netherlands) containing 1µl/10ml 10000x concentrated SybrGold (Invitrogen, The Netherlands). Staining was for 20 min at room temperature. These procedures allowed staining of the organisms on the anopore surface through the pores without disruption of the microcolonies. Strips were then imaged directly (without coverslip, immersion oil, or fixative) using an Olympus BX-41 fluorescence microscope equipped with U-MWIBA filters (excitation spectrum of 460 to 490 nm, diachronic mirror splitting at 505 nm, and an emission spectrum of 515 to 550 nm, used for SybrGold) (Olympus, Japan). Scanning and examination of microcolonies were performed with 4x and 10x UMPlanF1 objective lenses, and observation of individual cells used a 50x UMPlanF1 objective (Olympus). Image capture was done with a charge-coupled device (CCD) camera controlled by Kappa Image Base software (Kappa, Germany).

Scanning electron microscopy (SEM) of biofilms.

Samples were cultured on anopore strips on milk agar plates for 2-4 days and fixed in situ by placing the anopore strips on MRS agar plates with 2.5% glutaraldehyde (w/v) for at least 2-3 hours. The strips were then washed three times with MQ water. Here after, samples were stained with 1% osmium in water for 15 min, and dehydrated through a series of ethanol/water mixtures with increasing ethanol content (10, 30, 50, 70, 90, 100%) for 10 min per treatment (twice with 100%) and then critical point dried.

Anopore strips cultured with bacteria were glued on a sample holder with conductive carbon cement (Leit-C, Neubauer Chemicalien) and frozen in liquid nitrogen. Samples were transferred under vacuum to the dedicated cryo-preparation chamber (Oxford Cryo-system, CT 1500 HF) onto a sample stage at -90°C. The samples were freeze-dried for 4 min at -90°C in a $3x10^{-7}$ Pa vacuum to remove water vapor contamination. Subsequently, the sample surface was sputter-coated with 10 nm platinum, and it was transferred to the cold sample stage (-190°C) inside the Cryo-FESEM (JEOL 6300F Field Emission SEM) and subsequently analyzed with an accelerating voltage of 5 kV. Images were digitally recorded (Orion, E.L.I.).

RESULTS

Population dynamics of *S. thermophilus* and *L. bulgaricus*.

To study the interaction of *S. thermophilus* and *L. bulgaricus* in milk growth and acid production in mono-cultures and in a mixed culture were compared (see Figure 2). While the *L. bulgaricus* mono-culture showed only one exponential phase, a typical 4 growth-phase behavior, following the lag phase, was observed in the *S. thermophilus* and the mixed cultures: (i) a first exponential phase, (ii) a transition phase at which the growth rate decreased, (iii) a second exponential phase, and (iv) a stationary phase (12, 24).

Intervention studies.

Several compounds are known or hypothesized to play a role in the interaction between the two yoghurt bacteria. We systematically analyzed the effect of these compounds on acidification and outgrowth in mono and mixed cultures. Therefore, mono and mixed cultures were prepared with single additions, a mixture of all components added, and all components with single omissions. The effects of these medium interventions on the evolution of pH were analyzed by scoring effect of key parameters of the acidification profile. That is, the maximal or minimal acidification rate in each growth phase (see Figure 2B) and the final pH. The final viable counts were also taken as measures for the paired comparisons (see Table 1). A higher cell count, lower final pH, higher acidification rate and a shorter time to reach this rate are considered stimulatory effect of the intervention compared to the control.

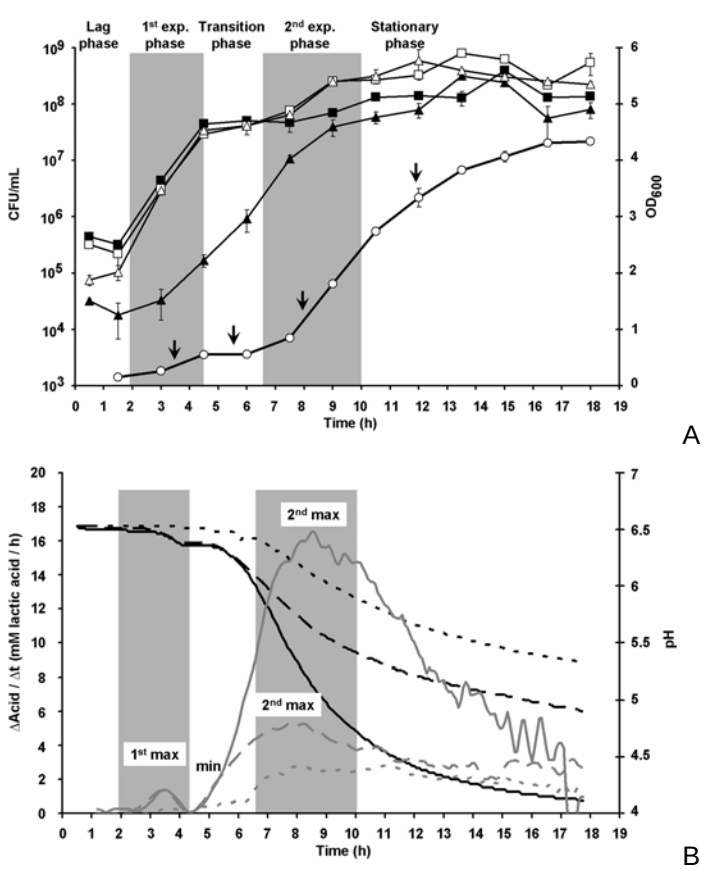
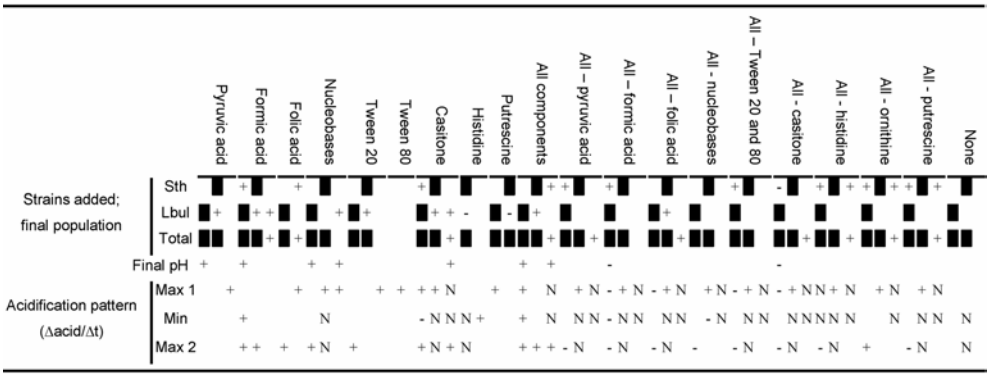


Figure 2. Growth and acidification of mono and mixed cultures grown in 10% reconstituted skim milk at 42 °C. Panel A, Colony-forming units per mL of *S. thermophilus* in mono-culture (■) and mixed culture (□), and *L. bulgaricus* in mono-culture (▲) and mixed culture (Δ), and the OD_{600} of the mixed culture (○). Error bars indicate standard deviations of triplicate measurements. Gray and white boxes indicate the five distinct growth phases of the mixed culture. Arrows indicate sampling points for transcriptomics. Panel B, pH (black lines) and acidification (ΔmM lactic acid / h) (grey lines) of typical milk fermentations of *S. thermophilus* (striped), *L. bulgaricus* (dotted) and the mixed culture (solid). The three distinct phases (1st exponential phase, decreased growth phase, 2nd exponential phase) with a maximal, minimal and another maximal acidification rate are present in the *S. thermophilus* and mixed cultures, but not in the *L. bulgaricus* culture.

Acidification by *S. thermophilus* was stimulated by the following compounds in decreasing order: formic acid, casitone, pyruvic acid, folic acid and Tween-20. *L. bulgaricus* acidification was stimulated the most by formic acid and nucleobases, whereas pyruvic acid, folic acid, Tween-20 and Tween-80 showed a small stimulatory effect. Acidification of the mixed cultures was still stimulated by pyruvic

acid, formic acid and casitone, but in all cases stimulatory effects were less than with the mono-cultures. Only formic acid and casitone led to a higher final cell count of *S. thermophilus*. *L. bulgaricus* cell counts were higher when pyruvic acid or Tween-20 was supplied. These effects were less evident in mixed cultures, indicating the importance of purine and AA acquisition and a possible role of LCFA in the interactions between the two bacteria.

Table 1. Stimulation of growth and acidification by components potentially involved in the interactions between *S. thermophilus* and *L. bulgaricus* in milk. The final populations presented were determined from duplicate plating of quadruplate cultures. The acidification rates and final pH values were calculated from the same quadruplicate cultures in which one, all, or all minus one compound(s) were added. The pH was monitored for 19 h where after dilutions were plated for cell enumeration. Significant effects were determined by a two-tailed t-test (p=0.05). Cultures containing one component were compared to cultures without additions (single additions). Cultures containing all components minus one were compared to the cultures containing all (single omissions). Components that showed no difference with the reference in any of the measurements are left out from the table (i.e. Tween-80 on both mono-cultures and ornithine on all three cultures). Sth, *S. thermophilus*; Lbul, *L. bulgaricus*; +, positive effect (higher cell count, lower end pH, higher acidification rate, earlier maximal rate); -, negative effect (lower cell count, higher end pH, lower acidification rate, later maximal rate); N, not present; ▮, this species is not added in the culture.



Transcriptome analysis of mono and mixed cultures

In order to identify genes that are differentially expressed in both species upon co-culture, we performed transcriptome profiling on mixed cultures and compared those to mono-cultures at four different growth phases, i.e. the first exponential phase (3.5 h after starting the fermentation), transition phase (5.5 h), second exponential phase (8 h) and stationary phase (12 h). Similarly, we compared these four distinct growth phases within a culture. Finally, transcriptome profiling was performed on cultures in early and mid second exponential phase mixed cultures supplemented with the interaction compounds formic acid and putrescine. These

studies allowed analysis of global regulatory responses and the development of the interactions throughout the fermentation. DNA micro arrays were used that contained probes targeting strain-specific sequences ensuring minimal cross-hybridization for the genomes of both *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365. An RNA extraction method based on quenching by rapid freezing the culture and clarification by citrate was specifically designed for these experiments and proved to be crucial for the acquisition of high quality RNA samples from yoghurt cultures. Although we defined genes that were two-fold or more up or down-regulated with a FDR value of lower than 0.05 as significantly differentially expressed, also the more general effects were considered (e.g. all genes in a pathway are significantly upregulated by 1.5-fold).

Differential expression between mixed and mono-cultures was high in all four growth stages (see Table 2, Supplementary Tables 1 and 2 provide an overview of all significantly differentially expressed genes). The interactions affected *S. thermophilus* mainly in the second exponential phase (23% of all genes was more than 2-fold differentially expressed), which is in agreement with the observation that only at this growth phase *S. thermophilus* is profoundly stimulated by *L. bulgaricus* (see Figure 2A) (19). The major functional groups affected included 'Amino acid transport and metabolism' (15-42% of the genes in the category), 'Inorganic ion transport and metabolism' (14-32%) and 'Nucleotide transport and metabolism' (10-47%). The presence of *S. thermophilus* stimulates *L. bulgaricus* growth already in the early stages of the fermentation, which is exemplified by the higher portion of differentially expressed genes in *L. bulgaricus* in the two early growth phases compared to *S. thermophilus* (24% versus 7% in the transition phase). A major part of the differential expression in both species could be attributed to the increased growth rate as is exemplified by the induction of primary metabolism including the genes involved in the production of important end products such as diacetyl, contributing to the typical yoghurt flavor. Indeed, this compound was present in larger quantities in mixed culture than in mono-culture (see Table 3). The major affected functional groups related to interactions included 'Amino acid transport and metabolism' (21-36% of the genes in the category), 'Inorganic ion transport and metabolism' (20-28%) and 'Nucleotide transport and metabolism' (18-44%).

Below we will first describe the global physiological responses of both strains. Subsequently, regulatory responses for the major pathways affected will be described in more detail.

Global regulatory responses analysis of *L. bulgaricus*. In the *L. bulgaricus* mono-culture there was little difference in gene expression between the different growth phases except that from 8 h on (growth slows down and the culture enters stationary phase, see Figure 2A) many pathways were down-regulated, especially those associated with the biosynthesis of folic acid, purines, LCFA and AA and genes related directly related to growth such as those encoding ribosomal proteins and enzymes involved in cell wall biosynthesis. In the mixed culture there was a clear lower expression of genes associated with folic acid and purine biosynthesis, LCFA biosynthesis and sulfur AA metabolism in the transition phase compared to the first exponential phase. This may be due to the lower growth rate in the transition phase. In the second exponential phase, however, expression of purine and LCFA biosynthesis genes remained at a low level despite the higher growth rate compared to the transition phase. Moreover, *LBUL_0106*, encoding 1-acyl-sn-glycerol-3-phosphate acyltransferase was expressed 13-fold higher, suggesting that this acyltransferase was loaded with LCFA harvested from the medium. In addition, genes involved in EPS and sulfur AA metabolism were higher expressed in the second exponential phase than in the transition phase.

Global regulatory responses in *S. thermophilus*. In the *S. thermophilus* mono-culture, the gene *pflA* (4.6-fold) for the production of formic acid and the pathway for purine biosynthesis were higher expressed in the transition phase compared to the first exponential phase despite the lower growth rate. Similarly, BCAA import and production genes were 2.9-3.0-fold higher expressed in the transition phase suggesting a shortage of these AA relatively early in the fermentation. Expression of genes for the production of other AA was in general lower in the transition phase compared to the first exponential phase. There was little difference in the second exponential phase compared to the transition phase except the up regulation of sulfur AA metabolism, as was also described by Hervé-Jimenez *et al.* (20). The trends in differential expression between the first exponential phase and the transition phase were comparable in *S. thermophilus* in mixed culture and the mono-culture, except for the fact that the higher expression of BCAA acquisition genes did not occur in the mixed culture. In the second exponential phase in mixed culture, purine biosynthesis genes were lower expressed than in the transition phase, but many pathways involved in AA acquisition were higher expressed, especially those for BCAA (2-3.1-fold) and sulfur AA (2.2-61.5-fold) suggesting an increased requirement for these AA. In the stationary phase, growth-related pathways were lower expressed. It is noteworthy that EPS biosynthesis genes of *S. thermophilus* were significantly higher

Table 2. Global patterns in differential gene expression. Numbers of genes two-fold or more up and down-regulated (FDR=0.05) in mixed culture compared to the mono-culture, cultures supplemented with formic acid compared to a culture without supplements. “All” refers to the number of genes that were differentially expressed at all four time-points. ‘Early exponential’ and ‘mid exponential’ correspond to 6.5 and 8 h in the fermentation without formic acid, respectively.

	S. thermophilus		*L. bulgaricus*	
	Up	Down	Up	Down
Mixed versus mono-culture				
3.5 h	96	103	77	431
5.5 h	58	93	96	702
8 h	270	223	342	344
12 h	246	514	489	411
All	4	4	6	0
Supplementation of formic acid				
Early exp.	105	122	53	80
Mid exp.	89	63	61	93
Both	5	7	8	20

Table 3. Production of volatile compounds important for yoghurt flavor by the different cultures (µM).

	acetaldehyde	methanethiol	2-propanone	dimethylsulfide	2-methylpropanal	diacetyl	2-butanone	bthylacetate	3-methylbutanal	2-methylbutanal	2-pentanone	2,3-pentanedione	dimethyldisulfide	2-heptanone	dimethyltrisulfide
S. thermophilus	410	9.87	1020	2.27	4.50	74.00	41.67	13.67	1.63	0.87	14.00	3.63	0.90	18.00	0.73
L. bulgaricus	108	2.07	317	6.17	2.00	730	38.00	11.67	0.30	0.20	1.33	41.67	0.13	1.77	0.23
Mixed culture	252	21.67	1290	9.37	7.63	2333	68.00	25.67	1.40	0.60	8.57	423	1.03	9.40	1.60

expressed in the second exponential phase and stationary phase compared to the earlier growth phases in mixed culture, but not in mono-culture.

Purine metabolism. In a proteome study of *S. thermophilus*, Dezelle *et al*. (14) found that pyruvate-formate lyase (PFL) was highly abundant during growth in milk. We also found that the two genes, *pfl* and *pflA* were higher expressed in mixed culture, especially in the first exponential phase (3.0 and 4.1-fold, respectively) compared to mono-cultures. Expression was down-regulated 3.8 and 5.7-fold when formic acid was supplied indicating that the physiological role of the enzyme was (in part) ensuring sufficient supply of formic acid. Expression of genes of the biosynthetic pathway for folic acid production was not affected, but expression of folic acid cycling genes (C1 pool) corresponded to the expression of genes for the production of purines. However, the incomplete folate biosynthetic

pathway in *L. bulgaricus* was lower expressed, especially at the first two growth stages. Genes in the purine biosynthesis pathway in *S. thermophilus* were higher expressed in the mixed culture in the two earlier growth stages, but, in accordance to the study by Hervé-Jimenez *et al*. (19), less expressed in the second exponential phase despite the higher growth rate. Similarly, purine metabolism in *L. bulgaricus* was lower expressed in mixed culture, especially after 5.5 h, potentially due to the lower growth rate in mixed culture at this phase (see Figure 2A). When formic acid was supplied, expression of genes involved in biosynthesis of purines and folic acid cycling was lower in the early (second) exponential phase but higher in the mid exponential phase in both species.

Amino acid and carbon dioxide metabolism. Interactions occur at the level of nitrogen metabolism (proteolysis (19) and carbon dioxide utilization(16)). Nitrogen metabolism was poorly affected in *L. bulgaricus* with few exceptions. In co-culture we observed considerable higher expression levels of the *prtB* gene, *LBUL_1105*, which was 8.9-fold higher expressed in the second exponential phase in co-culture. This can be explained by the fact that peptides generated upon casein hydrolysis by the protease are more rapidly consumed when *S. thermophilus* is also present. This demands higher protease activity to sustain growth of both bacteria. In addition, genes involved in the biosynthesis of the sulfur AA cysteine and methionine were highly upregulated in mixed culture, e.g. the gene that converts O-acetyl-L-serine into cysteine, *LBUL_1235*, was expressed 23.1-fold higher in the mixed culture during the second exponential phase (see Figure 3A). This indicates that the proteolysis of casein does not allow the supply of sufficient cysteine for both organisms. Indeed, the cysteine content of casein is only 0.35% (41). Moreover, the free methionine content of a milk culture is negligible and the free cysteine is rapidly consumed, i.e. cysteine does not accumulate in *L. bulgaricus* mono-culture and mixed culture, while several other AA do (for an overview, see Supplementary Table 1). In *S. thermophilus*, the higher peptide abundance due to the proteolysis executed by the protease that is produced by *L. bulgaricus* led to the upregulation of peptide import systems, such as the ABC transport system encoded by *amiC*, *amiD*, *amiE* and *amiF1* (2.5-2.8-fold), and peptidolysis, as exemplified by the upregulation of the gene encoding peptidase PepN (2.4-fold) in the second exponential phase. In addition, genes encoding the biosynthesis of the three BCAA (2.0-fold) and uptake (1.0-1.3-fold) were slightly higher expressed in mixed culture. Similarly, in *L. bulgaricus* in mixed culture *LBUL_0431*, encoding a branched-chain amino acid permease, was 2.3-fold higher expressed during the second exponential phase. That was anticipated since especially the *S. thermophilus* mono-culture and the mixed culture displayed

a very low BCAA content, in particular of isoleucine. Similarly, in *S. thermophilus* there was a higher expression of pathways that convert serine into cysteine and methionine (1.5-1.9-fold). The pathways for *de novo* production of arginine out of glutamine and glutamate were upregulated in mixed culture. Glutamate is converted into ornithine mediated by four genes, *argJ*, *argB*, *argC* and *argD*, which were all 1.8-3.3-fold higher expressed in mixed culture at the second exponential phase. In addition, *carA*, one of the genes responsible for the conversion of glutamine into carbamoyl phosphase, was 1.8 fold higher expressed. This all indicates that the urea cycle is running faster in *S. thermophilus* in the second exponential phase when grown in co-culture with *L. bulgaricus*. Moreover, *cah,* encoding carbonate dehydratase in *S. thermophilus*, was 3.8 to 15.8-fold higher expressed in mixed culture, in particular in the earlier growth phases. By liberating CO_2 from carbonate this enzyme may play a role in providing the CO_2 required for biosynthesis of aspartate, glutamate, arginine and nucleotides (4, 27, 49) in both species. These results are in accordance with the results described by Hervé-Jimenez *et al*. (19), who argued that BCAA and arginine metabolism in *S. thermophilus* were upregulated in presence of *L. bulgaricus*.

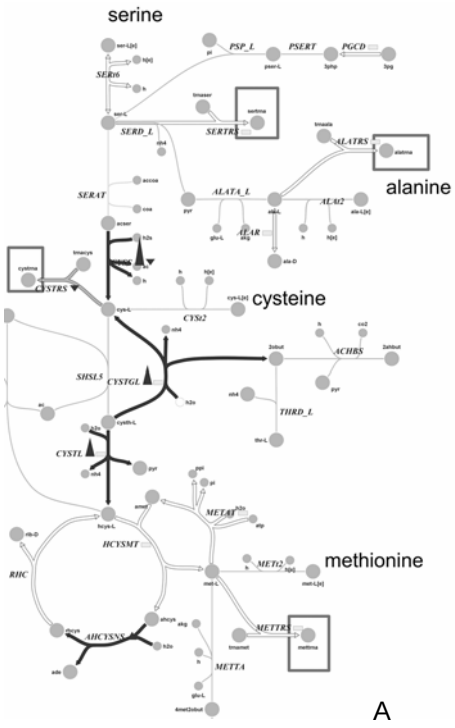


Figure 3. Differential gene expression in *L. bulgaricus* between mixed cultures and mono-cultures in the second exponential phase plotted on SimPheny metabolic maps. These maps are based on the gene content of the presented organism. Circles represent metabolites. Thick arrows indicate reactions catalyzed by proteins present in this species; thin arrows indicate that the genes encoding the protein catalyzing have not been identified in the genome. Dark arrows indicate a higher expression in the mixed culture; lighter arrows a higher expression in mono-culture and white arrows indicate no differential expression. Pyramids indicate the extent of differential expression. Multiple pyramids indicate that there are multiple genes associated with this reaction. Panel A, Sulfur AA metabolism of *L. bulgaricus*. acser, O-acetyl-L-serine; CYSS, cysteine synthase; CYSTRS, cystenyl-tRNA synthetase; CYSTGL, cystathionine g-lyase; CYSTL, cystathionine b-lyase; HCYSMT, homocysteine s-methyltransferase; AHCYSNS, adenosylhomocysteine nucleosidase. Panel B, Fatty acid biosynthesis of *L. bulgaricus* in mixed culture compared to mono-culture at the second exponential phase. apoACP, apoprotein [acyl carrier protein]; accoa, acetyl-coA; acp, acyl carrier protein; tdeacp, tetradecanoyl-acp; hdeacp, hexadecanoyl-acp; 2chdeacp, cis-hexadec-2-eonyl-acp; ocdacp, octadecanoyl-acp; 2cocdacp, cis-octadec-2-eonyl-acp; cpocdacp, cyclopropanol octadecanoyl-acp; AGAT-LPL, 1-acyl-glycerol-3-phosphate acyltransferase; pa_LPL, phosphatydic acid.

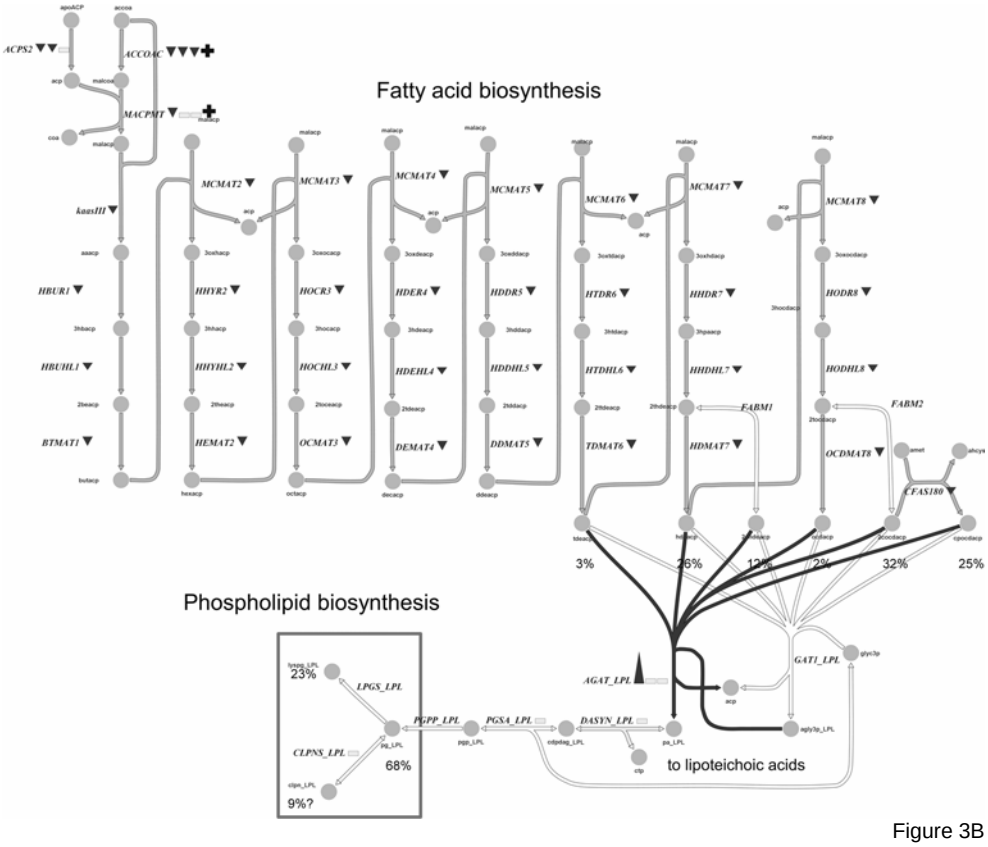


Figure 3B

Iron metabolism and H_2O_2 resistance. We found that 6 of 7 genes involved in iron transport in *S. thermophilus* were differentially expressed in mixed culture compared to mono-culture. In contrast to the findings of Hervé-Jimenez *et al*. (19), the iron complex ABC transporter consisting of *fatA*, *fatB*, *fatC* and *fatD* was expressed 2.7-4.5-fold higher in mixed culture during the second exponential phase. However, the putative iron transport regulator *fur* and the iron chelator *dpr* were 2.4 and 2.9-fold lower expressed in the mixed culture in this growth phase. In stationary phase, these genes were 1.5 and 1.2-fold higher expressed, which is in accordance with a previous study (19), in which it was concluded that *S. thermophilus* lowers its iron uptake in presence of *L. bulgaricus* in order to minimize the production of ROS from H_2O_2 produced by *L bulgaricus* (26). Indeed, the expression of *fur* and *dpr* is correlated to the expression of *LBUL_2034*, the homologue of *pox1* in *L. bulgaricus* LMG11842, but not with *LBUL_1421* and *LBUL_1955*, the homologues of *pyrD2* and *pyrD1* (19).

EPS biosynthesis. Sugar nucleotide precursors that are used for EPS biosynthesis are also used as intermediates in sugar catabolism, the assembly of peptidoglycan and other glycan-containing cellular polymer, both important for growth. EPS is mainly produced in the later stages of fermentation (5). We observed that *S. thermophilus* EPS genes (*epsA* - *epsM*) were higher expressed in the second exponential phase and especially in the stationary phase compared to the earlier growth phases. In addition, all these genes were higher expressed at these two later phases in the mixed culture compared to the mono-culture, i.e. 2.1 - 5.1-fold in the second exponential phase and 2.2-4.0-fold in stationary phase (the transcriptional activator *epsA* 2.0 and 1.9-fold). Similarly, in the *L. bulgaricus* (poly)saccharide metabolism many genes were higher expressed in mixed culture compared to mono-culture at the two later growth phases, including several genes in EPS synthesis, indicating that more EPS is produced in mixed cultures. Indeed, the amount of EPS increased from 0.80±0.02 g/L in a *S. thermophilus* mono-culture and 1.18±0.12 g/L in a *L. bulgaricus* mono-culture to 1.46±0.04 g/L in the mixed culture. However, this increase in EPS production in the mixed culture compared to the mono-cultures (1.3-1.8-fold) is less than would be expected based on the increase in total biomass (4.5-8.5-fold).

Fatty acid metabolism in *L. bulgaricus*. In the three later phases of fermentation, the genes encoding LCFA synthesis by *L. bulgaricus* were 3.3-9.6-fold lower expressed in mixed culture, while in the second exponential and the stationary phase *LBUL_0106* and *LBUL_1256* (both 1-acyl-sn-glycerol-3-phosphate acyltransferase) were 3.1 and 15-fold higher expressed in mixed culture, respectively (see Figure 3B). Therefore, it is likely that this acyltransferase is loaded with fatty acids from the medium in presence of *S. thermophilus*, e.g. liberated from milk fat by its lipolytic activity (33).

Biofilm studies

In order to explore whether the responses to mixed culture growth in milk identified above are generic, we studied interactions with cells residing in a different environment while growing on the same substrate. It has been well documented that cells growing in biofilms are in a physiological state very different from that during planctonic growth (17, 38). In addition, in mixed culture biofilms the high proximity and/or physical contact is likely to amplify interaction response and the phenotypic responses can be easily explored by staining and microscopy or SEM. A series of experiments was performed on mono and mixed cultures growing in biofilms on anopore placed on top of milk agar which aimed to explore the effect on growth as well as regulatory responses at the transcriptome level. Figures 4A-C

show SEM photographs of *S. thermophilus*, *L. bulgaricus* and mixed biofilms. In the mixed biofilm, *L. bulgaricus* cells in particular showed a remarkable increase in cell length compared to mono-culture biofilms. We also observed elongated cells of both species in mixed liquid cultures. This elongation is due to incomplete septation (21) as is exemplified by a transmission electron microscopy photograph of a *S. thermophilus* cell (see Figure 4D).

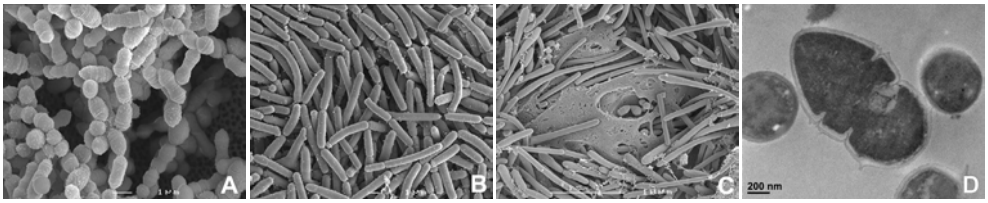


Figure 4. Minicolonies in biofilms grown on anopore slides placed on top of milk agar plates. A-C, SEM images of mono-cultures of *S. thermophilus* (A), *L. bulgaricus* (B) and the mixed culture (C). D, Cross section of a *S. thermophilus* cell displaying the characteristic ring pattern associated with incomplete septation.

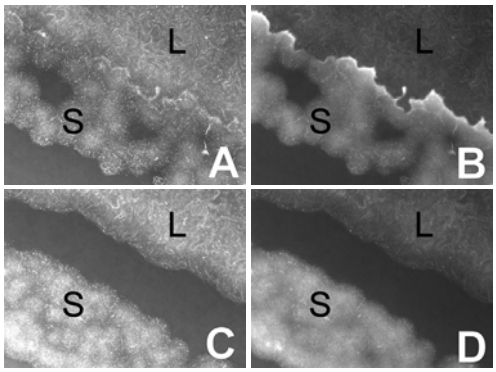


Figure 5. Minicolonies grown in biofilms on anopore slides on milk plates stained with SybrGold (A, C) and Wheat Germ Agglutin (WGA) (B, D). In A and B, colonies of *S. thermophilus* and *L. bulgaricus* are in physical contact leading to a higher staining of WGA at the interphase compared to C and D. S, *S. thermophilus*; L, *L. bulgaricus*.

In addition, there was a large quantity of extracellular matrix present in the mixed biofilm, which was only present at low levels in the mono-culture biofilms. It was hypothesized that this matrix mostly consist of EPS for the following 3 reasons: (i) it is specifically stained with Wheat Germ Agglutin (WGA), a lectin that is suitable for specific staining of EPS in yoghurt (18); (ii) if not protected by osmium tetroxide, the structure collapses as is typically observed with a highly hydrated biopolymer. (iii) Moreover, colonies of *S. thermophilus* and *L. bulgaricus*

in physical contact led to an increase of WGA stain at the interface relative to the interface of two colonies of the same species or to the colonies that are not in contact (see Figure 5). Additional support is provided by a transcription profiling study of mono-culture and mixed culture biofilms (data not shown). There was little differential expression between mono and mixed culture biofilms. However, most of the EPS biosynthesis genes were higher expressed in the mixed culture biofilm (1.2-3.4-fold for *S. thermophilus* and 2.0-5.1-fold for *L. bulgaricus*).

DISCUSSION

The growth pattern with the four distinct growth phases following the lag phase in our study was similar to what was described before (12, 19). Differences may be due to strain-to-strain variation and to our relative low inoculation level (10^4-10^5 CFU/mL in stead of 10^6 CFU/mL in the two cited references) that was chosen in order to follow the complete fermentation and to be able to identify when in the fermentation interactions between the bacteria become of importance, i.e. around 10^5-10^6. Growth and acidification was mainly stimulated by formic acid, folic acid, casitone (*S. thermophilus* only) and Tween-20. That is in accordance with literature (11, 13, 14, 16, 29).

The physiology of mixed culture fermentation and strain interaction was further explored with transcriptome analysis in mono and mixed cultures. Transcriptome analysis of *S. thermophilus* in mono (20) and in mixed culture (19) has been reported previously, but only at the second exponential phase. We analyzed gene expression related to the interactions between the yoghurt bacteria through the fermentation. Also the analysis of *L. bulgaricus* is a novel aspect of our study. Gene expression data are supported by the intervention studies and by physiological data (see below). We confirmed that interactions are based on the exchange of folic acid and purine (precursors) and on proteolysis. Moreover, we found that availability of LCFA to *L. bulgaricus* by lipolytic action of *S. thermophilus* is likely a novel mode of interaction and that the genes for EPS synthesis are induced in both strains in co-culture leading to a higher EPS content.

Formic acid and folic acid support growth of *L. bulgaricus*. The high abundance of PFL in *S. thermophilus* in milk and the stimulatory effect of formic acid and folic acid on acidification growth of both species show that purine availability in milk is too low to sustain optimal growth. The folic acid and purine biosynthesis pathways were very highly expressed in *L. bulgaricus* mono-culture, indicating that it was difficult to acquire sufficient purines. The down regulation of

these pathways in combination with the up regulation of PFL and folic acid production in *S. thermophilus* show that formic acid and folic acid were indeed provided by *S. thermophilus* for *L. bulgaricus*. These two components support *L. bulgaricus* growth already during the first exponential phase. The stimulatory effect of nucleobases is evident as they are the end products of purine (and pyrimidine) metabolism.

Amino acid and carbon dioxide metabolism. The proteolytic activity of *L. bulgaricus* plays a key role in the co-fermentation of *S. thermophilus* and *L. bulgaricus* as is exemplified by the large stimulatory effect of addition of hydrolyzed casein to a *S. thermophilus* culture and the smaller effect to a mixed culture. *L. bulgaricus* has a very degraded AA synthesis system, which makes the bacterium highly dependent on its proteolytic activity when growing in milk (45) both in mono-culture and in mixed culture. That may explain the relatively small number of changes in the expression of AA biosynthetic genes between mono-culture and mixed culture. The cleavage of casein into peptides by PrtB and the higher expression of peptidases in *S. thermophilus* support higher growth rates of both species in mixed culture. However, the upregulation of BCAA (present 6-7% w/w in casein), arginine (4%) and cysteine (0.35%) biosynthesis pathways shows that the proteolysis by PrtB did not supply a sufficient amount of these AA, as they are relatively highly abundant in the predicted proteins of *S. thermophilus* (19) and *L. bulgaricus* (http://www.cbs.dtu.dk/services/GenomeAtlas) compared to their content in casein (41). It was reported before that sulfur AA become limiting for growth of *S. thermophilus* in the second exponential phase in mono-culture (20) and BCAA in mixed culture as well (19). Our study confirms this observation for *S. thermophilus*. In addition, cysteine and methionine biosynthesis were overexpressed by both organisms in mixed culture indicating a shortage of these AA in mixed culture. This may also explain why BCAA and sulfur AA pathways remained functional in *L. bulgaricus* unlike many other AA biosynthetic pathways. The up regulation of the urea cycle and carbonate dehydratase shows that CO_2 production is stimulated in mixed culture which favors growth of *L. bulgaricus* (16).

Iron metabolism and H_2O_2 resistance. In stationary phase of the mixed culture, *dpr* and *fur* were higher expressed than in the *S. thermophilus* mono-culture. It was shown before that this relates to the expression of *pyrD1* and *pyrD2* and the concomitant production of H_2O_2 by *L. bulgaricus* LMG11842 (19). In our study differential expression of *fur* and *dpr* was comparable to the differential expression of pyruvate oxidase, *LBUL_2034*, in strain ATCC BAA-365. Thus, the intake of iron by *S. thermophilus* is negatively correlated to H_2O_2 production by *L. bulgaricus*, minimizing the damage caused by ROS.

EPS biosynthesis increases upon co-culture. Previously, it was found that EPS production increases in the late exponential and stationary phase in fermentation in mono-culture as well as in mixed culture, but no induction of expression of EPS related genes was observed (20), contradicting our results. We clearly observed the upregulation of genes coding for EPS biosynthesis and secretion in both *S. thermophilus* and in *L. bulgaricus* in liquid mixed culture compared to mono-culture. This was confirmed in biofilm growth using polysaccharide staining, SEM and transcriptome profiling. The higher EPS synthesis may be a direct response to the lower pH in the mixed species systems, since low pH was shown to trigger EPS production by *L. bulgaricus* (31). To date, there has not been assigned a biological function for EPS production by these microbes in yoghurt, but it is not unlikely that EPS causes increased acid resistance (28) or facilitates exchange of metabolites by forcing close proximities or even physical contact between the two species, as is the case in kefir (8, 35).

In conclusion, we have performed a gene expression study of the whole yoghurt system and provided evidence for the involvement of purine metabolism (formic acid and folic acid), proteolysis and LCFA in the interactions between the two consortium members. In addition, we showed that in mixed culture genes for EPS formation are induced leading to a higher EPS content of yoghurt. Finally, iron uptake by *S. thermophilus* is correlated to its growth rate, but decreased in the later stages of co-fermentation due to the increased production of H_2O_2 by *L. bulgaricus*. The stimulatory effect of pyruvic acid can be easily explained as this compound plays a central role in the primary metabolism and many other pathways but we found no evidence that the exchange of pyruvic acid is a mode of interaction. Putrescine and ornithine did not show any positive effect on acidification and outgrowth. Neither did we find any obvious differential regulation in transport or processing related to these two compounds between both mono-cultures and the mixed culture, nor any differential expression when putrescine was or was not added to the culture. However, it can not be excluded that there is exchange of these compounds despite the absence of regulatory responses at the RNA level.

Our study provides important information on the molecular basis of this mixed culture fermentation in an industrial or natural environment rather than a laboratory medium. These findings may help to understand other mixed culture systems and provide prospects for engineering interacting mixed cultures, for instance by introducing nutritional dependencies in dairy systems containing probiotics.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Free amino acid content (µM) of milk and three different cultures after 12 and 24 h of fermentation. ND, not determined, below detection limit.

	Time (h)	Asp	Glu	Asn	Gln	Ser	Thr	Gly	Arg	Ala	Pro	Val	Met	Ile	Leu	Trp	Phe	Cys	Lys	His	Tyr
Milk		61	361	1114	541	174	601	145	ND	132	31	ND	ND	19	14	5	10	354	35	24	12
S. thermophilus	12	219	389	1083	868	84	554	74	ND	47	61	30	ND	ND	36	ND	42	285	9	21	16
	24	161	301	1118	1006	339	438	118	49	53	75	44	ND	ND	49	ND	36	234	14	27	20
L. bulgaricus	12	291	633	ND	931	1555	286	960	55	319	486	426	ND	129	371	17	110	394	318	330	303
	24	136	632	ND	1122	ND	1447	ND	63	ND	532	378	ND	87	473	16	115	190	331	262	226
Mixed culture	12	108	456	2000	187	1096	1061	262	161	302	372	108	26	19	154	10	80	250	141	71	122
	24	603	1059	2196	115	584	626	98	170	146	741	342	55	152	266	ND	111	248	245	129	175

Supplementary Table 2. Significant (≥2-fold FDR≤0.05) differential expression of *S. thermophilus* genes between mixed and mono-cultures and between cultures supplemented with formic acid and cultures without any supplement, per functional category. Values represent ratios between the first and the second condition. Columns 3-6 contain ratios of mixed cultures over mono-cultures in the first exponential phase (3.5 h), reduced growth phase (5.5 h), second exponential phase (8 h) and stationary phase (12 h), respectively. Columns 7 and 8 contain ratios of a culture supplemented with formic acid over one without supplement in the early exponential phase and mid exponential phase, respectively.

		Mixed culture over mono-culture				Formic acid over none		
Locus ID	Locus	1st exp phase	Transition phase	2nd exp. phase	Stationary phase	1st exp. phase	2nd exp. phase	Product
Amino acid transport and metabolism								
str0023	prsA1			0.424	0.202	0.283		ribose-phosphate pyrophosphokinase
str0026	araT				0.317		2.099	hypothetical protein
str0063	pepS				0.392	0.483		aminopeptidase PepS
str0125	amiA2				0.253			oligopeptide ABC transporter substrate-binding protein
str0158	-				2.038			amino acid (glutamine) ABC transporter ATP-binding protein
str0159	-			3.273	2.047	0.405		amino acid (glutamine) ABC transporter substrate binding protein/permease protein
str0172	-			0.446			0.420	dipeptide/oligopeptide ABC trasporter permease protein, truncated
str0229	pepC				0.248			cysteine aminopeptidase C
str0283	ureC				0.416			urea amidohydrolase (urease) alpha subunit
str0296	-	0.361	0.056		0.082	6.942		amino acid ABC transporter substrate binding protein, putative
str0298	-		0.149		0.011			succinyl-diaminopimelic descuccinlyasadipeptidase, truncated
str0300	-		0.117		0.013	2.665		succinyl-diaminopimelic descuccinlyasadipeptidase, truncated
str0303	dctA	0.470	0.269		0.174	3.492		dicarboxylate/amino acid:cation (Na+ or H+) symporter
str0352	metB1	0.092	0.025		0.052	7.589		cystathionine gamma-synthase
str0353	-	0.111	0.045		0.117	7.405		aminotransferase (class II)
str0360	livH					0.477		branched-chain amino acid ABC transporter permease protein
str0366	cysM1	0.445	0.100		0.314	6.449		cysteine synthase
str0377	asnA				0.269			asparagine synthetase AsnA
str0430	gdhA				0.500			glutamate dehydrogenase
str0454	pepB				0.165			oligopeptidase
str0463	aspC3		0.113			7.519		hypothetical protein
str0464	argC		0.219				0.384	N-acetyl-gamma-glutamyl-phosphate reductase
str0466	argB		0.251				0.355	acetylglutamate kinase
str0467	argD				2.578			acetylornithine aminotransferase
str0469	hom			2.148				homoserine dehydrogenase
str0470	thrB					2.034		homoserine kinase
str0526	carA				2.121	0.445		carbamoyl-phosphate synthase small subunit
str0527	carB				2.145	0.428		carbamoyl-phosphate synthase large subunit
str0555	-	0.169	0.365	3.100		2.874		alanine dehydrogenase, truncated
str0556	-	0.319		2.301		8.274		alanine dehydrogenase, truncated
str0583	-	0.468			0.240	6.095		amino acid permease
str0584	mmuM	0.401	0.449		0.317	7.337		homocysteine methyltransferase
str0590	bcaT	2.012			0.304			branched-chain amino acid aminotransferase
str0629	pepQ				2.015			dipeptidase
str0645	aroA				2.798			3-phosphoshikimate 1-carboxyvinyltransferase
str0646	aroK				4.274			shikimate kinase
str0647	pheA				3.613			prephenate dehydratase
str0771	pabB				3.038			para-aminobenzoate synthetase component I
str0785	metE	0.036	0.016		0.019	8.497		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
str0786	metF	0.043	0.027		0.045	7.964		5,10-methylenetetrahydrofolate reductase
str0846	cysM2				0.415			cysteine synthase
str0877	-					0.367	0.321	amino acid (glutamine) ABC transporter, substrate binding protein
str0904	-					4.197		hypothetical protein
str0905	-	0.309	0.227			7.913		hypothetical protein
str0908	-	0.435	0.273			3.666		ABC transporter substrate binding protein
str0923	als			2.128	3.987			alpha-acetolactate synthase
str0970	dtpT		0.331		0.144		2.192	di-/tripeptide transporter
str0974	-			2.771	2.366	0.297		ABC transporter substrate binding protein, truncated
str0975	-			3.287	4.384			ABC transporter substrate binding protein, truncated
str0976	-			3.198	2.549			ABC transporter permease protein, truncated
str0977	-				3.949			ABC transporter permease protein, truncated
str0984	dagA			3.153		0.389	2.263	sodium/alanine glycine symporter
str0987	cysD	0.278	0.201	3.232	0.089	7.222	2.300	O-acetylhomoserine sulfhydrylase
str1007	pepN			2.374	0.451		2.050	lysyl-aminopeptidase, aminopeptidase N
str1061	-			0.353	2.485			hypothetical protein

str1127	pepV				0.489			dipeptidase
str1161	-						0.481	amino acid (glutamine) ABC transporter ATP-binding protein
str1163	-				2.379			amino acid (glutamine) ABC transporter permease protein
str1164	-				2.715			amino acid (glutamine) ABC transporter permease protein
str1181	aroH				0.306			hypothetical protein
str1203	leuA				0.333			2-isopropylmalate synthase
str1211	-				2.060			hypothetical protein
str1222	metA	0.284	0.263		0.130	2.066		homoserine O-succinyltransferase
str1287	-			2.523			3.974	transcriptional regulator, putative
str1297	dapA			2.506				dihydrodipicolinate synthase
str1298	asd				0.429			aspartate-semialdehyde dehydrogenase
str1309	proWX	0.372				18.333		proline/glycine betaine ABC transporter
str1310	proV	0.424		2.816		16.210		proline/glycine betaine ABC transporter ATP-binding protein
str1312	proWZ	0.468		2.173	0.291	29.876		proline/glycine betaine ABC transporter substrate-binding protein
str1317	sdaA				2.109			L-serine dehydratase alpha subunit
str1346	-		0.263					amino acid (glutamine) ABC transporter ATP-binding protein
str1347	-			0.353				ABC transporter amino acid permease protein
str1357	brnQ				0.217		2.850	branched chain amino acid transport system II carrier protein
str1389	-						4.027	amino-acid efflux protein, putative
str1438	amiF1	0.488	0.389	2.748		3.921		oligopeptide ABC transporter ATP-binding protein
str1439	amiE		0.229	2.451		2.141		oligopeptide ABC transporter ATP-binding protein
str1440	amiD	0.447	0.139	2.720		2.667		oligopeptide ABC transporter membrane-binding protein
str1441	amiC		0.301	2.784	0.393	3.075		oligopeptide ABC transporter membrane-binding protein
str1442	-				0.239			oligopeptide ABC transporter substrate-binding protein, truncated
str1443	-		0.438	2.350	0.423	2.564		oligopeptide ABC transporter substrate-binding protein, truncated
str1445	amiA3				0.323		2.127	oligopeptide ABC transporter substrate-binding protein
str1447	-		0.432	3.331		4.711		oligopeptide ABC transporter ATP-binding protein, truncated
str1448	-			0.440	0.249			oligopeptide ABC transporter ATP-binding protein, truncated
str1461	nifS3	2.109						aminotransferase (class V), putative
str1519	serB				4.050			phosphoserine phosphatase
str1527	serA			2.089	0.169		2.536	D-3-phosphoglycerate dehydrogenase
str1529	serC		0.373	2.445	0.196			phosphoserine aminotransferase
str1579	-	0.305	0.158	2.084	0.236	12.993		amino acid (glutamine) ABC transporter substrate binding protein
str1580	-	0.249	0.138		0.140	18.328		amino acid (glutamine) ABC transporter ATP-binding protein
str1581	-	0.263	0.158		0.208	13.125		amino acid (glutamine) ABC transporter permease protein
str1582	-	0.268	0.176		0.140	16.197		amino acid (glutamine) ABC transporter permease protein
str1589	trpF					23.120		N-(5'-phosphoribosyl)anthranilate isomerase
str1590	trpC					2.615		indole-3-glycerol-phosphate synthase
str1591	trpD					23.842		anthranilate phosphoribosyltransferase
str1592	trpG					9.455		anthranilate synthase component II
str1593	trpE					2.894		anthranilate synthase component I
str1636	aspC2				0.463			aspartate aminotransferase
str1652	-	0.461	0.425	2.091	0.405	2.352		ABC transporter ATP-binding protein, amino acid
str1653	-	0.467	0.423	2.354	0.489			ABC transporter amino acid permease protein
str1654	-		0.380	2.320	0.331			ABC transporter substrate-binding protein, amino acid
str1710	proA				0.180			gamma-glutamyl phosphate reductase
str1711	proB				0.192			gamma-glutamyl kinase
str1812	argH			0.218				argininosuccinate lyase
str1813	argG			0.445				argininosuccinate synthase
str1839	dapD				0.342			2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase, putative
str1871	ilvC				0.351		2.069	ketol-acid reductoisomerase
str1872	ilvN				0.279			acetolactate synthase small subunit
str1873	ilvB				0.231			acetolactate synthase large subunit
str1972	-				0.393			hypothetical protein
str1973	-				0.394	3.484		hypothetical protein
Carbohydrate transport and metabolism								
str0114	gpmB	2.969		0.487	0.487			phosphoglycerate mutase
str0189	-					0.376		PTS glucose-specific enzyme IIABC components, truncated
str0190	-				0.433			PTS glucose-specific enzyme IIABC components, truncated
str0191	-				0.422			PTS glucose-specific enzyme IIABC components, truncated
str0194	pgi				0.420			glucose-6-phosphate isomerase
str0219	-			2.855				transcriptional regulator, DeoR family
str0312	tkt				0.369			transketolase
str0399	fruR			0.475				transcriptional repressor
str0400	fruB			0.335				fructose-1-phosphate kinase
str0401	-			0.324				PTS fructose-specific enzyme IIABC components, truncated
str0405	-			0.307				PTS fructose-specific enzyme IIABC components, truncated
str0407	-		2.297	0.332				PTS fructose-specific enzyme IIABC components, truncated
str0488	tpiA				0.372			triosephosphate isomerase
str0501	nagA				0.498			N-acetylglucosamine-6-phosphate deacetylase
str0512	-					0.377	0.314	beta-glucoside-specific PTS system IIABC component, truncated
str0513	-						0.357	beta-glucoside-specific PTS system IIABC component, truncated
str0541	nagB				0.448			glucosamine-6-phosphate isomerase
str0635	eno		2.230	0.458				phosphopyruvate hydratase
str0697	gpmC		2.020		7.232			phosphoglycerate mutase
str0895	-				10.016			transcriptional regulator
str0999	suhB			2.082				inositol monophosphatase family protein
str1012	glgP				2.175	0.413		glycogen phosphorylase
str1017	-						0.500	maltose/maltodextrin ABC transporter permease protein, truncated
str1069	-						3.862	hypothetical protein
str1077	epsL			2.629				exopolysaccharide polymerization protein
str1120	deoB	0.450						phosphopentomutase
str1121	rpiA	0.437						ribose-5-phosphate isomerase A
str1204	gpmA				0.250		2.348	phosphoglyceromutase
str1230	-				2.514			hypothetical protein
str1251	-				2.914			phospho-sugar mutase, putative
str1265	ptsH		0.471					phosphocarrier protein HPr (histidine-containing protein)
str1358	-				0.353			mannose-6-phosphate isomerase, truncated
str1359	-			0.482	0.366			mannose-6-phosphate isomerase, truncated
str1398	lacS			0.437			2.185	sodium:beta-glucoside symporter
str1457	ppnK			3.367	2.705			inorganic polyphosphate/ATP-NAD kinase
str1469	rgpD	0.455		3.622				polysaccharide ABC transporter ATP-binding protein
str1470	rgpC	0.364		4.327				polysaccharide ABC transporter membrane-spanning protein
str1610	hit	7.289	2.048					cell cycle regulation histidine triad (HIT) protein
str1732	pmi1						0.390	mannose-6-phosphate isomerase
str1733	scrK			0.484		0.417	0.465	fructokinase
str1734	scrA			0.498		0.480		sucrose-specific PTS permease, enzyme II
str1788	gapA1				0.498			glyceraldehyde-3-phosphate dehydrogenase
str1797	rpe			0.450				ribulose-phosphate 3-epimerase
str1851	pepA	2.998			0.398			glutamyl-aminopeptidase
Cell division and chromosome partitioning								
str1109	epsD				2.224			exopolysaccharide biosynthesis protein
str1144	ftsX				0.488			cell division ABC transporter, permease
str1295	smc			3.334	4.901			chromosome segregation SMC protein
str1520	ezrA				3.215			septation ring formation regulator EzrA
Cell envelope biogenesis, outer membrane								
str0020	mreC				0.325	0.373		rod shape-determining protein MreC
str0078	-		2.062			0.456		D-alanyl-D-alanine carboxypeptidase, truncated
str0079	-	2.646	2.608					D-alanyl-D-alanine carboxypeptidase, truncated
str0110	-			0.396	0.272			hypothetical protein
str0128	cfa					0.455		cyclopropane-fatty-acyl-phospholipid synthase

str0131	-				0.363			mechanosensitive ion channel, putative
str0163	rgpG				0.193			polysaccharide biosynthesis protein
str0349	murE	2.269	2.484		4.340			UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase
str0563	gcaD			2.064				UDP-N-acetylglucosamine pyrophosphorylase
str0569	-				0.451			glycosyl transferase, family 1
str0636	-			3.109	6.616			hypothetical protein
str0699	-				4.189			endolysin, putative
str0721	-				4.380			hypothetical protein
str0733	divIB				2.539			cell division protein
str0762	dltB			2.551	2.312			integral membrane protein
str0764	dltD			2.119	2.627			extramembranal protein
str0873	glmS			2.988			4.304	D-fructose-6-phosphate amidotransferase
str1046	-			0.357			0.253	endolysin, putative, truncated
str1073	epsX				0.075			lipopolysaccharide 1,6-galactosyltransferase
str1077	epsL			2.629				exopolysaccharide polymerization protein
str1080	epsI			4.044	3.657			exopolysaccharide biosynthesis protein, sugar transferase
str1082	epsG	0.456		5.117	3.457			exopolysaccharide biosynthesis protein, glycosyltransferase
str1083	-			2.507	2.470			exopolysaccharide biosynthesis protein, UDP-galactopyranose mutase, truncated
str1085	epsE			2.178				exopolysaccharide biosynthesis protein
str1167	murA				0.401			UDP-N-acetylglucosamine 1-carboxyvinyltransferase
str1242	rmlB				3.221			dTDP-glucose-4,6-dehydratase
str1297	dapA			2.506				dihydrodipicolinate synthase
str1301	-						2.110	phosphinothricin acetyltransferase, putative
str1311	proWY	0.401	0.392	2.415		15.697		proline/glycine betaine ABC transporter membrane-spanning protein
str1400	galE1				0.477			UDP-glucose 4-epimerase
str1413	-	5.956					2.766	putative glutamate--cysteine ligase
str1467	rgpF			2.689				polysaccharide biosynthesis protein
str1468	rgpE	0.478		4.034				polysaccharide biosynthesis protein/putative glycosyltransferase
str1469	rgpD	0.455		3.622				polysaccharide ABC transporter ATP-binding protein
str1470	rgpC	0.364		4.327				polysaccharide ABC transporter membrane-spanning protein
str1471	rgpB			2.627				polysaccharide biosynthesis protein/putative rhamnosyl transferase
str1472	rgpA			3.881				polysaccharide biosynthesis protein/putative rhamnosyl transferase
str1476	-			2.267				glycosyl transferase
str1478	-	0.486		2.508				glycosyl transferase, truncated
str1479	-	0.493		6.948				glycosyl transferase
str1485	-			2.031				glycosyl transferase, family 2
str1560	murZ				0.347	0.485		UDP-N-acetylglucosamine 1-carboxyvinyltransferase
str1576	murF			3.757				UDP-N-acetylmuramoylalanine-D-glutamyl-lysine--D-alanyl-D-alanine ligase
str1607	-				0.486			hypothetical protein
str1629	-	0.272				8.226		hypothetical protein
str1869	pbp1B				0.350			penicillin-binding protein 1B
str2006	-				0.028			conserved hypothetical protein, LysM domain protein
Cell motility and secretion								
str0356	clpP					0.386		ATP-dependent Clp protease proteolytic subunit
str0722	pilD			0.335				prepilin peptidase type IV
str0889	ffh						3.346	signal recognition particle
str0896	dprA			0.339	3.075			DNA processing protein, Smf family
str1730	secA			2.020				translocase
str1860	-			0.222				competence protein, putative
str1862	comGD	2.599		0.174				competence protein
str1863	comGC			0.180				late competence protein, exogenous DNA-binding protein
str1864	comGB			0.168				late competence protein, ABC transporter subunit
str1865	comGA	0.348		0.054	0.237			late competence protein, ABC transporter subunit
str1914	secY			2.036				preprotein translocase SecY
Coenzyme metabolism								
str0092	panE		0.337		0.116			2-dehydropantoate 2-reductase (ketopantoate reductase)
str0123	thiD				2.113			phosphomethylpyrimidine kinase
str0226	pncB				0.398			nicotinate phosphoribosyltransferase
str0227	nadE	2.105			0.434			NAD(+) synthetase
str0409	folC1	3.888			3.675		0.439	folylpolyglutamate synthase / dihydrofolate synthase
str0545	-				0.273		7.274	molybdopterin biosynthesis protein (HesA/MoeB/ThiF family protein), putative, truncated
str0590	bcaT	2.012			0.304			branched-chain amino acid aminotransferase
str0771	pabB				3.038			para-aminobenzoate synthetase component I
str0789	dfp1	2.110						phosphopantothenoylcysteine synthase/decarboxylase
str0790	dfp2	2.124						hypothetical protein
str0826	ptpS			2.044	0.215		2.600	6-pyruvoyl tetrahydrobiopterin synthase, putative
str0923	als			2.128	3.987			alpha-acetolactate synthase
str1009	lplA			0.230	2.122			lipoate-protein ligase
str1172	metK	0.404			0.056			S-adenosylmethionine synthetase
str1284	pdxK				0.195			pyridoxine kinase
str1413	-	5.956					2.766	putative glutamate--cysteine ligase
str1527	serA			2.089	0.169		2.536	D-3-phosphoglycerate dehydrogenase
str1529	serC		0.373	2.445	0.196			phosphoserine aminotransferase
str1592	trpG					9.455		anthranilate synthase component II
str1593	trpE					2.894		anthranilate synthase component I
str1871	ilvC				0.351		2.069	ketol-acid reductoisomerase
str1873	ilvB				0.231			acetolactate synthase large subunit
str1944	mccB					3.726	3.868	bacteriocin biosynthesis protein, putative
Defense mechanisms								
str0009	-			0.453				hypothetical protein
str0071	-					2.533	5.179	bacteriocin self-immunity protein, putative, truncated
str0072	-		0.310		0.260		8.024	bacteriocin self-immunity protein, putative, truncated
str0272	-				0.304			peptide ABC transporter ATP binding/permease protein, truncated
str0273	-				0.391			peptide ABC transporter ATP binding/permease protein, truncated
str0324	-			0.387			8.181	peptide ABC transporter ATP binding/permease protein, putative
str0539	-			4.012				ABC transporter ATP binding/permease protein
str0546	-				0.411		5.256	ABC transporter ATP binding protein
str0708	hsdS1				3.145	4.408	0.378	type I restriction-modification system specificty subunit
str0711	hsdM1				4.882			type I restriction-modification system methyltransferase subunit
str0759	-			3.575				ABC transporter ATP-binding/permease protein
str0927	-				2.657			ABC transporter permease protein
str0991	hsdS2					2.749		type I restriction-modification system specificty subunit
str1155	murM				2.492			peptidoglycan branched peptide synthesis protein, alanine adding enzyme
str1328	-		3.858	0.270	2.991	0.122	0.356	ABC transporter ATP binding protein
str1342	norM					5.864		MATE efflux family protein (Na(+)/drug antiporter)
str1411	-			2.338		0.460		ABC transporter, truncated
str1436	-			2.277	2.112			ABC transporter ATP binding protein
str1609	-				0.435			ABC transporter ATP binding protein
str1660	-				2.025	0.120		type I restriction-modification system specificty subunit, truncated
str1693	-				2.449			ABC transporter ATP-binding/permease protein
str1694	-					0.460	0.490	ABC transporter ATP-binding/permease protein
str1713	-				0.381			ABC transporter ATP binding protein, truncated
str1952	-				0.201			ABC transporter ATP binding/permease protein
DNA replication, recombination and repair								
str0001	dnaA			0.430				chromosomal replication initiation protein
str0006	trcF					0.489		transcription repair coupling factor
str0016	-				0.390			IS861, transposase (orf1), IS3 family, truncated
str0018	-				0.423			IS861, transposase (orf2), IS3 family, truncated
str0019	-				0.444			IS861, transposase (orf2), IS3 family, truncated
str0027	recO				0.405		2.121	DNA repair protein RecO

str0036	-			0.454				IS861, transposase (orf1), IS3 family, truncated
str0056	ruvA				0.442			Holliday junction DNA helicase motor protein
str0060	recA				0.382			recombinase A
str0062	polC			3.019		0.296		DNA polymerase III subunit alpha
str0067	-			0.474	0.432			ISSpn1, transposase, IS3 family, truncated
str0069	-				0.394			ISSpn1, transposase, IS3 family, truncated
str0105	tnp1239				0.394		2.206	IS1239 transposase
str0107	-		2.028					truncated IS1216 transposase
str0126	-				0.434			truncated IS1193 transposase
str0206	-			0.464	0.353			IS861, transposase (orf2), IS3 family, truncated
str0207	-				0.353			IS861, transposase (orf2), IS3 family, truncated
str0223	tnp1239				0.492			IS1239 transposase
str0234	-			2.033				hypothetical protein
str0244	-				0.398			ISSpn1, transposase, IS3 family, truncated
str0259	xer2				4.244			tyrosine recombinase
str0320	dnaB				0.330			chromosome replication initiation / membrane attachment protein DnaB
str0321	dnaI				0.445			primosomal protein DnaI
str0368	comFA				2.482		0.376	late competence protein required for DNA uptake
str0490	dnaH			0.491	0.477			DNA polymerase III subunit delta
str0588	parE			2.701				DNA topoisomerase IV subunit B
str0589	parC			3.330	4.738	0.428		DNA topoisomerase IV subunit A
str0601	-	2.531						conserved hypothetical protein, MutT/nudix family protein
str0614	recR			2.501		0.314		recombination protein RecR
str0658	-				0.356			hypothetical protein
str0679	-				2.898			MutT/nudix family protein
str0742	-					127.988		truncated IS1193 transposase
str0743	-				0.444			truncated IS1193 transposase
str0765	-				0.444			IS861, transposase (orf2), IS3 family, truncated
str0783	int2				0.330			integrase/recombinase, phage associated
str0813	dinG	0.459		2.941				ATP-dependent DNA helicase
str0822	-				0.417			truncated IS1193 transposase
str0841	-			0.365				truncated IS1191 transposase
str0849	tnp1191			0.412				IS1191 transposase
str0879	-						0.344	SOS responce UmuC protein, truncated
str0885	sthIM			5.974	5.172			type III restriction-modification system methylation subunit
str0894	rnhB				5.545			ribonuclease HII
str0896	dprA			0.339	3.075			DNA processing protein, Smf family
str0897	topA			2.948	3.771			DNA topoisomerase I
str0956	-				0.355			hypothetical protein
str0986	pcrA			2.036				ATP-dependent DNA helicase
str1035	-			0.458				truncated IS1191 transposase
str1045	tnp1193				0.493			IS1193 transposase
str1074	-				0.339			truncated IS1216 transposase
str1126	-	0.406						hypothetical protein
str1198	dnaE			2.156	3.573			DNA polymerase III subunit alpha
str1213	recN				2.044			DNA repair and genetic recombination protein
str1220	nth	0.259	0.303		0.260	3.137		endonuclease III, DNA repair
str1221	dnaD	0.223	0.329		0.199	3.214		DNA replication protein dnaD
str1224	recJ			2.222				single strand DNA-specific exonuclease
str1300	alkD		2.234					DNA alkylation repair protein
str1304	-				0.355			truncated IS1193 transposase
str1385	umuC1			2.900			0.104	SOS responce UmuC protein
str1394	sbcC		2.851		4.240			ATP-dependent dsDNA exonuclease
str1395	sbcD			0.439				ATP-dependent dsDNA exonuclease
str1425	pknB			2.757				protein kinase
str1449	-			0.448	0.195			hypothetical protein
str1489	dnaG			2.384				DNA primase
str1496	-				0.451			MutT/nudix family protein
str1521	gyrB				3.237			DNA gyrase subunit B
str1550	exoA	2.539						3'-exo-deoxyribonuclease
str1567	-				2.796	0.434		hypothetical protein
str1568	rheA			2.454				ATP-dependent RNA helicase
str1601	dnaQ			0.314		0.313		DNA polymerase III subunit epsilon
str1638	-	3.762		0.166	2.414			ISSth3, transposase, IS30 family, truncated
str1656	dinP					4.585		DNA polymerase IV
str1699	rheB		2.174					ATP-dependent RNA helicase
str1725	recG						0.483	ATP-dependent DNA helicase
str1753	ssbB			2.584	0.328		2.392	single-strand DNA-binding protein
str1757	mutY	2.824						A/G-specific adenine glycosylase
str1762	mutS2	2.529	2.568	2.312	2.220			DNA mismatch repair protein
str1765	rnhB			2.047				ribonuclease HIII
str1767	recD		2.933	2.955				exodeoxyribonuclease V
str1783	-				0.426			IS657, transposase, IS200 family, truncated
str1784	-				0.466			IS861, transposase (orf1), IS3 family, truncated
str1807	tnp657				0.419	0.215		IS657, transposase, IS200 family
str1845	ssbA		0.458		0.273	10.252		single-strand DNA-binding protein
str1897	-			0.474				ISSth4, transposase, IS30 family, truncated
str1904	-			0.353				ISSth4, transposase, IS30 family, truncated
str1945	-				0.493			truncated IS1191 transposase
str1948	-				0.329			hypothetical protein
str1957	cshA			0.486				chromosome segregation helicase
str1988	-				0.396	0.429		conserved hypothetical protein, MutT/nudix family
str1999	holB				0.470			replicative DNA helicase
str2015	recF			2.036				recombination protein F
Energy production and conversion								
str0246	acyP	3.406						acylphosphatase
str0478	atpE				0.112			proton-translocating ATPase, c subunit
str0480	atpF				0.198			ATP synthase subunit B
str0481	atpH				0.249			ATP synthase subunit D
str0482	atpA				0.399			ATP synthase subunit A
str0557	-	2.525		0.309	2.436			pyridine nucleotide-disulfide oxidoreductase
str0562	idi				2.882		0.338	isopentenyl pyrophosphate isomerase
str0644	hdhL				3.233			L-2-hydroxyisocaproate dehydrogenase
str1049	acoC				0.491			dihydrolipoamide acetyltransferase
str1050	acoB				0.268			acetoin dehydrogenase complex, E1 component, beta subunit
str1051	acoA			0.456	0.261			acetoin dehydrogenase complex, E1 component, alpha subunit
str1149	nfrA	3.210				0.224	0.204	nitro/flavin reductase
str1266	icd			0.412				isocitrate dehydrogenase
str1280	ldh				0.423			L-lactate dehydrogenase
str1353	-			0.343	0.415			glycerol dehydrogenase, truncated
str1369	-	2.270			0.240			NAD(P)H nitroreductase, putative
str1455	pta				2.180			phosphate acetyltransferase
str1657	pfl	3.035				0.265	2.189	pyruvate formate-lyase
str1803	trxA1				0.129			thioredoxin
str1805	bta				0.277			bacteriocin transport accessory protein, putative
str1849	trxA2				0.340			thioredoxin
str1857	ackA			0.151				acetate kinase
str1879	-			0.264	0.479			alcohol-acetaldehyde dehydrogenase, truncated
str1881	-			0.184	0.323			alcohol-acetaldehyde dehydrogenase, truncated
str1882	-			0.225	0.425			alcohol-acetaldehyde dehydrogenase, truncated
str1884	-	5.783		0.276	0.351	0.263		alcohol-acetaldehyde dehydrogenase, truncated

Function unknown								
str0022	pcsB				0.095	0.398		glucan binding protein
str0064	-				0.301			hypothetical protein
str0113	-				0.241		2.223	hypothetical protein
str0124	-				2.424			hypothetical protein
str0260	scpA				6.205			segregation and condensation protein A
str0268	-			4.060		0.282		hypothetical protein
str0276	-				0.118			membrane protein
str0306	-	0.382	0.208	2.008	0.165	4.699		hypothetical protein
str0340	-			2.176				hypothetical protein
str0491	sip			0.485	0.430			signal-petidase-like protein
str0492	-				0.407			hypothetical protein
str0504	-				0.459			hypothetical protein, truncated
str0552	-				0.136	3.069		hypothetical protein
str0595	-					0.500		hypothetical protein
str0632	-			0.484				hypothetical protein
str0634	-	2.374	2.351		2.283			hypothetical protein
str0643	-				3.300			hypothetical protein
str0652	mip			3.699		24.134		macrophage infectivity potentiator-related protein, putative
str0657	-				0.231			hypothetical protein
str0661	-	3.089						hypothetical protein
str0673	-		2.817		2.615			hypothetical protein
str0676	-			0.496				hypothetical protein
str0698	-		2.158		6.101	0.470		transcriptional regulator
str0884	sthIR			2.535	9.595			DNA endonuclease, type III restriction and modification system
str1063	-			0.221				hypothetical protein
str1157	-				4.334			hypothetical protein
str1248	-			0.434				hypothetical protein
str1252	-				2.431			hypothetical protein
str1256	-				3.261			hypothetical protein
str1377	-	2.025						hypothetical protein
str1422	-			2.457		0.426		conserved hypothetical protein, putative transporter
str1425	pknB			2.757				protein kinase
str1466	-			2.535				hypothetical protein
str1506	-				0.384			hypothetical protein
str1551	-			0.438				hypothetical protein, citrulline cluster-linked gene
str1723	-				0.381			conserved hypothetical protein, Cof family
str1961	-				0.361			hypothetical protein
str1967	-					0.499		hypothetical protein
str1980	-				0.343			hypothetical protein
str1982	-						0.494	hypothetical protein
str1993	-				0.206	0.358		hypothetical protein
str1996	-				0.358	0.313		hypothetical protein
str2014	-			2.063	0.415	0.394		hypothetical protein
str2019	-				0.416			hypothetical protein
str2023	-				0.349	2.633		hypothetical protein
General function prediction only								
str0022	pcsB				0.095	0.398		glucan binding protein
str0059	cinA				0.365	0.384		competence damage-inducible protein A
str0066	-					0.476		hypothetical protein
str0188	-				0.270	0.357		ABC transporter permease protein, putative malate permease
str0193	-				0.435			hypothetical protein
str0217	-				2.829			hypothetical protein
str0218	-				2.985			hypothetical protein
str0231	recU			2.026	0.433			hypothetical protein
str0248	-			0.405				conserved hypothetical protein, putative hydrolase
str0249	-			0.378				conserved hypothetical protein, membrane protein
str0257	-				3.826			hypothetical protein
str0258	-				4.330			hypothetical protein
str0316	-				0.191			hypothetical protein
str0334	-	0.082	0.147		0.202	34.356		hypothetical protein
str0335	-	2.179						conserved hypothetical protein, Cof family
str0336	-						2.711	conserved hypothetical protein, xanthine/uracil permease family
str0337	-					0.483		hypothetical protein
str0346	-			2.118				hypothetical protein, truncated
str0347	-			2.296	2.268			hypothetical protein, truncated
str0364	-	2.210			2.464			acetoin utilization protein, truncated
str0369	comFC					0.258		late competence protein
str0375	recX				2.466			RecA regulator RecX
str0379	-	2.499						beta-phosphoglucomutase, putative
str0443	-				0.137	0.341		glucan binding protein
str0451	metG				0.365			methionine--tRNA ligase
str0453	coiA				0.221			competence protein, transcription factor
str0455	-			2.029	0.431			methyltransferase, putative
str0462	-	0.290	0.188			4.264		hypothetical protein
str0474	-			2.222	2.739			hypothetical protein
str0493	-	3.480		0.298	0.266			tetrapyrrole methylase family protein
str0554	hipO1	0.227	0.291	3.927	0.203	4.536		aminoacylase/N-acyl-L-amino acid amidohydrolase/hippurate hydrolase
str0601	-	2.531						conserved hypothetical protein, MutT/nudix family protein
str0679	-				2.898			MutT/nudix family protein
str0696	-				3.211	0.451		hypothetical protein
str0704	-	2.975			2.658			oxidoreductase, short chain dehydrogenase/reductase family
str0748	-	5.745						conserved hypothetical protein, DHH subfamily
str0825	-	0.381	0.479	2.222	0.285		3.038	hypothetical protein
str0828	-		0.385	2.197	0.326		2.874	hypothetical protein
str0860	adcA				0.202		6.875	zinc ABC transporter substrate binding protein
str0880	-				3.419		0.432	alcohol dehydrogenase I, truncated
str0881	-			0.319	2.038		0.296	alcohol dehydrogenase I, truncated
str0882	-				2.051			alcohol dehydrogenase I, truncated
str0893	-				4.926			GTP-binding protein
str0909	-	0.380	0.432			4.592		acetoin reductase, truncated
str0910	-			2.767		10.053		acetoin reductase, truncated
str0929	-				0.226			oxidoreductase, short chain dehydrogenase/reductase family
str0937	-				4.179			hypothetical protein, truncated
str0938	-	4.074	2.834	2.922	8.925		0.469	hypothetical protein, truncated
str0942	-				0.294		2.483	phosphatase, putative
str0983	thdF	3.407						tRNA modification GTPase
str0988	-	0.200	0.281	4.644	0.108	6.769		hypothetical protein
str0989	-	0.212	0.255	4.784	0.185	5.930		hypothetical protein
str1060	-			0.119			0.099	conserved hypothetical protein, putative phosphoesterase
str1062	-			0.241				transporter, putative
str1068	-				0.217			hypothetical protein
str1076	epsM	0.436		2.418	4.000		2.856	exopolysaccharide biosynthesis protein
str1081	epsH			3.515	2.287			exopolysaccharide biosynthesis protein, acetyltransferase
str1142	-				0.431			hypothetical protein
str1152	-				6.513			conserved hypothetical protein, acetyltransferase, truncated
str1158	vicX				2.342			hypothetical protein
str1186	estA			2.753				esterase
str1189	-			0.316	0.410			ABC transporter permease protein
str1190	-			2.807				ABC transporter substrate binding protein
str1225	-			2.286				oxidoreductase, short chain dehydrogenase/reductase family
str1226	elaC				0.464			ribonuclease Z
str1228	hflX				0.262			GTP-binding protein

str1255	cobQ	2.017						cobyric acid synthase
str1257	-				2.345			hypothetical protein
str1259	-	0.450			2.407			ABC transporter ATP binding protein
str1282	-						2.493	ABC transporter ATP binding protein
str1292	pheT			2.178				phenylalanyl-tRNA synthetase beta subunit
str1307	-					6.168		hydrolase, haloacid dehalogenase-like family
str1308	bioY2						0.197	biotin synthase
str1319	-			0.478				ABC transporter permease protein
str1320	-			0.353				hypothetical protein
str1321	-				0.264			hypothetical protein
str1324	-			0.217				ABC transporter ATP binding protein, truncated
str1330	-		0.369					Na+-dependent transporter, putative
str1354	hipO2	0.210			0.291			aminoacylase/N-acyl-L-amino acid amidohydrolase/hippurate hydrolase
str1365	-			0.164	0.397			hypothetical protein
str1371	-				0.188			oxidoreductase, aldo/keto reductase family
str1425	pknB			2.757				protein kinase
str1464	-		2.130	3.220	2.471			glutamine amidotransferase (class I), putative
str1480	-			3.039	0.330			polysaccharide/teichoic acid transporter, putative
str1496	-				0.451			MutT/nudix family protein
str1522	-				2.083			hydrolase, haloacid dehalogenase-like family
str1566	-	3.679	3.507	2.108	3.248			hypothetical protein
str1605	-				2.125			hypothetical protein
str1610	hit	7.289	2.048					cell cycle regulation histidine triad (HIT) protein
str1621	-	2.027						hypothetical protein
str1641	hlyX				0.105			hemolysin, putative
str1665	-					0.497		hypothetical protein
str1666	-			2.046				ABC transporter ATP binding protein
str1696	-					0.461		hypothetical protein
str1715	-			0.359				hypothetical protein
str1723	-				0.381			conserved hypothetical protein, Cof family
str1775	-				0.497	0.447		glucan-binding protein
str1802	-				0.173			hypothetical protein
str1809	jag			2.008				conserved hypothetical protein, Jag protein
str1840	-			0.440			0.411	hypothetical protein
str1847	-		0.290		0.168	7.326		permease, putative
str1848	-				0.337			phenylalanyl-tRNA synthetase homolog
str1855	-			0.306				hypothetical protein, truncated
str1875	-				0.465			hypothetical protein
str1948	-				0.329			hypothetical protein
str1951	-				0.302			hypothetical protein
str1965	-			2.814			2.381	acetyltransferase, GNAT family
str1978	-				0.459			hypothetical protein
str1979	-				0.351			hypothetical protein
str1988	-				0.396	0.429		conserved hypothetical protein, MutT/nudix family
str2012	-				0.433			peptidase
str2013	-				0.254			protease, putative
str2016	guaB				0.237			inositol-5-monophosphate dehydrogenase
Inorganic ion transport and metabolism								
str0061	spxA				0.202	0.344		transcriptional regulator Spx
str0172	-			0.446			0.420	dipeptide/oligopeptide ABC trasporter permease protein, truncated
str0187	adcB	0.200	0.356	0.208	0.308			zinc ABC transporter permease protein
str0265	trkA1			2.361				potassium Trk transporter NAD+ binding protein
str0288	cbiM		0.439					cobalt transport protein CbiM
str0289	cbiQ		0.479					cobalt ABC transporter permease protein
str0297	-		0.146		0.009	2.081		ABC transporter substrate binding protein
str0301	-	0.420	0.065		0.020	3.504		ABC transporter ATP binding protein
str0302	-	0.468	0.069		0.026	5.868		ABC transporter permease protein
str0308	-	0.304	0.200		0.217	3.809		ABC transporter ATP binding protein
str0310	-	0.234	0.111		0.195	11.303		ABC transporter permease protein
str0351	-				0.461			cation transporter P-type ATPase, truncated
str0608	feoB				0.418			ferrous ion transport protein B
str0677	mntH				0.344			manganese transport protein, NRAMP family
str0723	dpr			0.349				peroxide resistance protein,non-heme iron-containing ferritin
str0724	fur			0.419				ferric transport regulator protein
str0745	-	2.146			0.210		11.473	hypothetical protein
str0840	copB	2.144		0.416				cation transporting ATPase, copper transport
str0860	adcA				0.202		6.875	zinc ABC transporter substrate binding protein
str0874	phnA			2.661			2.371	conserved hypothetical protein, PhnA protein
str0932	-				2.277			cation efflux protein
str0943	-				0.353			ferrichrome ABC transporter, substrate-binding protein, truncated
str1002	pstC1			2.201				phosphate ABC transporter permease protein
str1003	pstC2			2.090	2.209			phosphate ABC transporter permease protein
str1004	pstB1			2.349	2.542			phosphate ABC transporter ATP-binding protein
str1005	pstB2				3.322			phosphate ABC transporter ATP-binding protein
str1006	phoU				2.603			phosphate uptake regulatory protein
str1023	-						4.030	hypothetical protein
str1025	fatB			4.336			3.752	ferrichrome ABC transporter, substrate-binding protein
str1026	fatA			4.513			3.694	ferrichrome ABC transporter, ATP-binding protein
str1027	fatC			3.941	0.338		3.235	ferrichrome ABC transporter, permease protein
str1028	fatD			2.737	0.088			ferrichrome ABC transporter, permease protein
str1056	-			2.067				cation efflux protein
str1116	-	0.364		2.206	2.329			chloride channel protein, truncated
str1150	pacL2				4.464			calcium transporter P-type ATPase
str1439	amiE		0.229	2.451		2.141		oligopeptide ABC transporter ATP-binding protein
str1440	amiD	0.447	0.139	2.720		2.667		oligopeptide ABC transporter membrane-binding protein
str1441	amiC		0.301	2.784	0.393	3.075		oligopeptide ABC transporter membrane-binding protein
str1569	corA1	5.627		0.299				cation transporter CorA family
str1584	copZ			0.462				copper chaperone - copper transport ATPase
str1585	copA			0.479	0.357			cation transporting ATPase, copper transport
str1596	ctpE					0.453		cation transporting ATPase
str1633	-	0.213	0.448		0.449	7.271		ABC transporter substrate binding protein
str1749	corA2			2.405				cation transporter CorA family
Lipid metabolism								
str0028	plsX			0.443	0.395			fatty acid/phospholipid synthesis protein
str0029	acpP1	22.301		0.476	0.367			acyl carrier protein
str0269	-			3.327		0.226		conserved hypothetical protein, PAP2 family
str0384	acpP2				0.470			acyl carrier protein
str0390	fabZ			2.020				(3R)-hydroxymyristoyl ACP dehydratase
str0560	mvaD		2.267		4.482			mevalonate pyrophosphate decarboxylase
str0561	mvaK2		2.044		2.423			phosphomevalonate kinase
str0576	mvaA	2.894	2.737	2.062	2.037			3-hydroxy-3-methylglutaryl-coenzyme A reductase
str0651	-		0.437	2.107		18.932		hypothetical protein, acyl-CoA dehydrogenase family
str0763	dltC				2.281			D-alanine--poly(phosphoribitol) ligase subunit 2
str0909	-	0.380	0.432			4.592		acetoin reductase, truncated
str0910	-			2.767		10.053		acetoin reductase, truncated
str0929	-				0.226			oxidoreductase, short chain dehydrogenase/reductase family
str1563	-						2.360	1-acyl-sn-glycerol-3-phosphate acyltransferase, putative
str1727	acpS				2.261			4'-phosphopantetheinyl transferase
str1978	-				0.459			hypothetical protein
str1979	-				0.351			hypothetical protein
str2010	pgsA				0.461			CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase

Not in COGs								
str0005	pth					0.414		peptidyl-tRNA hydrolase
str0017	-			0.464	0.401			IS861, transposase (orf1), IS3 family, truncated
str0021	mreD			2.118	0.340		2.015	rod shape-determining protein MreD
str0030	purC	2.742		0.271	0.098	0.318	3.644	phosphoribosylaminoimidazole-succinocarboxamide synthase
str0034	purN				0.480	0.483	2.288	phosphoribosylglycinamide formyltransferase
str0035	purH		2.176	0.473		0.471		bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
str0038	-				0.358			IS861, transposase (orf2), IS3 family, truncated
str0043	-	2.382		0.468	0.204		2.457	hypothetical protein
str0044	-	2.446		0.493	0.347		2.469	hypothetical protein
str0057	-				0.466			3-methyladenine DNA glycosylase I, truncated
str0058	-				0.467			3-methyladenine DNA glycosylase I, truncated
str0073	rpsB			2.472				30S ribosomal protein S2
str0082	-				0.466			hypothetical protein
str0089	-					0.494		hypothetical protein
str0093	rplM				0.314			50S ribosomal protein L13
str0094	rpsI				0.264			30S ribosomal protein S9
str0095	-				0.364			Integrase/recombinase, putative, truncated
str0097	-	2.912			0.224			hypothetical protein, putative bacteriocin
str0098	-				0.394			lantibiotic biosynthesis protein, truncated
str0109	-			0.399	0.280			hypothetical protein, truncated
str0111	-			0.127	0.216			hypothetical protein
str0116	dacB	2.764						D-alanyl-D-alanine-carboxypeptidase
str0130	comX				0.327			transcriptional regulator, competence factor,RNA polymerase sigma factor
str0135	-				0.403			hypothetical protein
str0149	-						0.197	1,6-alpha-glucanhydrolase (dextranase), truncated
str0151	def					0.418		peptide deformylase
str0153	msf				0.474			major facilitator superfamily transporter, efflux protein
str0154	rpsO			2.247				30S ribosomal protein S15
str0155	-				0.306	0.369		hypothetical protein
str0156	-				0.387			hypothetical protein
str0157	-				0.456			hypothetical protein
str0161	-				3.432			hypothetical protein
str0162	mecA				0.197			adaptor protein
str0179	-			0.369				hypothetical protein
str0184	-		0.411					conserved hypothetical protein, peptide-efflux protein homolog
str0208	-	2.445						hypothetical protein
str0209	-				0.453			hypothetical protein
str0210	-				0.442			hypothetical protein
str0232	-					0.441		hypothetical protein
str0240	-				2.214			acetyltransferase, GNAT family
str0241	pabC					0.497		aminodeoxychorismate lyase
str0251	-			0.380				hypothetical protein
str0261	scpB				5.715		0.437	segregation and condensation protein B
str0263	-				6.288			hypothetical protein
str0270	plcR			2.162		0.468		transcriptional regulator
str0277	-				0.200			urease cluster protein, truncated
str0278	-				0.243			urease cluster protein, truncated
str0279	-				0.215			urease cluster protein, truncated
str0280	ureI				0.242			urease accessory protein, putatuve transport protein
str0281	ureA		0.475		0.265			urea amidohydrolase (urease) gamma subunit
str0282	ureB		0.473		0.270			urea amidohydrolase (urease) beta subunit
str0287	ureD				0.477			urease accessory protein
str0299	-	0.485	0.112		0.014	3.372		succinyl-diaminopimelic descuccinlyasadipeptidase, truncated
str0304	-	0.351	0.187		0.134	4.978		hypothetical protein
str0319	-				0.205			hypothetical protein
str0328	-			0.437		0.195		hypothetical protein
str0330	-			0.424	2.291			hypothetical protein
str0331	manN				3.047			mannose PTS system component IID
str0332	manM				3.207			mannose PTS system component IIC
str0354	-	0.072	0.057		0.181	10.544		hypothetical protein
str0357	-				0.441			hypothetical protein
str0365	-	2.315			3.391			acetoin utilization protein, truncated
str0373	-				2.260			hypothetical protein
str0394	luxS	2.678		0.489				S-ribosylhomocysteinase
str0402	-		2.001	0.383				PTS fructose-specific enzyme IIABC components, truncated
str0403	-			0.390				PTS fructose-specific enzyme IIABC components, truncated
str0404	-			0.476				PTS fructose-specific enzyme IIABC components, truncated
str0410	-	3.762	2.578					hypothetical protein
str0414	-				2.181			hypothetical protein
str0417	rplU				0.420			50S ribosomal protein L21
str0435	-			3.146				hypothetical protein
str0441	-			2.542				hypothetical protein
str0444	-				0.418			truncated IS1193 transposase
str0446	-						2.867	surface immunogenic protein, truncated
str0448	-		3.014					hypothetical protein
str0456	prtM				0.266			protease maturation protein precursor
str0458	-				3.656			cell wall protein precursor, similar to choline binding protein, truncated
str0459	-				4.377			cell wall protein precursor, similar to choline binding protein, truncated
str0460	-				2.600			cell wall protein precursor, similar to choline binding protein, truncated
str0461	-	0.344						cell wall protein precursor, similar to choline binding protein, truncated
str0465	argJ		0.473				0.357	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase protein
str0468	-			2.457				hypothetical protein
str0473	-				3.434			hypothetical protein
str0479	atpB				0.160			ATP synthase subunit A
str0494	amtB			0.305	0.295			ammonium transporter, Amp/Mep/NrgA family
str0505	glyQ	2.258			2.642			glycyl-tRNA synthetase alpha subunit
str0506	-				2.551			hypothetical protein
str0508	-				2.277			hypothetical protein
str0510	-				0.496	0.377	0.361	hypothetical protein
str0511	-						0.480	beta-glucoside-specific PTS system IIABC component, truncated
str0514	-					0.263	0.398	beta-glucoside-specific PTS system IIABC component, truncated
str0528	-			2.137				hypothetical protein
str0530	-			2.934				hypothetical protein
str0540	queA			2.689	2.381			S-adenosylmethionine:tRNA ribosyltransferase-isomerase
str0544	-				0.347		6.133	hypothetical protein
str0547	-			2.221			3.826	ABC transporter permease protein
str0550	-		0.415	0.355				hypothetical protein
str0558	-				2.164			hypothetical protein
str0564	-				0.435			conserved hypothetical protein, MutT/nudix family
str0565	-			2.112				hypothetical protein
str0571	-			2.381	2.519			hypothetical protein
str0573	-			3.267	3.822			conserved hypothetical protein, putative hydrolase
str0574	-				2.352			hypothetical protein
str0578	thyA			0.382				thymidylate synthase
str0580	-	2.192						hypothetical protein
str0585	-			2.700				hypothetical protein
str0586	-	0.459		3.311				hypothetical protein
str0591	-				0.278			hypothetical protein
str0600	-	3.094						hypothetical protein
str0607	feoA				0.297			ferrous ion transport protein A
str0633	grk			0.388			0.483	glycerate kinase, putative
str0648	-				7.987			negative transcriptional regualtor-PBP synthesis, truncated
str0650	eetB					36.430		exfoliative exotoxin B, putative

str0660	-				0.444			hypothetical protein
str0664	-				8.452	0.139		hypothetical protein
str0670	-			2.086				hypothetical protein
str0672	-				3.332			peptidase, U32 family
str0682	-			0.336				hypothetical protein
str0685	-		0.373			3.279		hypothetical protein
str0688	-			0.375				hypothetical protein
str0694	-				2.115			hypothetical protein
str0700	-				3.929			hypothetical protein
str0702	-				3.444			hypothetical protein
str0703	-				2.341			hypothetical protein
str0706	-			2.782				hypothetical protein
str0707	-			2.503	2.510			hypothetical protein
str0709	-	0.293		2.132	4.389			hypothetical protein
str0710	-				4.003			hypothetical protein
str0714	lemA			0.482	0.336			cytoplasmic membrane protein
str0730	-				3.087			hypothetical protein
str0738	-				2.084			hypothetical protein
str0744	-				0.433			truncated IS1193 transposase
str0747	apbE	4.266						lipoprotein involved thiamine biosynthesis
str0756	-			2.442				hypothetical protein
str0757	-			2.352				hypothetical protein, RAS-related protein
str0760	dltX			2.273				component involved in D-alanylation of teichoic acids, putative
str0766	-				0.356			IS861, transposase (orf2), IS3 family, truncated
str0769	-			0.461				IS861, transposase (orf1), IS3 family, truncated
str0772	-	0.262	0.328	0.163	0.240			unknown protein, phage associated
str0777	-			0.363				hypothetical protein, phage-plasmid associated
str0784	-			0.455				unknown protein, phage associated
str0793	-			0.181	0.193			sensor histidine kinase (homolog to ciaH Spn), truncated
str0794	-			0.166	0.215			sensor histidine kinase (homolog to ciaH Spn), truncated
str0798	rpsT				0.470	3.191	9.283	30S ribosomal protein S20
str0821	-				0.403			truncated IS1193 transposase
str0861	-				0.407			hypothetical protein
str0862	-				2.969			hypothetical protein
str0890	-				2.385	4.078		hypothetical protein
str0891	-				4.269			transcriptional regulator, biotin repressor family
str0913	-			0.380				hypothetical protein
str0914	-			0.426				hypothetical protein
str0930	-				3.443			hypothetical protein
str0939	-			0.229				hypothetical protein
str0944	-				0.355			hypothetical protein
str0951	-				0.408			hypothetical protein
str0965	-	0.254			3.351			hypothetical protein
str0969	-			0.254	2.788			amidase, truncated
str0973	-			3.169				amidase, truncated
str0978	-			6.464	9.009			hypothetical protein
str0980	-			3.026	5.273			hypothetical protein
str0981	-				6.056			transcriptional regulator, AraC family, truncated
str0982	-			0.348	2.748			transcriptional regulator, AraC family, truncated
str1011	-				2.712			hypothetical protein
str1013	malQ					0.344		4-alpha-glucanotransferase
str1030	-			3.351				alkaline amylopullulanase, truncated
str1037	-			0.403				hypothetical protein
str1038	-	3.030		0.457				hypothetical protein
str1044	-				0.500			positive transcriptional regulator MutR family
str1047	-	6.855					0.337	hypothetical protein
str1066	-				0.485			6-phospho-beta-glucosidase, truncated
str1078	epsK	0.400		3.770	3.925			exopolysaccharide gene claster protein
str1079	epsJ			4.121	3.543			exopolysaccharide polymerization protein
str1118	-	0.457			2.116			hypothetical protein, truncated
str1125	-	0.459		2.465				hypothetical protein
str1130	-				0.398			IS657, transposase, IS200 family, truncated
str1132	rplT			2.408				50S ribosomal protein L20
str1140	sipB			4.471	5.585			signal peptidase I
str1151	-				7.041			hypothetical protein
str1153	-				4.952			hypothetical protein, acetyltransferase, truncated
str1156	-			0.425	2.875			hypothetical protein
str1165	endA						10.932	competence associated membrane nuclease
str1168	-				0.401			hypothetical protein
str1170	-				0.254			hypothetical protein
str1179	rplS	0.461			0.179		5.261	50S ribosomal protein L19
str1184	-			2.098				hypothetical protein
str1191	-				2.607			hypothetical protein
str1192	-				2.770		0.215	hypothetical protein
str1193	-				3.263			surface-associated protein cshA precursor, truncated
str1194	-				5.310			hypothetical protein
str1195	-				3.742			hypothetical protein
str1209	hstH			0.462				histone-like DNA-binding protein
str1210	-				2.776			hypothetical protein
str1219	-				0.494	2.102		hypothetical protein
str1227	-				0.461			hypothetical protein
str1231	-				2.893			hypothetical protein
str1237	-				2.599			hypothetical protein
str1239	-	2.824			3.413		0.484	hypothetical protein
str1240	-				3.357			hypothetical protein
str1241	-				3.632			hypothetical protein
str1250	-				2.750			hypothetical protein, GtrA family
str1258	-				3.419			hypothetical protein
str1283	-				0.096			hypothetical protein
str1285	-				0.153			hypothetical protein
str1286	-			3.007	0.344		2.009	hypothetical protein
str1290	-			3.339	3.510			hypothetical protein
str1291	-			4.090	3.030			hypothetical protein
str1293	-			2.032				transcriptional regulator
str1313	hutH	0.448		2.021	0.131	23.396		histidine ammonia-lyase
str1314	-	0.499	0.379		0.306	19.004		hypothetical protein
str1315	hutU					25.795		urocanate hydratase
str1327	-			0.385	3.930	0.124	0.357	ABC transporter permease protein
str1329	-				2.494	0.092	0.421	hypothetical protein
str1332	-				0.252			hypothetical protein
str1343	msrA1			0.165	2.850		0.438	bifunctional methionine sulfoxide reductase A/B protein
str1348	-			0.293				hypothetical protein
str1349	-				0.295			hypothetical protein
str1360	-	3.249		0.490	0.122	2.721		hypothetical protein
str1362	-			0.364				hypothetical protein
str1364	-			0.191	0.312			hypothetical protein
str1370	-		0.473		0.215			hypothetical protein
str1378	-			0.344	4.782		0.399	hypothetical protein
str1383	-						0.473	hypothetical protein
str1387	-				0.349			hypothetical protein
str1406	-					0.362	0.301	zinc metalloprotease ZmpB, truncated
str1410	-			2.943				ABC transporter, putative ATP binding protein, truncated
str1412	-			3.037				ABC transporter, truncated
str1423	-			2.315				potassium channel protein, truncated

str1424	-			2.247				potassium channel protein, truncated
str1437	-	2.160						ABC transporter permease protein
str1450	oxlT	3.031		0.260	0.021	0.220	3.256	oxalate:formate antiporter
str1462	-				2.054			hypothetical protein
str1475	-	0.485		2.260				hypothetical protein
str1477	-			2.998				glycosyl transferase, truncated
str1487	-			2.168				hypothetical protein
str1499	-				0.325			hypothetical protein
str1500	-				0.336			hypothetical protein
str1507	-				0.357			hypothetical protein
str1511	-					0.480		positive transcriptional regulator MutR family
str1518	-				2.078			hypothetical protein
str1528	-		0.494	3.211	0.162		2.194	acetyltransferase, GNAT family
str1548	rpsP			2.165				30S ribosomal protein S16
str1552	-				3.974			ISSth2, transposase, IS5 family, truncated
str1565	-				4.173			peptidoglycan GlcNAc deacetylase, truncated
str1602	-	2.107				0.396		hypothetical protein
str1604	ilvD1				0.249			dihydroxy-acid dehydratase
str1608	-				0.288			ABC transporter permease protein
str1611	-	5.489						hypothetical protein
str1628	-	0.187		2.164		6.350		hypothetical protein
str1630	-	0.176				8.857		6-phospho-beta-glucosidase, truncated
str1631	-	0.182		2.079		10.418		6-phospho-beta-glucosidase, truncated
str1632	msrA2	0.285	0.488		0.457	6.990		methionine sulfoxide reductase A
str1639	-			0.217				hypothetical protein
str1658	cah	15.750	3.729			0.181	0.312	carbonate dehydratase
str1659	-	7.936	3.818			0.443	0.396	hypothetical protein
str1662	-			2.825	2.967	0.233		plasmid mobilization protein, truncated
str1663	int3			2.808		0.353		integrase/recombinase plasmid associated, putative
str1671	gla				0.208			glycerol uptake facilitator protein
str1672	pepXP	3.292			2.770	0.446		x-prolyl-dipeptidyl aminopeptidase
str1685	blpK			0.398				pore-forming peptide, putative bacteriocin
str1688	blpC			0.324	0.303			signal peptide, putative
str1689	-				0.414			peptide ABC transporter, truncated
str1690	-				0.331			peptide ABC transporter accessory protein, truncated
str1712	-			0.353	0.298			hypothetical protein
str1718	-				0.189			hypothetical protein
str1719	-				0.238			hypothetical protein
str1724	ansB	2.075						L-asparaginase
str1731	-						0.414	hypothetical protein
str1735	scrB				2.720			sucrose-6-phosphate hydrolase
str1743	-			0.344	0.333			hypothetical protein
str1746	-	0.294						hypothetical protein
str1747	-			0.411				hypothetical protein
str1750	-			2.012				hypothetical protein
str1752	rpsR			2.287	0.335		2.272	30S ribosomal protein S18
str1756	-					0.379		hypothetical protein
str1759	-			0.457				hypothetical protein
str1760	-			0.464				hypothetical protein
str1764	-				0.489			hypothetical protein
str1776	gnlA				0.253			glutamine synthetase
str1782	pgk				0.201			phosphoglycerate kinase
str1791	rpsL			2.786				30S ribosomal protein S12
str1808	rpmH				0.181			50S ribosomal protein L34
str1816	-			2.121		0.411		oligopeptide ABC transporter substrate-binding protein, truncated
str1817	rplA			3.112	0.437			50S ribosomal protein L1
str1818	rplK			3.358	0.274			50S ribosomal protein L11
str1820	-					0.480		hypothetical protein
str1829	-			0.469				formate-nitrate transporter, truncated
str1841	-			0.461			0.436	hypothetical protein
str1843	-			0.468	0.351			hypothetical protein
str1850	-				0.242			hypothetical protein
str1854	-			0.370				hypothetical protein
str1859	-			0.190				hypothetical protein
str1861	-			0.250				hypothetical protein
str1866	-			0.217				hypothetical protein
str1874	-				0.371			dihydroxy-acid dehydratase, truncated
str1876	asp				0.388			alkaline-shock protein
str1883	-			0.235	0.395	0.380		alcohol-acetaldehyde dehydrogenase, truncated
str1886	-		2.530	0.158	0.403		0.443	trehalose-6-phosphate hydrolase, truncated
str1887	-		2.083	0.119	0.451			trehalose-6-phosphate hydrolase, truncated
str1888	-	5.878	2.455	0.101	0.475	0.218	0.460	trehalose-6-phosphate hydrolase, truncated
str1889	-			0.072				PTS trehalose-specific IIBC component, truncated
str1890	-	10.738	2.192	0.077	0.286			PTS trehalose-specific IIBC component, truncated
str1891	-	7.187	2.023	0.096	0.261			PTS trehalose-specific IIBC component, truncated
str1894	-	2.074		0.343				hypothetical protein
str1895	-			0.407				hypothetical protein
str1901	-			0.440	0.402			glutamate--cysteine ligase, putative, truncated
str1902	-			0.359		149.748		glutamate--cysteine ligase, putative, truncated
str1903	-			0.394	0.401			glutamate--cysteine ligase, putative, truncated
str1920	rpsH				0.479			30S ribosomal protein S8
str1924	rplN				0.495			50S ribosomal protein L14
str1926	rpmC			2.502				50S ribosomal protein L29
str1929	rplV			2.364				50S ribosomal protein L22
str1933	rplD			2.645	0.429			50S ribosomal protein L4
str1934	rplC			2.239	0.335			50S ribosomal protein L3
str1943	pmrB						3.167	multidrug-efflux transporter, putative
str1950	-				0.423			positive transcriptional regulator MutR family, truncated
str1953	rip				0.060			replication initiator protein
str1954	-				0.399			hypothetical protein
str1955	int4			0.380	0.287			integrase/recombinase, phage associated
str1956	-				0.140			hypothetical protein
str1959	-				0.254			hypothetical protein
str1960	-				0.278			Holliday junction resolvase-like protein
str1964	-			3.817	2.058		2.913	hypothetical protein
str1970	-				0.321			hypothetical protein
str1976	-				0.381			hypothetical protein
str1983	-					0.435		hypothetical protein
str1987	-			0.487				hypothetical protein
str1990	-				0.262			hypothetical protein
str1991	-			0.436	0.163			hypothetical protein
str1992	-			0.406	0.214			hypothetical protein
str2004	-			0.443	0.434	0.437		hypothetical protein
str2005	-				0.068	0.485		hypothetical protein
str2022	-			0.420	0.347			hypothetical protein
Nucleotide transport and metabolism								
str0023	prsA1			0.424	0.202	0.283		ribose-phosphate pyrophosphokinase
str0031	purL			0.400	0.310	0.478	2.594	phosphoribosylformylglycinamidine synthase II (FGAM synthetase)
str0032	purF			0.452	0.425	0.491	2.604	amidophosphoribosyltransferase
str0033	purM			0.480	0.470	0.489	2.570	phosphoribosylaminoimidazole synthetase
str0040	purD	4.899	2.561		0.147		2.407	phosphoribosylamine--glycine ligase
str0041	purE	5.768	4.046		0.199		2.515	phosphoribosylaminoimidazole carboxylase catalytic subunit
str0042	purK	5.416	3.899		0.307		2.524	phosphoribosylaminoimidazole carboxylase

str0045	purB1	2.228	2.080		0.374			adenylosuccinate lyase
str0134	pyrG				0.321			CTP synthetase
str0139	-	4.852	2.078					cyclo-nucleotide phosphodiesterase, truncated
str0141	-					0.395		cyclo-nucleotide phosphodiesterase, truncated
str0142	-	3.740	2.152			0.407		cyclo-nucleotide phosphodiesterase, truncated
str0144	nrdI			3.002			2.012	hypothetical protein
str0256	-				2.923		0.485	putative deoxyribonucleotide triphosphate pyrophosphatase/unknown domain fusion protein
str0438	pyrH			2.164				uridylate kinase
str0489	tmk	3.269			0.397			thymidylate kinase
str0523	pyrR				0.270			pyrimidine regulatory protein PyrR
str0526	carA				2.121	0.445		carbamoyl-phosphate synthase small subunit
str0527	carB				2.145	0.428		carbamoyl-phosphate synthase large subunit
str0551	-		0.353		0.221			phosphorylase, Pnp/Udp family, putative
str0566	pfs			2.902				5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
str0750	add				0.472			adenosine deaminase
str0751	tdk				0.409			thymidine kinase
str0791	fhs			0.389	0.137		2.355	formate-tetrahydrofolate ligase
str0803	-				2.317			pyrimidine-nucleoside phosphorylase, truncated
str0804	-				2.471			pyrimidine-nucleoside phosphorylase, truncated
str0805	-				2.644			deoxyribose-phosphate aldolase, truncated
str0806	-				2.846		0.484	deoxyribose-phosphate aldolase, truncated
str0807	cdd				3.090			cytidine deaminase
str0886	guaA				2.237			bifunctional GMP synthase/glutamine amidotransferase protein
str0941	-				0.258			phosphorylase, Pnp/Udp family
str0967	pyrF			0.460		0.279		orotidine 5'-phosphate decarboxylase
str0979	-				3.842			5'-nucleotidase, putative
str1113	deoD	0.482			2.455			purine nucleoside phosphorylase (family 1)
str1114	gat	0.350			2.457			glutamine amidotransferase
str1117	punA	0.376						purine nucleoside phosphorylase
str1207	pyrDa			2.401				dihydroorotate dehydrogenase
str1223	apt				0.329			adenine phosphoribosyltransferase
str1260	udk				2.305			uridine kinase
str1270	nrdE			3.567			4.742	ribonucleotide-diphosphate reductase alpha subunit
str1331	-			0.360	0.366			cytidine/deoxycytidylate deaminase family protein, putative
str1339	-			0.391				nucleobase:cation symporter for xanthine, truncated
str1341	-			0.500				nucleobase:cation symporter for xanthine, truncated
str1610	hit	7.289	2.048					cell cycle regulation histidine triad (HIT) protein
str1844	-			0.497	0.236			cytidine/deoxycytidylate deaminase family protein, putative
str1913	adk	0.494		4.704	0.459	0.479		adenylate kinase
str1941	flaR				0.482			topology modulation protein
str1963	nrdD			3.618			3.391	anaerobic ribonucleoside triphosphate reductase
str2016	guaB				0.237			inositol-5-monophosphate dehydrogenase
Posttranslational modification, protein turnover, chaperones								
str0119	grpE		2.033	0.444			0.483	heat shock protein, chaperonin
str0120	dnaK			0.404			0.438	molecular chaperone DnaK
str0121	dnaJ				2.787		0.440	heat shock protein, chaperonin
str0132	tig	2.176			0.095	0.448		trigger factor
str0203	groES			0.377				co-chaperonin GroES
str0204	groEL			0.351			0.465	chaperonin GroEL
str0284	ureE				0.368			urease accessory protein
str0285	ureF				0.475			urease accessory protein
str0356	clpP					0.386		ATP-dependent Clp protease proteolytic subunit
str0515	ppiA				0.293	2.041	2.835	peptidyl-prolyl cis-trans isomerase
str0602	clpE	3.441						ATP-dependent Clp protease
str0715	htpX			0.468				heat shock protein HtpX
str0722	pilD			0.335				prepilin peptidase type IV
str0749	-	3.842			0.204			glutathione S-transferase family
str0827	-			2.601	0.264		2.887	hypothetical protein, coenzyme PQQ synthesis homologue
str0990	tpx		0.352	3.370	0.294	8.235		thiol peroxidase
str1269	nrdH			3.082	0.233		6.174	glutaredoxin
str1614	clpL			0.188	2.162			ATP-dependent proteinase ATP-binding subunit
str1640	pflA	4.059			0.469	0.176		pyruvate-formate lyase activating enzyme
str1803	trxA1				0.129			thioredoxin
str1805	bta				0.277			bacteriocin transport accessory protein, putative
str1846	-		0.171		0.286	6.616		transcription regulator, putative
str1849	trxA2				0.340			thioredoxin
str1885	pepO				0.407			endopeptidase O
str1966	nrdG			2.377			2.641	anaerobic ribonucleotide reductase activator
str2024	htrA			0.350	0.187			exported serine protease
Secondary metabolites biosynthesis, transport and catabolism								
str0029	acpP1	22.301		0.476	0.367			acyl carrier protein
str0384	acpP2				0.470			acyl carrier protein
str0763	dltC				2.281			D-alanine--poly(phosphoribitol) ligase subunit 2
str0909	-	0.380	0.432			4.592		acetoin reductase, truncated
str0910	-			2.767		10.053		acetoin reductase, truncated
str0924	aldB				3.226			alpha-acetolactate decarboxylase
str0929	-				0.226			oxidoreductase, short chain dehydrogenase/reductase family
str1634	entB			0.342				pyrazinamidase/nicotinamidase, putative
Signal transduction mechanisms								
str0112	-				0.126		2.319	hypothetical protein
str0152	-	2.401						transcriptional regulator, putative
str0159	-			3.273	2.047	0.405		amino acid (glutamine) ABC transporter substrate binding protein/permease protein
str0296	-	0.361	0.056		0.082	6.942		amino acid ABC transporter substrate binding protein, putative
str0317	rr01				0.291			response regulator (homolog to csrR/covR Spy)
str0318	hk01				0.445			sensor histidine kinase (homolog to csrS/covS Spy)
str0401	-			0.324				PTS fructose-specific enzyme IIABC components, truncated
str0447	phoH				0.261			phosphate starvation-induced protein
str0543	rr02				0.279			response regulator
str0665	-			0.421				hypothetical protein
str0729	typA				2.601	0.406		GTP-binding protein TypA/BipA (tyrosine phosphorylated protein A)
str0792	-			0.186	0.154			response regulator (homolog to ciaR Spn), truncated
str0795	-			0.146	0.261			sensor histidine kinase (homolog to ciaH Spn), truncated
str0877	-					0.367	0.321	amino acid (glutamine) ABC transporter, substrate binding protein
str0908	-	0.435	0.273			3.666		ABC transporter substrate binding protein
str0974	-			2.771	2.366	0.297		ABC transporter substrate binding protein, truncated
str0975	-			3.287	4.384			ABC transporter substrate binding protein, truncated
str1175	-				0.220			hypothetical protein
str1325	rr06		2.554		4.326			response regulator (homolog to RR11 Spn)
str1326	hk06				2.469			sensor histidine kinase (homolog to HK11 Spn)
str1380	-			0.236	2.060			response regulator (homolog to RR08 Spn) uncoupled, truncated
str1381	-			0.253			0.415	response regulator (homolog to RR08 Spn) uncoupled, truncated
str1420	rr08					0.419		response regulator (homolog to RR03 Spn)
str1421	hk08					0.387		sensor histidine kinase (homolog to HK03 Spn)
str1425	pknB			2.757				protein kinase
str1426	pppL			2.297				phosphoprotein phosphatase
str1579	-	0.305	0.158	2.084	0.236	12.993		amino acid (glutamine) ABC transporter substrate binding protein
str1637	-			0.217				hypothetical protein
str1654	-		0.380	2.320	0.331			ABC transporter substrate-binding protein, amino acid
str1948	-				0.329			hypothetical protein
str2001	-			2.169	0.275			hypothetical protein

Transcription								
str0006	trcF					0.489		transcription repair coupling factor
str0065	-				0.374			transcriptional regulator, MarR family, truncated
str0096	-				0.126			transcriptional regulator, putative, truncated
str0118	hrcA					0.408	0.485	heat-inducible transcription repressor
str0133	rpoE				0.153	0.361		DNA-directed RNA polymerase subunit delta
str0185	adcR			0.394				zinc transport transcriptional repressor
str0219	-			2.855				transcriptional regulator, DeoR family
str0242	greA				2.364			transcription elongation factor GreA
str0317	rr01				0.291			response regulator (homolog to csrR/covR Spy)
str0341	nusA			2.871				transcription elongation factor NusA
str0342	-			4.082				hypothetical protein
str0381	-				0.498			transcriptional regulator, MarR family
str0399	fruR			0.475				transcriptional repressor
str0452	-	0.366	0.205			9.072		transcriptional regulator, LysR family
str0543	rr02				0.279			response regulator
str0549	-				0.377			transcriptional repressor, putative
str0615	-		2.126					transcriptional antiterminator (BglG family), truncated
str0625	rnr			2.092				exoribonuclease R
str0630	ccpA			0.489				catabolite control protein
str0649	-			2.296	7.315			negative transcriptional regualtor-PBP synthesis, truncated
str0663	tex			2.434	4.710			transcriptional regulator, putative
str0665	-			0.421				hypothetical protein
str0689	-				2.093			restriction-modification system regulatory protein, putative
str0792	-			0.186	0.154			response regulator (homolog to ciaR Spn), truncated
str0813	dinG	0.459		2.941				ATP-dependent DNA helicase
str0895	-				10.016			transcriptional regulator
str0916	-						2.077	transcriptional regulator
str0931	-				3.830			transcriptional regulator, TetR family
str0950	fbp		2.039					fibronectin-binding protein-like protein A
str1214	-				2.503			transcriptional repressor protein, putative
str1287	-			2.523			3.974	transcriptional regulator, putative
str1296	rncS			2.876	5.487			ribonuclease III
str1325	rr06		2.554		4.326			response regulator (homolog to RR11 Spn)
str1366	-			0.315				conserved hypothetical protein, Rrf2 family protein
str1380	-			0.236	2.060			response regulator (homolog to RR08 Spn) uncoupled, truncated
str1381	-			0.253			0.415	response regulator (homolog to RR08 Spn) uncoupled, truncated
str1420	rr08					0.419		response regulator (homolog to RR03 Spn)
str1425	pknB			2.757				protein kinase
str1435	-	2.459						transcriptional regulator, GntR family, putative
str1488	rpoD			2.319				RNA polymerase sigma factor
str1568	rheA			2.454				ATP-dependent RNA helicase
str1586	copY			0.370	0.314			negative transcriptional regulator - copper transport operon
str1600	-			0.373		0.359		transcriptional regulator, MerR family
str1699	rheB		2.174					ATP-dependent RNA helicase
str1725	recG						0.483	ATP-dependent DNA helicase
str1733	scrK			0.484		0.417	0.465	fructokinase
str1736	-				4.534			sucrose regulon regulatory protein, truncated
str1777	glnR				0.212			transcriptional regulator, repressor of the glutamine synthetase, MerR family
str1892	-		2.642	0.422				trehalose operon transcriptional repressor GntR family, truncated
str1908	rpoA				0.296			DNA-directed RNA polymerase alpha subunit
str1948	-				0.329			hypothetical protein
str1989	-				0.220			hypothetical protein
str1995	-				0.426			transcriptional regulator, TetR/AcrR family
str2025	spoJ			0.378	0.241			chromosome segregation protein
Translation, ribosomal structure and biogenesis								
str_r01			2.649	0.487	0.402			
str_r03			2.292	0.465	0.375			
str_r04			2.526	0.376	0.458			
str_r05		0.397		0.298	0.464			
str_r09			2.224	0.459	0.364			
str_t01					0.186			
str_t02		0.415			0.192			
str_t03					0.333		2.839	
str_t05					0.148			
str_t06					0.343			
str_t07		0.316			0.268			
str_t08		0.467		0.470	0.189		2.699	
str_t09					0.142			
str_t10					0.077			
str_t11		0.129	0.370	0.057	0.078			
str_t13					0.195			
str_t14				0.323	0.370			
str_t15					0.438			
str_t16				0.439	0.224		2.838	
str_t17		0.306	0.404	0.465	0.226			
str_t18				0.474	0.172			
str_t23					0.243			
str_t27		0.447		0.413	0.185			
str_t30					0.198			
str_t44				0.479	0.244			
str_t46		0.280		0.444	0.187			
str_t52		0.426			0.187			
str_t54			0.421	0.278	0.287			
str_t55					0.357			
str_t59				0.346				
str0007	-			0.445				conserved hypothetical protein, S4 domain protein
str0047	argS				2.825			arginyl-tRNA synthetase
str0081	pnpA				0.383			polynucleotide phosphorylase, (PNPase)
str0211	-	2.672	2.662					ribosomal large subunit pseudouridine synthase, RluD subfamily
str0220	leuS	2.136		2.164				leucyl-tRNA synthetase
str0262	rluB				7.165			ribosomal large subunit pseudouridine synthase B
str0267	-			4.484		0.298		rRNA methyltransferase, TrmH family
str0329	serS				4.154	0.334		seryl-tRNA synthetase
str0343	-			2.554	2.125			hypothetical protein
str0344	infB			3.451				translation initiation factor IF-2
str0419	-	2.571			0.247	0.441		acetyltransferase, GNAT family, truncated
str0420	-	2.415			0.278	0.498		acetyltransferase, GNAT family, truncated
str0451	metG				0.365			methionine--tRNA ligase
str0476	-			2.785	4.433			acetyltransferase, GNAT family
str0477	valS			3.588	4.888			valyl-tRNA synthetase
str0487	tuf		2.359					elongation factor Tu
str0507	glyS			2.070	4.584			glycyl-tRNA synthetase beta subunit
str0536	rplJ			5.891				50S ribosomal protein L10
str0537	rplL			4.078				50S ribosomal protein L7/L12
str0572	thrS			2.612	3.756			threonyl-tRNA synthetase
str0592	rpsA				0.260			30S ribosomal protein S1
str0692	lysS	2.021	2.067					lysyl-tRNA synthetase
str0741	ileS			2.176	2.626			isoleucyl-tRNA synthetase
str0746	rpmE				0.440			50S ribosomal protein L31
str0752	prfA				0.420			peptide chain release factor 1
str0753	hemK			2.178	0.426			protoporphyrinogen oxidase
str0824	-					2.049		conserved hypothetical protein, translation initiation inhibitor protein

str1000	-			2.521				rRNA methyltransferase, putative
str1133	rpmI			3.648				50S ribosomal protein L35
str1134	infC			3.801	0.481			translation initiation factor IF-3
str1146	prfB				0.355			peptide chain release factor 2
str1177	miaA				0.223			tRNA delta(2)-isopentenylpyrophosphate transferase
str1292	pheT			2.178				phenylalanyl-tRNA synthetase beta subunit
str1294	pheS			2.226				phenylalanyl-tRNA synthetase alpha subunit
str1428	fmt			2.353				methionyl-tRNA formyltransferase
str1490	rpsU			2.196	0.354			30S ribosomal protein S21
str1505	rsuA2				0.381			ribosomal small subunit pseudouridine synthase A
str1559	-				0.479			acetyltransferase, GNAT family
str1568	rheA			2.454				ATP-dependent RNA helicase
str1574	prfC			2.600				peptide chain release factor 3
str1625	gatB	3.336						aspartyl/glutamyl-tRNA amidotransferase subunit B
str1627	gatC			2.131	0.431			aspartyl/glutamyl-tRNA amidotransferase subunit C
str1699	rheB	2.174						ATP-dependent RNA helicase
str1754	rpsF			2.760	0.350		2.605	30S ribosomal protein S6
str1790	rpsG			3.698				30S ribosomal protein S7
str1806	tgt				0.367			queuine tRNA-ribosyltransferase
str1844	-			0.497	0.236			cytidine/deoxycytidylate deaminase family protein, putative
str1870	tyrS			2.520	0.418			tyrosyl-tRNA synthetase
str1907	rplQ				0.346			50S ribosomal protein L17
str1909	rpsK				0.293			30S ribosomal protein S11
str1910	rpsM				0.268			30S ribosomal protein S13
str1911	rpmJ				0.278			50S ribosomal protein L36
str1912	infA				0.221			translation initiation factor IF-1
str1919	rplF			2.307				50S ribosomal protein L6
str1921	rpsN			2.100				30S ribosomal protein S14
str1922	rplE				0.480			50S ribosomal protein L5
str1925	rpsQ				0.442			30S ribosomal protein S17
str1927	rplP				0.492			50S ribosomal protein L16
str1928	rpsC			2.036				30S ribosomal protein S3
str1930	rpsS				0.393			30S ribosomal protein S19
str1931	rplB			2.228	0.471			50S ribosomal protein L2
str1932	rplW			2.218	0.385			50S ribosomal protein L23
str1935	rpsJ			2.301	0.282			30S ribosomal protein S10
str1958	rpmB			2.708				50S ribosomal protein L28
str1969	aspS				0.487			aspartyl-tRNA synthetase
str1971	hisS				0.486		2.120	histidyl-tRNA synthetase
str1974	rpmF			2.440	0.209		2.013	50S ribosomal protein L32
str1975	rpmGB			2.868	0.211			50S ribosomal protein L33
str1997	rpsD			2.011				30S ribosomal protein S4
str2003	trmU				0.387	0.337		tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
str2018	trpS				0.354			tryptophanyl-tRNA synthetase

Supplementary Table 3. Significant (≥2-fold FDR≤0.05) differential expression of *L. bulgaricus* genes between mixed and mono-cultures and between cultures supplemented with formic acid and cultures without any supplement, per functional category. Values represent ratios between the first and the second condition. Columns 3-6 contain ratios of mixed cultures over by mono-cultures in the first exponential phase (3.5 h), reduced growth phase (5.5 h), second exponential phase (8 h) and stationary phase (12 h), respectively. Columns 7 and 8 contain ratios of a culture supplemented with formic acid over one without supplement in the early exponential phase and mid exponential phase, respectively.

		Mixed culture over mono-culture				Formic acid over none		
Locus ID	Locus	1st exp. phase	Transition phase	2nd exp. phase	Stationary phase	1st exp. phase	2nd exp. phase	Product
Amino acid transport and metabolism								
LBUL_0065	TesA				0.402			Lysophospholipase L1 and related esterase
LBUL_0084	SerA		3.717			0.262		Phosphoglycerate dehydrogenase and related dehydrogenase
LBUL_0089	MetA		0.390		0.212			Homoserine trans-succinylase
LBUL_0112	TyrR				0.476			Transcriptional regulator of aromatic amino acids metabolism
LBUL_0129	LivG		2.794					ABC-type branched-chain amino acid transport systems ATPase component
LBUL_0145	HisM			5.749				ABC-type amino acid transport system permease component
LBUL_0156	PrsA	2.071		0.441		0.312	0.433	Phosphoribosylpyrophosphate synthetase
LBUL_0161	CarB		0.475					Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0165	DAP2		0.138					Dipeptidyl aminopeptidase/acylaminoacyl-peptidase
LBUL_0176	HisJ				0.438			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0177	GlnQ	0.331	0.347	2.596				ABC-type polar amino acid transport system ATPase component
LBUL_0178	OpuBB	0.294	0.350	2.695				ABC-type proline/glycine betaine transport systems permease component
LBUL_0179	OpuBB	0.153	0.186				3.599	ABC-type proline/glycine betaine transport systems permease component
LBUL_0180	OppA				0.249			ABC-type oligopeptide transport system periplasmic component
LBUL_0184	CarB			0.350				Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0211	MetH		0.372					Methionine synthase I cobalamin-binding domain
LBUL_0214	HisM	0.229	0.117		0.140			ABC-type amino acid transport system permease component
LBUL_0215	HisM	0.202	0.155		0.117			ABC-type amino acid transport system permease component
LBUL_0216	GlnQ	0.386	0.055		0.159			ABC-type polar amino acid transport system ATPase component
LBUL_0217	HisJ	0.412	0.171		0.224			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0225	ArgE				0.144			Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase and related deacylase
LBUL_0233	OppA			2.226		0.251		ABC-type oligopeptide transport system periplasmic component
LBUL_0235	OppA				2.491		0.251	ABC-type oligopeptide transport system periplasmic component
LBUL_0238	DppD				2.013			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0239	DppF				3.705			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0241	PepC					0.398	0.479	Aminopeptidase C
LBUL_0242	PepC		2.070					Aminopeptidase C
LBUL_0251	GltB	2.639				0.290	0.458	Glutamate synthase domain 2
LBUL_0252	PabA	2.315						Anthranilate/para-aminobenzoate synthase component II
LBUL_0256	OpuBA		0.347	6.884				ABC-type proline/glycine betaine transport systems ATPase components
LBUL_0261	HisJ	0.421	0.335	2.825	0.088			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0262	GlnQ	0.380	0.268		0.077			ABC-type polar amino acid transport system ATPase component
LBUL_0263	HisM	0.357	0.268		0.085			ABC-type amino acid transport system permease component
LBUL_0273	AnsB			0.186				L-asparaginase/ Glu-tRNAGln amidotransferase subunit D
LBUL_0303	CysE		0.392		2.057		0.432	Serine acetyltransferase
LBUL_0326	PepD			2.960				Di- and tripeptidase
LBUL_0399	ARO8		2.272	0.487				Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_0427	SerB		3.059					Phosphoserine phosphatase
LBUL_0431	BrnQ		0.241					Branched-chain amino acid permease
LBUL_0445	DppF		0.333					ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0457	DppD		0.368		6.995			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0479	ProV			0.430				ABC-type proline/glycine betaine transport system ATPase component

LBUL_0480	PrsA				0.202			Phosphoribosylpyrophosphate synthetase
LBUL_0481	PotE	0.273	0.210					Amino acid transporter
LBUL_0488	CsdB			0.224				Selenocysteine lyase
LBUL_0496	PepP		0.492		2.524			Xaa-Pro aminopeptidase
LBUL_0500	DAP2		0.293					Dipeptidyl aminopeptidase/acylaminoacyl-peptidase
LBUL_0506	MetH				4.576			Methionine synthase I cobalamin-binding domain
LBUL_0529	SdaC		0.349	2.661				Amino acid permease
LBUL_0548	GltD				0.425			NADPH-dependent glutamate synthase beta chain and related oxidoreductase
LBUL_0550	LivG		2.433	0.243	0.098			ABC-type branched-chain amino acid transport systems ATPase component
LBUL_0553	DmpA		2.448	0.284	0.181			L-aminopeptidase/D-esterase
LBUL_0572	SerB				2.538			Phosphoserine phosphatase
LBUL_0578	PotA		0.448					ABC-type spermidine/putrescine transport systems ATPase components
LBUL_0579	PotB	0.442	0.396					ABC-type spermidine/putrescine transport system permease component I
LBUL_0580	PotC		0.268					ABC-type spermidine/putrescine transport system permease component II
LBUL_0581	PotD		0.402					Spermidine/putrescine-binding periplasmic protein
LBUL_0585	SerB	2.582		0.484				Phosphoserine phosphatase
LBUL_0589	SerB	0.358						Phosphoserine phosphatase
LBUL_0646	EutJ				0.459			Ethanolamine utilization protein possible chaperonin
LBUL_0663	EutJ		0.383		3.612			Ethanolamine utilization protein possible chaperonin
LBUL_0686	NifS	0.402	0.328		5.142			Cysteine sulfinate desulfinase/cysteine desulfurase
LBUL_0688	ArgG		2.445		8.246			Argininosuccinate synthase
LBUL_0717	OppA		0.254					ABC-type oligopeptide transport system periplasmic component
LBUL_0726	DadA			0.047	0.130	4.189	3.051	Glycine/D-amino acid oxidase (deaminating)
LBUL_0737	ARO8		4.440	3.248	0.065	0.125		Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_0812	HisZ		0.288		6.877			ATP phosphoribosyltransferase involved in histidine biosynthesis
LBUL_0823	GcvH		0.397	0.211	0.345		2.227	Glycine cleavage system H protein (lipoate-binding)
LBUL_0825	CarB		0.295	0.132	0.391			Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0869	PotC	0.300						ABC-type spermidine/putrescine transport system permease component II
LBUL_0870	GlnQ				0.363			ABC-type polar amino acid transport system ATPase component
LBUL_0882	PotA		0.216					ABC-type spermidine/putrescine transport systems ATPase components
LBUL_0883	PotA		0.350		5.201			ABC-type spermidine/putrescine transport systems ATPase components
LBUL_0895	SerB				2.029			Phosphoserine phosphatase
LBUL_0904	ThrB		0.179	0.456				Homoserine kinase
LBUL_0905	ThrB	0.232	0.157					Homoserine kinase
LBUL_0906	ThrB	0.156	0.171		3.061			Homoserine kinase
LBUL_0914	SerB		4.041	0.139	0.138			Phosphoserine phosphatase
LBUL_0917	SerA			0.343			0.498	Phosphoglycerate dehydrogenase and related dehydrogenase
LBUL_0918	DAP2				4.671			Dipeptidyl aminopeptidase/acylaminoacyl-peptidase
LBUL_0931	CarA	3.078			0.038	0.230		Carbamoylphosphate synthase small subunit
LBUL_0932	CarB	2.215			0.059	0.369		Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0933	GcvR				0.117	0.322		Glycine cleavage system regulatory protein
LBUL_0961	ArgE		0.270		10.350			Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase and related deacylase
LBUL_0975	CarA			16.246				Carbamoylphosphate synthase small subunit
LBUL_0988	PotA	0.408			4.992			ABC-type spermidine/putrescine transport systems ATPase components
LBUL_1067	SerA	0.410			3.027			Phosphoglycerate dehydrogenase and related dehydrogenase
LBUL_1069	OpuBA			7.779				ABC-type proline/glycine betaine transport systems ATPase components
LBUL_1070	PotA			7.598				ABC-type spermidine/putrescine transport systems ATPase components
LBUL_1096	ProA		0.123	0.177				Gamma-glutamyl phosphate reductase
LBUL_1103	MalY	0.301						Bifunctional PLP-dependent enzyme with beta-cystathionase and maltose regulon repressor activities
LBUL_1110	AsnA				10.118			Asparagine synthetase A
LBUL_1126	PepD	0.089	0.141		8.033			Di- and tripeptidase
LBUL_1138	DadA			2.770				Glycine/D-amino acid oxidase (deaminating)
LBUL_1157	SerB	0.432		0.328		7.961	0.290	Phosphoserine phosphatase
LBUL_1180	COG0436					0.224		Aspartate/tyrosine/aromatic aminotransferase
LBUL_1189	GltD			0.412				NADPH-dependent glutamate synthase beta chain and related oxidoreductase
LBUL_1204	PotA			62.155				ABC-type spermidine/putrescine transport systems ATPase components
LBUL_1213	GlnQ			2.998				ABC-type polar amino acid transport system ATPase component
LBUL_1214	HisM				4.248			ABC-type amino acid transport system permease component
LBUL_1219	IlvE				4.736		0.225	Branched-chain amino acid aminotransferase/4-amino-4-deoxychorismate lyase
LBUL_1227	EutJ	0.367	0.479					Ethanolamine utilization protein possible chaperonin
LBUL_1231	MetF	0.393	0.371					5 10-methylenetetrahydrofolate reductase
LBUL_1232	MetE	0.488			2.229			Methionine synthase II (cobalamin-independent)
LBUL_1235	CysK	9.381	3.000	23.087	2.835			Cysteine synthase
LBUL_1236	MetC	6.367	2.770	13.936	4.313			Cystathionine beta-lyase/cystathionine gamma-synthase
LBUL_1241	ArgK			3.477				Putative periplasmic protein kinase ArgK and related GTPase of G3E family
LBUL_1246	HIS2				4.074			Histidinol phosphatase and related hydrolase of the PHP family
LBUL_1257	Asd						3.733	Aspartate-semialdehyde dehydrogenase
LBUL_1261	DppF	0.137						ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1267	HisJ	0.201	0.345					ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_1285	ArgK		0.366					Putative periplasmic protein kinase ArgK and related GTPase of G3E family
LBUL_1288	DdpA		0.415					ABC-type dipeptide transport system periplasmic component
LBUL_1289	DdpA	0.383		2.321	0.356			ABC-type dipeptide transport system periplasmic component
LBUL_1291	DppB	0.330	0.229	2.883	0.179			ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_1292	DppD	0.324	0.262	2.588	0.193			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1293	DppD	0.282	0.278	2.768	0.148			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1308	EutQ			0.462				Ethanolamine utilization protein
LBUL_1330	CarB		0.339		0.063			Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_1334	AsnB		0.371		0.052		2.314	Asparagine synthase (glutamine-hydrolyzing)
LBUL_1336	HisH		0.442		0.039			Glutamine amidotransferase
LBUL_1339	ArgH				0.165	0.332		Argininosuccinate lyase
LBUL_1340	CarB				0.105	0.303		Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_1353	MetA	0.491	0.233		0.081			Homoserine trans-succinylase
LBUL_1354	CysK		0.157	0.356	0.034			Cysteine synthase
LBUL_1368	GlnA		0.345	0.328				Glutamine synthetase
LBUL_1423	PrsA						2.350	Phosphoribosylpyrophosphate synthetase
LBUL_1426	AroK		0.364					Shikimate kinase
LBUL_1466	GltD		0.500					NADPH-dependent glutamate synthase beta chain and related oxidoreductase
LBUL_1474	PepP	2.063		0.402				Xaa-Pro aminopeptidase
LBUL_1502	ArgA			0.363				N-acetylglutamate synthase and related acetyltransferase
LBUL_1562	DppD	0.212					0.162	ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1563	ARO8	0.247	0.459					Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_1567	CarB			3.600				Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_1581	ProA			3.670	0.096			Gamma-glutamyl phosphate reductase
LBUL_1588	DppF				0.442			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1604	PepC			0.454	2.248			Aminopeptidase C
LBUL_1619	ArgE			0.248	0.386			Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase and related deacylase
LBUL_1630	ThrB			0.381	0.121			Homoserine kinase
LBUL_1645	LdcC		0.324					Arginine/lysine/ornithine decarboxylase
LBUL_1664	LYS9	0.485						Saccharopine dehydrogenase and related protein
LBUL_1668	AnsP				0.185			Gamma-aminobutyrate permease and related permease
LBUL_1691	CsdB		0.365		2.204			Selenocysteine lyase
LBUL_1693	LivG				7.298			ABC-type branched-chain amino acid transport systems ATPase component
LBUL_1700	GlnQ			0.272	0.323			ABC-type polar amino acid transport system ATPase component
LBUL_1751	DAP2			0.156				Dipeptidyl aminopeptidase/acylaminoacyl-peptidase
LBUL_1753	MetC		0.158		3.998			Cystathionine beta-lyase/cystathionine gamma-synthase
LBUL_1762	MET2			5.544				Homoserine acetyltransferase
LBUL_1772	DppF				2.425			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1780	TesA	4.522						Lysophospholipase L1 and related esterase
LBUL_1810	DadA			2.745	4.943			Glycine/D-amino acid oxidase (deaminating)
LBUL_1868	SerA		3.201	3.363				Phosphoglycerate dehydrogenase and related dehydrogenase
LBUL_1870	LivG		0.429					ABC-type branched-chain amino acid transport systems ATPase component
LBUL_1880	COG1164	2.236						Oligoendopeptidase F

LBUL_1885	SerB			0.499				Phosphoserine phosphatase
LBUL_1890	COG4126				2.236			Hydantoin racemase
LBUL_1909	PepD			0.263				Dipeptidase
LBUL_1930	AspA				0.016	0.254	3.051	Aspartate ammonia-lyase
LBUL_1942	HisH				3.898			Glutamine amidotransferase
LBUL_1950	CarB						7.197	Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_1954	PrsA	0.368	0.393	2.979	5.512			Phosphoribosylpyrophosphate synthetase
LBUL_1955	TrpA			2.592				Tryptophan synthase alpha chain
LBUL_1959	RhaT		0.206		0.417			Permease of the drug/metabolite transporter (DMT) superfamily
LBUL_1961	SerB				0.424			Phosphoserine phosphatase
LBUL_2003	GlnQ			2.851				ABC-type polar amino acid transport system ATPase component
LBUL_2004	LivH		0.416	3.269				Branched-chain amino acid ABC-type transport system permease components
LBUL_2005	LivH	0.300	0.241	3.352				Branched-chain amino acid ABC-type transport system permease components
LBUL_2020	GlnQ					4.257		ABC-type polar amino acid transport system ATPase component
LBUL_2034	IlvB	0.419	0.253		11.093			Thiamine pyrophosphate-requiring protein
Carbohydrate transport and metabolism								
LBUL_0033	RbsK			2.484	0.249			Sugar kinase ribokinase family
LBUL_0039	COG0702	3.297	15.670	2.025				Predicted nucleoside-diphosphate-sugar epimerase
LBUL_0099	NagC	0.363	0.354		2.571			Transcriptional regulator/sugar kinase
LBUL_0129	TagH		2.794					ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0145	UgpA			5.749				ABC-type sugar transport systems permease components
LBUL_0173	PtsN			0.212	0.441			Phosphotransferase system mannitol/fructose-specific IIA domain (Ntr-type)
LBUL_0177	MalK	0.331	0.347	2.596				ABC-type sugar transport systems ATPase components
LBUL_0215	MalG	0.202	0.155		0.117			ABC-type maltose transport systems permease component
LBUL_0216	MalK	0.386	0.055		0.159			ABC-type sugar transport systems ATPase components
LBUL_0238	MalK				2.013			ABC-type sugar transport systems ATPase components
LBUL_0239	MalK				3.705			ABC-type sugar transport systems ATPase components
LBUL_0246	Eda	0.221	0.285		0.053			2-keto-3-deoxy-6-phosphogluconate aldolase
LBUL_0251	NanE	2.639				0.290	0.458	Putative N-acetylmannosamine-6-phosphate epimerase
LBUL_0256	MalK		0.347	6.884				ABC-type sugar transport systems ATPase components
LBUL_0262	MalK	0.380	0.268		0.077			ABC-type sugar transport systems ATPase components
LBUL_0263	UgpA	0.357	0.268		0.085			ABC-type sugar transport systems permease components
LBUL_0266	GlpR	0.318						Transcriptional regulators of sugar metabolism
LBUL_0303	GlgC		0.392		2.057		0.432	ADP-glucose pyrophosphorylase
LBUL_0326	FrvX			2.960				Cellulase M and related protein
LBUL_0327	NanE		0.461					Putative N-acetylmannosamine-6-phosphate epimerase
LBUL_0399	GlpR		2.272	0.487				Transcriptional regulators of sugar metabolism
LBUL_0445	MglA		0.333					ABC-type sugar transport system ATPase component
LBUL_0498	GlgC		0.448		5.087			ADP-glucose pyrophosphorylase
LBUL_0532	WcaG		0.451					Nucleoside-diphosphate-sugar epimerase
LBUL_0550	MglA		2.433	0.243	0.098			ABC-type sugar transport system ATPase component
LBUL_0578	MalK		0.448					ABC-type sugar transport systems ATPase components
LBUL_0579	UgpA	0.442	0.396					ABC-type sugar transport systems permease components
LBUL_0580	UgpE		0.268					ABC-type sugar transport system permease component
LBUL_0581	UgpB		0.402					ABC-type sugar transport system periplasmic component
LBUL_0585	NagD	2.582		0.484				Predicted sugar phosphatase of the HAD superfamily
LBUL_0674	COG1819	0.499	0.220					Glycosyl transferase related to UDP-glucuronosyltransferase
LBUL_0676	XylB		0.434					Sugar (pentulose and hexulose) kinase
LBUL_0821	WcaG		0.318	0.258	0.196			Nucleoside-diphosphate-sugar epimerase
LBUL_0869	UgpE	0.300						ABC-type sugar transport system permease component
LBUL_0870	MalK				0.363			ABC-type sugar transport systems ATPase components
LBUL_0882	MalK		0.216					ABC-type sugar transport systems ATPase components
LBUL_0883	MalK		0.350		5.201			ABC-type sugar transport systems ATPase components
LBUL_0904	GalK		0.179	0.456				Galactokinase
LBUL_0905	GalK	0.232	0.157					Galactokinase
LBUL_0906	GalK	0.156	0.171		3.061			Galactokinase
LBUL_0919	COG5309				4.804			Exo-beta-1 3-glucanase
LBUL_0989	MalK	0.408			4.992			ABC-type sugar transport systems ATPase components
LBUL_1069	MalK			7.779				ABC-type sugar transport systems ATPase components
LBUL_1070	MalK			7.598				ABC-type sugar transport systems ATPase components
LBUL_1148	TagH	0.413	0.206	0.267				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1204	MglA			62.155				ABC-type sugar transport system ATPase component
LBUL_1213	MalK			2.998				ABC-type sugar transport systems ATPase components
LBUL_1214	UgpA				4.248			ABC-type sugar transport systems permease components
LBUL_1261	MalK	0.137						ABC-type sugar transport systems ATPase components
LBUL_1292	MalK	0.324	0.262	2.588	0.193			ABC-type sugar transport systems ATPase components
LBUL_1293	MalK	0.282	0.278	2.768	0.148			ABC-type sugar transport systems ATPase components
LBUL_1297	DAK1			0.284	0.412			Dihydroxyacetone kinase
LBUL_1419	Hit			0.375				Diadenosine tetraphosphate (Ap4A) hydrolase and other HIT family hydrolase
LBUL_1422	SgbH						2.359	3-hexulose-6-phosphate synthase and related protein
LBUL_1562	MalK	0.212					0.162	ABC-type sugar transport systems ATPase components
LBUL_1588	MalK				0.442			ABC-type sugar transport systems ATPase components
LBUL_1630	GalK			0.381	0.121			Galactokinase
LBUL_1639	RbsB			31.959				ABC-type sugar transport system periplasmic component
LBUL_1652	UhpC					2.471		Sugar phosphate permease
LBUL_1658	UhpC	2.845	2.903		0.017	0.058		Sugar phosphate permease
LBUL_1664	WcaG	0.485						Nucleoside-diphosphate-sugar epimerase
LBUL_1693	MglA				7.298			ABC-type sugar transport system ATPase component
LBUL_1700	MalK			0.272	0.323			ABC-type sugar transport systems ATPase components
LBUL_1710	GlgA				5.122			Glycogen synthase
LBUL_1772	MglA				2.425			ABC-type sugar transport system ATPase component
LBUL_1807	GlgA	0.393	0.294	2.498				Glycogen synthase
LBUL_1835	WcaG			6.551				Nucleoside-diphosphate-sugar epimerase
LBUL_1854	COG1819			2.298				Glycosyl transferase related to UDP-glucuronosyltransferase
LBUL_1857	CapC	0.282						Capsular polysaccharide biosynthesis protein
LBUL_1870	COG2074		0.429					2-phosphoglycerate kinase
LBUL_1959	RhaT		0.206		0.417			Permease of the drug/metabolite transporter (DMT) superfamily
LBUL_2001	RbsB	0.484						ABC-type sugar transport system periplasmic component
LBUL_2002	RbsB				0.308	0.330		ABC-type sugar transport system periplasmic component
LBUL_2003	MglA			2.851				ABC-type sugar transport system ATPase component
LBUL_2004	AraH		0.416	3.269				Ribose/xylose/arabinose/galactoside ABC-type transport systems permease components
LBUL_2005	AraH	0.300	0.241	3.352				Ribose/xylose/arabinose/galactoside ABC-type transport systems permease components
LBUL_2020	MalK					4.257		ABC-type sugar transport systems ATPase components
LBUL_2034	COG3961	0.419	0.253		11.093			Pyruvate decarboxylase
Cell division and chromosome partitioning								
LBUL_0129	FtsE		2.794					Predicted ATPase involved in cell division
LBUL_0177	FtsE	0.331	0.347	2.596				Predicted ATPase involved in cell division
LBUL_0216	FtsE	0.386	0.055		0.159			Predicted ATPase involved in cell division
LBUL_0238	FtsE				2.013			Predicted ATPase involved in cell division
LBUL_0239	FtsE				3.705			Predicted ATPase involved in cell division
LBUL_0252	MesJ	2.315						Predicted ATPase of the PP-loop superfamily protein implicated in cell cycle control
LBUL_0256	FtsE		0.347	6.884				Predicted ATPase involved in cell division
LBUL_0262	FtsE	0.380	0.268		0.077			Predicted ATPase involved in cell division
LBUL_0320	COG2919		0.398					Septum formation initiator
LBUL_0322	MesJ		0.484		0.260			Predicted ATPase of the PP-loop superfamily protein implicated in cell cycle control
LBUL_0445	FtsE		0.333					Predicted ATPase involved in cell division
LBUL_0529	FtsK		0.349	2.661				DNA segregation ATPase FtsK/SpoIIIE and related protein
LBUL_0548	GidA				0.425			NAD/FAD-utilizing enzyme apparently involved in cell division
LBUL_0550	FtsE		2.433	0.243	0.098			Predicted ATPase involved in cell division
LBUL_0578	FtsE		0.448					Predicted ATPase involved in cell division
LBUL_0591	CrcB			6.545		2.785		Integral membrane protein possibly involved in chromosome condensation
LBUL_0646	MreB				0.459			Actin-like ATPase involved in cell morphogenesis

LBUL_0655	EzrA			0.451				Negative regulator of septation ring formation
LBUL_0663	MreB		0.383		3.612			Actin-like ATPase involved in cell morphogenesis
LBUL_0676	FtsA		0.434					Actin-like ATPase involved in cell division
LBUL_0677	FtsZ				2.291			Cell division GTPase
LBUL_0688	MesJ		2.445		8.246			Predicted ATPase of the PP-loop superfamily protein implicated in cell cycle control
LBUL_0697	FtsW		0.382		2.050			Bacterial cell division membrane protein
LBUL_0712	CDC3			0.330				Septin family protein
LBUL_0870	FtsE				0.363			Predicted ATPase involved in cell division
LBUL_0882	FtsE		0.216					Predicted ATPase involved in cell division
LBUL_0883	FtsE		0.350		5.201			Predicted ATPase involved in cell division
LBUL_0989	FtsE	0.408			4.992			Predicted ATPase involved in cell division
LBUL_1060	Smc			0.352				Chromosome segregation ATPase
LBUL_1069	FtsE			7.779				Predicted ATPase involved in cell division
LBUL_1070	FtsE			7.598				Predicted ATPase involved in cell division
LBUL_1189	GidA			0.412				NAD/FAD-utilizing enzyme apparently involved in cell division
LBUL_1204	FtsE			62.155				Predicted ATPase involved in cell division
LBUL_1213	FtsE			2.998				Predicted ATPase involved in cell division
LBUL_1227	MreB	0.367	0.479					Actin-like ATPase involved in cell morphogenesis
LBUL_1261	FtsE	0.137						Predicted ATPase involved in cell division
LBUL_1282	Mrp		0.358		4.280			ATPase involved in chromosome partitioning
LBUL_1285	Smc		0.366					Chromosome segregation ATPase
LBUL_1286	Smc		0.326					Chromosome segregation ATPase
LBUL_1292	FtsE	0.324	0.262	2.588	0.193			Predicted ATPase involved in cell division
LBUL_1293	FtsE	0.282	0.278	2.768	0.148			Predicted ATPase involved in cell division
LBUL_1315	Smc				4.141		0.219	Chromosome segregation ATPase
LBUL_1562	FtsE	0.212					0.162	Predicted ATPase involved in cell division
LBUL_1588	FtsE				0.442			Predicted ATPase involved in cell division
LBUL_1693	FtsE				7.298			Predicted ATPase involved in cell division
LBUL_1700	FtsE			0.272	0.323			Predicted ATPase involved in cell division
LBUL_1772	FtsE				2.425			Predicted ATPase involved in cell division
LBUL_1900	Soj		0.321	2.324				ATPase involved in chromosome partitioning
LBUL_2003	FtsE			2.851				Predicted ATPase involved in cell division
LBUL_2020	FtsE					4.257		Predicted ATPase involved in cell division
LBUL_2035	GidA	0.201					3.583	NAD/FAD-utilizing enzyme apparently involved in cell division
Cell envelope biogenesis, outer membrane								
LBUL_0031	Acm				3.249		0.131	Lyzozyme M1 (1 4-beta-N-acetylmuramidase)
LBUL_0033	RfaE			2.484	0.249			ADP-heptose synthase bifunctional sugar kinase/adenylyltransferase
LBUL_0039	COG0702	3.297	15.670	2.025				Predicted nucleoside-diphosphate-sugar epimerase
LBUL_0061	WcaA				40.987			Glycosyltransferase involved in cell wall biogenesis
LBUL_0118	Prc						0.415	Periplasmic protease
LBUL_0129	TagH		2.794					ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0140	Spr		0.404		8.677		0.297	Cell wall-associated hydrolase (invasion-associated protein)
LBUL_0145	COG4623			5.749				Predicted soluble lytic transglycosylase fused to an ABC-type amino acid-binding protein
LBUL_0161	DdlA		0.475					D-alanine-D-alanine ligase and related ATP-grasp protein
LBUL_0177	TagH	0.331	0.347	2.596				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0184	DdlA			0.350				D-alanine-D-alanine ligase and related ATP-grasp protein
LBUL_0206	MdoB	0.181	0.150	2.028				Phosphoglycerol transferase and related protein alkaline phosphatase superfamily
LBUL_0210	MurE	0.480	0.335					UDP-N-acetylmuramyl tripeptide synthase
LBUL_0216	TagH	0.386	0.055		0.159			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0217	COG4623	0.412	0.171		0.224			Predicted soluble lytic transglycosylase fused to an ABC-type amino acid-binding protein
LBUL_0238	TagH				2.013			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0239	TagH				3.705			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0256	TagH		0.347	6.884				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0261	COG4623	0.421	0.335	2.825	0.088			Predicted soluble lytic transglycosylase fused to an ABC-type amino acid-binding protein
LBUL_0262	TagH	0.380	0.268		0.077			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0303	GlmU		0.392		2.057		0.432	N-acetylglucosamine-1-phosphate uridyltransferase (contains nucleotidyltransferase and I-patch acetyltransferase domains)
LBUL_0348	LepA	0.379	0.465					Membrane GTPase LepA
LBUL_0404	OCH1		0.476					Mannosyltransferase OCH1
LBUL_0445	TagH		0.333					ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0451	MurI				2.251		0.335	Glutamate racemase
LBUL_0493	WecB			0.357				UDP-N-acetylglucosamine 2-epimerase
LBUL_0498	GalU		0.448		5.087			UDP-glucose pyrophosphorylase
LBUL_0506	LepA				4.576			Membrane GTPase LepA
LBUL_0532	DltE		0.451					Short-chain dehydrogenase involved in D-alanine esterification of lipoteichoic acid and wall teichoic acid (D-alanine transfer protein)
LBUL_0546	Lgt		0.385					Prolipoprotein diacylglyceryltransferase
LBUL_0577	MurB		0.442					UDP-N-acetylmuramate dehydrogenase
LBUL_0578	TagH		0.448					ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0623	MdoB			0.258				Phosphoglycerol transferase and related protein alkaline phosphatase superfamily
LBUL_0631	MurE		0.467		2.449			UDP-N-acetylmuramyl tripeptide synthase
LBUL_0660	MurE		0.419		4.814			UDP-N-acetylmuramyl tripeptide synthase
LBUL_0664	MreC	0.350			5.486			Cell shape-determining protein
LBUL_0671	FtsI	0.342	0.297					Cell division protein FtsI/penicillin-binding protein 2
LBUL_0672	Rfe	0.303	0.242					UDP-N-acetylmuramyl pentapeptide phosphotransferase/UDP-N- acetylglucosamine-1-phosphate transferase
LBUL_0673	MurD		0.382					UDP-N-acetylmuramoylalanine-D-glutamate ligase
LBUL_0674	MurG	0.499	0.220					UDP-N-acetylglucosamine:LPS N-acetylglucosamine transferase
LBUL_0675	FtsQ				2.251			Cell division septal protein
LBUL_0696	LepA	0.448	0.332		2.191			Membrane GTPase LepA
LBUL_0700	TagD		0.332		2.182			Cytidylyltransferase
LBUL_0701	Prc				2.301			Periplasmic protease
LBUL_0709	LepA		3.587	2.430	2.048			Membrane GTPase LepA
LBUL_0726	Glf			0.047	0.130	4.189	3.051	UDP-galactopyranose mutase
LBUL_0776	LepA		0.278		3.234			Membrane GTPase LepA
LBUL_0821	DltE		0.318	0.258	0.196			Short-chain dehydrogenase involved in D-alanine esterification of lipoteichoic acid and wall teichoic acid (D-alanine transfer protein)
LBUL_0825	DdlA		0.295	0.132	0.391			D-alanine-D-alanine ligase and related ATP-grasp protein
LBUL_0870	TagH				0.363			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0882	TagH		0.216					ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0883	TagH		0.350		5.201			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0919	MrcB				4.804			Membrane carboxypeptidase (penicillin-binding protein)
LBUL_0929	LspA	3.422		0.481	0.021	0.269		Lipoprotein signal peptidase
LBUL_0989	TagH	0.408			4.992			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_0990	LolE	0.381	0.148		4.118			ABC-type transport system involved in lipoprotein release permease component
LBUL_1060	COG3264			0.352				Small-conductance mechanosensitive channel
LBUL_1069	TagH			7.779				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1070	TagH			7.598				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1083	COG1247			0.120				Sortase and related acyltransferase
LBUL_1148	TagH	0.413	0.206	0.267				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1180	WecE					0.224		Predicted pyridoxal phosphate-dependent enzyme apparently involved in regulation of cell wall biogenesis
LBUL_1204	TagH			62.155				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1213	TagH			2.998				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1214	COG4623				4.248			Predicted soluble lytic transglycosylase fused to an ABC-type amino acid-binding protein
LBUL_1224	SrtA		0.269					Sortase (surface protein transpeptidase)
LBUL_1225	LepA	0.341						Membrane GTPase LepA
LBUL_1230	RfaE				4.575			ADP-heptose synthase bifunctional sugar kinase/adenylyltransferase
LBUL_1236	WecE	6.367	2.770	13.936	4.313			Predicted pyridoxal phosphate-dependent enzyme apparently involved in regulation of cell wall biogenesis
LBUL_1241	LepA			3.477				Membrane GTPase LepA
LBUL_1261	TagH	0.137						ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1267	COG4623	0.201	0.345					Predicted soluble lytic transglycosylase fused to an ABC-type amino acid-binding protein
LBUL_1285	TolA		0.366					Membrane protein involved in colicin uptake
LBUL_1286	COG3264		0.326					Small-conductance mechanosensitive channel

LBUL_1292	TagH	0.324	0.262	2.588	0.193			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1293	TagH	0.282	0.278	2.768	0.148			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1308	Cfa			0.462				Cyclopropane fatty acid synthase and related methyltransferase
LBUL_1330	DdlA		0.339		0.063			D-alanine-D-alanine ligase and related ATP-grasp protein
LBUL_1334	GlmS		0.371		0.052		2.314	Glucosamine 6-phosphate synthetase contains amidotransferase and phosphosugar isomerase domains
LBUL_1340	DdlA				0.105	0.303		D-alanine-D-alanine ligase and related ATP-grasp protein
LBUL_1356	Cfa		0.301		0.051			Cyclopropane fatty acid synthase and related methyltransferase
LBUL_1413	MurC		0.383	0.387	2.190			UDP-N-acetylmuramate-alanine ligase
LBUL_1515	COG3264				3.376			Small-conductance mechanosensitive channel
LBUL_1542	WcaA		0.376					Glycosyltransferase involved in cell wall biogenesis
LBUL_1562	TagH	0.212					0.162	ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1588	TagH				0.442			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1600	RfaJ				2.219			Lipopolysaccharide biosynthesis protein LPS:glycosyltransferase
LBUL_1618	GutQ			0.320				Predicted sugar phosphate isomerase involved in capsule formation
LBUL_1664	GalE	0.485						UDP-glucose 4-epimerase
LBUL_1667	Spr			4.745				Cell wall-associated hydrolase (invasion-associated protein)
LBUL_1691	WecE		0.365		2.204			Predicted pyridoxal phosphate-dependent enzyme apparently involved in regulation of cell wall biogenesis
LBUL_1693	TagH				7.298			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1700	TagH			0.272	0.323			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1707	MdoB				4.510			Phosphoglycerol transferase and related protein alkaline phosphatase superfamily
LBUL_1709	RfaG		0.140	2.453	12.066			Glycosyltransferase
LBUL_1710	RfaG				5.122			Glycosyltransferase
LBUL_1772	TagH				2.425			ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_1800	TagD	0.210	0.119					Cytidylyltransferase
LBUL_1801	COG3475	0.236	0.154	2.304				LPS biosynthesis protein
LBUL_1807	RfaG	0.393	0.294	2.498				Glycosyltransferase
LBUL_1808	WcaA	0.472		3.024	2.394			Glycosyltransferase involved in cell wall biogenesis
LBUL_1810	Glf			2.745	4.943			UDP-galactopyranose mutase
LBUL_1812	WcaJ	0.447			4.090			Sugar transferase involved in lipopolysaccharide synthesis
LBUL_1835	RfbD			6.551				dTDP-4-dehydrorhamnose reductase
LBUL_1843	COG1215	0.288	0.304					Glycosyltransferase probably involved in cell wall biogenesis
LBUL_1848	WcaA	0.191	0.480					Glycosyltransferase involved in cell wall biogenesis
LBUL_1853	OCH1		0.440					Mannosyltransferase OCH1
LBUL_1854	MurG			2.298				UDP-N-acetylglucosamine:LPS N-acetylglucosamine transferase
LBUL_1856	WcaJ	0.319						Sugar transferase involved in lipopolysaccharide synthesis
LBUL_1857	CapC	0.282						Capsular polysaccharide biosynthesis protein
LBUL_1866	Spr		0.474	3.163				Cell wall-associated hydrolase (invasion-associated protein)
LBUL_1890	RacX				2.236			Aspartate racemase
LBUL_1902	GidB		0.434					Predicted S-adenosylmethionine-dependent methyltransferase involved in cell division
LBUL_1950	DdlA						7.197	D-alanine-D-alanine ligase and related ATP-grasp protein
LBUL_1984	DltB					0.421		Predicted membrane protein involved in D-alanine export
LBUL_2003	TagH			2.851				ABC-type polysaccharide/polyol phosphate transport system ATPase component
LBUL_2020	TagH					4.257		ABC-type polysaccharide/polyol phosphate transport system ATPase component
Cell motility and secretion								
LBUL_0111	CheB	0.373						Chemotaxis response regulator containing a CheY-like receiver domain and a methylesterase domain
LBUL_0112	Tar				0.476			Methyl-accepting chemotaxis protein
LBUL_0140	FimV		0.404		8.677		0.297	Tfp pilus assembly protein FimV
LBUL_0197	FlgJ				6.229			Muramidase (flagellum-specific)
LBUL_0412	FlgJ				2.460			Muramidase (flagellum-specific)
LBUL_0515	MecA			0.451	0.471			Negative regulator of genetic competence sporulation and motility
LBUL_0535	FlaH						0.359	Predicted ATPase involved in biogenesis of flagella
LBUL_0610	PulE	7.998		18.355				Type II secretory pathway ATPase PulE/Tfp pilus assembly pathway ATPase PilB
LBUL_0612	PulG		0.445					Type II secretory pathway pseudopilin PulG
LBUL_0621	CheB			0.403				Chemotaxis response regulator containing a CheY-like receiver domain and a methylesterase domain
LBUL_0646	PilM				0.459			Tfp pilus assembly protein ATPase PilM
LBUL_0663	PilM		0.383		3.612			Tfp pilus assembly protein ATPase PilM
LBUL_0676	PilM		0.434					Tfp pilus assembly protein ATPase PilM
LBUL_0872	CheB	0.378			2.434			Chemotaxis response regulator containing a CheY-like receiver domain and a methylesterase domain
LBUL_1070	VirB11			7.598				Type IV secretory pathway VirB11 protein involved in flagella biosynthesis
LBUL_1282	FlhF		0.358		4.280			Flagellar GTP-binding protein
LBUL_1285	FlhF		0.366					Flagellar GTP-binding protein
LBUL_1601	FlaH			2.872				Predicted ATPase involved in biogenesis of flagella
LBUL_1756	FlhB				4.797			Flagellar biosynthesis pathway component FlhB
LBUL_1757	Tar	0.198		4.153				Methyl-accepting chemotaxis protein
LBUL_1944	FlhF	2.021	2.121			0.449	0.488	Flagellar GTP-binding protein
Chromatin structure and dynamics								
LBUL_1285	COG5137		0.366					Histone chaperone involved in gene silencing
LBUL_1474	COG5406	2.063		0.402				Nucleosome binding factor SPN SPT16 subunit
Coenzyme metabolism								
LBUL_0033	PdxK			2.484	0.249			Pyridoxal/pyridoxine/pyridoxamine kinase
LBUL_0084	LdhA		3.717			0.262		Lactate dehydrogenase and related dehydrogenase
LBUL_0129	FepC		2.794					ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0161	RimK		0.475					Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_0177	FepC	0.331	0.347	2.596				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0208	FolB		0.430					Dihydroneopterin aldolase
LBUL_0209	FolE	0.386	0.311					GTP cyclohydrolase I
LBUL_0210	FolC	0.480	0.335					Folylpolyglutamate synthase
LBUL_0211	FolP		0.372					Dihydropteroate synthase
LBUL_0216	FepC	0.386	0.055		0.159			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0238	FepC				2.013			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0239	FepC				3.705			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0252	PabA	2.315						Anthranilate/para-aminobenzoate synthase component II
LBUL_0256	FepC		0.347	6.884				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0262	FepC	0.380	0.268		0.077			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0294	RibD				5.175			Pyrimidine deaminase
LBUL_0303	MobA		0.392		2.057		0.432	Molybdopterin-guanine dinucleotide biosynthesis protein A
LBUL_0371	CoaE	0.308	0.329	2.217	4.203			Dephospho-CoA kinase
LBUL_0405	NadE			0.487	0.483		0.472	NAD synthase
LBUL_0421	CobL	0.359			0.480			Precorrin-6B methylase 2
LBUL_0445	FepC		0.333					ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0457	CoaA		0.368		6.995			Panthothenate kinase
LBUL_0488	BioF			0.224				7-keto-8-aminopelargonate synthetase
LBUL_0501	CobL		0.195					Precorrin-6B methylase 2
LBUL_0548	THI4				0.425			Flavoprotein involved in thiazole biosynthesis
LBUL_0550	FepC		2.433	0.243	0.098			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0578	FepC		0.448					ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0579	TupA	0.442	0.396					ABC-type tungstate transport system periplasmic component
LBUL_0580	TupA		0.268					ABC-type tungstate transport system periplasmic component
LBUL_0581	TbpA		0.402					ABC-type thiamine transport system periplasmic component
LBUL_0634	CobL		0.441					Precorrin-6B methylase 2
LBUL_0660	FolC		0.419		4.814			Folylpolyglutamate synthase
LBUL_0688	NadE		2.445		8.246			NAD synthase
LBUL_0700	CoaD		0.332		2.182			Phosphopantetheine adenylyltransferase
LBUL_0721	FolA			0.138	0.322			Dihydrofolate reductase
LBUL_0726	NadB			0.047	0.130	4.189	3.051	Aspartate oxidase
LBUL_0825	RimK		0.295	0.132	0.391			Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_0829	BirA		0.257	0.303	0.249			Biotin-(acetyl-CoA carboxylase) ligase
LBUL_0869	TupA	0.300						ABC-type tungstate transport system periplasmic component
LBUL_0870	FepC				0.363			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0882	FepC		0.216					ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0883	FepC		0.350		5.201			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components

LBUL_0904	COG1685		0.179	0.456				shikimate kinase
LBUL_0905	COG1685	0.232	0.157					shikimate kinase
LBUL_0906	COG1685	0.156	0.171		3.061			shikimate kinase
LBUL_0917	LdhA			0.343			0.498	Lactate dehydrogenase and related dehydrogenase
LBUL_0931	PabA	3.078			0.038	0.230		Anthranilate/para-aminobenzoate synthase component II
LBUL_0975	PabA			16.246				Anthranilate/para-aminobenzoate synthase component II
LBUL_0989	FepC	0.408			4.992			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1067	LdhA	0.410			3.027			Lactate dehydrogenase and related dehydrogenase
LBUL_1069	FepC			7.779				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1070	FepC			7.598				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1089	UbiE		0.350					Methylase involved in ubiquinone/menaquinone biosynthesis
LBUL_1128	CobL	0.180	0.255		3.841			Precorrin-6B methylase 2
LBUL_1138	THI4			2.770				Flavoprotein involved in thiazole biosynthesis
LBUL_1180	BioF					0.224		7-keto-8-aminopelargonate synthetase
LBUL_1189	UbiH			0.412				2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase
LBUL_1204	FepC			62.155				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1213	FepC			2.998				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1219	IlvE				4.736		0.225	Branched-chain amino acid aminotransferase/4-amino-4-deoxychorismate lyase
LBUL_1230	RibF				4.575			FAD synthase
LBUL_1261	FepC	0.137						ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1285	CobT		0.366					Cobalamin biosynthesis protein CobT (nicotinate-mononucleotide:5 6-dimethylbenzimidazole phosphoribosyltransferase)
LBUL_1292	FepC	0.324	0.262	2.588	0.193			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1293	FepC	0.282	0.278	2.768	0.148			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1304	THI80		4.434	5.059				Thiamine pyrophosphokinase
LBUL_1308	CobL			0.462				Precorrin-6B methylase 2
LBUL_1330	RimK		0.339		0.063			Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_1333	ThiL		0.334		0.045		2.116	Thiamine monophosphate kinase
LBUL_1335	ThiL		0.453		0.073		2.397	Thiamine monophosphate kinase
LBUL_1336	PDX2		0.442		0.039			Predicted glutamine amidotransferase involved in pyridoxine biosynthesis
LBUL_1340	LdhA				0.105	0.303		Lactate dehydrogenase and related dehydrogenase
LBUL_1356	UbiG		0.301		0.051			2-polyprenyl-3-methyl-5-hydroxy-6-metoxy-1 4-benzoquinol methylase
LBUL_1375	COG0212	0.420	0.245					5-formyltetrahydrofolate cyclo-ligase
LBUL_1405	CoaE			0.369				Dephospho-CoA kinase
LBUL_1413	FolC		0.383	0.387	2.190			Folylpolyglutamate synthase
LBUL_1466	THI4		0.500					Flavoprotein involved in thiazole biosynthesis
LBUL_1507	CobJ			0.482				Precorrin-3B methylase
LBUL_1532	UbiB	4.058						2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductase
LBUL_1562	FepC	0.212					0.162	ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1567	RimK			3.600				Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_1588	FepC				0.442			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1630	COG1685			0.381	0.121			shikimate kinase
LBUL_1693	FepC				7.298			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1700	FepC			0.272	0.323			ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1772	ThiQ				2.425			ABC-type thiamine transport system ATPase component
LBUL_1800	CoaD	0.210	0.119					Phosphopantetheine adenylyltransferase
LBUL_1810	HemY			2.745	4.943			Protoporphyrinogen oxidase
LBUL_1868	LdhA		3.201	3.363				Lactate dehydrogenase and related dehydrogenase
LBUL_1900	CobB		0.321	2.324				Cobyrinic acid a c-diamide synthase
LBUL_1902	CobL		0.434					Precorrin-6B methylase 2
LBUL_1942	CobB				3.898			Cobyrinic acid a c-diamide synthase
LBUL_1950	RimK						7.197	Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_1955	ThiE			2.592				Thiamine monophosphate synthase
LBUL_2003	FepC			2.851				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_2020	FepC					4.257		ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_2034	IlvB	0.419	0.253		11.093			Thiamine pyrophosphate-requiring protein
LBUL_2035	UbiH	0.201					3.583	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase
Cytoskeleton								
LBUL_0663	COG5277		0.383		3.612			Actin and related protein
LBUL_0712	CDC3			0.330				Septin family protein
Defense mechanisms								
LBUL_0129	CcmA		2.794					ABC-type multidrug transport system ATPase component
LBUL_0177	SalX	0.331	0.347	2.596				ABC-type antimicrobial peptide transport system ATPase component
LBUL_0178	SapC	0.294	0.350	2.695				ABC-type antimicrobial peptide transport system permease component
LBUL_0179	SapC	0.153	0.186				3.599	ABC-type antimicrobial peptide transport system permease component
LBUL_0216	SalX	0.386	0.055		0.159			ABC-type antimicrobial peptide transport system ATPase component
LBUL_0238	SapD				2.013			ABC-type antimicrobial peptide transport system ATPase component
LBUL_0239	SapF				3.705			ABC-type antimicrobial peptide transport system ATPase component
LBUL_0255	LcnDR2			4.400				Lantibiotic modifying protein
LBUL_0256	MdlB		0.347	6.884				ABC-type multidrug transport system ATPase and permease components
LBUL_0262	SalX	0.380	0.268		0.077			ABC-type antimicrobial peptide transport system ATPase component
LBUL_0422	Mrr	0.466			0.450			Restriction endonuclease
LBUL_0445	CcmA		0.333					ABC-type multidrug transport system ATPase component
LBUL_0523	BacA			0.373				Uncharacterized bacitracin resistance protein
LBUL_0549	HsdR			0.095	0.085			Type I site-specific restriction-modification system R (restriction) subunit and related helicase
LBUL_0550	CcmA		2.433	0.243	0.098			ABC-type multidrug transport system ATPase component
LBUL_0578	CcmA		0.448					ABC-type multidrug transport system ATPase component
LBUL_0617	HsdM		0.329	0.296				Type I restriction-modification system methyltransferase subunit
LBUL_0624	VanZ	0.208		0.214	0.470			Glycopeptide antibiotics resistance protein
LBUL_0629	AmpC		0.385		6.937			Beta-lactamase class C and other penicillin binding protein
LBUL_0634	HsdM		0.441					Type I restriction-modification system methyltransferase subunit
LBUL_0870	CcmA				0.363			ABC-type multidrug transport system ATPase component
LBUL_0882	CcmA		0.216					ABC-type multidrug transport system ATPase component
LBUL_0883	CcmA		0.350		5.201			ABC-type multidrug transport system ATPase component
LBUL_0978	NorM				5.106			Na+-driven multidrug efflux pump
LBUL_0989	SalX	0.408			4.992			ABC-type antimicrobial peptide transport system ATPase component
LBUL_0990	SalY	0.381	0.148		4.118			ABC-type antimicrobial peptide transport system permease component
LBUL_1069	MdlB			7.779				ABC-type multidrug transport system ATPase and permease components
LBUL_1070	MdlB			7.598				ABC-type multidrug transport system ATPase and permease components
LBUL_1088	LdcA		0.499					Uncharacterized protein homologs of microcin C7 resistance protein MccF
LBUL_1147	COG3440			3.468				Predicted restriction endonuclease
LBUL_1204	MdlB			62.155				ABC-type multidrug transport system ATPase and permease components
LBUL_1213	SalX			2.998				ABC-type antimicrobial peptide transport system ATPase component
LBUL_1261	MdlB	0.137						ABC-type multidrug transport system ATPase and permease components
LBUL_1286	SunT		0.326					ABC-type bacteriocin/lantibiotic exporter contain an N-terminal double-glycine peptidase domain
LBUL_1291	SapB	0.330	0.229	2.883	0.179			ABC-type antimicrobial peptide transport system permease component
LBUL_1292	SapF	0.324	0.262	2.588	0.193			ABC-type antimicrobial peptide transport system ATPase component
LBUL_1293	SapD	0.282	0.278	2.768	0.148			ABC-type antimicrobial peptide transport system ATPase component
LBUL_1450	NorM				2.425			Na+-driven multidrug efflux pump
LBUL_1523	AmpC			0.410	2.709			Beta-lactamase class C and other penicillin binding protein
LBUL_1524	AmpC				2.395		0.381	Beta-lactamase class C and other penicillin binding protein
LBUL_1562	CcmA	0.212					0.162	ABC-type multidrug transport system ATPase component
LBUL_1588	CcmA				0.442			ABC-type multidrug transport system ATPase component
LBUL_1693	CcmA				7.298			ABC-type multidrug transport system ATPase component
LBUL_1700	SalX			0.272	0.323			ABC-type antimicrobial peptide transport system ATPase component
LBUL_1701	SalY		2.593	0.303	0.363			ABC-type antimicrobial peptide transport system permease component
LBUL_1757	AcrB	0.198		4.153				Cation/multidrug efflux pump
LBUL_1772	MdlB				2.425			ABC-type multidrug transport system ATPase and permease components
LBUL_1841	NorM	0.273	0.426					Na+-driven multidrug efflux pump
LBUL_2003	CcmA			2.851				ABC-type multidrug transport system ATPase component
LBUL_2020	SalX					4.257		ABC-type antimicrobial peptide transport system ATPase component

DNA replication, recombination, and repair								
LBUL_0002	DnaN			2.479				DNA polymerase sliding clamp subunit (PCNA homolog)
LBUL_0008	Ssb	0.464	0.346	2.714		2.349		Single-stranded DNA-binding protein
LBUL_0012	DnaB	0.371		2.381				Replicative DNA helicase
LBUL_0075	XthA		0.382					Exonuclease III
LBUL_0152	RnhA			0.297	0.247			Ribonuclease HI
LBUL_0177	UvrA	0.331	0.347	2.596				Excinuclease ATPase subunit
LBUL_0212	MutT		0.274					NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0216	SbcC	0.386	0.055		0.159			ATPase involved in DNA repair
LBUL_0238	UvrA				2.013			Excinuclease ATPase subunit
LBUL_0239	UvrA				3.705			Excinuclease ATPase subunit
LBUL_0247	DinP			0.125	0.037			Nucleotidyltransferase/DNA polymerase involved in DNA repair
LBUL_0255	SPS1			4.400				Serine/threonine protein kinase
LBUL_0256	SbcC		0.347	6.884				ATPase involved in DNA repair
LBUL_0262	UvrA	0.380	0.268		0.077			Excinuclease ATPase subunit
LBUL_0288	UvrA			11.010				Excinuclease ATPase subunit
LBUL_0290	SPS1				3.405	5.086		Serine/threonine protein kinase
LBUL_0298	TatD				3.074			Mg-dependent DNase
LBUL_0299	COG1658	0.434	0.249		4.861			Small primase-like protein (Toprim domain)
LBUL_0339	MGS1				3.118			ATPase related to the helicase subunit of the Holliday junction resolvase
LBUL_0413	UvrD		0.435					Superfamily I DNA and RNA helicase
LBUL_0414	Lig		0.227		9.293			NAD-dependent DNA ligase (contains BRCT domain type II)
LBUL_0421	COG0742	0.359			0.480			N6-adenine-specific methylase
LBUL_0433	NPY1					2.654		NTP pyrophosphohydrolase containing a Zn-finger probably nucleic-acid-binding
LBUL_0445	UvrA		0.333					Excinuclease ATPase subunit
LBUL_0446	COG0675			0.222	0.047			Transposase and inactivated derivatives
LBUL_0501	COG0742		0.195					N6-adenine-specific methylase
LBUL_0510	MGS1			0.381				ATPase related to the helicase subunit of the Holliday junction resolvase
LBUL_0535	RecA						0.359	RecA/RadA recombinase
LBUL_0549	UvrB			0.095	0.085			Helicase subunit of the DNA excision repair complex
LBUL_0550	UvrA		2.433	0.243	0.098			Excinuclease ATPase subunit
LBUL_0573	Ung				0.333			Uracil DNA glycosylase
LBUL_0578	UvrA		0.448					Excinuclease ATPase subunit
LBUL_0617	COG0827		0.329	0.296				Adenine-specific DNA methylase
LBUL_0634	COG0742		0.441					N6-adenine-specific methylase
LBUL_0662	RadC				5.978			DNA repair protein
LBUL_0683	MutT				5.121			NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0699	COG0742	0.147	0.226		3.769			N6-adenine-specific methylase
LBUL_0704	HolA			0.264				DNA polymerase III delta subunit
LBUL_0744	RecQ	4.095						Superfamily II DNA helicase
LBUL_0777	HimA		0.472		3.323			Bacterial nucleoid DNA-binding protein
LBUL_0797	COG1688		0.477					Uncharacterized protein predicted to be involved in DNA repair (RAMP superfamily)
LBUL_0870	UvrA				0.363			Excinuclease ATPase subunit
LBUL_0880	MutT	4.334						NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0883	UvrA		0.350		5.201			Excinuclease ATPase subunit
LBUL_0900	GyrB	0.255	0.318		0.325			Type IIA topoisomerase (DNA gyrase/topo II topoisomerase IV) B subunit
LBUL_0901	GyrA	0.245	0.242		0.209			Type IIA topoisomerase (DNA gyrase/topo II topoisomerase IV) A subunit
LBUL_0909	DinG			0.349				Rad3-related DNA helicase
LBUL_0912	DnaD		2.171	0.324	0.163			Putative primosome component and related protein
LBUL_0913	Nth		2.081	0.229	0.234			Predicted EndoIII-related endonuclease
LBUL_0924	COG0116				4.098			Predicted N6-adenine-specific DNA methylase
LBUL_0982	XerD			0.163	0.210			Site-specific recombinase XerD
LBUL_0989	UvrA	0.408			4.992			Excinuclease ATPase subunit
LBUL_1060	SbcC			0.352				ATPase involved in DNA repair
LBUL_1069	UvrA			7.779				Excinuclease ATPase subunit
LBUL_1070	UvrA			7.598				Excinuclease ATPase subunit
LBUL_1089	COG1041		0.350					Predicted DNA modification methylase
LBUL_1123	XerD				0.393			Site-specific recombinase XerD
LBUL_1129	MutT	0.258						NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_1142	Dcm				0.417			Site-specific DNA methylase
LBUL_1145	Vsr			0.373				DNA G:T-mismatch repair endonuclease
LBUL_1160	COG3547	0.403	0.479					Transposase and inactivated derivatives
LBUL_1165	DnaG		0.333		2.490			DNA primase
LBUL_1168	RecO		0.323		2.051			Recombinational DNA repair protein (RecF pathway)
LBUL_1172	DnaC	0.493	0.357					DNA replication protein
LBUL_1186	MGS1			0.432				ATPase related to the helicase subunit of the Holliday junction resolvase
LBUL_1188	XerD			0.416				Site-specific recombinase XerD
LBUL_1190	TopA				2.803			Topoisomerase IA
LBUL_1192	RnhB	0.279	0.313		2.369			Ribonuclease HII
LBUL_1204	UvrA			62.155				Excinuclease ATPase subunit
LBUL_1213	UvrA			2.998				Excinuclease ATPase subunit
LBUL_1223	RecJ		0.325					Single-stranded DNA-specific exonuclease
LBUL_1246	PolC				4.074			DNA polymerase III alpha subunit (gram-positive type)
LBUL_1255	COG2827				3.622			Predicted endonuclease containing a URI domain
LBUL_1261	UvrA	0.137						Excinuclease ATPase subunit
LBUL_1269	SrmB		0.254		0.116			Superfamily II DNA and RNA helicase
LBUL_1285	COG5406		0.366					Nucleosome binding factor SPN SPT16 subunit
LBUL_1286	SbcC		0.326					ATPase involved in DNA repair
LBUL_1292	UvrA	0.324	0.262	2.588	0.193			Excinuclease ATPase subunit
LBUL_1293	UvrA	0.282	0.278	2.768	0.148			Excinuclease ATPase subunit
LBUL_1296	RecG			0.290				RecG-like helicase
LBUL_1308	COG1041			0.462				Predicted DNA modification methylase
LBUL_1315	RecN				4.141		0.219	ATPase involved in DNA repair
LBUL_1318	XseB				2.170			Exonuclease VII small subunit
LBUL_1362	COG3547	0.321	0.273			11.118	2.409	Transposase and inactivated derivatives
LBUL_1402	DnaC			0.362	2.109			DNA replication protein
LBUL_1403	DnaB				2.056			Replication initiation/membrane attachment protein
LBUL_1407	PolA			0.416				DNA polymerase I - 3'-5' exonuclease and polymerase domains
LBUL_1474	COG5406	2.063		0.402				Nucleosome binding factor SPN SPT16 subunit
LBUL_1484	COG0816			0.342				Predicted endonuclease involved in recombination (possible Holliday junction resolvase in Mycoplasmas and B. subtilis)
LBUL_1487	SrmB				2.234			Superfamily II DNA and RNA helicase
LBUL_1489	DinP				2.232			Nucleotidyltransferase/DNA polymerase involved in DNA repair
LBUL_1495	MutS			0.355				Mismatch repair ATPase (MutS family)
LBUL_1515	DnaX				3.376			DNA polymerase III gamma/tau subunits
LBUL_1539	COG0675			0.339	0.274			Transposase and inactivated derivatives
LBUL_1555	MutT				3.346			NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_1601	DnaB			2.872				Replicative DNA helicase
LBUL_1700	UvrA			0.272	0.323			Excinuclease ATPase subunit
LBUL_1772	UvrA				2.425			Excinuclease ATPase subunit
LBUL_1842	COG3328	0.160						Transposase and inactivated derivatives
LBUL_1944	MGS1	2.021	2.121			0.449	0.488	ATPase related to the helicase subunit of the Holliday junction resolvase
LBUL_1999	SPS1				0.359			Serine/threonine protein kinase
LBUL_2003	UvrA			2.851				Excinuclease ATPase subunit
LBUL_2020	UvrA					4.257		Excinuclease ATPase subunit
Energy production and conversion								
LBUL_0010	PPX1			2.087	2.099			Inorganic pyrophosphatase/exopolyphosphatase
LBUL_0078	Tas						0.199	Predicted oxidoreductase (related to aryl-alcohol dehydrogenase)
LBUL_0084	LdhA		3.717			0.262		Lactate dehydrogenase and related dehydrogenase
LBUL_0099	AraB	0.363	0.354		2.571			Ribulose kinase
LBUL_0100	Mdh				0.452			Malate/lactate dehydrogenase
LBUL_0117	FpaA				0.335			Uncharacterized flavoprotein
LBUL_0129	NatA		2.794					ABC-type Na+ transport system ATPase component

LBUL_0177	CydD	0.331	0.347	2.596				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0216	CydC	0.386	0.055		0.159			ABC-type transport system involved in cytochrome bd biosynthesis fused ATPase and permease components
LBUL_0238	NatA				2.013			ABC-type Na+ transport system ATPase component
LBUL_0239	NatA				3.705			ABC-type Na+ transport system ATPase component
LBUL_0246	LldD	0.221	0.285		0.053			L-lactate dehydrogenase (FMN-dependent) and related alpha-hydroxy acid dehydrogenase
LBUL_0251	LldD	2.639				0.290	0.458	L-lactate dehydrogenase (FMN-dependent) and related alpha-hydroxy acid dehydrogenase
LBUL_0256	CydD		0.347	6.884				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0262	CydD	0.380	0.268		0.077			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0327	NemA		0.461					NADH:flavin oxidoreductase Old Yellow Enzyme family
LBUL_0445	CydD		0.333					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0457	PrkB		0.368		6.995			Phosphoribulokinase
LBUL_0495	FldA	0.342	0.380		2.657			Flavodoxins
LBUL_0548	Lpd				0.425			Pyruvate/2-oxoglutarate dehydrogenase complex dihydrolipoamide dehydrogenase (E3)
LBUL_0550	CydD		2.433	0.243	0.098			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0577	GlcD		0.442					FAD/FMN-containing dehydrogenase
LBUL_0578	NatA		0.448					ABC-type Na+ transport system ATPase component
LBUL_0618	ackA		0.230	0.274	4.235			Acetate kinase
LBUL_0642	AtpG		0.493					F0F1-type ATP synthase gamma subunit
LBUL_0644	AtpC				2.054			F0F1-type ATP synthase epsilon subunit (mitochondrial delta subunit)
LBUL_0700	CitC		0.332		2.182			Citrate lyase synthetase
LBUL_0716	NfnB			0.191	2.746			Nitroreductase
LBUL_0726	SdhA			0.047	0.130	4.189	3.051	Succinate dehydrogenase/fumarate reductase flavoprotein subunit
LBUL_0823	PycA		0.397	0.211	0.345		2.227	Pyruvate carboxylase
LBUL_0825	PycA		0.295	0.132	0.391			Pyruvate carboxylase
LBUL_0870	CydD				0.363			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0882	CydD		0.216					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0883	CydD		0.350		5.201			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0902	PPX1			0.352				Inorganic pyrophosphatase/exopolyphosphatase
LBUL_0917	LdhA			0.343			0.498	Lactate dehydrogenase and related dehydrogenase
LBUL_0943	NfnB				2.945			Nitroreductase
LBUL_0989	CydD	0.408			4.992			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1060	NtpI			0.352				H+-ATPase subunit I
LBUL_1067	LdhA	0.410			3.027			Lactate dehydrogenase and related dehydrogenase
LBUL_1069	CydC			7.779				ABC-type transport system involved in cytochrome bd biosynthesis fused ATPase and permease components
LBUL_1070	CydD			7.598				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1096	PutA		0.123	0.177				NAD-dependent aldehyde dehydrogenase
LBUL_1138	SdhA			2.770				Succinate dehydrogenase/fumarate reductase flavoprotein subunit
LBUL_1189	FixC			0.412				Dehydrogenase (flavoprotein)
LBUL_1204	CydD			62.155				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1213	CydD			2.998				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1228	TtdA	0.302	0.477					Tartrate dehydratase alpha subunit/Fumarate hydratase class I N-terminal domain
LBUL_1261	CydD	0.137						ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1286	CydD		0.326					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1292	NatA	0.324	0.262	2.588	0.193			ABC-type Na+ transport system ATPase component
LBUL_1293	CydD	0.282	0.278	2.768	0.148			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1315	CydD				4.141		0.219	ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1339	FumC				0.165	0.332		Fumarase
LBUL_1340	LdhA				0.105	0.303		Lactate dehydrogenase and related dehydrogenase
LBUL_1356	Qor		0.301		0.051			NADPH:quinone reductase and related Zn-dependent oxidoreductase
LBUL_1386	AcyP			0.192				Acylphosphatase
LBUL_1466	Lpd		0.500					Pyruvate/2-oxoglutarate dehydrogenase complex dihydrolipoamide dehydrogenase (E3)
LBUL_1481	TrxA			0.179	0.422			Thiol-disulfide isomerase and thioredoxins
LBUL_1488	PPX1				3.076			Inorganic pyrophosphatase/exopolyphosphatase
LBUL_1532	UbiB	4.058						2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductase
LBUL_1560	NtpI	0.123	0.022		4.359			H+-ATPase subunit I
LBUL_1562	NatA	0.212					0.162	ABC-type Na+ transport system ATPase component
LBUL_1581	PutA			3.670	0.096			NAD-dependent aldehyde dehydrogenase
LBUL_1588	NatA				0.442			ABC-type Na+ transport system ATPase component
LBUL_1680	NhaC		0.390			31.248		Na+/H+ antiporter
LBUL_1690	IscU	0.288						NifU homolog involved in Fe-S cluster formation
LBUL_1693	CydD				7.298			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1700	CydD			0.272	0.323			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1772	CydD				2.425			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1787	UgpQ			0.224	0.411			Glycerophosphoryl diester phosphodiesterase
LBUL_1810	UbiH			2.745	4.943			2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase
LBUL_1823	GpsA	0.404		0.119			0.434	Glycerol-3-phosphate dehydrogenase
LBUL_1868	LdhA		3.201	3.363				Lactate dehydrogenase and related dehydrogenase
LBUL_1900	COG1149		0.321	2.324				MinD superfamily P-loop ATPase containing an inserted ferredoxin domain
LBUL_1915	GlcD			3.429				FAD/FMN-containing dehydrogenase
LBUL_1930	FumC				0.016	0.254	3.051	Fumarase
LBUL_1950	PycA						7.197	Pyruvate carboxylase
LBUL_1955	NemA			2.592				NADH:flavin oxidoreductase Old Yellow Enzyme family
LBUL_2003	NatA			2.851				ABC-type Na+ transport system ATPase component
LBUL_2018	COG1149				0.181			MinD superfamily P-loop ATPase containing an inserted ferredoxin domain
LBUL_2020	CydD					4.257		ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_2035	FixC	0.201					3.583	Dehydrogenase (flavoprotein)
Function unknown								
LBUL_0034	COG4720			2.081	0.259			Predicted membrane protein
LBUL_0060	COG5617		0.321		2.133			Predicted integral membrane protein
LBUL_0081	COG4720		0.471			0.207	0.200	Predicted membrane protein
LBUL_0082	COG2096						0.221	Uncharacterized conserved protein
LBUL_0116	COG4853	0.192			0.444			Uncharacterized protein
LBUL_0119	COG1576					24.895		Uncharacterized conserved protein
LBUL_0137	LemA					0.388		Uncharacterized conserved protein
LBUL_0140	XkdP		0.404		8.677		0.297	Uncharacterized protein containing LysM domain
LBUL_0153	COG4086				0.237		0.400	Predicted secreted protein
LBUL_0163	COG5438	0.308	0.094					Predicted multitransmembrane protein
LBUL_0164	COG5438		0.277				0.382	Predicted multitransmembrane protein
LBUL_0176	COG3181				0.438			Uncharacterized protein
LBUL_0200	COG3273				3.628			Uncharacterized conserved protein
LBUL_0205	COG0344	0.289	0.250					Predicted membrane protein
LBUL_0207	COG3274		0.235					Uncharacterized protein
LBUL_0218	COG3274			8.702				Uncharacterized protein
LBUL_0254	COG1598				0.301			Uncharacterized conserved protein
LBUL_0266	COG2512	0.318						Uncharacterized membrane-associated protein/domain
LBUL_0286	COG1511			9.611				Predicted membrane protein
LBUL_0307	COG4506			0.316	0.457			Uncharacterized protein
LBUL_0319	COG2302	0.268	0.407					Uncharacterized conserved protein contains S4-like domain
LBUL_0438	COG3759				0.413			Predicted membrane protein
LBUL_0454	COG4721	0.241			2.078			Predicted membrane protein
LBUL_0465	COG4320				2.228			Uncharacterized protein
LBUL_0494	COG2246	0.291	0.428		3.447			Predicted membrane protein
LBUL_0497	Rbn				2.261			Predicted membrane protein
LBUL_0510	COG3880			0.381				Uncharacterized protein with conserved CXXC pairs
LBUL_0518	COG4116				0.399			Uncharacterized protein
LBUL_0519	COG2357				0.307			Uncharacterized protein
LBUL_0529	HdeD		0.349	2.661				Uncharacterized conserved protein
LBUL_0535	COG4544						0.359	Uncharacterized conserved protein
LBUL_0551	HdeD			0.165	0.113			Uncharacterized conserved protein
LBUL_0557	COG0391			0.340			0.371	Uncharacterized conserved protein
LBUL_0558	COG1481			0.198				Uncharacterized protein

LBUL_0582	COG1624		0.290					Uncharacterized conserved protein
LBUL_0583	COG4856		0.439					Uncharacterized protein
LBUL_0608	COG5652				2.888			Predicted integral membrane protein
LBUL_0609	COG0217			0.439	2.166			Uncharacterized conserved protein
LBUL_0610	SpoIIAA	7.998		18.355				Uncharacterized protein
LBUL_0645	COG4836				0.216			Predicted membrane protein
LBUL_0653	COG5506	0.360		0.206				Uncharacterized conserved protein
LBUL_0654	COG2302	0.441						Uncharacterized conserved protein contains S4-like domain
LBUL_0658	COG2302		0.358					Uncharacterized conserved protein contains S4-like domain
LBUL_0671	COG2815	0.342	0.297					Uncharacterized protein
LBUL_0678	COG1799				2.468			Uncharacterized protein
LBUL_0680	COG2302		0.422		3.940			Uncharacterized conserved protein contains S4-like domain
LBUL_0698	COG4471	0.323	0.359		3.627			Uncharacterized protein
LBUL_0713	HdeD			0.443				Uncharacterized conserved protein
LBUL_0722	COG4359			0.147				Uncharacterized conserved protein
LBUL_0756	COG4475			0.124	0.427			Uncharacterized protein
LBUL_0769	COG1354	0.272						Uncharacterized conserved protein
LBUL_0807	COG2966				0.131			Uncharacterized conserved protein
LBUL_0815	COG4907			0.322	3.462			Predicted membrane protein
LBUL_0822	COG3199		0.322	0.224	0.222			Uncharacterized conserved protein
LBUL_0828	COG3007		0.288	0.104	0.321		2.147	Uncharacterized paraquat-inducible protein B
LBUL_0871	COG3273	0.213						Uncharacterized conserved protein
LBUL_0910	COG5353			0.467				Uncharacterized protein
LBUL_0930	COG2302	2.875		0.448	0.046	0.334		Uncharacterized conserved protein contains S4-like domain
LBUL_0934	COG2848				0.112	0.394		Uncharacterized conserved protein
LBUL_0936	DegV			0.135				Uncharacterized protein
LBUL_0969	COG2013						0.347	Uncharacterized conserved protein
LBUL_1011	GepA			0.493				Uncharacterized phage-associated protein
LBUL_1057	COG4924		0.290					Uncharacterized protein
LBUL_1060	COG4717			0.352				Uncharacterized conserved protein
LBUL_1089	COG3270		0.350					Uncharacterized conserved protein
LBUL_1127	COG0327	0.180			3.312			Uncharacterized conserved protein
LBUL_1138	COG3976			2.770				Uncharacterized protein
LBUL_1145	COG2852			0.373				Uncharacterized protein
LBUL_1148	COG4694	0.413	0.206	0.267				Uncharacterized protein
LBUL_1152	COG1479			0.293				Uncharacterized conserved protein
LBUL_1189	COG3349			0.412				Uncharacterized conserved protein
LBUL_1194	COG4479					2.910		Uncharacterized protein
LBUL_1208	DegV			0.378				Uncharacterized protein
LBUL_1211	COG4269			0.491	0.392			Predicted membrane protein
LBUL_1223	COG4199		0.325					Uncharacterized protein
LBUL_1245	COG0779	0.476						Uncharacterized protein
LBUL_1263	COG3763		2.328	0.329	0.329			Uncharacterized protein
LBUL_1264	COG4224		3.451	0.183	0.238	0.217		Uncharacterized protein
LBUL_1283	COG2739		0.441		2.088			Uncharacterized protein
LBUL_1286	COG4372		0.326					Uncharacterized protein with the myosin-like domain
LBUL_1298	COG1302			0.260	0.355			Uncharacterized protein
LBUL_1304	COG4825		4.434	5.059				Uncharacterized membrane-anchored protein
LBUL_1322	COG1302			0.382				Uncharacterized protein
LBUL_1368	COG4018		0.345	0.328				Uncharacterized protein
LBUL_1373	COG4483	0.103	0.098					Uncharacterized protein
LBUL_1378	COG4485		0.453					Predicted membrane protein
LBUL_1382	COG3382		0.340		4.159			Uncharacterized conserved protein
LBUL_1434	ErfK		0.337		2.907			Uncharacterized protein
LBUL_1442	ErfK			0.226				Uncharacterized protein
LBUL_1447	COG4372			0.123				Uncharacterized protein with the myosin-like domain
LBUL_1468	COG4478			0.297				Predicted membrane protein
LBUL_1470	COG4470			0.383				Uncharacterized protein
LBUL_1483	COG3906			0.403				Uncharacterized protein
LBUL_1485	COG4472			0.436				Uncharacterized protein
LBUL_1514	COG0718				2.115			Uncharacterized protein
LBUL_1559	COG2855	2.155	3.993					Predicted membrane protein
LBUL_1580	COG4720				2.118			Predicted membrane protein
LBUL_1670	COG4687			0.189				Uncharacterized protein
LBUL_1708	COG0392		0.390					Predicted integral membrane protein
LBUL_1750	COG0398			0.301	0.383			Uncharacterized conserved protein
LBUL_1757	COG1511	0.198		4.153				Predicted membrane protein
LBUL_1777	COG1426			0.282				Uncharacterized protein
LBUL_1797	COG2246		0.246					Predicted membrane protein
LBUL_1810	COG3349			2.745	4.943			Uncharacterized conserved protein
LBUL_1822	COG1426		8.143		0.338			Uncharacterized protein
LBUL_1854	COG5017			2.298				Uncharacterized conserved protein
LBUL_1875	COG3610			2.281				Uncharacterized conserved protein
LBUL_1876	COG2966	0.257	0.310		0.371			Uncharacterized conserved protein
LBUL_1896	COG4858		0.364		2.491			Uncharacterized membrane-bound protein
LBUL_1919	ErfK			0.305	0.129			Uncharacterized protein
General function prediction only								
LBUL_0010	COG0618			2.087	2.099			Exopolyphosphatase-related protein
LBUL_0029	COG0390				0.388			ABC-type uncharacterized transport system permease component
LBUL_0039	COG2910	3.297	15.670	2.025				Putative NADH-flavin reductase
LBUL_0042	COG1942	2.092						Uncharacterized protein 4-oxalocrotonate tautomerase homolog
LBUL_0061	COG1216				40.987			Predicted glycosyltransferase
LBUL_0075	ElsH		0.382					Metal-dependent hydrolase
LBUL_0078	ARA1						0.199	Aldo/keto reductase related to diketogulonate reductase
LBUL_0084	LdhA		3.717			0.262		Lactate dehydrogenase and related dehydrogenase
LBUL_0117	PhnP				0.335			Metal-dependent hydrolase of the beta-lactamase superfamily I
LBUL_0118	COG3975						0.415	Predicted protease with the C-terminal PDZ domain
LBUL_0123	COG3576			0.138				Predicted flavin-nucleotide-binding protein structurally related to pyridoxine 5'-phosphate oxidase
LBUL_0129	COG4586		2.794					ABC-type uncharacterized transport system ATPase component
LBUL_0131	COG3694				0.424			ABC-type uncharacterized transport system permease component
LBUL_0136	COG4783				2.487			Putative Zn-dependent protease contains TPR repeats
LBUL_0140	COG3858		0.404		8.677		0.297	Predicted glycosyl hydrolase
LBUL_0152	COG3341			0.297	0.247			Predicted double-stranded RNA/RNA-DNA hybrid binding protein
LBUL_0156	ComFC	2.071		0.441		0.312	0.433	Predicted amidophosphoribosyltransferase
LBUL_0160	COG1754			0.285	0.287			Uncharacterized C-terminal domain of topoisomerase IA
LBUL_0165	COG0400		0.138					Predicted esterase
LBUL_0176	Imp				0.438			TRAP-type uncharacterized transport system periplasmic component
LBUL_0177	COG1123	0.331	0.347	2.596				ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0180	COG4533				0.249			ABC-type uncharacterized transport system periplasmic component
LBUL_0184	COG3919			0.350				Predicted ATP-grasp protein
LBUL_0187	PaaD	4.362						Predicted metal-sulfur cluster biosynthetic protein
LBUL_0197	Bax				6.229			Uncharacterized FlgJ-related protein
LBUL_0206	COG2194	0.181	0.150	2.028				Predicted membrane-associated metal-dependent hydrolase
LBUL_0212	MutT		0.274					NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0215	COG4239	0.202	0.155		0.117			ABC-type uncharacterized transport system permease component
LBUL_0216	COG1123	0.386	0.055		0.159			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0223	COG3576		2.215	0.074				Predicted flavin-nucleotide-binding protein structurally related to pyridoxine 5'-phosphate oxidase
LBUL_0225	AbgB				0.144			Metal-dependent amidase/aminoacylase/carboxypeptidase
LBUL_0230	COG1418	0.280	0.473					Predicted HD superfamily hydrolase
LBUL_0233	COG4533			2.226		0.251		ABC-type uncharacterized transport system periplasmic component
LBUL_0235	COG4533				2.491		0.251	ABC-type uncharacterized transport system periplasmic component
LBUL_0238	COG1123				2.013			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0239	COG1123				3.705			ATPase components of various ABC-type transport systems contain duplicated ATPase

LBUL_0246	COG2070	0.221	0.285		0.053			Dioxygenase related to 2-nitropropane dioxygenase
LBUL_0251	COG2070	2.639				0.290	0.458	Dioxygenase related to 2-nitropropane dioxygenase
LBUL_0252	COG2071	2.315						Predicted glutamine amidotransferase
LBUL_0255	SPS1			4.400				Serine/threonine protein kinase
LBUL_0256	ArpD		0.347	6.884				ABC-type protease/lipase transport system ATPase and permease components
LBUL_0262	COG1123	0.380	0.268		0.077			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0282	COG4111		2.411					Uncharacterized conserved protein
LBUL_0290	SPS1				3.405	5.086		Serine/threonine protein kinase
LBUL_0295	COG1512				4.915			Beta-propeller domains of methanol dehydrogenase type
LBUL_0296	COG4087		0.168					Soluble P-type ATPase
LBUL_0297	ARC1				2.240			EMAP domain
LBUL_0298	COG1099				3.074			Predicted metal-dependent hydrolase with the TIM-barrel fold
LBUL_0303	LpxD		0.392		2.057		0.432	UDP-3-O-(3-hydroxymyristoyl)
LBUL_0306	COG1078		0.328					HD superfamily phosphohydrolase
LBUL_0326	AbgB			2.960				Metal-dependent amidase/aminoacylase/carboxypeptidase
LBUL_0339	COG0714				3.118			MoxR-like ATPase
LBUL_0348	COG1160	0.379	0.465					Predicted GTPase
LBUL_0393	PfoR			5.484				Predicted membrane protein putative toxin regulator
LBUL_0412	Bax				2.460			Uncharacterized FlgJ-related protein
LBUL_0413	COG3973		0.435					Superfamily I DNA and RNA helicase
LBUL_0414	COG5275		0.227		9.293			BRCT domain type II
LBUL_0419	LCB5				2.183			Sphingosine kinase
LBUL_0421	COG1092	0.359			0.480			Predicted SAM-dependent methyltransferase
LBUL_0422	COG4889	0.466			0.450			Predicted helicase
LBUL_0427	COG4087		3.059					Soluble P-type ATPase
LBUL_0431	PutP		0.241					Na+/proline symporter
LBUL_0433	MutT					2.654		NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0445	Uup		0.333					ATPase components of ABC transporter with duplicated ATPase domains
LBUL_0450	COG0670				4.139		0.116	Integral membrane protein interacts with FtsH
LBUL_0454	COG4449	0.241			2.078			Predicted protease of the Abi (CAAX) family
LBUL_0457	COG4240		0.368		6.995			Predicted kinase
LBUL_0479	TlyC			0.430				Hemolysins and related protein containing CBS domains
LBUL_0480	COG2236				0.202			Predicted phosphoribosyltransferase
LBUL_0486	PfoR			5.679				Predicted membrane protein putative toxin regulator
LBUL_0498	COG2068		0.448		5.087			Uncharacterized MobA-related protein
LBUL_0500	COG1073		0.293					Hydrolase of the alpha/beta superfamily
LBUL_0501	COG1092		0.195					Predicted SAM-dependent methyltransferase
LBUL_0505	TlyC		0.480					Hemolysins and related protein containing CBS domains
LBUL_0506	COG1160				4.576			Predicted GTPase
LBUL_0510	COG0714			0.381				MoxR-like ATPase
LBUL_0516	CoiA			0.384				Competence protein
LBUL_0530	PqqL		0.340					Predicted Zn-dependent peptidase
LBUL_0531	PqqL		0.261		3.543			Predicted Zn-dependent peptidase
LBUL_0532	FabG		0.451					Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_0548	GltD				0.425			NADPH-dependent glutamate synthase beta chain and related oxidoreductase
LBUL_0549	Lhr			0.095	0.085			Lhr-like helicase
LBUL_0550	COG1123		2.433	0.243	0.098			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0551	COG2194			0.165	0.113			Predicted membrane-associated metal-dependent hydrolase
LBUL_0556	COG1660				0.334			Predicted P-loop-containing kinase
LBUL_0572	Cof				2.538			Predicted hydrolase of the HAD superfamily
LBUL_0578	COG1123		0.448					ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0579	COG4132	0.442	0.396					ABC-type uncharacterized transport system permease component
LBUL_0580	COG4132		0.268					ABC-type uncharacterized transport system permease component
LBUL_0581	COG4134		0.402					ABC-type uncharacterized transport system periplasmic component
LBUL_0582	COG1623		0.290					Predicted nucleic-acid-binding protein (contains the HHH domain)
LBUL_0585	Cof	2.582		0.484				Predicted hydrolase of the HAD superfamily
LBUL_0589	Gph	0.358						Predicted phosphatase
LBUL_0606	COG4667			13.802	8.345			Predicted esterase of the alpha-beta hydrolase superfamily
LBUL_0610	COG1855	7.998		18.355				ATPase (PilT family)
LBUL_0623	COG3083			0.258				Predicted hydrolase of alkaline phosphatase superfamily
LBUL_0634	COG4123		0.441					Predicted O-methyltransferase
LBUL_0661	COG0637		0.448	2.433	8.747			Predicted phosphatase/phosphohexomutase
LBUL_0674	COG4671	0.499	0.220					Predicted glycosyl transferase
LBUL_0683	MutT				5.121			NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0686	COG1103	0.402	0.328		5.142			pyridoxal phosphate-dependent protein
LBUL_0688	COG1365		2.445		8.246			Predicted ATPase (PP-loop superfamily)
LBUL_0696	Era	0.448	0.332		2.191			GTPase
LBUL_0699	COG1092	0.147	0.226		3.769			Predicted SAM-dependent methyltransferase
LBUL_0701	COG1750				2.301			serine protease
LBUL_0707	COG0595				2.687			Predicted hydrolase of the metallo-beta-lactamase superfamily
LBUL_0709	GTPBP1		3.587	2.430	2.048			GTPase
LBUL_0712	COG0218			0.330				Predicted GTPase
LBUL_0717	COG4533		0.254					ABC-type uncharacterized transport system periplasmic component
LBUL_0722	COG4087			0.147				Soluble P-type ATPase
LBUL_0726	COG2081			0.047	0.130	4.189	3.051	Predicted flavoprotein
LBUL_0742	COG2159		0.317		2.395			Predicted metal-dependent hydrolase of the TIM-barrel fold
LBUL_0744	COG1205	4.095						Distinct helicase family with a unique C-terminal domain including a metal-binding cysteine cluster
LBUL_0747	Obg		0.472					Predicted GTPase
LBUL_0749	ElaC		0.339					Metal-dependent hydrolase of the beta-lactamase superfamily III
LBUL_0776	COG1160		0.278		3.234			Predicted GTPase
LBUL_0804	FabH	0.215	0.145					3-oxoacyl-(acyl-carrier-protein)
LBUL_0814	COG3889			0.428				Predicted solute binding protein
LBUL_0816	PerM			0.294	3.126			Predicted permease
LBUL_0818	FabH		0.371	0.262	0.200			3-oxoacyl-(acyl-carrier-protein)
LBUL_0821	FabG		0.318	0.258	0.196			Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_0825	COG2232			0.295	0.132	0.391		Predicted ATP-dependent carboligase related to biotin carboxylase
LBUL_0828	FabI		0.288	0.104	0.321		2.147	Enoyl-(acyl-carrier-protein)
LBUL_0869	COG4590	0.300						ABC-type uncharacterized transport system permease component
LBUL_0870	COG1123				0.363			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0879	COG3858		0.315			2.214		Predicted glycosyl hydrolase
LBUL_0880	MutT	4.334						NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_0882	COG1123		0.216					ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0883	COG1123		0.350		5.201			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_0895	Cof				2.029			Predicted hydrolase of the HAD superfamily
LBUL_0902	COG0618			0.352				Exopolyphosphatase-related protein
LBUL_0906	COG2605	0.156	0.171		3.061			Predicted kinase related to galactokinase and mevalonate kinase
LBUL_0914	Cof		4.041	0.139	0.138			Predicted hydrolase of the HAD superfamily
LBUL_0917	LdhA			0.343			0.498	Lactate dehydrogenase and related dehydrogenase
LBUL_0918	COG2936				4.671			Predicted acyl esterase
LBUL_0920	PrfA				4.083			Penicillin-binding protein-related factor A putative recombinase
LBUL_0924	COG4123				4.098			Predicted O-methyltransferase
LBUL_0961	AbgB		0.270		10.350			Metal-dependent amidase/aminoacylase/carboxypeptidase
LBUL_0975	COG2071			16.246				Predicted glutamine amidotransferase
LBUL_0978	RfbX				5.106			Membrane protein involved in the export of O-antigen and teichoic acid
LBUL_0989	COG1123	0.408			4.992			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_1000	COG3981	0.228						Predicted acetyltransferase
LBUL_1067	LdhA	0.410			3.027			Lactate dehydrogenase and related dehydrogenase
LBUL_1069	ArpD			7.779				ABC-type protease/lipase transport system ATPase and permease components
LBUL_1070	ArpD			7.598				ABC-type protease/lipase transport system ATPase and permease components
LBUL_1076	COG2388	0.252						Predicted acetyltransferase
LBUL_1089	COG4123		0.350					Predicted O-methyltransferase
LBUL_1101	COG4783				2.363			Putative Zn-dependent protease contains TPR repeats
LBUL_1126	AbgB	0.089	0.141		8.033			Metal-dependent amidase/aminoacylase/carboxypeptidase
LBUL_1128	COG2384	0.180	0.255		3.841			Predicted SAM-dependent methyltransferase

LBUL_1129	MutT	0.258						NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_1138	COG3380			2.770				Predicted NAD/FAD-dependent oxidoreductase
LBUL_1148	Uup	0.413	0.206	0.267				ATPase components of ABC transporter with duplicated ATPase domains
LBUL_1157	COG4087	0.432		0.328		7.961	0.290	Soluble P-type ATPase
LBUL_1169	Era	0.256	0.499		2.176			GTPase
LBUL_1171	COG0319	0.371	0.315					Predicted metal-dependent hydrolase
LBUL_1186	COG1223			0.432				Predicted ATPase (AAA+ superfamily)
LBUL_1189	GltD			0.412				NADPH-dependent glutamate synthase beta chain and related oxidoreductase
LBUL_1193	COG1161	0.188	0.137		4.149			Predicted GTPase
LBUL_1204	ArpD			62.155				ABC-type protease/lipase transport system ATPase and permease components
LBUL_1213	COG1123			2.998				ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_1214	COG0603				4.248			Predicted PP-loop superfamily ATPase
LBUL_1223	COG0618		0.325					Exopolyphosphatase-related protein
LBUL_1225	COG1160	0.341						Predicted GTPase
LBUL_1235	COG1350	9.381	3.000	23.087	2.835			Predicted alternative tryptophan synthase beta-subunit (paralog of TrpB)
LBUL_1241	COG1160			3.477				Predicted GTPase
LBUL_1246	COG0613				4.074			Predicted metal-dependent phosphoesterase (PHP family)
LBUL_1249	COG4589		0.454					Predicted CDP-diglyceride synthetase/phosphatidate cytidylyltransferase
LBUL_1255	COG4123				3.622			Predicted O-methyltransferase
LBUL_1260	PheB	0.366						ACT domain-containing protein
LBUL_1261	ArpD	0.137						ABC-type protease/lipase transport system ATPase and permease components
LBUL_1269	Lhr		0.254		0.116			Lhr-like helicase
LBUL_1285	MDN1		0.366					AAA ATPase containing von Willebrand factor type A (vWA) domain
LBUL_1286	COG1106		0.326					Predicted ATPase
LBUL_1291	COG4174	0.330	0.229	2.883	0.179			ABC-type uncharacterized transport system permease component
LBUL_1292	COG1123	0.324	0.262	2.588	0.193			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_1293	COG1123	0.282	0.278	2.768	0.148			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_1296	COG1205			0.290				Distinct helicase family with a unique C-terminal domain including a metal-binding cysteine cluster
LBUL_1297	COG1461			0.284	0.412			Predicted kinase related to dihydroxyacetone kinase
LBUL_1305	COG1162	0.315						Predicted GTPase
LBUL_1308	COG4122			0.462				Predicted O-methyltransferase
LBUL_1315	COG4637				4.141		0.219	Predicted ATPase
LBUL_1330	COG1759		0.339		0.063			ATP-utilizing protein of ATP-grasp superfamily (probably carboligase)
LBUL_1333	COG2144		0.334		0.045		2.116	Selenophosphate synthetase-related protein
LBUL_1334	COG0121		0.371		0.052		2.314	Predicted glutamine amidotransferase
LBUL_1335	COG2144		0.453		0.073		2.397	Selenophosphate synthetase-related protein
LBUL_1336	ThiJ		0.442		0.039			Putative intracellular protease/amidase
LBUL_1340	LdhA				0.105	0.303		Lactate dehydrogenase and related dehydrogenase
LBUL_1341	COG1691				0.136	0.286		NCAIR mutase (PurE)-related protein
LBUL_1351	COG2936		0.500		5.195			Predicted acyl esterase
LBUL_1354	COG1350		0.157	0.356	0.034			Predicted alternative tryptophan synthase beta-subunit (paralog of TrpB)
LBUL_1356	COG4976		0.301		0.051			Predicted methyltransferase (contains TPR repeat)
LBUL_1358	SsnA			2.801				Cytosine deaminase and related metal-dependent hydrolase
LBUL_1368	COG3968		0.345	0.328				Uncharacterized protein related to glutamine synthetase
LBUL_1374	GlpG	0.299						Uncharacterized membrane protein (homolog of Drosophila rhomboid)
LBUL_1382	ARC1		0.340		4.159			EMAP domain
LBUL_1390	COG1399	0.353	0.477					Predicted metal-binding possibly nucleic acid-binding protein
LBUL_1414	ARC1			0.415				EMAP domain
LBUL_1419	Hit			0.375				Diadenosine tetraphosphate (Ap4A) hydrolase and other HIT family hydrolase
LBUL_1423	ComFC						2.350	Predicted amidophosphoribosyltransferase
LBUL_1450	RfbX				2.425			Membrane protein involved in the export of O-antigen and teichoic acid
LBUL_1466	HcaD		0.500					Uncharacterized NAD(FAD)-dependent dehydrogenase
LBUL_1477	COG4768			0.278				Uncharacterized protein containing a divergent version of the methyl-accepting chemotaxis-like domain
LBUL_1486	COG2872				2.597			Predicted metal-dependent hydrolase related to alanyl-tRNA synthetase HxxxH domain
LBUL_1487	Lhr				2.234			Lhr-like helicase
LBUL_1488	COG0618				3.076			Exopolyphosphatase-related protein
LBUL_1502	RimI			0.363				Acetyltransferase
LBUL_1506	FcbC			0.298				Predicted thioesterase
LBUL_1507	COG0313			0.482				Predicted methyltransferase
LBUL_1542	COG1216		0.376					Predicted glycosyltransferase
LBUL_1555	MutT				3.346			NTP pyrophosphohydrolase including oxidative damage repair protein
LBUL_1562	COG1123	0.212					0.162	ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_1588	COG4152				0.442			ABC-type uncharacterized transport system ATPase component
LBUL_1606	COG4639				2.903			Predicted kinase
LBUL_1635	RimI				0.347			Acetyltransferase
LBUL_1658	COG2270	2.845	2.903		0.017	0.058		Permease of the major facilitator superfamily
LBUL_1664	COG1090	0.485						Predicted nucleoside-diphosphate sugar epimerase
LBUL_1680	COG2056		0.390			31.248		Predicted permease
LBUL_1691	COG1103		0.365		2.204			pyridoxal phosphate-dependent protein
LBUL_1693	YhbG				7.298			ABC-type (unclassified) transport system ATPase component
LBUL_1700	COG1123			0.272	0.323			ATPase components of various ABC-type transport systems contain duplicated ATPase
LBUL_1707	COG2194				4.510			Predicted membrane-associated metal-dependent hydrolase
LBUL_1749	RimI			0.355	0.253			Acetyltransferase
LBUL_1751	COG1647			0.156				Esterase/lipase
LBUL_1756	COG1645				4.797			Uncharacterized Zn-finger containing protein
LBUL_1757	COG2409	0.198		4.153				Predicted drug exporter of the RND superfamily
LBUL_1762	MhpC			5.544				Predicted hydrolase or acyltransferase (alpha/beta hydrolase superfamily)
LBUL_1763	RimI	0.308			4.319			Acetyltransferase
LBUL_1772	ArpD				2.425			ABC-type protease/lipase transport system ATPase and permease components
LBUL_1775	MhpC			0.408	2.682			Predicted hydrolase or acyltransferase (alpha/beta hydrolase superfamily)
LBUL_1785	COG1913			0.173	0.396			Predicted Zn-dependent protease
LBUL_1800	COG1019	0.210	0.119					Predicted nucleotidyltransferase
LBUL_1802	RfbX	0.074	0.109			4.640		Membrane protein involved in the export of O-antigen and teichoic acid
LBUL_1808	COG1216	0.472		3.024	2.394			Predicted glycosyltransferase
LBUL_1810	HcaD			2.745	4.943			Uncharacterized NAD(FAD)-dependent dehydrogenase
LBUL_1823	COG2085	0.404		0.119			0.434	Predicted dinucleotide-binding protein
LBUL_1835	FabG			6.551				Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_1841	RfbX	0.273	0.426					Membrane protein involved in the export of O-antigen and teichoic acid
LBUL_1843	COG1216	0.288	0.304					Predicted glycosyltransferase
LBUL_1848	COG1216	0.191	0.480					Predicted glycosyltransferase
LBUL_1861	HflX			0.267	0.170			GTPase
LBUL_1868	LdhA		3.201	3.363				Lactate dehydrogenase and related dehydrogenase
LBUL_1885	Cof			0.499				Predicted hydrolase of the HAD superfamily
LBUL_1897	Obg		0.284					Predicted GTPase
LBUL_1906	COG0431	0.244	0.171					Predicted flavoprotein
LBUL_1907	COG0431	0.394	0.253					Predicted flavoprotein
LBUL_1936	COG2110			0.230	0.193			Predicted phosphatase homologous to the C-terminal domain of histone macroH2A1
LBUL_1942	COG3442				3.898			Predicted glutamine amidotransferase
LBUL_1944	COG0714	2.021	2.121			0.449	0.488	MoxR-like ATPase
LBUL_1950	COG2232						7.197	Predicted ATP-dependent carboligase related to biotin carboxylase
LBUL_1954	ComFC	0.368	0.393	2.979	5.512			Predicted amidophosphoribosyltransferase
LBUL_1955	COG1646			2.592				Predicted phosphate-binding protein with TIM-barrel fold
LBUL_1959	RarD		0.206		0.417			Predicted permease
LBUL_1961	Cof				0.424			Predicted hydrolase of the HAD superfamily
LBUL_1962	COG4814	0.328	0.310					Uncharacterized protein with an alpha/beta hydrolase fold
LBUL_1996	Med	0.328			0.420			Uncharacterized ABC-type transport system periplasmic component/surface lipoprotein
LBUL_1999	SPS1				0.359			Serine/threonine protein kinase
LBUL_2001	Med	0.484						Uncharacterized ABC-type transport system periplasmic component/surface lipoprotein
LBUL_2002	Med				0.308	0.330		Uncharacterized ABC-type transport system periplasmic component/surface lipoprotein
LBUL_2003	COG3845			2.851				ABC-type uncharacterized transport systems ATPase components
LBUL_2004	COG4603		0.416	3.269				ABC-type uncharacterized transport system permease component
LBUL_2005	COG1079	0.300	0.241	3.352				Uncharacterized ABC-type transport system permease component
LBUL_2017	COG0802				0.132	0.169		Predicted ATPase or kinase
LBUL_2020	COG1123					4.257		ATPase components of various ABC-type transport systems contain duplicated ATPase

LBUL_2021	COG2252				0.077			Permease
LBUL_2034	COG3960	0.419	0.253		11.093			Glyoxylate carboligase
LBUL_2035	COG2081	0.201					3.583	Predicted flavoprotein
LBUL_2036	ThdF	0.267		6.275				Predicted GTPase
Inorganic ion transport and metabolism								
LBUL_0125	CbiQ	0.155	0.302					ABC-type cobalt transport system permease component CbiQ and related transporter
LBUL_0129	NatA		2.794					ABC-type Na+ transport system ATPase component
LBUL_0138	ArsB	0.232	0.257	0.267	6.179			Na+/H+ antiporter NhaD and related arsenite permease
LBUL_0145	AbcD			5.749				ABC-type metal ion transport system permease component
LBUL_0176	PhnD				0.438			ABC-type phosphate/phosphonate transport system periplasmic component
LBUL_0177	COG3638	0.331	0.347	2.596				ABC-type phosphate/phosphonate transport system ATPase component
LBUL_0178	COG3639	0.294	0.350	2.695				ABC-type phosphate/phosphonate transport system permease component
LBUL_0179	COG3639	0.153	0.186				3.599	ABC-type phosphate/phosphonate transport system permease component
LBUL_0192	Kup				0.349		0.489	K+ transporter
LBUL_0200	KefB				3.628			Kef-type K+ transport systems membrane components
LBUL_0214	AbcD	0.229	0.117		0.140			ABC-type metal ion transport system permease component
LBUL_0215	AbcD	0.202	0.155		0.117			ABC-type metal ion transport system permease component
LBUL_0216	AbcC	0.386	0.055		0.159			ABC-type metal ion transport system ATPase component
LBUL_0217	TauA	0.412	0.171		0.224			ABC-type nitrate/sulfonate/bicarbonate transport systems periplasmic components
LBUL_0238	DppD				2.013			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0239	DppF				3.705			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0249	SUL1				0.308	0.215		Sulfate permease and related transporter (MFS superfamily)
LBUL_0256	ZnuC		0.347	6.884				ABC-type Mn/Zn transport systems ATPase component
LBUL_0261	PhnD	0.421	0.335	2.825	0.088			ABC-type phosphate/phosphonate transport system periplasmic component
LBUL_0262	AbcC	0.380	0.268		0.077			ABC-type metal ion transport system ATPase component
LBUL_0263	AbcD	0.357	0.268		0.085			ABC-type metal ion transport system permease component
LBUL_0296	MgtA		0.168					Cation transport ATPase
LBUL_0348	CysN	0.379	0.465					GTPase - Sulfate adenylate transferase subunit 1
LBUL_0427	ZntA		3.059					Cation transport ATPase
LBUL_0445	FepC		0.333					ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_0457	DppD		0.368		6.995			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0463	GppA				3.773			Exopolyphosphatase
LBUL_0479	CorB			0.430				Putative Mg2+ and Co2+ transporter CorB
LBUL_0488	COG4100			0.224				Cystathionine beta-lyase family protein involved in aluminum resistance
LBUL_0495	CysJ	0.342	0.380		2.657			Sulfite reductase alpha subunit (flavoprotein)
LBUL_0505	CorC		0.480					Putative Mg2+ and Co2+ transporter CorC
LBUL_0506	CysN				4.576			GTPase - Sulfate adenylate transferase subunit 1
LBUL_0514	ArsC			0.372				Arsenate reductase and related protein glutaredoxin family
LBUL_0529	FepD		0.349	2.661				ABC-type Fe3+-siderophore transport system permease component
LBUL_0548	TrkA				0.425			Predicted flavoprotein involved in K+ transport
LBUL_0550	ZnuC		2.433	0.243	0.098			ABC-type Mn/Zn transport systems ATPase component
LBUL_0578	CysA		0.448					ABC-type sulfate/molybdate transport systems ATPase component
LBUL_0579	ThiP	0.442	0.396					ABC-type Fe3+ transport system permease component
LBUL_0580	ThiP		0.268					ABC-type Fe3+ transport system permease component
LBUL_0581	AfuA		0.402					ABC-type Fe3+ transport system periplasmic component
LBUL_0589	ZntA	0.358						Cation transport ATPase
LBUL_0623	AslA			0.258				Arylsulfatase A
LBUL_0646	GppA				0.459			Exopolyphosphatase
LBUL_0696	CysN	0.448	0.332		2.191			GTPase - Sulfate adenylate transferase subunit 1
LBUL_0709	CysN		3.587	2.430	2.048			GTPase - Sulfate adenylate transferase subunit 1
LBUL_0712	FeoB			0.330				Fe2+ transport system protein B
LBUL_0722	MgtA			0.147				Cation transport ATPase
LBUL_0723	NlpA	3.144		8.266		3.928		ABC-type metal ion transport system periplasmic component/surface antigen
LBUL_0747	FeoB		0.472					Fe2+ transport system protein B
LBUL_0776	FeoB		0.278		3.234			Fe2+ transport system protein B
LBUL_0867	PstS	0.356						ABC-type phosphate transport system periplasmic component
LBUL_0869	PstA	0.300						ABC-type phosphate transport system permease component
LBUL_0870	PstB				0.363			ABC-type phosphate transport system ATPase component
LBUL_0871	PhoU	0.213						Phosphate uptake regulator
LBUL_0882	CbiO		0.216					ABC-type cobalt transport system ATPase component
LBUL_0883	CbiO		0.350		5.201			ABC-type cobalt transport system ATPase component
LBUL_0884	CbiQ	2.167	0.322					ABC-type cobalt transport system permease component CbiQ and related transporter
LBUL_0885	MgtE		0.355					Mg/Co/Ni transporter MgtE (contains CBS domain)
LBUL_0989	AbcC	0.408			4.992			ABC-type metal ion transport system ATPase component
LBUL_1069	CbiO			7.779				ABC-type cobalt transport system ATPase component
LBUL_1070	CbiO			7.598				ABC-type cobalt transport system ATPase component
LBUL_1138	TrkA			2.770				Predicted flavoprotein involved in K+ transport
LBUL_1157	ZntA	0.432		0.328		7.961	0.290	Cation transport ATPase
LBUL_1169	FeoB	0.256	0.499		2.176			Fe2+ transport system protein B
LBUL_1193	FeoB	0.188	0.137		4.149			Fe2+ transport system protein B
LBUL_1204	FepC			62.155				ABC-type cobalamin/Fe3+-siderophores transport systems ATPase components
LBUL_1213	AbcC			2.998				ABC-type metal ion transport system ATPase component
LBUL_1214	TauC				4.248			ABC-type nitrate/sulfonate/bicarbonate transport system permease component
LBUL_1220	CzcD	0.424	0.472	0.331				Co/Zn/Cd efflux system component
LBUL_1225	CysN	0.341						GTPase - Sulfate adenylate transferase subunit 1
LBUL_1236	COG4100	6.367	2.770	13.936	4.313			Cystathionine beta-lyase family protein involved in aluminum resistance
LBUL_1241	CysN			3.477				GTPase - Sulfate adenylate transferase subunit 1
LBUL_1261	CbiO	0.137						ABC-type cobalt transport system ATPase component
LBUL_1285	CysC		0.366					Adenylylsulfate kinase and related kinase
LBUL_1286	CbiO		0.326					ABC-type cobalt transport system ATPase component
LBUL_1291	DppB	0.330	0.229	2.883	0.179			ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_1292	DppD	0.324	0.262	2.588	0.193			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1293	DppD	0.282	0.278	2.768	0.148			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1327	ArsC		2.498	0.293	0.494			Arsenate reductase and related protein glutaredoxin family
LBUL_1372	PspE	0.337	0.425		2.875			Rhodanese-related sulfurtransferase
LBUL_1466	TrkA		0.500					Predicted flavoprotein involved in K+ transport
LBUL_1562	ZnuC	0.212					0.162	ABC-type Mn/Zn transport systems ATPase component
LBUL_1588	NatA				0.442			ABC-type Na+ transport system ATPase component
LBUL_1652	CynX					2.471		Cyanate permease
LBUL_1658	NarK	2.845	2.903		0.017	0.058		Nitrate/nitrite transporter
LBUL_1693	DppF				7.298			ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1700	COG3638			0.272	0.323			ABC-type phosphate/phosphonate transport system ATPase component
LBUL_1707	AslA				4.510			Arylsulfatase A
LBUL_1772	TauB				2.425			ABC-type nitrate/sulfonate/bicarbonate transport system ATPase component
LBUL_1810	TrkA			2.745	4.943			K+ transport systems NAD-binding component
LBUL_1861	FeoB			0.267	0.170			Fe2+ transport system protein B
LBUL_1870	COG3709		0.429					Uncharacterized component of phosphonate metabolism
LBUL_1897	FeoB		0.284					Fe2+ transport system protein B
LBUL_1900	NifH		0.321	2.324				Nitrogenase subunit NifH (ATPase)
LBUL_1929	CitT				0.030	0.274		Di- and tricarboxylate transporter
LBUL_2003	COG3638			2.851				ABC-type phosphate/phosphonate transport system ATPase component
LBUL_2020	AbcC					4.257		ABC-type metal ion transport system ATPase component
LBUL_2021	SUL1				0.077			Sulfate permease and related transporter (MFS superfamily)
LBUL_2036	FeoB	0.267		6.275				Fe2+ transport system protein B
Intracellular trafficking and secretion								
LBUL_0140	FimV		0.404		8.677		0.297	Tfp pilus assembly protein FimV
LBUL_0197	FlgJ				6.229			Muramidase (flagellum-specific)
LBUL_0370	SecY	0.337	0.313	2.524	2.739	2.994		Preprotein translocase subunit SecY
LBUL_0412	FlgJ				2.460			Muramidase (flagellum-specific)
LBUL_0535	FlaH						0.359	Predicted ATPase involved in biogenesis of flagella
LBUL_0559	ClpP	0.411		0.444				Protease subunit of ATP-dependent Clp protease
LBUL_0610	PulE	7.998		18.355				Type II secretory pathway ATPase PulE/Tfp pilus assembly pathway ATPase PilB

LBUL_0612	ComGC		0.445					Competence protein ComGC
LBUL_0646	PilM				0.459			Tfp pilus assembly protein ATPase PilM
LBUL_0663	PilM		0.383		3.612			Tfp pilus assembly protein ATPase PilM
LBUL_0676	PilM		0.434					Tfp pilus assembly protein ATPase PilM
LBUL_0929	LspA	3.422		0.481	0.021	0.269		Lipoprotein signal peptidase
LBUL_1069	YidC			7.779				Preprotein translocase subunit YidC
LBUL_1070	VirB11			7.598				Type IV secretory pathway VirB11 protein involved in flagella biosynthesis
LBUL_1124	LepB			0.337				Signal peptidase I
LBUL_1204	TadG			62.155				Flp pilus assembly protein TadG
LBUL_1226	SEC63		2.857		2.867			Preprotein translocase subunit Sec63
LBUL_1282	Ffh		0.358		4.280			Signal recognition particle GTPase
LBUL_1285	FtsY		0.366					Signal recognition particle GTPase
LBUL_1286	COG5391		0.326					Phox homology (PX) domain protein
LBUL_1296	SecA			0.290				Preprotein translocase subunit SecA (ATPase RNA helicase)
LBUL_1417	EcsB		0.458					Predicted ABC-type exoprotein transport system permease component
LBUL_1491	YajC				3.319			Preprotein translocase subunit YajC
LBUL_1546	SecE		0.464		2.743			Preprotein translocase subunit SecE
LBUL_1601	FlaH			2.872				Predicted ATPase involved in biogenesis of flagella
LBUL_1756	FlhB				4.797			Flagellar biosynthesis pathway component FlhB
LBUL_1757	SecD	0.198		4.153				Preprotein translocase subunit SecD
LBUL_1900	CpaE		0.321	2.324				Flp pilus assembly protein ATPase CpaE
LBUL_2037	YidC			3.726				Preprotein translocase subunit YidC
Lipid metabolism								
LBUL_0099	COG1924	0.363	0.354		2.571			Activator of 2-hydroxyglutaryl-CoA dehydratase (HSP70-class ATPase domain)
LBUL_0106	PlsC			3.130				1-acyl-sn-glycerol-3-phosphate acyltransferase
LBUL_0150	Cls				2.993			Phosphatidylserine/phosphatidylglycerophosphate/cardiolipin synthase
LBUL_0161	AccC		0.475					Biotin carboxylase
LBUL_0165	Aes		0.138					Esterase/lipase
LBUL_0303	IspD		0.392		2.057		0.432	4-diphosphocytidyl-2-methyl-D-erithritol synthase
LBUL_0419	LCB5				2.183			Sphingosine kinase
LBUL_0433	Idi					2.654		Isopentenyldiphosphate isomerase
LBUL_0498	IspD		0.448		5.087			4-diphosphocytidyl-2-methyl-D-erithritol synthase
LBUL_0500	PldB		0.293					Lysophospholipase
LBUL_0534	PgsA		0.386					Phosphatidylglycerophosphate synthase
LBUL_0700	TagD		0.332		2.182			Cytidylyltransferase
LBUL_0748	COG1835		0.496					Predicted acyltransferase
LBUL_0804	PaaJ	0.215	0.145					Acetyl-CoA acetyltransferase
LBUL_0805	HMG1		0.386					Hydroxymethylglutaryl-CoA reductase
LBUL_0818	PksG		0.371	0.262	0.200			3-hydroxy-3-methylglutaryl CoA synthase
LBUL_0819	AcpP			0.322	0.405			Acyl carrier protein
LBUL_0820	FabD		0.259	0.188	0.275			(acyl-carrier-protein) S-malonyltransferase
LBUL_0822	FabB		0.322	0.224	0.222			3-oxoacyl-(acyl-carrier-protein) synthase
LBUL_0823	AccB		0.397	0.211	0.345		2.227	Biotin carboxyl carrier protein
LBUL_0824	FabA		0.303	0.288	0.469			3-hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratase
LBUL_0825	COG4770		0.295	0.132	0.391			Acetyl/propionyl-CoA carboxylase alpha subunit
LBUL_0826	AccD		0.280	0.243	0.281		2.010	Acetyl-CoA carboxylase beta subunit
LBUL_0827	AccA		0.261		0.367			Acetyl-CoA carboxylase alpha subunit
LBUL_0880	Idi	4.334						Isopentenyldiphosphate isomerase
LBUL_0904	ERG12		0.179	0.456				Mevalonate kinase
LBUL_0905	MVD1	0.232	0.157					Mevalonate pyrophosphate decarboxylase
LBUL_0906	ERG12	0.156	0.171		3.061			Mevalonate kinase
LBUL_0918	Aes				4.671			Esterase/lipase
LBUL_0932	AccC	2.215			0.059	0.369		Biotin carboxylase
LBUL_1067	MmsB	0.410			3.027			3-hydroxyisobutyrate dehydrogenase and related beta-hydroxyacid dehydrogenase
LBUL_1189	FadB			0.412				3-hydroxyacyl-CoA dehydrogenase
LBUL_1249	CdsA		0.454					CDP-diglyceride synthetase
LBUL_1256	PlsC		0.237		14.967			1-acyl-sn-glycerol-3-phosphate acyltransferase
LBUL_1294	AcpP			0.288				Acyl carrier protein
LBUL_1295	PlsX			0.416				Fatty acid/phospholipid biosynthesis enzyme
LBUL_1330	AccC		0.339		0.063			Biotin carboxylase
LBUL_1340	AccC				0.105	0.303		Biotin carboxylase
LBUL_1506	FatA			0.298				Acyl-ACP thioesterase
LBUL_1549	PldB	2.842						Lysophospholipase
LBUL_1567	AccC			3.600				Biotin carboxylase
LBUL_1607	FabA		0.438		4.364		2.378	3-hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratase
LBUL_1630	ERG12			0.381	0.121			Mevalonate kinase
LBUL_1751	Aes			0.156				Esterase/lipase
LBUL_1762	PldB			5.544				Lysophospholipase
LBUL_1775	PldB			0.408	2.682			Lysophospholipase
LBUL_1800	TagD	0.210	0.119					Cytidylyltransferase
LBUL_1950	AccC						7.197	Biotin carboxylase
Nucleotide transport and metabolism								
LBUL_0079	NrdA					0.271	0.172	Ribonucleotide reductase alpha subunit
LBUL_0156	PrsA	2.071		0.441		0.312	0.433	Phosphoribosylpyrophosphate synthetase
LBUL_0161	CarB		0.475					Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0172	COG3613		0.262					Nucleoside 2-deoxyribosyltransferase
LBUL_0184	CarB			0.350				Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0185	NrdD				0.356		0.272	Oxygen-sensitive ribonucleoside-triphosphate reductase
LBUL_0212	COG1051		0.274					ADP-ribose pyrophosphatase
LBUL_0246	GuaB	0.221	0.285		0.053			IMP dehydrogenase/GMP reductase
LBUL_0249	UraA				0.308	0.215		Xanthine/uracil permease
LBUL_0250	Apt			2.210		0.211		Adenine/guanine phosphoribosyltransferase and related PRPP-binding protein
LBUL_0251	GuaB	2.639				0.290	0.458	IMP dehydrogenase/GMP reductase
LBUL_0252	GuaA	2.315						GMP synthase PP-ATPase domain/subunit
LBUL_0264	Udp	0.252	0.186					Uridine phosphorylase
LBUL_0282	COG1051		2.411					ADP-ribose pyrophosphatase
LBUL_0294	ComEB				5.175			Deoxycytidylate deaminase
LBUL_0302	Apt		0.331					Adenine/guanine phosphoribosyltransferase and related PRPP-binding protein
LBUL_0306	Dgt		0.328					dGTP triphosphohydrolase
LBUL_0327	PyrD		0.461					Dihydroorotate dehydrogenase
LBUL_0371	Adk	0.308	0.329	2.217	4.203			Adenylate kinase and related kinase
LBUL_0433	COG1051					2.654		ADP-ribose pyrophosphatase
LBUL_0457	Udk		0.368		6.995			Uridine kinase
LBUL_0463	GppA				3.773			Exopolyphosphatase
LBUL_0473	URH1					0.273		Inosine-uridine nucleoside N-ribohydrolase
LBUL_0480	Hpt				0.202			Hypoxanthine-guanine phosphoribosyltransferase
LBUL_0632	Tdk	0.413	0.353		4.287			Thymidine kinase
LBUL_0646	GppA				0.459			Exopolyphosphatase
LBUL_0683	COG1051				5.121			ADP-ribose pyrophosphatase
LBUL_0685	Pfs	0.126	0.364	4.168	5.420			Nucleoside phosphorylase
LBUL_0688	GuaA		2.445		8.246			GMP synthase PP-ATPase domain/subunit
LBUL_0720	ThyA			0.335				Thymidylate synthase
LBUL_0745	URH1			0.253	0.149			Inosine-uridine nucleoside N-ribohydrolase
LBUL_0752	UshA		2.767					5'-nucleotidase/2' 3'-cyclic phosphodiesterase and related esterase
LBUL_0774	Cmk	0.459	0.486		2.305			Cytidylate kinase
LBUL_0825	CarB		0.295	0.132	0.391			Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0880	COG1051	4.334						ADP-ribose pyrophosphatase
LBUL_0928	MIS1	4.269	2.707		0.053	0.102		Formyltetrahydrofolate synthetase
LBUL_0931	CarA	3.078			0.038	0.230		Carbamoylphosphate synthase small subunit
LBUL_0932	CarB	2.215			0.059	0.369		Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0933	PurU				0.117	0.322		Formyltetrahydrofolate hydrolase
LBUL_0975	CarA			16.246				Carbamoylphosphate synthase small subunit

LBUL_1129	COG1051	0.258						ADP-ribose pyrophosphatase
LBUL_1170	Cdd		0.461					Cytidine deaminase
LBUL_1213	Gmk			2.998				Guanylate kinase
LBUL_1222	Apt			0.467	0.429			Adenine/guanine phosphoribosyltransferase and related PRPP-binding protein
LBUL_1272	UshA				0.057	0.418		5'-nucleotidase/2' 3'-cyclic phosphodiesterase and related esterase
LBUL_1330	PurD		0.339		0.063			Phosphoribosylamine-glycine ligase
LBUL_1331	PurH		0.412		0.047		2.100	AICAR transformylase/IMP cyclohydrolase PurH (only IMP cyclohydrolase domain in Aful)
LBUL_1332	PurN				0.061			Folate-dependent phosphoribosylglycinamide formyltransferase PurN
LBUL_1333	PurM		0.334		0.045		2.116	Phosphoribosylaminoimidazole (AIR) synthetase
LBUL_1334	PurF		0.371		0.052		2.314	Glutamine phosphoribosylpyrophosphate amidotransferase
LBUL_1335	PurL		0.453		0.073		2.397	Phosphoribosylformylglycinamidine (FGAM) synthase synthetase domain
LBUL_1336	PurL		0.442		0.039			Phosphoribosylformylglycinamidine (FGAM) synthase glutamine amidotransferase domain
LBUL_1337	PurS				0.029		2.216	Phosphoribosylformylglycinamidine (FGAM) synthase PurS component
LBUL_1338	PurC				0.034		2.177	Phosphoribosylaminoimidazolesuccinocarboxamide (SAICAR) synthase
LBUL_1339	PurB				0.165	0.332		Adenylosuccinate lyase
LBUL_1340	PurK				0.105	0.303		Phosphoribosylaminoimidazole carboxylase (NCAIR synthetase)
LBUL_1341	PurE				0.136	0.286		Phosphoribosylcarboxyaminoimidazole (NCAIR) mutase
LBUL_1358	Add			2.801				Adenosine deaminase
LBUL_1405	Udk			0.369				Uridine kinase
LBUL_1419	Hit			0.375				Diadenosine tetraphosphate (Ap4A) hydrolase and other HIT family hydrolase
LBUL_1422	PyrF						2.359	Orotidine-5'-phosphate decarboxylase
LBUL_1423	PyrE						2.350	Orotate phosphoribosyltransferase
LBUL_1426	Adk		0.364					Adenylate kinase and related kinase
LBUL_1471	UshA			0.212			0.314	5'-nucleotidase/2' 3'-cyclic phosphodiesterase and related esterase
LBUL_1479	COG0127			0.323			0.498	Xanthosine triphosphate pyrophosphatase
LBUL_1555	COG1051				3.346			ADP-ribose pyrophosphatase
LBUL_1567	PurK			3.600				Phosphoribosylaminoimidazole carboxylase (NCAIR synthetase)
LBUL_1606	COG4088				2.903			Predicted nucleotide kinase
LBUL_1753	COG3613		0.158		3.998			Nucleoside 2-deoxyribosyltransferase
LBUL_1870	Gmk		0.429					Guanylate kinase
LBUL_1930	PurB				0.016	0.254	3.051	Adenylosuccinate lyase
LBUL_1950	CarB						7.197	Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_1954	PyrR	0.368	0.393	2.979	5.512			Pyrimidine operon attenuation protein/uracil phosphoribosyltransferase
LBUL_1955	PyrD			2.592				Dihydroorotate dehydrogenase
LBUL_2017	COG1428				0.132	0.169		Deoxynucleoside kinase
LBUL_2018	COG1428				0.181			Deoxynucleoside kinase
LBUL_2021	UraA				0.077			Xanthine/uracil permease
Posttranslational modification, protein turnover, chaperones								
LBUL_0118	DegQ						0.415	Trypsin-like serine protease typically periplasmic contain C-terminal PDZ domain
LBUL_0129	CydD		2.794					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0136	HtpX				2.487			Zn-dependent protease with chaperone function
LBUL_0145	CysU			5.749				ABC-type sulfate transport system permease component
LBUL_0177	CydD	0.331	0.347	2.596				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0179	CysU	0.153	0.186				3.599	ABC-type sulfate transport system permease component
LBUL_0216	CydC	0.386	0.055		0.159			ABC-type transport system involved in cytochrome bd biosynthesis fused ATPase and permease components
LBUL_0238	CydC				2.013			ABC-type transport system involved in cytochrome bd biosynthesis fused ATPase and permease components
LBUL_0239	CydD				3.705			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0241	COG4870					0.398	0.479	Cysteine protease
LBUL_0243	IbpA			0.210	0.443		0.312	Molecular chaperone (small heat shock protein)
LBUL_0256	CydD		0.347	6.884				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0262	CydD	0.380	0.268		0.077			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0263	CysU	0.357	0.268		0.085			ABC-type sulfate transport system permease component
LBUL_0339	ClpA				3.118			ATPase with chaperone activity ATP-binding subunit
LBUL_0445	CydD		0.333					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0510	ClpA			0.381				ATPase with chaperone activity ATP-binding subunit
LBUL_0514	GrxC			0.372				Glutaredoxin and related protein
LBUL_0515	MecA			0.451	0.471			Negative regulator of genetic competence sporulation and motility
LBUL_0531	Ptr		0.261		3.543			Secreted/periplasmic Zn-dependent peptidase insulinase-like
LBUL_0535	Sms						0.359	Predicted ATP-dependent serine protease
LBUL_0548	TrxB				0.425			Thioredoxin reductase
LBUL_0550	CydD		2.433	0.243	0.098			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0559	ClpP	0.411		0.444				Protease subunit of ATP-dependent Clp protease
LBUL_0578	CydD		0.448					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0579	CysU	0.442	0.396					ABC-type sulfate transport system permease component
LBUL_0580	CysU		0.268					ABC-type sulfate transport system permease component
LBUL_0605	MsrA				2.234			Peptide methionine sulfoxide reductase
LBUL_0635	HypF		0.240					Hydrogenase maturation factor
LBUL_0646	DnaK				0.459			Molecular chaperone
LBUL_0663	DnaK		0.383		3.612			Molecular chaperone
LBUL_0701	Lon				2.301			ATP-dependent Lon protease
LBUL_0710	Tig	0.413	0.493		2.583			FKBP-type peptidyl-prolyl cis-trans isomerase (trigger factor)
LBUL_0726	TrxB			0.047	0.130	4.189	3.051	Thioredoxin reductase
LBUL_0869	CysU	0.300						ABC-type sulfate transport system permease component
LBUL_0870	CydD				0.363			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0882	CydD		0.216					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0883	CydD		0.350		5.201			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_0989	CydD	0.408			4.992			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1004	PpiB			0.333				Peptidyl-prolyl cis-trans isomerase (rotamase) - cyclophilin family
LBUL_1069	CydC			7.779				ABC-type transport system involved in cytochrome bd biosynthesis fused ATPase and permease components
LBUL_1070	ATM1			7.598				ABC-type transport system involved in Fe-S cluster assembly permease and ATPase components
LBUL_1089	Pcm		0.350					Protein-L-isoaspartate carboxylmethyltransferase
LBUL_1093	Pcp	0.433	0.500					Pyrrolidone-carboxylate peptidase (N-terminal pyroglutamyl peptidase)
LBUL_1101	HtpX				2.363			Zn-dependent protease with chaperone function
LBUL_1105	AprE	0.451	0.392	8.884	0.419	2.796		Subtilisin-like serine protease
LBUL_1124	TraF			0.337				Type IV secretory pathway protease TraF
LBUL_1138	TrxB			2.770				Thioredoxin reductase
LBUL_1186	HslU			0.432				ATP-dependent protease HslVU (ClpYQ) ATPase subunit
LBUL_1204	CydD			62.155				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1213	CydD			2.998				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1214	CysU				4.248			ABC-type sulfate transport system permease component
LBUL_1226	DnaJ		2.857		2.867			DnaJ-class molecular chaperone with C-terminal Zn finger domain
LBUL_1227	DnaK	0.367	0.479					Molecular chaperone
LBUL_1228	GrpE	0.302	0.477					Molecular chaperone GrpE (heat shock protein)
LBUL_1261	CydD	0.137						ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1286	CydD		0.326					ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1292	CydD	0.324	0.262	2.588	0.193			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1293	CydD	0.282	0.278	2.768	0.148			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1315	CydD				4.141		0.219	ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1333	HypE		0.334		0.045		2.116	Hydrogenase maturation factor
LBUL_1335	HypE		0.453		0.073		2.397	Hydrogenase maturation factor
LBUL_1386	HypF			0.192				Hydrogenase maturation factor
LBUL_1424	SurA		0.481					Parvulin-like peptidyl-prolyl isomerase
LBUL_1466	TrxB		0.500					Thioredoxin reductase
LBUL_1481	COG3118			0.179	0.422			Thioredoxin domain-containing protein
LBUL_1497	GroS	0.489		0.456				Co-chaperonin GroES (HSP10)
LBUL_1503	COG1214			0.437				Inactive homolog of metal-dependent protease putative molecular chaperone
LBUL_1515	CDC6				3.376			Cdc6-related protein AAA superfamily ATPase
LBUL_1562	CydD	0.212					0.162	ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1588	CydC				0.442			ABC-type transport system involved in cytochrome bd biosynthesis fused ATPase and permease components

LBUL_1601	Sms			2.872				Predicted ATP-dependent serine protease
LBUL_1689	SufB				2.957			ABC-type transport system involved in Fe-S cluster assembly permease component
LBUL_1692	SufB				3.794			ABC-type transport system involved in Fe-S cluster assembly permease component
LBUL_1693	SufC				7.298			ABC-type transport system involved in Fe-S cluster assembly ATPase component
LBUL_1700	CydD			0.272	0.323			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1710	Spy				5.122			Predicted O-linked N-acetylglucosamine transferase SPINDLY family
LBUL_1772	CydD				2.425			ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_1785	COG5549			0.173	0.396			Predicted Zn-dependent protease
LBUL_1833	COG0229		0.201	0.161				Conserved domain frequently associated with peptide methionine sulfoxide reductase
LBUL_1944	ClpA	2.021	2.121			0.449	0.488	ATPase with chaperone activity ATP-binding subunit
LBUL_2003	CydD			2.851				ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_2020	CydD					4.257		ABC-type transport system involved in cytochrome bd biosynthesis ATPase and permease components
LBUL_2035	TrxB	0.201					3.583	Thioredoxin reductase
Secondary metabolites biosynthesis, transport, and catabolism								
LBUL_0129	Ttg2A		2.794					ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0177	Ttg2A	0.331	0.347	2.596				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0216	Ttg2A	0.386	0.055		0.159			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0238	Ttg2A				2.013			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0239	Ttg2A				3.705			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0249	BenE				0.308	0.215		Uncharacterized protein involved in benzoate metabolism
LBUL_0256	Ttg2A		0.347	6.884				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0262	Ttg2A	0.380	0.268		0.077			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0279	PncA				2.013			Amidase related to nicotinamidase
LBUL_0339	AcoR				3.118			Transcriptional activator of acetoin/glycerol metabolism
LBUL_0445	Ttg2A		0.333					ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0500	COG0412		0.293					Dienelactone hydrolase
LBUL_0532	FabG		0.451					Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_0548	COG1233				0.425			Phytoene dehydrogenase and related protein
LBUL_0550	Ttg2A		2.433	0.243	0.098			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0553	DmpA		2.448	0.284	0.181			L-aminopeptidase/D-esterase
LBUL_0578	Ttg2A		0.448					ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0726	COG1233			0.047	0.130	4.189	3.051	Phytoene dehydrogenase and related protein
LBUL_0818	BcsA		0.371	0.262	0.200			Predicted naringenin-chalcone synthase
LBUL_0819	AcpP			0.322	0.405			Acyl carrier protein
LBUL_0820	COG3321		0.259	0.188	0.275			Polyketide synthase modules and related protein
LBUL_0821	FabG		0.318	0.258	0.196			Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_0822	FabB		0.322	0.224	0.222			3-oxoacyl-(acyl-carrier-protein) synthase
LBUL_0828	FabG		0.288	0.104	0.321		2.147	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_0870	Ttg2A				0.363			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0882	Ttg2A		0.216					ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0883	Ttg2A		0.350		5.201			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_0918	COG3458				4.671			Acetyl esterase (deacetylase)
LBUL_0989	COG4181	0.408			4.992			Predicted ABC-type transport system involved in lysophospholipase L1 biosynthesis ATPase component
LBUL_0990	COG3127	0.381	0.148		4.118			Predicted ABC-type transport system involved in lysophospholipase L1 biosynthesis permease component
LBUL_1069	Ttg2A			7.779				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1070	Ttg2A			7.598				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1138	COG1233			2.770				Phytoene dehydrogenase and related protein
LBUL_1189	COG1233			0.412				Phytoene dehydrogenase and related protein
LBUL_1204	Ttg2A			62.155				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1213	Ttg2A			2.998				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1261	Ttg2A	0.137						ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1292	Ttg2A	0.324	0.262	2.588	0.193			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1293	Ttg2A	0.282	0.278	2.768	0.148			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1294	AcpP			0.288				Acyl carrier protein
LBUL_1335	DIT1		0.453		0.073		2.397	Pyoverdine/dityrosine biosynthesis protein
LBUL_1358	HutI			2.801				Imidazolonepropionase and related amidohydrolase
LBUL_1466	COG1233		0.500					Phytoene dehydrogenase and related protein
LBUL_1562	Ttg2A	0.212					0.162	ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1588	Ttg2A				0.442			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1664	FabG	0.485						Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_1693	Ttg2A				7.298			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1700	COG4181			0.272	0.323			Predicted ABC-type transport system involved in lysophospholipase L1 biosynthesis ATPase component
LBUL_1751	COG0412			0.156				Dienelactone hydrolase
LBUL_1772	Ttg2A				2.425			ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_1810	COG1233			2.745	4.943			Phytoene dehydrogenase and related protein
LBUL_1835	FabG			6.551				Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenase)
LBUL_1944	AcoR	2.021	2.121			0.449	0.488	Transcriptional activator of acetoin/glycerol metabolism
LBUL_2003	Ttg2A			2.851				ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_2020	Ttg2A					4.257		ABC-type transport system involved in resistance to organic solvents ATPase component
LBUL_2035	COG1233	0.201					3.583	Phytoene dehydrogenase and related protein
Signal transduction mechanisms								
LBUL_0010	COG3887			2.087	2.099			Predicted signaling protein consisting of a modified GGDEF domain and a DHH domain
LBUL_0012	RAD55	0.371		2.381				RecA-superfamily ATPase implicated in signal transduction
LBUL_0064	Rtn			12.958				FOG: EAL domain
LBUL_0088	LuxS		0.327		0.466			LuxS protein involved in autoinducer AI2 synthesis
LBUL_0111	OmpR	0.373						Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain
LBUL_0112	VicK				0.476			Signal transduction histidine kinase
LBUL_0118	SdrC						0.415	Predicted secreted protein containing a PDZ domain
LBUL_0145	HisJ			5.749				ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0159	Crp			0.255				cAMP-binding protein - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinase
LBUL_0173	PtsN			0.212	0.441			Phosphotransferase system mannitol/fructose-specific IIA domain (Ntr-type)
LBUL_0176	HisJ				0.438			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0217	HisJ	0.412	0.171		0.224			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0255	SPS1			4.400				Serine/threonine protein kinase
LBUL_0261	HisJ	0.421	0.335	2.825	0.088			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0290	SPS1				3.405	5.086		Serine/threonine protein kinase
LBUL_0339	AtoC				3.118			Response regulator containing CheY-like receiver AAA-type ATPase and DNA-binding domains
LBUL_0348	TypA	0.379	0.465					Predicted membrane GTPase involved in stress response
LBUL_0434	COG5001			11.955				Predicted signal transduction protein containing a membrane domain an EAL and a GGDEF domain
LBUL_0479	COG3448			0.430				CBS-domain-containing membrane protein
LBUL_0506	TypA				4.576			Predicted membrane GTPase involved in stress response
LBUL_0510	PspF			0.381				Transcriptional regulators containing an AAA-type ATPase domain and a DNA-binding domain
LBUL_0515	MecA			0.451	0.471			Negative regulator of genetic competence sporulation and motility
LBUL_0519	SpoT				0.307			Guanosine polyphosphate pyrophosphohydrolase/synthetase
LBUL_0535	RAD55						0.359	RecA-superfamily ATPase implicated in signal transduction
LBUL_0594	COG2199		0.386					FOG: GGDEF domain
LBUL_0621	OmpR			0.403				Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain
LBUL_0650	UspA			0.166				Universal stress protein UspA and related nucleotide-binding protein
LBUL_0674	COG1819	0.499	0.220					Glycosyl transferase related to UDP-glucuronosyltransferase
LBUL_0696	TypA	0.448	0.332		2.191			Predicted membrane GTPase involved in stress response
LBUL_0701	SdrC				2.301			Predicted secreted protein containing a PDZ domain
LBUL_0709	TypA		3.587	2.430	2.048			Predicted membrane GTPase involved in stress response
LBUL_0776	TypA		0.278		3.234			Predicted membrane GTPase involved in stress response
LBUL_0872	OmpR	0.378			2.434			Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain
LBUL_0885	LytS		0.355					Putative regulator of cell autolysis
LBUL_0902	COG3887			0.352				Predicted signaling protein consisting of a modified GGDEF domain and a DHH domain
LBUL_0919	ROM1				4.804			RhoGEF Guanine nucleotide exchange factor for Rho/Rac/Cdc42-like GTPase
LBUL_0933	COG3830				0.117	0.322		ACT domain-containing protein
LBUL_1172	PhoH	0.493	0.357					Phosphate starvation-inducible protein PhoH predicted ATPase
LBUL_1201	COG2199	0.172			2.038			FOG: GGDEF domain
LBUL_1214	HisJ				4.248			ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_1225	TypA	0.341						Predicted membrane GTPase involved in stress response

LBUL_1233	LuxS				3.277			LuxS protein involved in autoinducer AI2 synthesis
LBUL_1241	TypA			3.477				Predicted membrane GTPase involved in stress response
LBUL_1265	LexA			0.390	0.378		0.375	SOS-response transcriptional repressors (RecA-mediated autopeptidase)
LBUL_1267	HisJ	0.201	0.345					ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_1401	SpoT				3.314			Guanosine polyphosphate pyrophosphohydrolase/synthetase
LBUL_1488	COG3887				3.076			Predicted signaling protein consisting of a modified GGDEF domain and a DHH domain
LBUL_1515	RAD55				3.376			RecA-superfamily ATPase implicated in signal transduction
LBUL_1577	COG2199			3.867				FOG: GGDEF domain
LBUL_1601	RAD55			2.872				RecA-superfamily ATPase implicated in signal transduction
LBUL_1757	Tar	0.198		4.153				Methyl-accepting chemotaxis protein
LBUL_1781	LytT			0.258				Response regulator of the LytR/AlgR family
LBUL_1854	COG1819			2.298				Glycosyl transferase related to UDP-glucuronosyltransferase
LBUL_1905	COG2365				0.415			Protein tyrosine/serine phosphatase
LBUL_1937	COG2365		2.433	0.181	0.324			Protein tyrosine/serine phosphatase
LBUL_1940	COG2365		0.468		0.361			Protein tyrosine/serine phosphatase
LBUL_1944	AtoC	2.021	2.121			0.449	0.488	Response regulator containing CheY-like receiver AAA-type ATPase and DNA-binding domains
LBUL_1999	SPS1				0.359			Serine/threonine protein kinase
LBUL_2018	PrkA				0.181			Putative Ser protein kinase
Transcription								
LBUL_0038	COG1959			0.271				Predicted transcriptional regulator protein
LBUL_0057	FliA			6.107				DNA-directed RNA polymerase specialized sigma subunit
LBUL_0095	LysR		0.332			6.050		Transcriptional regulator
LBUL_0099	NagC	0.363	0.354		2.571			Transcriptional regulator/sugar kinase
LBUL_0104	LysR				0.269			Transcriptional regulator
LBUL_0111	OmpR	0.373						Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain
LBUL_0112	RocR				0.476			Transcriptional regulator containing PAS AAA-type ATPase and DNA-binding domains
LBUL_0140	NOT5		0.404		8.677		0.297	CCR4-NOT transcriptional regulation complex NOT5 subunit
LBUL_0157	AcrR		2.127	0.405			0.347	Transcriptional regulator
LBUL_0204	LytR	0.268	0.423					Transcriptional regulator
LBUL_0244	GreA			0.327				Transcription elongation factor
LBUL_0255	SPS1			4.400				Serine/threonine protein kinase
LBUL_0266	COG2865	0.318						Predicted transcriptional regulator protein
LBUL_0290	SPS1				3.405	5.086		Serine/threonine protein kinase
LBUL_0339	AcoR				3.118			Transcriptional activator of acetoin/glycerol metabolism
LBUL_0343	RpoC		0.291					DNA-directed RNA polymerase beta' subunit/160 kD subunit
LBUL_0376	RpoA			2.246				DNA-directed RNA polymerase alpha subunit/40 kD subunit
LBUL_0399	PhnF		2.272	0.487				Transcriptional regulators
LBUL_0479	COG4109			0.430				Predicted transcriptional regulator protein containing CBS domains
LBUL_0488	ARO8			0.224				Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_0510	PspF			0.381				Transcriptional regulators containing an AAA-type ATPase domain and a DNA-binding domain
LBUL_0519	SpoT				0.307			Guanosine polyphosphate pyrophosphohydrolase/synthetase
LBUL_0549	Mfd			0.095	0.085			Transcription-repair coupling factor (superfamily II helicase)
LBUL_0566	DeoR					0.430		Transcriptional regulator contains sigma factor-related N-terminal domain
LBUL_0587	COG3682				0.459			Predicted transcriptional regulator protein
LBUL_0621	OmpR			0.403				Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain
LBUL_0682	CspC				4.766			Cold shock protein
LBUL_0737	ARO8		4.440	3.248	0.065	0.125		Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_0738	LysR	0.385						Transcriptional regulator
LBUL_0740	ArgR	0.494						Arginine repressor
LBUL_0744	SmrB	4.095						Superfamily II DNA and RNA helicase
LBUL_0770	COG1386		0.403		0.481			Predicted transcriptional regulator protein containing the HTH domain
LBUL_0783	LysR				0.189			Transcriptional regulator
LBUL_0872	OmpR	0.378			2.434			Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain
LBUL_0897	MarR	8.757	4.324	3.438				Transcriptional regulators
LBUL_0909	DinG			0.349				Rad3-related DNA helicase
LBUL_0979	SoxR				3.260			Predicted transcriptional regulator protein
LBUL_0981	COG2865			6.701				Predicted transcriptional regulator protein
LBUL_0997	COG3620			0.348				Predicted transcriptional regulator protein
LBUL_1010	COG3620			0.369				Predicted transcriptional regulator protein
LBUL_1068	MarR			0.340				Transcriptional regulators
LBUL_1103	ARO8	0.301						Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_1124	COG2932			0.337				Predicted transcriptional regulator protein
LBUL_1163	RpoD		0.319					DNA-directed RNA polymerase sigma subunit (sigma70/sigma32)
LBUL_1164	RpoD				2.537			DNA-directed RNA polymerase sigma subunit (sigma70/sigma32)
LBUL_1180	ARO8					0.224		Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_1205	LysR				6.441			Transcriptional regulator
LBUL_1221	COG1777			6.447				Predicted transcriptional regulator protein
LBUL_1229	HrcA	0.306	0.487					Transcriptional regulator of heat shock gene
LBUL_1236	ARO8	6.367	2.770	13.936	4.313			Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_1265	LexA			0.390	0.378		0.375	SOS-response transcriptional repressors (RecA-mediated autopeptidase)
LBUL_1269	SmrB		0.254		0.116			Superfamily II DNA and RNA helicase
LBUL_1285	COG5137		0.366					Histone chaperone involved in gene silencing
LBUL_1286	COG1293		0.326					Predicted RNA-binding protein homologous to eukaryotic snRNP
LBUL_1287	Rnc	0.121	0.044	3.323	4.226			dsRNA-specific ribonuclease
LBUL_1296	RecG			0.290				RecG-like helicase
LBUL_1308	NusB			0.462				Transcription termination factor
LBUL_1311	RpoZ				2.105			DNA-directed RNA polymerase subunit K/omega
LBUL_1321	NusB			0.463				Transcription termination factor
LBUL_1381	GreA	0.460	0.437		2.990			Transcription elongation factor
LBUL_1384	COG1733			0.255				Predicted transcriptional regulator protein
LBUL_1401	SpoT				3.314			Guanosine polyphosphate pyrophosphohydrolase/synthetase
LBUL_1404	COG1327	2.617						Predicted transcriptional regulator protein consists of a Zn-ribbon and ATP-cone domains
LBUL_1474	COG5406	2.063		0.402				Nucleosome binding factor SPN SPT16 subunit
LBUL_1487	SrmB				2.234			Superfamily II DNA and RNA helicase
LBUL_1515	TIP49				3.376			DNA helicase TIP49 TBP-interacting protein
LBUL_1535	TenA		0.366					Putative transcription activator
LBUL_1545	NusG	0.355	0.376					Transcription antiterminator
LBUL_1551	RpoE			0.321				DNA-directed RNA polymerase specialized sigma subunit sigma24 homolog
LBUL_1563	COG1725	0.247	0.459					Predicted transcriptional regulator protein
LBUL_1618	RpiR			0.320				Transcriptional regulators
LBUL_1639	PurR			31.959				Transcriptional regulators
LBUL_1665	HipB			0.389				Predicted transcriptional regulator protein
LBUL_1691	ARO8		0.365		2.204			Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_1758	AcrR	0.270			0.200			Transcriptional regulator
LBUL_1781	LytT			0.258				Response regulator of the LytR/AlgR family
LBUL_1815	LytR				2.808			Transcriptional regulator
LBUL_1822	COG1476		8.143		0.338			Predicted transcriptional regulator protein
LBUL_1860	LytR	0.352					0.223	Transcriptional regulator
LBUL_1925	DeoR			3.137				Transcriptional regulator contains sigma factor-related N-terminal domain
LBUL_1944	PspF	2.021	2.121			0.449	0.488	Transcriptional regulators containing an AAA-type ATPase domain and a DNA-binding domain
LBUL_1957	CspC			2.744	3.871			Cold shock protein
LBUL_1996	PurR	0.328			0.420			Transcriptional regulators
LBUL_1999	SPS1				0.359			Serine/threonine protein kinase
Translation, ribosomal structure and biogenesis								
LBUL_0007	RpsF	0.354	0.326	2.347				Ribosomal protein S6
LBUL_0009	RpsR	0.193	0.246		3.275	5.922		Ribosomal protein S18

LBUL_0011	RplI			2.147				Ribosomal protein L9
LBUL_0117	YSH1				0.335			Predicted exonuclease of the beta-lactamase fold involved in RNA processing
LBUL_0161	RimK		0.475					Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_0219	TyrS				3.698			Tyrosyl-tRNA synthetase
LBUL_0251	COG0042	2.639				0.290	0.458	tRNA-dihydrouridine synthase
LBUL_0252	TrmU	2.315						Predicted tRNA(5-methylaminomethyl-2-thiouridylate) methyltransferase contains the PP-loop ATPase domain
LBUL_0273	AnsB			0.186				L-asparaginase/ Glu-tRNAGln amidotransferase subunit D
LBUL_0293	TrpS				4.823			Tryptophanyl-tRNA synthetase
LBUL_0294	CumB				5.175			Cytosine/adenosine deaminase
LBUL_0297	MetG				2.240			Methionyl-tRNA synthetase
LBUL_0300	KsgA		0.464		3.318			Dimethyladenosine transferase (rRNA methylation)
LBUL_0303	GCD1		0.392		2.057		0.432	Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharide biosynthesis/translation initiation factor 2B gamma/epsilon subunits (eIF-2Bgamma/eIF-2Bepsilon)
LBUL_0311	RpmE			2.715	2.847			Ribosomal protein L31
LBUL_0319	COG1188	0.268	0.407					Ribosome-associated heat shock protein implicated in the recycling of the 50S subunit (S4 paralog)
LBUL_0327	COG0042		0.461					tRNA-dihydrouridine synthase
LBUL_0328	LysU		0.484					Lysyl-tRNA synthetase (class II)
LBUL_0346	RpsL	0.389						Ribosomal protein S12
LBUL_0347	RpsG	0.372						Ribosomal protein S7
LBUL_0348	FusA	0.379	0.465					Translation elongation factors (GTPase)
LBUL_0349	RpsJ	0.429	0.353	2.733	3.095			Ribosomal protein S10
LBUL_0350	RplC	0.454	0.364	2.467	2.792			Ribosomal protein L3
LBUL_0351	RplD	0.400	0.326	2.371	2.825			Ribosomal protein L4
LBUL_0352	RplW	0.378	0.302	2.116	2.767			Ribosomal protein L23
LBUL_0353	RplB	0.393	0.316	2.018	2.457			Ribosomal protein L2
LBUL_0354	RpsS	0.406	0.335	2.647	2.826			Ribosomal protein S19
LBUL_0355	RplV	0.376	0.373	2.036	2.296			Ribosomal protein L22
LBUL_0356	RpsC	0.423	0.370	2.201	2.528			Ribosomal protein S3
LBUL_0357	RplP			2.632	2.560			Ribosomal protein L16/L10E
LBUL_0358	RpmC	0.498		2.213	2.669			Ribosomal protein L29
LBUL_0359	RpsQ			2.665	2.903			Ribosomal protein S17
LBUL_0360	RplN	0.434	0.400	2.263	2.442	2.666		Ribosomal protein L14
LBUL_0361	RplX	0.464	0.460	2.485	2.853			Ribosomal protein L24
LBUL_0362	RplE		0.461	2.609	2.987			Ribosomal protein L5
LBUL_0363	RpsN	0.414	0.427	2.180	2.835			Ribosomal protein S14
LBUL_0364	RpsH	0.453	0.441	2.424	2.695			Ribosomal protein S8
LBUL_0365	RplF	0.484	0.457	2.295	3.608			Ribosomal protein L6P/L9E
LBUL_0366	RplR		0.483	3.090	2.697			Ribosomal protein L18
LBUL_0367	RpsE	0.376	0.351	2.188	2.640	2.808		Ribosomal protein S5
LBUL_0368	RpmD	0.331	0.328	2.400	3.397			Ribosomal protein L30/L7E
LBUL_0369	RplO	0.407	0.362	3.318	2.708			Ribosomal protein L15
LBUL_0372	InfA			2.045				Translation initiation factor 1 (IF-1)
LBUL_0373	RpmJ	0.419		2.305				Ribosomal protein L36
LBUL_0374	RpsM	0.462						Ribosomal protein S13
LBUL_0375	RpsK			2.288				Ribosomal protein S11
LBUL_0377	RplQ	0.430		2.139				Ribosomal protein L17
LBUL_0382	RplM	0.325	0.442					Ribosomal protein L13
LBUL_0383	RpsI	0.415	0.421					Ribosomal protein S9
LBUL_0421	TrmA	0.359			0.480			SAM-dependent methyltransferase related to tRNA (uracil-5-)-methyltransferase
LBUL_0496	Map		0.492		2.524			Methionine aminopeptidase
LBUL_0498	GCD1		0.448		5.087			Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharide biosynthesis/translation initiation factor 2B gamma/epsilon subunits (eIF-2Bgamma/eIF-2Bepsilon)
LBUL_0501	TrmA		0.195					SAM-dependent methyltransferase related to tRNA (uracil-5-)-methyltransferase
LBUL_0506	PrfC				4.576			Peptide chain release factor RF-3
LBUL_0521	RluA	0.292		0.447				Pseudouridylate synthase 23S RNA-specific
LBUL_0541	COG1544			0.424				Ribosome-associated protein Y (PSrp-1)
LBUL_0549	SrmB			0.095	0.085			Superfamily II DNA and RNA helicase
LBUL_0633	PrfA				3.038			Protein chain release factor A
LBUL_0634	HemK		0.441					Methylase of polypeptide chain release factors
LBUL_0635	SUA5		0.240					Putative translation factor (SUA5)
LBUL_0654	RpsD	0.441						Ribosomal protein S4 and related protein
LBUL_0658	RsuA		0.358					16S rRNA uridine-516 pseudouridylate synthase and related pseudouridylate synthase
LBUL_0659	ValS		0.457		6.459			Valyl-tRNA synthetase
LBUL_0680	RluA		0.422		3.940			Pseudouridylate synthase 23S RNA-specific
LBUL_0681	IleS				4.516			Isoleucyl-tRNA synthetase
LBUL_0688	TrmU		2.445		8.246			Predicted tRNA(5-methylaminomethyl-2-thiouridylate) methyltransferase contains the PP-loop ATPase domain
LBUL_0695	Def		0.392		2.313			N-formylmethionyl-tRNA deformylase
LBUL_0696	FusA	0.448	0.332		2.191			Translation elongation factors (GTPase)
LBUL_0699	TRM1	0.147	0.226		3.769			N2 N2-dimethylguanosine tRNA methyltransferase
LBUL_0706	RpsO	0.235	0.377		2.775	3.616		Ribosomal protein S15P/S13E
LBUL_0707	YSH1				2.687			Predicted exonuclease of the beta-lactamase fold involved in RNA processing
LBUL_0709	TufB		3.587	2.430	2.048			GTPase - translation elongation factors
LBUL_0712	InfB			0.330				"Translation initiation factor 2 (IF-2; GTPase)"
LBUL_0744	SrmB	4.095						Superfamily II DNA and RNA helicase
LBUL_0747	COG0012		0.472					Predicted GTPase probable translation factor
LBUL_0749	YSH1		0.339					Predicted exonuclease of the beta-lactamase fold involved in RNA processing
LBUL_0771	RsuA	0.499						16S rRNA uridine-516 pseudouridylate synthase and related pseudouridylate synthase
LBUL_0776	InfB		0.278		3.234			"Translation initiation factor 2 (IF-2; GTPase)"
LBUL_0812	HisS		0.288		6.877			Histidyl-tRNA synthetase
LBUL_0813	AspS				7.026			Aspartyl-tRNA synthetase
LBUL_0825	RimK		0.295	0.132	0.391			Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_0842	SpoU		2.513		2.164			rRNA methylase
LBUL_0870	BMS1				0.363			GTP-binding protein required for 40S ribosome biogenesis
LBUL_0916	AsnS		0.496		6.099			Aspartyl/asparaginyl-tRNA synthetase
LBUL_0919	COG5099				4.804			RNA-binding protein of the Puf family translational repressor
LBUL_0924	HemK				4.098			Methylase of polypeptide chain release factors
LBUL_0930	RluA	2.875		0.448	0.046	0.334		Pseudouridylate synthase 23S RNA-specific
LBUL_1000	RimL	0.228						Acetyltransferase including N-acetylase of ribosomal protein
LBUL_1083	RimL			0.120				Acetyltransferase including N-acetylase of ribosomal protein
LBUL_1089	Sun		0.350					tRNA and rRNA cytosine-C5-methylase
LBUL_1110	AsnS				10.118			Aspartyl/asparaginyl-tRNA synthetase
LBUL_1111	AsnS		0.460		2.030			Aspartyl/asparaginyl-tRNA synthetase
LBUL_1166	GlyS	0.378	0.288		4.216			Glycyl-tRNA synthetase beta subunit
LBUL_1167	GlyQ		0.347		3.204			Glycyl-tRNA synthetase alpha subunit
LBUL_1169	SelB	0.256	0.499		2.176			Selenocysteine-specific translation elongation factor
LBUL_1173	RpsU				4.343			Ribosomal protein S21
LBUL_1189	Gid			0.412				NAD(FAD)-utilizing enzyme possibly involved in translation
LBUL_1197	PcnB				2.657			tRNA nucleotidyltransferase/poly(A) polymerase
LBUL_1225	FusA	0.341						Translation elongation factors (GTPase)
LBUL_1226	ZUO1		2.857		2.867			Ribosome-associated chaperone zuotin
LBUL_1239	TruB			4.501				Pseudouridine synthase
LBUL_1241	InfB			3.477				"Translation initiation factor 2 (IF-2; GTPase)"
LBUL_1253	Tsf	0.408		2.089				Translation elongation factor Ts
LBUL_1254	RpsB	0.357	0.276	3.002	5.257			Ribosomal protein S2
LBUL_1255	HemK				3.622			Methylase of polypeptide chain release factors
LBUL_1269	SrmB		0.254		0.116			Superfamily II DNA and RNA helicase
LBUL_1273	RplS	0.206						Ribosomal protein L19
LBUL_1279	TrmD	0.191	0.458		0.335			tRNA-(guanine-N1)-methyltransferase
LBUL_1280	RimM	0.242	0.448		0.243			RimM protein required for 16S rRNA processing
LBUL_1281	RpsP	0.415	0.391					Ribosomal protein S16
LBUL_1296	SrmB			0.290				Superfamily II DNA and RNA helicase
LBUL_1299	RpmB						2.098	Ribosomal protein L28

LBUL_1308	Sun			0.462				tRNA and rRNA cytosine-C5-methylase
LBUL_1316	COG1189			2.259	2.022			Predicted rRNA methylase
LBUL_1323	Efp			0.423				Translation elongation factor P (EF-P)/translation initiation factor 5A (eIF-5A)
LBUL_1325	RpmA	0.378	0.345		2.595			Ribosomal protein L27
LBUL_1326	RplU	0.444	0.371		2.086			Ribosomal protein L21
LBUL_1330	RimK		0.339		0.063			Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_1332	Fmt				0.061			Methionyl-tRNA formyltransferase
LBUL_1356	RsmC		0.301		0.051			16S RNA G1207 methylase RsmC
LBUL_1382	PheT		0.340		4.159			Phenylalanyl-tRNA synthetase beta subunit
LBUL_1383	PheS		0.364		3.688			Phenylalanyl-tRNA synthetase alpha subunit
LBUL_1385	SpoU		0.495		2.338			rRNA methylase
LBUL_1396	RplT	0.217		2.507	3.058	3.195		Ribosomal protein L20
LBUL_1397	RpmI	0.319	0.326	2.374		2.535		Ribosomal protein L35
LBUL_1398	InfC	0.299	0.364	2.181		2.402		Translation initiation factor 3 (IF-3)
LBUL_1401	ThrS				3.314			Threonyl-tRNA synthetase
LBUL_1451	LeuS		0.409		3.541			Leucyl-tRNA synthetase
LBUL_1474	Map	2.063		0.402				Methionine aminopeptidase
LBUL_1486	AlaS				2.597			Alanyl-tRNA synthetase
LBUL_1487	SrmB				2.234			Superfamily II DNA and RNA helicase
LBUL_1502	RimL			0.363				Acetyltransferase including N-acetylase of ribosomal protein
LBUL_1536	RplL	0.336			6.202			Ribosomal protein L7/L12
LBUL_1537	RplJ	0.461	0.335		2.711	4.031		Ribosomal protein L10
LBUL_1543	RplA			0.483				Ribosomal protein L1
LBUL_1547	RpmG		0.432		3.293			Ribosomal protein L33
LBUL_1554	CysS			0.351				Cysteinyl-tRNA synthetase
LBUL_1560	GlnS	0.123	0.022		4.359			Glutamyl- and glutaminyl-tRNA synthetase
LBUL_1567	RimK			3.600				Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_1635	RimL				0.347			Acetyltransferase including N-acetylase of ribosomal protein
LBUL_1749	RimL			0.355	0.253			Acetyltransferase including N-acetylase of ribosomal protein
LBUL_1778	Efp	0.441	0.186					Translation elongation factor P (EF-P)/translation initiation factor 5A (eIF-5A)
LBUL_1897	COG0012		0.284					Predicted GTPase probable translation factor
LBUL_1902	HemK		0.434					Methylase of polypeptide chain release factors
LBUL_1950	RimK						7.197	Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase)
LBUL_1955	COG0042			2.592				tRNA-dihydrouridine synthase
LBUL_2035	Gid	0.201					3.583	NAD(FAD)-utilizing enzyme possibly involved in translation
LBUL_2036	COG0012	0.267		6.275				Predicted GTPase probable translation factor
LBUL_2038	RnpA	0.264		3.257				RNase P protein component

Chapter 5

Experimental evolution of yoghurt cultures improves co-culture growth and interactions

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ABSTRACT

Most microbial ecosystems consist of many different species that influence each other. The evolutionary driving forces that shape the concomitant microbial interaction networks are poorly understood and little experimental information exists on the co-evolution of microbes. Yoghurt is milk fermented by the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* It is one of the best studied mixed cultures and represents a simple and attractive model system for ecological and evolutionary studies. *L. bulgaricus* and *S. thermophilus* have co-evolved towards optimal growth in milk, where they stimulate each other's growth through multiple nutritional interactions. However, how, with what speed and to what extent these nutritional interactions evolve is not known yet. To address this evolution process, we selected genomically characterized strains of *S. thermophilus* and *L. bulgaricus* that had not been grown together before and therefore are expected not to have optimized their metabolism to co-cultivation. These strains were cultivated in co-culture in milk for more than 1000 generations in sequential batch cultures under a constant growth regime. The evolved mixed culture showed improved acidification rate, survival and exopolysaccharide production, which are all important industrial traits. Moreover, combinatorial co-cultivation of the parental and evolved strains showed that co-evolution of these strains resulted in increased mutual stimulatory effects. Sequencing of the genomes and transcriptome profiling of the parental and the evolved strains revealed mutations that affected the acquisition of sulfur amino acid and branched-chain amino acids, folic acid and long-chain fatty acids. These results indicate that the canonical nutritional dependencies of *S. thermophilus* and *L. bulgaricus* in amino acid, purine and fatty acid metabolism were strengthened in this short adaptation period. As the resulting adapted co-culture has useful industrial properties, this approach not only shows that experimental evolution can be used to study microbial interaction networks but can also be a powerful tool to improve industrial traits paving the way for creating new starter cultures with improved characteristics

INTRODUCTION

Most microbial ecosystems, including industrial fermentations, consist of many different species that have complex interactions contributing to the ecological structure of the community (7, 16). Mutual effects on fitness between interacting

microbes are an effective means of classifying these interactions (reviewed in Sieuwerts *et al*. (27)). A further unraveling of these interactions is the key in understanding and rationally improving industrial fermentations. Important questions are how stable communities of interacting microorganisms evolve and how the interactions influence the consortium members. Recent advances in the fields of microbial evolution (2, 34) and functional genomics (14) shed light on the mechanisms driving the evolution of interactions. Low complexity microbial ecosystems are attractive models to further study the evolution of interacting organisms. Food fermentations offer this opportunity as they are typically carried out by a small number of dominant strains. Yoghurt can be regarded as the paradigm. It is (bovine) milk fermented by the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and has great industrial relevance; the worldwide turnover of yoghurt was 34 billion US $ in 2007 (20). The two lactic acid bacteria stimulate each other's growth and acid production when grown together in milk, in a process referred to as proto-cooperation. Their interactions are mainly related to purine and amino acid (AA) metabolism. *L. bulgaricus* provides *S. thermophilus* with amino acids and peptides through the action of its exoprotease PrtB (4, 25, 35). In turn, *S. thermophilus* provides formic acid, folic acid (6) and carbon dioxide (9), all involved in purine and arginine metabolism. Exopolysaccharides (EPS) are also suggested to play a role in the interactions, but their exact function remains to be established (29).

In this work, we applied experimental evolution to adapt a novel strain combination of genomically characterized *S. thermophilus* (3) and *L. bulgaricus* (17) under a strict growth regime. In addition, we addressed the molecular mechanisms and phenotypical traits that form the basis of their stable proto-cooperation. For this purpose, we used a combination of genomics approaches, i.e. genome re-sequencing and transcriptome profiling, and physiological characterization, i.e. testing of acidification, growth and survival, end metabolite production and EPS production. We observed that the experimental evolution improved important functionalities, such as acid resistance and viscosity, that resemble those of a commercial starter. We linked these improvements to mutations in genes in pathways that were previously shown to be involved in the proto-cooperation between the two bacteria (29). As the resulting adapted co-culture has useful industrial properties, this approach not only shows that experimental evolution can be used to study microbial interaction networks but can also be a powerful tool to improve industrial traits paving the way for creating new starter cultures with improved characteristics.

MATERIALS AND METHODS

Strains and culture conditions.

Streptococcus thermophilus CNRZ1066 (3) and Sts (from the commercial starter I-St) were maintained in M17 broth (Oxoid) containing 22% glycerol (Scharlau) at -80°C, *Lactobacillus delbrueckii* subsp *bulgaricus* ATCC BAA-365 (17) and lb (from I-St) in MRS broth (Merck) with 22% glycerol at -80°C. Pre-cultures of both species were made as mono cultures in UHT-treated 10% (w/v) reconstituted skim milk (Nilac, NIZO food research), referred to as milk in the rest of the paper, for 24 h at 37°C. Mixed cultures for the experimental evolution studies were obtained by inoculating 10 mL of milk with pre-cultures to a density of approximately 10^6 cells per mL per strain. The experiments were carried out in duplicate. The cultures were propagated by transferring 1.5% (v/v) daily until a theoretical number of 1000 generations was reached (167 transfers). Acidification and final cell counts were monitored with regular intervals of approximately 3 weeks (i.e. 127 generations). After 49, 97, 133, 219, 304, 365, 456, 517, 602, 699, 809, 930 generations freezer stocks were prepared for later experimentation by mixing of 1 volume 60% glycerol with 2 volumes yoghurt. From one final culture, single colony isolates were obtained that were designated *S. thermophilus* NIZO3938 and *L. bulgaricus* NIZO3939. Single colony isolate freezer stocks were obtained by selective plating (M17 agar (Oxoid) supplemented with 1% glucose at 45°C for *S. thermophilus* and MRS agar (Merck) at 45°C anaerobic for *L. bulgaricus*). Colonies of these plates were streaked onto new plates. Single colonies from the second plates were grown in liquid media and visually inspected with a microscope for infections.

Pre-cultures in follow-up experiments were made as described above. After 24 h, the optical density at 600 nm was measured in duplicate using 1 volume of milk culture mixed with 9 volumes of a 0.2% NaEDTA (w/v) / 0.2% NaOH (w/v) solution. Final cultures were inoculated to a start OD_{600} of 0,005 per strain (corresponding to approximately 10^6 cells per mL).

For monitoring of acidification activity during the evolution process, 10 mL milk was inoculated with 1% of a 24 h old mixed culture and pH was followed at 37°C for 24 h using the Cinac system (Ysebaert, France). *S. thermophilus* and *L. bulgaricus* were enumerated by spotting serial dilutions onto agar plates as described before (28). *S. thermophilus* was plated onto TY agar supplemented with 1% (w/v) glucose and *L. bulgaricus* was plated onto MRS agar. Both were

incubated at 37°C for 6 h before being transferred to 45°C for another 20 h, *L. bulgaricus* anaerobically.

The acidification rate (∆lactic acid/∆t in mM/h) was calculated as follows. A calibration curve of lactic acid concentration (known y values) in milk versus pH (known x values) was prepared. Using the GROWTH function in Microsoft Excel, the measured pH values in the culture (new x values) were calculated into lactic acid concentration.

Significant differences in acidification rate and CFU counts between samples were calculated with a two-tailed Students t-test (p=0.05).

Viscosity measurements.

For viscosity measurements 120 mL cultures were grown in milk at 37°C for 48 h. Viscosity was measured using a viscosimeter M5 (Haake Fisons) with a rotation speed of 0 increasing to 400 rotations per s at 37°C. Each strain combination was measured in duplicate. Significant differences were determined using a Students t-test (p=0.05) on the average measurements between 50 and 55 rotations per sec.

EPS isolation.

EPS were isolated from 275 mL cultures grown for 24 h at 37°C as described before (29). All isolations were performed in duplicate.

Metabolite analysis with HPLC and GC-MS.

Duplicate samples for HPLC analysis were taken from cultures and frozen at -20°C in 2 mL eppendorf tubes until analysis. For acid analysis, proteins and fat were precipitated with perchloric acid. After filtration using a Spartan 30/B (Schleicher & Schuell) filter, the liquid was used for high pressure liquid chromatography. In a HPX-87H column (BioRad 300 x 7.8 mm) column at 60°C with a flow of 0.6 mL/min 0.005 M sulfuric acid 25 µL of the solution was injected. For sugar analysis, 1 g of culture was mixed with 10 mL of a solution composed of 4.55 g/L $Zn(CH_3COOH)_2.2H_2O$ (BDH Analar), 2.73 g/L $H_3[P(W_3O_{10})]_4.xH_2O$ (BDH Analar) and 2.9 mL/L 99.8% acetic acid (BDH Analar). After filtering this solution with a Spartan 30/B filter, was used for chromatography. In a HPX-87H column with a flow of 0.4 mL/min 0.01 (w/v) NaCl, 25 µL of the solution was injected. Components were detected using a RID-10A (Shimadzu, Japan) and ERC-7510 (Erma optical works Ltd, Japan) refraction index meter at 40°C for organic acids and sugars, respectively. Data were analyzed using Chromeleon (Dionex) and compared to standard series of desired components.

GC-MS analyses were performed as described before (29).

Resequencing and sequence analysis.
The parental strains *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365 and their evolved variants NIZO3938 and NIZO3939 were sequenced using Illumina Solexa sequencing (GATC Biotech AG, Konstanz, Germany). The sequences of the reads were 36 base pairs and covered the genomes 80-120 times (see Supplementary Table 1). Single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) were detected by comparing the acquired sequences with the published genome sequences (3, 17) using in-house developed software (van Hijum *et al.*, unpublished). The reads (trimmed at 5' and/or 3' side to various sizes and untrimmed) were aligned to the reference (repetitive sequences of 20 bp were masked by N nucleotides). The alignments of the reads to the repeat-masked reference were filtered based on various cutoffs (e.g. minimal ratio 1/10 minor / major nucleotides; in other words, in order for a SNP / insertion to be selected the major nucleotide in the reads should at least be 10x more frequent than the alternative nucleotides). Only mutations that were present in at least 10 reads and a ratio of major / minor nucleotide of 10 were used for further analysis. The mutations were confirmed by PCR amplification of a 500 bp fragment carrying the mutation site and subsequent sequence analysis. (BaseClear, Leiden, the Netherlands). These fragments were also amplified and sequenced in the duplicate final evolved culture to identify possible mutations in the same regions.

The potential impact of the mutations was determined by assessing the deduced amino acid changes using protein alignment tools and by inspection of 3D structures (http://www.ncbi.nlm.nih.gov/Structure/) of orthologous proteins where relevant and available (18). Intergenic regions were checked for promoter sequences with PPP (http://bamics2.cmbi.ru.nl/websoftware/).

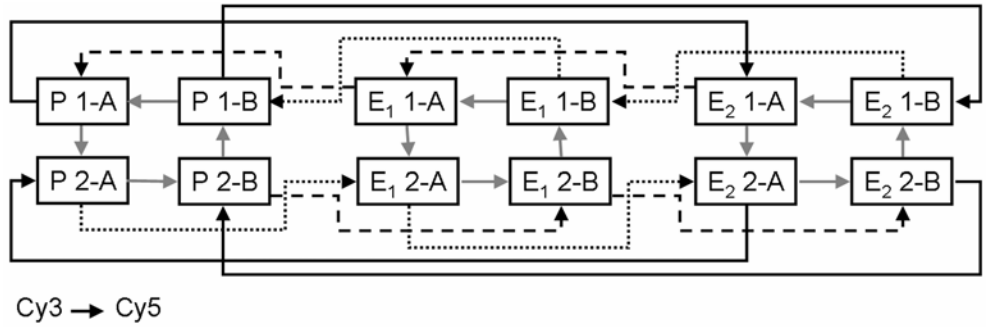

Figure 1. Experimental design of the hybridizations for transcriptome profiling. 1 and 2 indicate the first en second exponential phase; A and B indicate two biological duplicates; P, E_1 and E_2 indicate the parental mixed culture, the first evolved mixed culture and the second evolved mixed culture, respectively.

Transcriptome profiling.
The effect of the experimental evolution on gene expression was tested for both evolved cultures. Therefore, parental and evolved mixed cultures were prepared and sampled in the first and second exponential phase (i.e. 4.5 h and 7.5 h for the parental culture and 4 h and 6.25 h for the evolved cultures). mRNA isolation, cDNA synthesis and DNA micro arrays were performed and data were analyzed as described before (29). Figure 1 depicts the experimental design of the hybridizations. Significant differential expression of genes was defined as a fold change of 2 or more and a FDR value of 0.05 or lower. However, also more general effects were taken into account, e.g. a fold change below 2 with a low FDR value.

RESULTS

Experimental evolution.
We selected the strains *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365 as they had been characterized at the genomic level and to the best of our knowledge had no history of co-cultivation. The selected strains both originate from French yoghurt starter cultures. Experimental evolution was applied to study the evolution of their interactions. For this purpose, the selected strains were cultured together in skim milk for more than 1000 generations by serial batch cultivation at 37°C to ensure an approximate ratio of 1 between both yoghurt strains and the possible application of mesophilic probiotics, such as *Lactobacillus plantarum* (discussed in Chapter 7). The evolution of the population composition and its activity were monitored by determining the colony-forming unit (CFU) counts and acidification kinetics at three week intervals. We observed an incremental increase of the acidification rate (see Figure 2A). This is also illustrated by comparison of the maximal acidification rate (Δlactic acid/Δt) and the time the adapted culture needed to reach this maximal rate (see Figure 2B). In addition, the acidification profile throughout the experiment and the final evolved cultures did not show the transition phase between the two exponential growth phases that is characterized by a temporary reduced acidification rate (29). Moreover, the final colony counts increased in the course of evolution. These step-wise increases suggest the involvement of multiple mutational events. All these observations were confirmed in an independent duplicate experiment (data not shown).

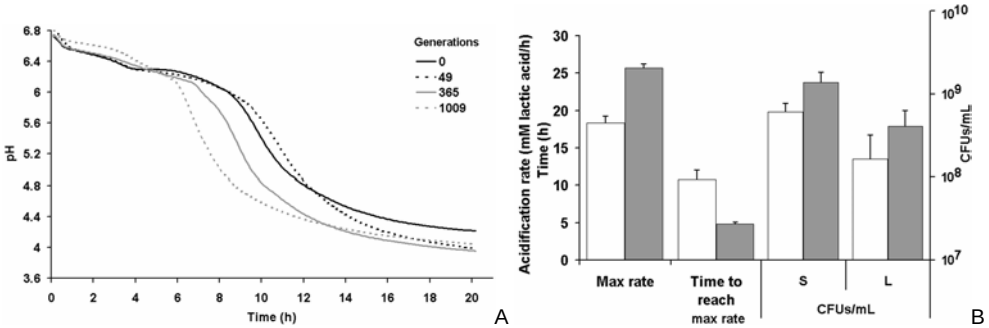

Figure 2. Experimental evolution of a mixed culture of *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365. The strain combination was allowed to adapt to milk for 1009 generations. A, the acidification profiles of the culture through evolutionary time after 0, 49, 365 and 1009 generations. B, maximal acidification rates, times within fermentations to reach these rates, and final CFU counts after 0 (white bars) and 1009 (grey bars) generations of the mixed culture. Error bars indicate standard deviations of sextuplicate experiments. S, *S. thermophilus*; L, *L. bulgaricus*.

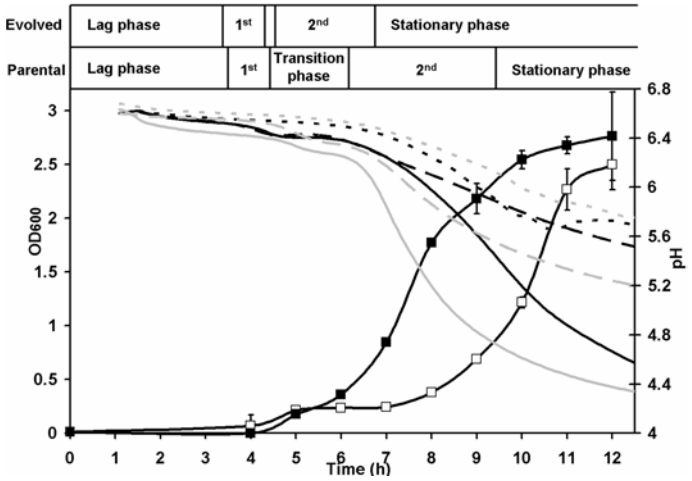

Figure 3. Acidification and growth of parental and evolved cultures. Error bars indicate standard deviations of duplicate measurements. The line with □ represents OD_{600} of the parental mixed culture; ■ represents OD_{600} of the reconstituted evolved mixed culture. Other lines indicate acidification curves: *S. thermophilus* parental strain CNRZ1066 and evolved strain NIZO3938 (striped black and striped grey, respectively), *L. bulgaricus* parental ATCC BAA-365 and evolved strain NIZO3939 (dashed black and dashed grey, respectively), and the parental and evolved mixed culture (solid black and solid grey, respectively). 1st and 2nd refer to the first and second exponential phase.

Phenotypic comparison of parental and evolved variants.

For further experimentation, 14 representative *S. thermophilus* and *L. bulgaricus* single colony isolates (see below) of the evolved cultures were selected. Of these, two were designated *S. thermophilus* NIZO3938 and *L. bulgaricus* NIZO3939,

hereafter referred to as evolved *S. thermophilus* and *L. bulgaricus*. These two strains combined will be referred to as the reconstituted evolved mixed culture that was subsequently cultivated in milk (see Figure 3). The acidification rate and growth showed global phenotypic changes that were in agreement with the observations described above for the final evolved culture (see Figure 2).

The mono and mixed parental and evolved cultures were analyzed for the levels of lactose, glucose, galactose, lactic acid, acetic acid and volatile flavor compounds. Additionally, the average and maximal growth rate (g of biomass/h), rate of product or substrate production (mmol/h) and the yield of produced biomass/product per consumed lactose (g/mmol or mmol/mmol) were calculated (see Table 2). Lactose uptake and lactic acid production rates were in accordance with the acidification curves in Figure 3. Interestingly, the biomass yield per mol of consumed lactose was higher in the evolved *S. thermophilus* and mixed cultures compared to their parental equivalents.

Of the four major flavor components in yoghurt, the production of acetaldehyde increased in the evolved mixed culture compared to the parental mixed culture, notably due to the faster growth of evolved *S. thermophilus* (see Table 1). The levels of 2-propanone remained equal while the levels of diacetyl and 2,3-pentadione decreased remarkably in the evolved culture.

Table 1. Analysis of volatile compounds in triplicate mono and mixed cultures. The used (combinations of) strains are *S. thermophilus* CNRZ1066, NIZO3938 and Sts, *L. bulgaricus* ATCC BAA-365, NIZO3939 and Ib, and the mixed cultures CNRZ1066/ATCC BAA-365, NIZO3938/NIZO3939 and Sts/Ib (I-St). P, parental strain(s); E, evolved strain(s). Significant differences were determined with a Students t-test (p=0,05) per component per type of culture (*S. thermophilus*, *L. bulgaricus* or mixed). The values within a row (i.e. for each component) that have the same letter (^{a},^{b},^{c}) are significantly different.

Component	Concentration in culture (µM)								
	S. thermophilus			*L. bulgaricus*			Mixed culture		
	P	E	Sts	P	E	Ib	P	E	I-St
Acetaldehyde	108.36^{a}	168.25	156.17^{a}	410.21^{a}	258.04	251.69^{a}	252.16^{ab}	367.05^{a}	350.15^{b}
Methanethiol	2.07	6.90	4.93	9.87^{a}	14.00^{b}	27.00^{ab}	21.67	28.33	21.67
2-Propanone	316.67^{a}	493.33^{b}	813.33^{ab}	1020.00^{a}	713.33^{b}	3666.67^{ab}	1290.00	1146.^{67}	1366.67
Dimetylsulfide (DMS)	6.17^{a}	6.67^{b}	11.67^{ab}	2.27^{a}	3.23	6.67^{a}	9.37	8.90	9.03
2-methyl-propanal	2.00^{a}	2.35^{b}	12.13^{ab}	4.50^{a}	6.75	3.67^{a}	7.63	7.00	14.47
Diacetyl	730.00^{ab}	360.00^{ac}	2466.67^{bc}	74.00	28.00	15.00	2333.33^{a}	296.67^{ab}	1600.00^{b}
2-Butanone	38.00^{a}	36.33^{b}	130.00^{ab}	41.67	35.00	39.33	68.00^{a}	53.67^{b}	130.00^{ab}
Ethylacetate	11.67^{a}	12.00^{b}	23.33^{ab}	13.67^{a}	12.33^{a}	13.67	25.67	17.67^{a}	28.00^{a}
3-methyl-butanal	0.30	0.35	0.43	1.63^{a}	33.50^{ab}	2.30^{b}	1.40^{ab}	4.90^{ac}	15.67^{bc}
2-methyl-butanal	0.20^{a}	3.50^{b}	1.07^{ab}	0.87	5.87	0.70	0.60^{a}	1.60	3.30^{a}
2-Pentanone	1.33^{a}	6.03^{b}	4.00^{ab}	14.00^{a}	11.23^{b}	47.67^{ab}	8.57	6.73	12.33
2,3-pentanedione	41.67^{ab}	54.40^{ac}	143.33^{bc}	3.63	1.63	1.50	423.33^{ab}	78.33^{a}	83.67^{b}
Dimethyldisulfide (DMDS)	0.13	0.47^{a}	0.20^{a}	0.90^{a}	0.90^{b}	1.43^{ab}	1.03^{a}	1.87	1.53^{a}
2-Heptanone	1.77^{a}	8.67^{b}	5.10^{ab}	18.00^{a}	16.00^{b}	62.33^{ab}	9.40^{a}	9.10^{b}	38.00^{ab}
Dimethyltrisulfide (DMTS)	0.23^{a}	0.53^{a}	0.20	0.73	0.77	1.10	1.60^{a}	2.87^{a}	1.93

Table 2. Primary carbohydrate metabolism of parental and evolves cultures. For each culture, the amount of produced biomass, the rate of sugar consumption and acid production and the yield of biomass per amount of substrate consumed are depicted for the major growth phase (i.e. second exponential in the *S. thermophilus* and mixed cultures). Standard deviations are in parentheses. Abbreviations: P, parental; E, evolved; Sts, strain Sts; Ib, strain Ib; I-St, mixed culture of Sts and Ib; N.A., not applicable because lower than detection limit. See also Supplementary Figure 1.

	Biomass production (g/h)	Produced substrate / product (mM/h)					Yield (/mmol lactose consumed per h)				
		Lactose	Glucose	Galactose	Lactic acid	Acetic acid	Biomass (g)	Glucose (mmol)	Galactose (mmol)	Lactic Acid (mmol)	Acetic Acid (mmol)
S. thermophilus											
P	0.019	-2.572	N.A.	1.213	2.98	0.019	0.007	0	0.472	1.158	0.007
	(0.007)	(0.423)		(0.323)	(0.991)	(0.005)	(0.003)		(0.137)	(0.42)	(0.002)
E	0.030	-2.034	N.A.	2.471	6.158	0.035	0.014	0	1.215	3.027	0.017
	(0.005)	(1.061)		(0.449)	(1.261)	(0.012)	(0.005)		(0.476)	(1.338)	(0.012)
Sts	0.046	-1.192	N.A.	1.572	3.358	0.079	0.039	0	1.318	2.816	0.066
	(0.011)	(0.977)		(0.228)	(0.582)	(0.018)	(0.011)		(0.223)	(0.569)	(0.018)
L. bulgaricus											
P	0.024	-2.676	0.768	1.874	2.604	0.094	0.009	0.287	0.700	0.973	0.035
	(0.001)	(0.476)	(1.798)	(1.262)	(0.357)	(0.012)	(0.001)	(0.856)	(0.601)	(0.17)	(0.006)
E	0.020	-2.259	2.686	2.654	2.233	0.107	0.009	1.189	1.175	0.988	0.047
	(0.004)	(0.464)	(1.164)	(0.085)	(0.459)	(0.029)	(0.002)	(0.541)	(0.039)	(0.213)	(0.013)
Ib	0.021	-1.433	0.188	1.174	2.109	0.151	0.014	0.131	0.820	1.472	0.105
	(0.001)	(1.424)	(0.163)	(0.096)	(0.045)	(0.004)	(0.001)	(0.232)	(0.136)	(0.064)	(0.005)
Mixed culture											
P	0.094	-4.076	-0.426	4.577	9.709	0.055	0.023	-0.105	1.123	2.382	0.013
	(0.013)	(0.253)	(0.2)	(0.316)	(0.806)	(0.004)	(0.003)	(0.051)	(0.080)	(0.204)	(0.001)
E	0.197	-5.849	-1.598	9.021	19.958	0.171	0.034	-0.273	1.542	3.412	0.029
	(0.062)	(1.059)	(0.923)	(2.257)	(4.138)	(0.042)	(0.066)	(0.977)	(2.390)	(4.381)	(0.045)
I-St	0.107	-4.175	N.A.	3.678	6.859	0.107	0.026	0	0.881	1.643	0.026
	(0.007)	(0.088)		(0.243)	(0.531)	(0.031)	(0.001)		(0.021)	(0.046)	(0.003)

Phenotypic and genetic heterogeneity.

Long-term propagation experiments with single-strain cultures may lead to a mixed culture of closely related variants carrying different mutation profiles (23). Therefore, heterogeneity of the evolved culture was tested on the 14 single-colony isolates of either species (see above). The different isolates were re-combined in mixed cultures of which acidification and outgrowth was tested. All behaved comparable to the final evolved mixed culture and different from the original mixed culture (data not shown). The same procedures were applied to cultures throughout the evolution process, which showed a step-wise improvement in acidification rate and final CFU counts similar as depicted in Figure 2. This indicates that there was no differentiation in phenotypically variable (sub-) strains in the evolved mixed culture in terms of acidification and growth.

Because not all mutations necessarily confer a benefit, it is possible that, although there was no apparent phenotypical heterogeneity in the evolved culture, there could still be genetic heterogeneity. The same 14 single colony isolates that were used for the growth test were tested for heterogeneity in a systematic analysis of all observed mutations (see below) by sequencing of PCR products.

There was no difference in any of the isolates, indicating that the final evolved culture was genetically homogeneous.

Reconstituted mixed cultures of parental and evolved strains.
To further explore the physiological changes in the evolved strains, different combinations of parental and evolved *S. thermophilus* and *L. bulgaricus* were prepared and compared to the parental strain combination and the reconstituted mixed culture. Acidification of triplicate cultures was followed for 24 h and the viable count was determined by selective plating (see Figure 4). The mixed culture consisting of evolved *S. thermophilus* and parental *L. bulgaricus* showed a similar acidification profile as the reconstituted evolved mixed culture. The mixed culture containing parental *S. thermophilus* and evolved *L. bulgaricus* acidified milk with the same kinetics as the culture with both parental strains. Thus, the increased acidification can mainly be attributed to the evolved *S. thermophilus*. This was also reflected in the final CFU counts: neither *S. thermophilus* strain was significantly stimulated more by the evolved *L. bulgaricus* than by the parental *L. bulgaricus*. The parental *L. bulgaricus* was stimulated more by the evolved *S. thermophilus* than by the parental *S. thermophilus*. Finally, the evolved *L. bulgaricus* was not significantly differently stimulated by the evolved *S. thermophilus* strain compared to the parental strain. Remarkably, the mutual stimulatory effect in the evolved mixed culture was larger than in the parental mixed culture for both species as is exemplified by the higher increase in CFUs due to co-cultivation in the reconstituted evolved mixed culture compared to the parental mixed culture.

Survival.
Cells were repeatedly challenged to low pH and high lactic acid concentrations during the propagation. We hypothesized that mutations leading to increased acid resistance improved survival during stationary phase and this may contribute to a fitness increase. Therefore, we grew duplicate parental and evolved mixed cultures in milk for 20 h and subsequently exposed all cultures to the lactic acid concentrations and pH 3.65 encountered during stationary phase in evolved mixed cultures. Viability was tested by plating in triplicate every half hour (see Figure 5). The viability decreased according to exponential decay, which allowed calculating viability coefficients, i.e. the proportion of cells that survives each h (0.19 and 0.69 for the parental and evolved *S. thermophilus* strains, respectively, and 0.65 and 0.59 for the parental and evolved *L. bulgaricus*, respectively). Using a pairwise two-tailed t-test, we determined that the evolved *S. thermophilus* was four times more

acid resistant at the used pH than the parental strain. The difference between the parental and evolved *L. bulgaricus* strains was not significant.

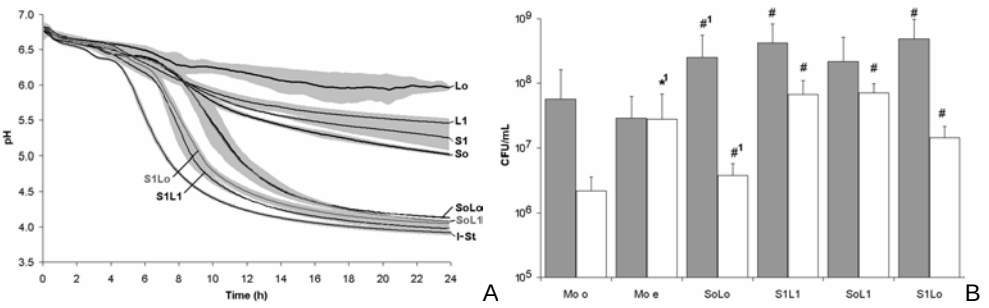

Figure 4. Acidification and final cell density (CFU) in reconstituted cultures. A, Acidification of reconstituted cultures. Grey zones indicate standard deviations of triplicate measurements. Abbreviations: So, parental *S. thermophilus*; S1, evolved *S. thermophilus*; Lo, parental *L. bulgaricus*; L1, evolved *L. bulgaricus*; I-St, commercial starter I-St. B, Colony-forming units after 24 h fermentation of *S. thermophilus* (grey bars) and *L. bulgaricus* (white bars) of the reconstituted cultures. Error bars indicate standard deviations of at least triplicate measurements. Abbreviations: Mo o, parental mono-cultures; Mo e, evolved mono-cultures; other abbreviations like in A. *, significantly different from the corresponding parental culture (two-tailed t-test, p=0.05); #, significantly different from the corresponding mono-culture; [1], not significant when p=0.05, but significant when p=0.1).

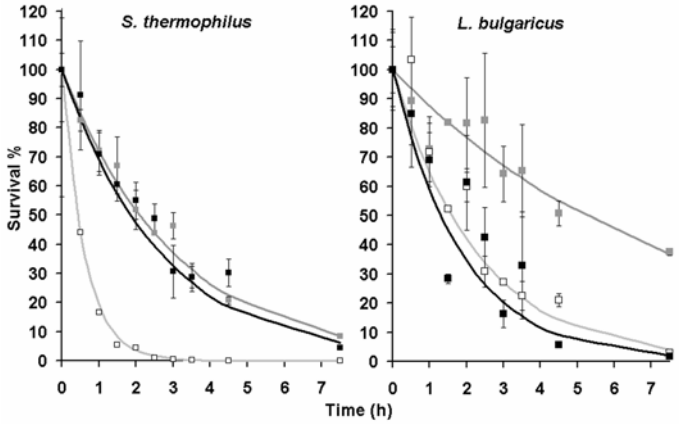

Figure 5. Acid tolerance of the evolved *S. thermophilus* (left) and *L. bulgaricus* (right) isolates compared to parental isolates and the industrial strains *S. thermophilus* Sts and *L. bulgaricus* Ib. The percentage of colony-forming unit counts in mixed cultures acidified to pH 3.65 with lactic acid was determined and compared to the reference (0 h): parental strains (□), evolved strains (■) and I-St (■). Error bars indicate standard deviations of sextuplicate measurements. Lines indicate fitted exponential decay curves used for calculating viability coefficients.

Viscosity and EPS production.

We noticed that the evolved mixed culture and that mono and mixed cultures with the evolved *S. thermophilus* strain were more viscous when grown in milk than the cultures with parental strains. Therefore, we determined the viscosity of duplicate reconstituted cultures of parental and evolved strains from the evolved culture discussed above and from strains isolated from the independent duplicate evolution experiment. These will be referred to as the second evolved *S. thermophilus* and *L. bulgaricus* below. The two evolved *S. thermophilus* strains displayed a higher viscosity than the parental strain (see Table 3). This was also reflected in the mixed cultures. There was no difference in viscosity between the parental and the two evolved *L. bulgaricus* mono cultures. In cultures consisting of one parental strain and one evolved strain, viscosity was enhanced by the application of the evolved *S. thermophilus*, but not by the evolved *L. bulgaricus*. These results strongly indicate that the increased viscosity in the evolved mixed culture can be attributed to a changed phenotype of the evolved *S. thermophilus* strains. Indeed, the amount of EPS produced after 24 h culturing was higher in the evolved *S. thermophilus* culture than in the parental culture. The concentration in the mixed evolved culture more than doubled compared to the reconstituted parental mixed culture. As the increase in biomass of the evolved mixed culture was less than twice that of the parental mixed culture (see Figure 3), the increase in EPS production can not only be attributed to a higher biomass. The evolved *L. bulgaricus* showed a slight increase when compared to the parental *L. bulgaricus* culture. It is evident that more EPS was produced in the mixed cultures than in the mono cultures and that in the evolved cultures, especially that of *S. thermophilus* and the mixed culture, more EPS was produced than in the parental cultures.

Table 3. Viscosity and EPS production in the parental and evolved cultures. P, parental strain(s); E_1, evolved strain(s) NIZO3938 and/or NIZO3939; E_2, evolved strain(s) from the duplicate evolved culture; N.D., not determined. Values within parentheses represent standard deviations of duplicate experiments.

	S. thermophilus				*L. bulgaricus*				Mixed culture			
	P	E_1	E_2	Sts	P	E_1	E_2	Ib	P	E_1	E_2	I-St
Viscosity (mPas)	62 (8)	158 (30)	341 (59)	146 (8)	194 (26)	243 (8)	229 (10)	187 (25)	107 (6)	278 (30)	300 (21)	253 (0)
EPS (g/L)	0.80 (0.02)	1.35 (0.26)	N.D.	N.D.	1.18 (0.12)	1.52 (0.03)	N.D.	N.D.	1.46 (0.04)	3.91 (0.48)	N.D.	N.D.

Comparative genome sequence analysis of parental and evolved isolates.

The data presented above clearly indicate that the phenotypic differences between the parental and evolved strains are stable and were caused by mutations in the genomic DNA sequence during the experimental evolution process. Therefore, the genomes of the parental and the evolved strains were sequenced using Illumina Solexa sequencing and compared to the reference sequence. Subsequently, mutations were detected by determining the (gaps in) overlap between the two SNP/INDEL lists (see Table 4).

Table 4. Mutations in the evolved strains *S. thermophilus* NIZO3938 and *L. bulgaricus* NIZO3939 as compared to their parental strains. Mutations that could have an effect based on protein structure are indicated in bold (see text for explanation). The primers used for the PCRs to confirm the presence of mutations in strains NIZO3938 and NIZO3939 and the strains from the duplicate evolved culture are depicted in Supplementary Table 2. N.D., not determined; [a], according to reference (31), the DegV family protein is involved in fatty acid transport or metabolism; [b], according to reference (15), the Fic family protein is involved in cell division and is suggested to be involved in the synthesis of 4-aminobenzoic acid or folic acid ; [c], annotation following (17).

						Mutation in duplicate culture		
Position	Base change	Locus ID	Total AA	Gene annotation (ERGO)	AA change	Position	Base change	AA change
S. thermophilus NIZO3938								
151195	G>T	*str0133 (rpoE)*	193	DNA-directed RNA polymerase delta chain (EC 2.7.7.6)	E178>STOP	No		
250442	**C>–**	***str0242 (greA)***	**160**	**Transcription elongation factor greA**	**Q15>N; STOP at 32**	**No**		
289618	C>A	*str0303 (dctA)*	402	Serine/threonine sodium symporter	L194>I	No		
385740	**C>T**	***str0408 (gor)***	**450**	**Glutathione reductase (EC 1.8.1.7)**	**A138>V**	**No**		
413374	G>T	*str0438 (pyrH)*	245	Uridylate kinase (EC 2.7.4.-)	M198>I	No		
586170	C>A	*str0625 (rnr)*	817	Exoribonuclease II (EC 3.1.13.1)	T180>N	No		
683939	**G>C**	***str0724 (fur)***	**145**	**Ferric uptake regulation protein**	**D46>H**	**683939 683994**	**G>A –>A**	**D46>N –64>D; STOP at 66**
725339	T>C	*str0770 (pacL1)*	878	Calcium-transporting ATPase (EC 3.6.3.8)	K339>E	No		
847292	**G>A**	***str0923 (als)***	**560**	**Acetolactate synthase (EC 2.2.1.6)**	**G417>E**	**No**		
1055098	C>A	*str1201 (leuC)*	464	3-isopropylmalate dehydratase large subunit (EC 4.2.1.33)	V172>L	N.D.		
1067308	C>T	*str1215*	288	Lantibotic precursor	G236>S	No		
1110735	**A>G**	***str1263 (gapN)***	**477**	**NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.9)**	**F228>L**	**1110738**	**T>C**	**N227>H**
1182279	G>A	*str1336 (rr07)*	226	Two-component response regulator	N155>N	No		
1371875	C>A	*str1546 (folC2)*	420	Folylpolyglutamate synthase (EC 6.3.2.17) / Dihydrofolate synthase (EC 6.3.2.12)	G21>V	No		
1425619	**G>A**	***str1601 (dnaQ)***	**196**	**DNA polymerase III, epsilon chain (EC 2.7.7.7)**	**A139>V**	**1425838**	**G>C**	**A66>G**
L. bulgaricus NIZO3939								
61050	**G>T**	***LBUL_0063***	**277**	**DegV family protein[a]**	**C62>S**	**N.D.**		
301103	C>T	*LBUL_0313*	483	ATP-dependent RNA helicase	R18>W	No		
333736	**G>T**	***LBUL_0343***	**1221**	**DNA-directed RNA polymerase beta' chain (EC 2.7.7.6)**	**R326>L**	**No**		
341010	G>A	*231* bp downstr. *LBUL_0348* *156* bp upstr. *LBUL_0349 (rpsJ)*		Protein Translation Elongation Factor G (EF-G); SSU ribosomal protein S10P		No		
998290	A>C	*LBUL_1085*	96	Fic family protein[b]	V28>F	N.D.		
1288314	T>C	*LBUL_1377*	682	Cell division protein FtsI[c]	E655>G	No		
1756309	G>A	*LBUL_1933*	141	Uncharacterized	R8>W	N.D.		

In *S. thermophilus*, 15 mutations were identified in coding regions, 14 single nucleotide substitutions and one single nucleotide deletion. In the evolved *L. bulgaricus*, there were 7 single nucleotide substitutions compared to the parental strain, of which 6 occurred in coding regions and one in an upstream region. Of all mutations there were 6 in *S. thermophilus* and 2 in *L. bulgaricus* that could have changed the functionality of the enzymes, either by introducing a stop codon or by changing the AA residue in the catalytic site. The discussed mutations are complemented with gene expression data. Global gene expression is discussed below.

Mutations in *S. thermophilus*. In *S. thermophilus*, there were four mutations in coding regions that were predicted to affect the catalytic sites of the encoded proteins (GreA, Fur, Als, GapN) and hence could contribute to the observed phenotype. The single nucleotide deletion in *greA* results in the introduction of a stop codon at the N-terminal part of the encoded transcription elongation factor GreA and renders it non-functional. No orthologues of *greB, gfh1* (8) or *dksA* (24), which are essential for the cleavage of RNA in order to release the transcription machinery when it is locked, have been identified in the genome of *S. thermophilus* CNRZ1066. Therefore, it is likely that the cleavage of RNA in order to relieve locked transcription processes will be impaired in the evolved strain. Interestingly, the *greA* gene was lower expressed in the evolved *S. thermophilus* strain that was isolated from the duplicate experimental evolution culture. The observed missense mutation in the iron-responsive genes transcriptional repressor *fur* in the evolved *S. thermophilus* strains may result in a lower affinity as it interfered with the metal binding site 2 of this enzyme (26). A multiple alignment showed that this AA residue is highly conserved among LAB. It is always an asparagine or a glutamate, indicating the importance of a polar hydrophylic AA at that site. If a mutation causes Fur to be less active, it would result in high expression of the genes involved in iron transport genes such as the *fatABCD* iron ABC transporter and the iron permease *feoAB*. These genes were much higher expressed in the evolved *S. thermophilus* compared to the parental strain (respectively 10.4-37.8-fold and 2.5-2.6-fold). Additional information comes from a mutation at the same location in the *S. thermophilus* isolated from the duplicate experimental evolution culture. Considering the limited number of SNPs in the strains it is highly unlikely that exactly the same nucleotide is mutated in two separate evolved strains. In addition, this strain carried a second mutation in this gene leading to an early stop codon rendering a truncated protein that is likely to be dysfunctional. In this strain, the *fatABCD* complex was also over expressed 4.7-13.8-fold and the *fur* gene was 2.3-3.3-fold higher expressed compared to the parental strain.

The gene *als* codes for acetolactate synthase that is involved in the branched-chain amino acid (BCAA) synthesis and the production of diacetyl and acetoin. The *als* gene of the evolved strain contained a missense mutation, changing a glycine in a glutamic acid, at the binding site for the co-factor thiamine pyrophosphate. The exact effect of this mutation is not clear, but it is noteworthy that despite the fact that this gene was 5.0-fold higher expressed in the evolved *S. thermophilus* compared to the parental strain, there was no increase in production of diacetyl (see Table 1). In addition, there was a lower expression of BCAA biosynthesis genes and a higher expression of BCAA import genes in the evolved *S. thermophilus* compared to the parental strain. This all indicates that the mutation in *als* made its gene product less functional.

The observed missense mutation in *gapN* results in a F228L substitution that is located in the NAD binding site of the enzyme in the evolved *S. thermophilus* strain. GapN catalyses an important step in the glycolysis and therefore a mutation in this gene may yield a higher glycolytic flux. Interestingly, the second evolved *S. thermophilus* strain was found to carry a mutation resulting in the N227H substitution, just in the preceding residue. Altogether, this indicates that this region in the *gapN* gene has an important function and is prone to mutation.

Mutations in *L. bulgaricus*. In *L. bulgaricus*, there was one mutation affecting the catalytic site of a protein that could be involved in the interaction between *S. thermophilus* and *L. bulgaricus.* This C62S mutation in *LBUL_0063* was in the fatty acid binding site of the corresponding protein (19). The exact function of this protein of the DegV family is not known, but it is suggested to play a role in fatty acid transport or synthesis as a phosphotransferase (13, 31). Interestingly, the fatty acid metabolism genes were 2.2-3.2-fold lower expressed in the evolved *L. bulgaricus* than in the parental *L. bulgaricus*, except for the 1-acyl-glycerol-3-phosphate acyltransferase *LBUL_0106*, which was 3-fold higher expressed. This also occurred in mixed culture compared to mono culture (29) and suggests that *LBUL_0063* has a function in the acquisition of fatty acids other than *de novo* synthesis. The gene *LBUL_0343* encoding the RNA polymerase beta chain contained a mutation close to its DNA binding site. It is not clear whether this had an effect on the transcription efficiency.

Global changes in gene expression.

Because the evolved mixed cultures grew faster and had mutations that caused this, the effect of the experimental evolution on the global gene expression was tested for both evolved cultures. Presented data focuses on the sequenced evolved culture and is – where appropriate – complemented with data from the

second evolved culture. For an overview of all significantly differential expressed genes, see Supplementary Tables 3 and 4.

***S. thermophilus*.** In the evolved *S. thermophilus* differential expression compared to the parental strain was mainly in genes involved in the purine and the AA metabolism. For instance, expression of *pfl* was 2.7-fold higher in the evolved strain in the second exponential phase. This was also the case for the second evolved *S. thermophilus* and indicates that a higher pyruvate-formate lyase activity is necessary tot sustain a higher growth rate. Interestingly, the gene responsible for CO_2 production from bicarbonate, *cah*, was lower expressed in both evolved strains compared to the parental strain. This indicates that there was possibly less production of CO_2 that aids in the purine metabolism in *L. bulgaricus* (see below) (9, 29). The lower CO_2 production is likely caused by a lower requirement for *de novo* biosynthesis of arginine and aspartate (1) due to an 8.2-fold higher expression of the *L. bulgaricus* exoprotease gene *prtB* already in the first exponential phase releasing more peptides from casein. The purine biosynthesis genes and those maintaining the C1 pool in the evolved *S. thermophilus* were higher expressed, but there was no difference in expression of folic acid production genes between the parental and evolved *S. thermophilus*. The higher expression of most genes in the purine production pathway may be due to the increased growth rate. Indeed genes for peptidoglycan synthesis were higher expressed indicating a higher growth rate. There were a number of remarkable changes in the AA metabolism. The glutamine ABC transport system encoded by *str1979*, *str1580*, *str1581* and *str1582* was higher expressed in the evolved culture compared to the parental culture. This suggests a higher flux through the arginine biosynthesis pathway / urea cycle, although there was no differential expression observed for that pathway. Similarly, the sulfur AA production pathways and BCAA import genes were higher expressed in the evolved strain compared to the parental *S. thermophilus*, especially in the first exponential phase. In fact, expression of BCAA import genes was reduced in the second exponential phase, probably due to the higher expression *L. bulgaricus prtB*. Interestingly, the expression of EPS production genes was higher in both evolved *S. thermophilus* and both evolved *L. bulgaricus* compared to their parental strains. This may explain the higher EPS production and higher viscosity of the evolved cultures, especially in the *S. thermophilus* and mixed cultures.

***L. bulgaricus*.** In the evolved *L. bulgaricus* the two lactate dehydrogenases genes were 2.9 and 3.3-fold lower expressed in the first exponential phase compared to the parental strain. In contrast, *LBUL_2034* (pyruvate oxidase), responsible for a large portion of H_2O_2 production by *L. bulgaricus* (10, 29), was

2.7-fold higher expressed. The resulting increased iron catalyzed production of reactive oxygen species (ROS) may be the explanation for the mutations in *fur,* likely to result in less iron uptake in both evolved *S. thermophilus* strains. The purine production pathway genes were higher expressed in the evolved strain compared to the parental strain, especially in the first exponential phase. This is in accordance with a higher growth rate resulting in a higher nucleobase requirement. Similarly, the genes for the (incomplete (32)) pathway for folic acid production were higher expressed throughout the fermentation of the evolved culture. We hypothesize that (i) the higher growth rate required more folic acid than could be acquired from the medium while there would be a sufficient amount otherwise (see (29)) or that (ii) the mutation in the evolved *S. thermophilus folC2* indirectly led to a lower secretion of folate and a concomitant lower availability to *L. bulgaricus*. There was a lower expression of sulfur AA biosynthesis genes in both evolved *L. bulgaricus* strains than in the parental strain. This may be a result of the higher expression of *prtB* liberating more sulfur AA form casein. BCAA metabolism also showed differential expression in evolved cultures. Adaptation clearly involved an increased supply of BCAA either through increased expression of BCAA biosynthetic genes (strain NIZO3939) or increased expression of the transporter encoded by *LBUL_0341* (second evolved *L. bulgaricus*) (10).

Finally, fatty acid metabolism was affected. The two evolved *L. bulgaricus* strains seem to have found different solutions to increase the supply of long-chain-fatty acids (LCFA) for lipid biosynthesis. The first evolved *L. bulgaricus* lowered the expression of the LCFA biosynthesis genes and increased the expression of 1-acyl-glycerol-3-phosphate acyltransferase gene compared with the parental *L. bulgaricus.* In the second evolved strain, in contrast, expression of the complete biosynthetic pathways increased. Both responses indicate that in the evolved cultures, *L. bulgaricus* required more LCFA in order to sustain a higher growth rate. However, in the first case, *L. bulgaricus* acquires a sufficient amount from the medium and in the second case *L. bulgaricus* acquires more LCFA by *de novo* production.

Comparison of parental and evolved cultures to a commercial yoghurt starter.

Important functionalities in yoghurt of the parental and evolved cultures were bench-marked by comparison with the representative commercial starter I-St. We tested acidification, survival, viscosity and end metabolite profiles (organic acids and flavor compounds) production of *S. thermophilus* Sts, *L. bulgaricus* Ib (both from the commercial starter I-St) and their mixed culture in the same conditions as

in which the other strains were tested. Acidification of I-St was slightly faster (8.5 h to reach pH 4.5) than the evolved mixed culture (10 h) and this was mainly due to the shorter lag phase as the acidification rates in the second exponential phase were comparable (see Figure 4A). In fact, the maximal acidification rate in the evolved culture was higher (25.69±1.28 compared to 21.33±1.03 for I-St). The biomass yield of the evolved strains on lactose improved greatly (see Table 2). Moreover, the yield is significantly higher than that of I-St. In addition, the evolved *S. thermophilus* became much more acid tolerant at pH 3.65 than the original strain and was comparable to Sts (see Figure 5). The production of acetaldehyde, 2-propanone and 2,3-pentatone of the evolved cultures was closer to that of Sts, Ib and I-St than to the corresponding original cultures.

DISCUSSION

In this study, two strains of *S. thermophilus* and *L. bulgaricus* were co-evolved. It was shown that the evolved culture acidified faster and produced more EPS than the parental culture. Mutation analysis and gene expression studies showed that the cooperative interactions between the bacteria improved by a few mutations in genes involved in the interactions and fine-tuning gene expression of pathways related to the interactions. It is evident that interactions between consortium members influence the performance of individual bacteria that structures the whole community. In the field of microbial ecology the evolution of stable networks of interacting microorganisms is of great interest. In particular because the occurrence of cooperation is difficult to explain from an evolutionary point of view (5, 34). It was suggested that cooperative behavior, such as that between *S. thermophilus* and *L. bulgaricus*, can only occur stably if there is a direct benefit of executing this behavior (34). Moreover, the physical structure of its environment is essential for the performance of a microorganism, i.e. the density of the culture (11) and the dispersal of common goods or secreted products define the extent of interactions (12, 22). In this case, there is a positive feedback loop between the yoghurt bacteria. Because the optimal extent of this cooperative behavior is dependent on the used strains and media (30), it was expected that co-evolving two unfamiliar strains would lead to the optimization of existing interactions (2). Indeed, co-evolving *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365 for 1009 generations led in two independent experiments from a mixed culture that grows relatively slow to a culture that grows and acidifies at rates similar to those of

a commercial starter while approximately maintaining the same ratio between both species.

There were three major improvements in important functionalities of the microorganisms that are likely to increase the fitness: (i) the yield on lactose of the evolved cultures had increased compared to the parental cultures, (ii) the stimulation between the yoghurt bacteria had increased, and (iii) the evolved *S. thermophilus* showed improved survival at a low pH. The evolved cultures also displayed differences in traits relevant for industrial applications: (i) the evolved mixed culture grew and acidified faster than the parental culture, (ii) the production of EPS was increased in the evolved culture, leading to a higher viscosity and (iii) the production of important flavor components by the evolved mixed culture more resembled that of the commercial starter I-St than that of the parental culture. These improvements could mainly be attributed to the evolved *S. thermophilus* as the evolved *L. bulgaricus* was not significantly different from its parental strain in any of the measured properties, except its EPS and volatile production and mutual stimulation with the evolved *S. thermophilus*. This lack of effective evolution in *L. bulgaricus* in this study is striking and may be related to the fact that evolution so far shaped a very reduced genome (32) that provides little possibilities for further improvement.

Genetic and regulatory base underlying the adaptations.

There were 15 mutations identified in the evolved *S. thermophilus* and 7 in the evolved *L. bulgaricus* compared to their parental strains. In several cases these mutations could be correlated to differential expression of pathways involved in (or affected by) the interactions and to the found differences in phenotype.

Iron metabolism. The upregulation of the H_2O_2-producing pyruvate oxidase gene in *L. bulgaricus* was likely the cause of the inactivating mutations in *fur* in *S. thermophilus*, leading to a higher expression of iron-responsive genes such as the *fatABCD* complex. Indeed, *fatA* was reported to be repressed by *fur* in *Vibrio anguillarum* (33). However, why the inactivation of *fur* is favorable remains unclear: a higher presence of ROS will increase the DNA-damaging Fenton reaction making high intracellular levels of iron unfavorable.

AA acquisition. BCAA availability for both bacteria is clearly a key determinant in the adaptation process as is demonstrated by the mutation in *als* in *S. thermophilus*, the increased expression of *prtB* and the BCAA import and *de novo* biosynthesis genes in *L. bulgaricus*. This was also reported before in mixed culture compared to mono culture (29). The higher expression of *prtB* appeared to supply a sufficient amount of sulfur AA for *L. bulgaricus*, but not for *S.*

thermophilus. While the former down-regulated sulfur AA production pathways in the evolved strain, the latter upregulated those pathways in order to achieve the higher growth rate. However, it is anticipated that the higher availability of peptides and of formic acid are the main reasons for the disappearance of the transition phase in co-culture growth (see Figure 3). Addition of these two components was shown to yield an acidification pattern of a parental mixed culture that resembles that of the evolved mixed culture.

LCFA acquisition. For the acquisition of more LCFA *L. bulgaricus* strains in both cultures found different solutions: (i) a higher expression of the LCFA production pathway genes and (ii) a lower expression of this pathways genes with a higher expression of genes involved in LCFA capture from the medium, which was also suggested to occur in mixed cultures compared to mono cultures (29). In the second case, there could be an important function for the mutated protein encoded by *LBUL_0063*, but an exact function of this protein other than fatty acid binding has not been established yet.

EPS production. The production of EPS increased in the evolved cultures as a result of higher expression of EPS production genes. However, it is unclear what triggered this induction as there is no mutation evidently related to EPS production. It could be that the higher EPS synthesis was a response associated with the higher acidification rate, i.e. the higher acidification rate caused a (local) lower pH leading to induction of EPS synthesis (21). That would also explain the lack of extra EPS production by the evolved *L. bulgaricus* in mono culture compared to its parental strain.

In conclusion, our results confirm that interactions between *S. thermophilus* and *L. bulgaricus* involve proteolysis, in particular sulfur AA and BCAA, formic acid, folic acid and EPS. It was shown that these interactions can be fine-tuned by a small number of mutations. This fine-tuning of interactions is relevant for the field of microbial ecology as well as for industry. Using experimental evolution, it is possible to acquire a mixed culture with improved functionalities, such as a higher biomass yield, increased mutual stimulation, a faster acidification, higher survival and more EPS production. It is anticipated that this methodology can be routinely applied as a strategy to improve new combinations of strains in order to develop new starter cultures with interesting traits. More specific, these findings open new possibilities for the development of industrially viable fermented foods containing poor performing probiotic strains or strains with prebiotic properties.

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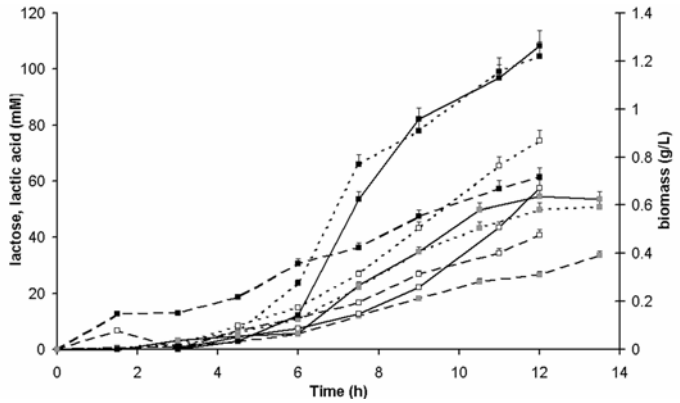
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Fermentation profiles of parental, evolved and I-St mixed cultures. The solid lines indicate biomass (g/L); the dashed lines indicate lactose consumption (mM); the dotted lines indicate lactic acid production (mM). □, Parental mixed culture; ■, evolved mixed culture; ■, I-St. Error bars indicate standard deviations.

Supplementary Table 1. Number of sequenced bases and their coverage as resulting from the Illumina sequencing. P, parental strain; E, evolved strain. *, The most abundant base in the reads was found only in ≤0.8 fraction.

	Reads	Bases	Referential bases	Average coverage	Coverage ≤10	Poor quality*	Not covered
S. thermophilus P	5984359	215436924	1796226	120	19	831	13
S. thermophilus E	5614347	202116492	1796226	113	30	929	15
L. bulgaricus P	5083083	182990988	1856951	99	2909	955	2708
L. bulgaricus E	4162688	149856768	1856951	81	5963	1044	5653

Supplementary Table 2. Primers used for confirming SNPs in evolved *S. thermophilus* and *L. bulgaricus* by sequencing of PCR products and for the detection of possible mutations in the strains selected from the duplicate evolved culture. Both primers were used for the PCR amplification and one of the primers for sequencing. The PCR program used was as follows: 1x(94°C, 5 min); 35x(94°C, 30 s; 58°C, 30 s; 72°C, 45 s); 1x(72°C, 5 min; 4°C, hold). N.D., not determined.

Position	Sequence primer 1	Tm	Sequence primer 2	Tm	Product size (bp)
S. thermophilus					
151195	AAATGCCTTCATGGATGGTG	60.7	CGTATTCGATAATCAAAATTGTCC	58.9	449
250442	CATGTACTTTGTGGCGGATG	60.0	AAGAACGGGCAATCTTGATG	60.1	317
289618	AGCTACTAAAGCGGCTGCAC	59.8	ACCCATCATCACAAATGCAA	59.8	385
385740	GCGAGTATGGCTTTGATGTG	59.3	ATGGGCGATCCTTACGAAC	59.9	405
413374	CTTTGAAAAAGGGCGTATCG	59.7	GCACGGATAGCTCCAAATTC	59.7	473
586170	CATTGATGGTGACACTGTTGC	60.0	GCCTCTGCTTCAGCCATAAC	60.0	408

683939	AAGAAGAAAGGCGGCGATA	59.9	AGTGGTCATGCCCCATAAAA	60.2	310
725339	ATCTGGAGCATCGGTTTCAC	60.1	CTCAATGGTGGCTCGATTTT	60.1	306
847292	GTCAGCCTGATGAAGGTACG	58.3	ACCAAAGTCAACCCCAGATG	59.8	401
1055098	N.D.		N.D.		
1067308	CCGTAGCCTGAGTAGCCTTC	59.1	AGGGCAAGTTGTTGCTCTTG	60.4	411
1110735	CGACCAAAGCTGTTACTTTTTC	58.1	TGACGTTGTTGCCTTCAAAC	59.7	413
1182279	TTATCCATCATGACGCAACC	59.4	CAATATGGGTGGGGACGAC	61.0	409
1371875	CGCTCAACCAAGCCAAGTAG	61.0	TGGTTGTAGCTAGCAATCTTTGG	60.7	412
1425619	GGGCAGCAAAAGGATTGTTA	60.1	ATAAGATTGCCAAGGCTCCA	59.7	378
L. bulgaricus					
61050	N.D.		N.D.		
301103	CAGGACATCGTTTTGCTGAA	59.8	GCCAGTTCTCTGGTTGGTTC	59.7	329
333736	TGCTGCAAGAAGCAGTTGAC	60.3	TAACTGAACGGCCGGAGTAG	60.3	171
341010	CCAAGGGAAAGCAATTCCA	61.0	TCATCGAGAATGCTGTGTTCA	60.4	300
998290	N.D.		N.D.		
1288314	ACTTCTGTCGCCACATTCG	59.9	GGCTCTACCAGGTCGTTCAC	59.7	454
1756309	ACTGCACTCTCCTGGTCAGC	60.6	GGCTAATTACCGATCCTTTGG	69.8	444

Supplementary Table 3. Significant (FDR≤0,05) differential expression of *S. thermophilus* genes between parental mixed cultures and evolved mixed cultures, per functional category. Values represent ratios between the first and the second condition. Loci that showed a mutation in the comparative genome sequence analysis are in bold. P, parental culture; E_1, evolved culture that is used in all analyses; E_2, duplicate evolved culture.

		1st exponential phase			2nd exponential phase			
Locus ID	Locus	E_1 over P	E_2 over P	E_2 over E_1	E_1 over P	E_2 over P	E_2 over E_1	Product
Amino acid transport and metabolism								
str0023	prsA1	0.266	0.198			0.453	0.414	ribose-phosphate pyrophosphokinase
str0026	araT	3.216				0.178	0.220	hypothetical protein
str0063	pepS					0.448		aminopeptidase PepS
str0083	cysE1	2.577	2.175			0.387	0.371	serine acetyltransferase
str0125	amiA2	4.728	5.356				0.358	oligopeptide ABC transporter substrate-binding protein
str0150	ilvA		4.225	2.467				threonine dehydratase
str0158	-	2.186	2.598		2.141			amino acid (glutamine) ABC transporter ATP-binding protein
str0159	-	5.305	5.739					amino acid (glutamine) ABC transporter substrate binding protein/permease protein
str0229	pepC				0.430			cysteine aminopeptidase C
str0283	ureC					0.468		urea amidohydrolase (urease) alpha subunit
str0296	-	7.213	7.525		8.622		0.248	amino acid ABC transporter substrate binding protein, putative
str0298	-		2.243		6.053		0.137	succinyl-diaminopimelic descuccinlyasadipeptidase, truncated
str0300	-	4.360	3.360		5.959		0.123	succinyl-diaminopimelic descuccinlyasadipeptidase, truncated
str0303	**dctA**					**0.315**	**0.238**	**dicarboxylate/amino acid:cation (Na+ or H+) symporter**
str0352	metB1		2.214					cystathionine gamma-synthase
str0353	-		2.382		0.348			aminotransferase (class II)
str0359	livJ		2.435			0.312		branched-chain amino acid ABC transporter substrate-binding protein
str0360	livH		3.123	2.688		0.371		branched-chain amino acid ABC transporter permease protein
str0361	livM		3.380	3.002		0.383		branched-chain amino acid ABC transporter permease protein
str0362	livG		3.958	2.425		0.457		branched-chain amino acid ABC transporter ATP binding protein
str0363	livF			2.206				branched chain amino acid ABC transporter ATP binding protein
str0366	cysM1	5.299	4.428		6.538		0.261	cysteine synthase
str0377	asnA					0.191	0.323	asparagine synthetase AsnA
str0378	lysC		4.757	3.161			0.495	aspartate kinase
str0411	nifS2	2.136						aminotransferase (class V), putative
str0430	gdhA		3.242	3.505				glutamate dehydrogenase
str0454	pepB	2.240				0.338	0.344	oligopeptidase
str0463	aspC3						0.275	hypothetical protein
str0464	argC					0.310		N-acetyl-gamma-glutamyl-phosphate reductase
str0466	argB				0.310	0.272		acetylglutamate kinase
str0467	argD					0.322		acetylornithine aminotransferase
str0469	hom	4.663	9.315				0.387	homoserine dehydrogenase
str0470	thrB	2.554	5.144	2.014			0.354	homoserine kinase
str0526	carA		4.560	4.807				carbamoyl-phosphate synthase small subunit
str0527	carB		4.485	3.841				carbamoyl-phosphate synthase large subunit
str0555	-		2.463		3.533		0.174	alanine dehydrogenase, truncated
str0583	-				6.247		0.219	amino acid permease
str0584	mmuM				8.742		0.162	homocysteine methyltransferase
str0590	bcaT	0.362		2.836	0.428	0.345		branched-chain amino acid aminotransferase
str0629	pepQ						2.290	dipeptidase
str0645	aroA		2.251					3-phosphoshikimate 1-carboxyvinyltransferase
str0646	aroK		2.397			2.230		shikimate kinase
str0755	glyA	4.310	3.666				0.322	serine hydroxymethyltransferase
str0771	pabB					2.503	2.212	para-aminobenzoate synthetase component I
str0785	metE		0.240			0.408		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
str0786	metF				0.332	0.364		5,10-methylenetetrahydrofolate reductase
str0846	cysM2		0.310	0.234	0.425	0.071	0.166	cysteine synthase
str0847	metB2		0.360	0.307	0.466	0.097	0.207	cystathionine beta-lyase
str0848	cysE2		0.425		0.450	0.086	0.191	serine acetyltransferase, putative
str0875	-					0.300	0.398	amino acid (glutamine) ABC transporter, permease protein
str0876	-					0.325	0.390	amino acid (glutamine) ABC transporter ATP-binding protein
str0877	-					0.492		amino acid (glutamine) ABC transporter, substrate binding protein
str0904	-						5.130	hypothetical protein
str0923	als	4.956	6.076				2.021	alpha-acetolactate synthase

str0936	adhB						2.627	alcohol dehydrogenase, zinc-containing
str0970	dtpT					0.095	0.115	di-/tripeptide transporter
str0975	-						2.045	ABC transporter substrate binding protein, truncated
str0976	-		2.566				2.167	ABC transporter permease protein, truncated
str0977	-		3.653				2.273	ABC transporter permease protein, truncated
str0984	dagA					0.197	0.130	sodium/alanine glycine symporter
str0987	cysD	8.639	13.675		6.101		0.274	O-acetylhomoserine sulfhydrylase
str1007	pepN		3.893			0.354	0.234	lysyl-aminopeptidase, aminopeptidase N
str1061	-					7.750	19.076	hypothetical protein
str1127	pepV		2.108					dipeptidase
str1139	pepT	0.479					2.078	peptidase T
str1161	-	0.493			0.463	2.035	4.392	amino acid (glutamine) ABC transporter ATP-binding protein
str1162	-				0.480		4.090	amino acid (glutamine) ABC transporter substrate-binding protein
str1163	-					2.310	4.490	amino acid (glutamine) ABC transporter permease protein
str1164	-					2.711	5.050	amino acid (glutamine) ABC transporter permease protein
str1181	aroH		0.344				0.356	hypothetical protein
str1200	leuD				0.357	0.390		isopropylmalate isomerase small subunit
str1202	leuB				0.323	0.281		3-isopropylmalate dehydrogenase
str1203	leuA				0.304	0.166		2-isopropylmalate synthase
str1222	metA		0.467			0.454	0.433	homoserine O-succinyltransferase
str1232	hisK	2.409	2.753					hypothetical protein
str1245	-	2.014						oxidoreductase, DadA family protein/D-amino acid oxidase
str1287	-					0.318		transcriptional regulator, putative
str1297	dapA	5.552	6.636				0.427	dihydrodipicolinate synthase
str1298	asd					0.227	0.259	aspartate-semialdehyde dehydrogenase
str1309	proWX	7.647	3.116		4.669		0.250	proline/glycine betaine ABC transporter
str1310	proV	9.053	6.126		15.640		0.127	proline/glycine betaine ABC transporter ATP-binding protein
str1312	proWZ	2.785			16.382	2.716	0.166	proline/glycine betaine ABC transporter substrate-binding protein
str1316	sdaB					2.064	2.451	L-serine dehydratase beta subunit
str1317	sdaA		0.422			3.204	3.240	L-serine dehydratase alpha subunit
str1357	brnQ	4.768		0.260		0.179	0.113	branched chain amino acid transport system II carrier protein
str1361	-		2.266		2.240		0.311	amino acid transporter
str1438	amiF1	10.487	11.719		2.134		0.281	oligopeptide ABC transporter ATP-binding protein
str1439	amiE	14.293	22.759		2.004	0.491	0.245	oligopeptide ABC transporter ATP-binding protein
str1440	amiD	18.279	24.126				0.280	oligopeptide ABC transporter membrane-binding protein
str1441	amiC	16.769	20.785		2.234		0.243	oligopeptide ABC transporter membrane-binding protein
str1443	-	3.384	3.767				0.450	oligopeptide ABC transporter substrate-binding protein, truncated
str1445	amiA3		2.001	2.506				oligopeptide ABC transporter substrate-binding protein
str1447	-	20.191	31.443		2.252		0.260	oligopeptide ABC transporter ATP-binding protein, truncated
str1492	-					0.277	0.450	amino acid (glutamine) ABC transporter substrate-binding protein
str1493	-		0.499					hypothetical protein
str1501	gnlP					0.488	0.407	glutamine ABC transporter permease protein
str1517	-		2.907	2.198		3.778	6.485	permease, putative
str1519	serB	0.388	0.439			2.122	2.465	phosphoserine phosphatase
str1527	serA	5.980	19.856	3.320	0.494	0.346		D-3-phosphoglycerate dehydrogenase
str1529	serC	6.387	27.583	4.319		0.464		phosphoserine aminotransferase
str1532	gloA					3.381	5.655	glyoxalase I/lactoylglutathione lyase
str1535	potD	2.113	2.794					spermidine/putrescine ABC transporter periplasmic protein
str1536	potC		2.614					spermidine/putrescine ABC transporter permease protein
str1537	potB		2.312					spermidine/putrescine ABC transporter permease protein
str1545	-					0.408		aminoacid specific permease
str1579	-	6.405	6.284		9.193		0.138	amino acid (glutamine) ABC transporter substrate binding protein
str1580	-	5.346	4.755		9.759		0.127	amino acid (glutamine) ABC transporter ATP-binding protein
str1581	-	7.747	7.211		3.921		0.321	amino acid (glutamine) ABC transporter permease protein
str1582	-				8.510		0.163	amino acid (glutamine) ABC transporter permease protein
str1587	trpA		6.597					tryptophan synthase subunit alpha
str1588	trpB		4.580					tryptophan synthase subunit beta
str1589	trpF		3.389					N-(5'-phosphoribosyl)anthranilate isomerase
str1590	trpC		11.702					indole-3-glycerol-phosphate synthase
str1636	aspC2					0.479		aspartate aminotransferase
str1652	-		4.195		2.066		0.310	ABC transporter ATP-binding protein, amino acid
str1653	-	3.008	4.122		2.231		0.283	ABC transporter amino acid permease protein
str1654	-	2.302	3.221				0.324	ABC transporter substrate-binding protein, amino acid
str1710	proA						0.317	gamma-glutamyl phosphate reductase
str1711	proB					0.318	0.265	gamma-glutamyl kinase
str1728	aroG		2.679					3-deoxy-7-phosphoheptulonate synthase
str1815	-					0.329	0.476	oligopeptide ABC transporter substrate-binding protein, truncated
str1839	dapD					0.266	0.435	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase, putative
str1852	proC	2.540						pyrroline-5-carboxylate reductase
str1871	ilvC		20.849	8.103				ketol-acid reductoisomerase
str1872	ilvN		9.738	6.235				acetolactate synthase small subunit
str1873	ilvB		11.322	8.882				acetolactate synthase large subunit
Carbohydrate transport and metabolism								
str0114	gpmB	0.339	0.261					phosphoglycerate mutase
str0191	-						0.495	PTS glucose-specific enzyme IIABC components, truncated
str0194	pgi	3.798	2.586				0.493	glucose-6-phosphate isomerase
str0312	tkt		2.124				0.443	transketolase
str0333	manL		2.071	2.275	0.341		3.555	mannose PTS system component IIAB
str0399	fruR	0.309		4.996				transcriptional repressor
str0400	fruB	0.463		2.936				fructose-1-phosphate kinase
str0401	-	0.393		3.731				PTS fructose-specific enzyme IIABC components, truncated
str0405	-		2.016	3.086		2.928	2.109	PTS fructose-specific enzyme IIABC components, truncated
str0407	-		2.092	5.339				PTS fructose-specific enzyme IIABC components, truncated
str0501	nagA		0.347					N-acetylglucosamine-6-phosphate deacetylase
str0512	-						0.485	beta-glucoside-specific PTS system IIABC component, truncated
str0612	-	2.078	3.291		3.190		0.469	hypothetical protein
str0635	eno						2.359	phosphopyruvate hydratase
str0697	gpmC					6.603	6.149	phosphoglycerate mutase
str0728	glcK					2.163	2.694	glucose kinase
str0895	-		5.840			4.662	2.523	transcriptional regulator
str0999	suhB	2.063	2.911					inositol monophosphatase family protein
str1012	glgP				0.387	2.365	6.113	glycogen phosphorylase
str1017	-					3.085		maltose/maltodextrin ABC transporter permease protein, truncated
str1069	-						2.509	hypothetical protein
str1077	epsL	6.693	8.815					exopolysaccharide polymerization protein
str1120	deoB		2.466					phosphopentomutase
str1264	ptsI		2.013					phosphoenolpyruvate:sugar phosphotransferase system enzyme I
str1265	ptsH		2.693				2.506	phosphocarrier protein HPr (histidine-containing protein)
str1368	celB	0.457	0.396					PTS cellobiose-specific IIC component
str1399	galM					2.041		aldose 1-epimerase
str1457	ppnK	3.149	5.091					inorganic polyphosphate/ATP-NAD kinase
str1469	rgpD	7.190	8.476					polysaccharide ABC transporter ATP-binding protein
str1470	rgpC	8.628	10.773				0.458	polysaccharide ABC transporter membrane-spanning protein
str1517	-		2.907	2.198		3.778	6.485	permease, putative
str1732	pmi1	0.207	0.308				3.192	mannose-6-phosphate isomerase
str1733	scrK					3.499	3.700	fructokinase
str1734	scrA					2.990	4.456	sucrose-specific PTS permease, enzyme II
str1788	gapA1						2.284	glyceraldehyde-3-phosphate dehydrogenase
str1797	rpe		0.499					ribulose-phosphate 3-epimerase
Cell division and chromosome partitioning								
str0008	divIC		0.486				2.955	cell-cycle protein, MesJ/Ycf62 family, putative
str0010	-						2.061	putative cell-cycle protein, MesJ/Ycf62 family

str0233	-					0.370		hypothetical protein
str1295	smc	3.200	4.782					chromosome segregation SMC protein
str2002	gidA					0.363	0.350	glucose-inhibited division protein A
Cell envelope biogenesis, outer membrane								
str0020	mreC					0.181	0.203	rod shape-determining protein MreC
str0110	-		0.415					hypothetical protein
str0131	-		0.405					mechanosensitive ion channel, putative
str0163	rgpG					0.175	0.217	polysaccharide biosynthesis protein
str0199	eep					0.485		conserved hypothetical protein, putative processing of a peptide sex pheromone
str0212	pbp2A			0.425				penicillin-binding protein 2A
str0230	pbp1A						0.496	penicillin-binding protein 1A
str0255	glr		0.479					glutamate racemase
str0349	murE						2.205	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase
str0413	-	2.304	2.033					glycosyltransferase, putative teichoic acid biosynthesis protein
str0563	gcaD	4.925	4.559					UDP-N-acetylglucosamine pyrophosphorylase
str0636	-	8.339	5.317					hypothetical protein
str0699	-					2.697	3.110	endolysin, putative
str0721	-	0.408	0.340					hypothetical protein
str0762	dltB	3.429	5.159					integral membrane protein
str0764	dltD		2.608					extramembranal protein
str0873	glmS	6.381	3.348				0.284	D-fructose-6-phosphate amidotransferase
str1046	-	0.428	0.107					endolysin, putative, truncated
str1058	-						0.489	D-alanyl-D-alanine-carboxypeptidase, penicillin-binding protein, truncated
str1073	epsX	0.421	0.307			0.339	0.488	lipopolysaccharide 1,6-galactosyltransferase
str1077	epsL	6.693	8.815					exopolysaccharide polymerization protein
str1080	epsI	3.632	3.946				0.491	exopolysaccharide biosynthesis protein, sugar transferase
str1082	epsG	3.687	5.565				0.453	exopolysaccharide biosynthesis protein, glycosyltransferase
str1083	-						0.480	exopolysaccharide biosynthesis protein, UDP-galactopyranose mutase, truncated
str1084	epsF		2.346					exopolysaccharide biosynthesis protein
str1085	epsE		2.001					exopolysaccharide biosynthesis protein
str1167	murA		0.413				0.372	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
str1169	galE2	0.367	0.350					UDP-glucose 4-epimerase
str1277	-	2.049	3.937					sortase, truncated
str1278	-	2.881	5.505					sortase, truncated
str1297	dapA	5.552	6.636				0.427	dihydrodipicolinate synthase
str1311	proWY	2.848			21.241		0.103	proline/glycine betaine ABC transporter membrane-spanning protein
str1373	-					3.654	2.848	UDP-glucose 4-epimerase, truncated
str1392	-	2.215	2.579					glycosyl transferase
str1467	rgpF	4.280	3.554					polysaccharide biosynthesis protein
str1468	rgpE	10.199	10.390				0.497	polysaccharide biosynthesis protein/putative glycosyltransferase
str1469	rgpD	7.190	8.476					polysaccharide ABC transporter ATP-binding protein
str1470	rgpC	8.628	10.773				0.458	polysaccharide ABC transporter membrane-spanning protein
str1471	rgpB	4.430	4.419				0.393	polysaccharide biosynthesis protein/putative rhamnosyl transferase
str1472	rgpA	2.553	2.237			0.345	0.289	polysaccharide biosynthesis protein/putative rhamnosyl transferase
str1476	-	2.792	2.207			0.406	0.384	glycosyl transferase
str1478	-	2.713	2.177			0.320	0.379	glycosyl transferase, truncated
str1479	-	5.892	4.978			0.288	0.283	glycosyl transferase
str1485	-					0.352	0.366	glycosyl transferase, family 2
str1560	murZ		2.156			0.272	0.281	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
str1576	murF	2.346	2.254					UDP-N-acetylmuramoylalanine-D-glutamyl-lysine--D-alanyl-D-alanine ligase
str1629	-				2.814		0.326	hypothetical protein
str1726	alr	2.263	2.408					alanine racemase
str1869	pbp1B	2.820	2.981					penicillin-binding protein 1B
str2006	-	0.463		2.314		0.456	0.469	conserved hypothetical protein, LysM domain protein
Cell motility and secretion								
str0117	mur3	0.427	0.349					peptidoglycan hydrolase
str0196	yajC	2.120				0.380	0.448	preprotein translocase, YajC subunit, putative
str0245	-					0.389	0.391	OxaA-like protein precursor
str0356	clpP				0.362		4.182	ATP-dependent Clp protease proteolytic subunit
str0496	mur1					0.439		peptidoglycan hydrolase
str0624	secG					0.340	0.401	translocase
str0889	ffh	5.131	4.181					signal recognition particle
str1730	secA	2.403	2.097			0.464		translocase
str1810	-	3.540	2.750			0.328	0.290	hypothetical protein
str1860	-		0.249					competence protein, putative
str1862	comGD		0.191				2.055	competence protein
str1863	comGC		0.177					late competence protein, exogenous DNA-binding protein
str1864	comGB		0.135		5.866	5.329		late competence protein, ABC transporter subunit
str1865	comGA	0.432	0.206					late competence protein, ABC transporter subunit
str1914	secY	3.057	3.463			0.389	0.371	preprotein translocase SecY
Coenzyme metabolism								
str0092	panE					0.336		2-dehydropantoate 2-reductase (ketopantoate reductase)
str0123	thiD				0.490			phosphomethylpyrimidine kinase
str0226	pncB					0.457	0.420	nicotinate phosphoribosyltransferase
str0227	nadE						0.429	NAD(+) synthetase
str0409	folC1					3.431	4.554	folylpolyglutamate synthase / dihydrofolate synthase
str0412	thiI		2.424					thiamine biosynthesis protein ThiI
str0545	-				0.357			molybdopterin biosynthesis protein (HesA/MoeB/ThiF family protein), putative, truncated
str0579	folA		0.323	0.441				dihydrofolate reductase
str0590	bcaT	0.362		2.836	0.428	0.345		branched-chain amino acid aminotransferase
str0611	folD		2.652		2.259		0.430	methylenetetrahydrofolate dehydrogenase / methenyltetrahydrofolate cyclohydrolase
str0771	pabB					2.503	2.212	para-aminobenzoate synthetase component I
str0826	ptpS					0.142	0.113	6-pyruvoyl tetrahydrobiopterin synthase, putative
str0923	als	4.956	6.076				2.021	alpha-acetolactate synthase
str0996	ribC					2.878		riboflavin kinase/flavin adenine dinucleotide synthase
str1009	lplA		0.392			3.076	4.610	lipoate-protein ligase
str1172	metK	2.339	2.176			0.181	0.189	S-adenosylmethionine synthetase
str1173	birA						2.186	biotin--protein ligase
str1527	serA	5.980	19.856	3.320	0.494	0.346		D-3-phosphoglycerate dehydrogenase
str1529	serC	6.387	27.583	4.319		0.464		phosphoserine aminotransferase
str1546	**folC2**					**0.365**		**folylpolyglutamate synthase / dihydrofolate synthase**
str1796	-		0.396					hypothetical protein
str1871	ilvC		20.849	8.103				ketol-acid reductoisomerase
str1873	ilvB		11.322	8.882				acetolactate synthase large subunit
Defense mechanisms								
str0009	-					2.545	3.376	hypothetical protein
str0071	-	4.438						bacteriocin self-immunity protein, putative, truncated
str0099	labC		0.199					lantibiotic biosynthesis protein
str0348	murO					0.440		peptidoglycan branched peptide synthesis protein, alanine adding enzyme, putative
str0434	-	8.263						ABC transporter ATP binding protein
str0538	-					0.204	0.219	ABC transporter ATP binding/permease protein
str0539	-					0.134	0.135	ABC transporter ATP binding/permease protein
str0546	-		2.459					ABC transporter ATP binding protein
str0705	hsdR1	11.232	8.490					type I restriction-modification system restriction subunit
str0708	hsdS1					2.545		type I restriction-modification system specificty subunit
str0711	hsdM1		3.871			3.267	2.486	type I restriction-modification system methyltransferase subunit
str0758	-	5.677	4.553				0.333	ABC transporter ATP-binding/permease protein
str0759	-	5.306	6.964		2.033	0.358	0.176	ABC transporter ATP-binding/permease protein
str0844	-	3.631						type I restriction-modification system restriction subunit, truncated
str0991	hsdS2		0.492					type I restriction-modification system specificty subunit

str1328	-					2.283		ABC transporter ATP binding protein
str1342	norM	0.482	0.456			0.356	0.270	MATE efflux family protein (Na(+)/drug antiporter)
str1375	sthII	3.868						type II restriction-modification system restriction subunit
str1411	-					0.284	0.326	ABC transporter, truncated
str1436	-						3.222	ABC transporter ATP binding protein
str1660	-		0.464			0.470	0.387	type I restriction-modification system specificty subunit, truncated
str1667	-			0.301			0.220	hypothetical protein
str1693	-					0.494	0.382	ABC transporter ATP-binding/permease protein
DNA replication, recombination and repair								
str0006	trcF	0.411	0.479					transcription repair coupling factor
str0013	-		3.579					IS1167, transposase, ISL3 family, truncated
str0019	-						4.077	IS861, transposase (orf2), IS3 family, truncated
str0027	recO	3.790		0.294		0.230	0.255	DNA repair protein RecO
str0050	mutS1		2.018					DNA mismatch repair protein
str0056	ruvA		0.491					Holliday junction DNA helicase motor protein
str0060	recA					0.351	0.437	recombinase A
str0062	polC					0.278		DNA polymerase III subunit alpha
str0067	-						5.257	ISSpn1, transposase, IS3 family, truncated
str0069	-					5.906		ISSpn1, transposase, IS3 family, truncated
str0107	-		0.401			0.371		truncated IS1216 transposase
str0108	-		0.356					truncated IS1216 transposase
str0126	-		3.180				2.492	truncated IS1193 transposase
str0136	-			0.449				MutT/nudix family protein, truncated
str0234	-					0.427	0.493	hypothetical protein
str0237	snf	2.074						ATP-dependent RNA helicase
str0259	xer2					2.915	2.917	tyrosine recombinase
str0320	dnaB		0.435			0.413	0.421	chromosome replication initiation / membrane attachment protein DnaB
str0321	dnaI					0.458	0.457	primosomal protein DnaI
str0326	-						2.573	truncated IS1191 transposase
str0396	tnp1216		0.185			0.221	0.379	IS1216 transposase
str0490	dnaH	0.495	0.306					DNA polymerase III subunit delta
str0588	parE	2.256				0.399	0.333	DNA topoisomerase IV subunit B
str0589	parC	5.397	5.515				0.390	DNA topoisomerase IV subunit A
str0601	-	0.480	0.289					conserved hypothetical protein, MutT/nudix family protein
str0614	recR					0.302	0.392	recombination protein RecR
str0620	mutM		2.314					formamidopyrimidine-DNA glycosylase
str0658	-					0.405		hypothetical protein
str0719	holA	0.371	0.223					DNA polymerase III subunit delta
str0783	int2	0.455	0.235					integrase/recombinase, phage associated
str0796	tnpSth1						2.425	ISSth1, transposase (orf2), IS3 family
str0813	dinG	4.221	4.125					ATP-dependent DNA helicase
str0822	-						2.041	truncated IS1193 transposase
str0885	sthIM	10.363	10.162					type III restriction-modification system methylation subunit
str0894	rnhB	2.637	3.148			2.428	2.214	ribonuclease HII
str0986	pcrA	2.358	2.218			0.396	0.399	ATP-dependent DNA helicase
str1055	ung		2.720	3.808				uracil-DNA glycosylase
str1126	-						0.438	hypothetical protein
str1198	dnaE					3.462	2.536	DNA polymerase III subunit alpha
str1221	dnaD		0.495				0.488	DNA replication protein dnaD
str1224	recJ	4.045	4.550			0.380	0.292	single strand DNA-specific exonuclease
str1261	deaD					2.910	2.279	ATP-dependent RNA helicase
str1274	-		2.063					ISSth5, transposase, IS110 family, truncated
str1279	gyrA	2.177	3.324					DNA gyrase subunit A
str1300	alkD					0.281	0.225	DNA alkylation repair protein
str1304	-					2.468	3.945	truncated IS1193 transposase
str1306	uvrC						2.045	excinuclease ABC subunit C
str1375	sthII	3.868						type II restriction-modification system restriction subunit
str1394	sbcC					2.759	2.342	ATP-dependent dsDNA exonuclease
str1425	pknB	2.344				0.477	0.407	protein kinase
str1489	dnaG					0.283	0.395	DNA primase
str1526	-		8.791	2.463	0.480	0.392		methylguanine-DNA methyltransferase, truncated
str1550	exoA			0.399				3'-exo-deoxyribonuclease
str1568	rheA		2.286			0.446	0.275	ATP-dependent RNA helicase
str1601	**dnaQ**	**0.082**	**0.045**		**0.495**			**DNA polymerase III subunit epsilon**
str1638	-	0.214	0.213		0.228	2.185	9.580	ISSth3, transposase, IS30 family, truncated
str1677	-						2.088	ISSth1, transposase (orf1), IS3 family, truncated
str1699	rheB					2.799	2.783	ATP-dependent RNA helicase
str1716	rexA					0.368	0.446	ATP-dependent exonuclease, subunit A
str1717	rexB					0.300	0.395	ATP-dependent exonuclease, subunit B
str1753	ssbB	6.554	11.995			0.397	0.311	single-strand DNA-binding protein
str1762	mutS2	2.013				2.076	2.415	DNA mismatch repair protein
str1765	rnhB		2.022			0.452	0.497	ribonuclease HIII
str1800	-	0.473	0.458					topoisomerase/primase
str1801	tatD		0.381					deoxyribonuclease, putative
str1807	tnp657	0.140	0.098		0.373			IS657, transposase, IS200 family
str1845	ssbA	2.507			4.025		0.152	single-strand DNA-binding protein
str1858	-		0.462	0.423				hypothetical protein
str1904	-					5.192		ISSth4, transposase, IS30 family, truncated
str1905	-					2.473	2.972	ISSth4, transposase, IS30 family, truncated
str1936	ruvB						2.210	Holliday junction DNA helicase RuvB
str1948	-	0.289	0.334					hypothetical protein
str1957	cshA						2.719	chromosome segregation helicase
str2015	recF				0.478	0.307		recombination protein F
Energy production and conversion								
str0246	acyP		0.230					acylphosphatase
str0372	ppaC	5.373	3.771				0.446	putative manganese-dependent inorganic pyrophosphatase
str0478	atpE					0.427		proton-translocating ATPase, c subunit
str0557	-					9.011	11.305	pyridine nucleotide-disulfide oxidoreductase
str0562	idi	2.274	2.158			3.813		isopentenyl pyrophosphate isomerase
str0718	ppc					0.439		phosphoenolpyruvate carboxylase
str1051	acoA	0.383	0.459					acetoin dehydrogenase complex, E1 component, alpha subunit
str1137	fer					2.312	2.294	ferredoxin
str1149	nfrA		0.335					nitro/flavin reductase
str1183	fld					0.230	0.324	flavodoxin
str1202	leuB				0.323	0.281		3-isopropylmalate dehydrogenase
str1263	**gapN**			**2.012**				**NADP-dependent glyceraldehyde-3-phosphate dehydrogenase**
str1268	citB		2.884					aconitate hydratase
str1369	-				0.322	0.352		NAD(P)H nitroreductase, putative
str1455	pta		8.495	2.073		2.440		phosphate acetyltransferase
str1657	pfl		0.352	0.247	2.747	2.152		pyruvate formate-lyase
str1803	trxA1		0.262			0.385	0.454	thioredoxin
str1805	bta		0.403			0.200	0.332	bacteriocin transport accessory protein, putative
str1849	trxA2					0.456	0.351	thioredoxin
str1879	-		0.134					alcohol-acetaldehyde dehydrogenase, truncated
str1881	-	0.250	0.234					alcohol-acetaldehyde dehydrogenase, truncated
str1882	-	0.364	0.198					alcohol-acetaldehyde dehydrogenase, truncated
str1884	-	0.194	0.201					alcohol-acetaldehyde dehydrogenase, truncated
Function unknown								
str0022	pcsB	0.384				0.289	0.389	glucan binding protein
str0085	-	3.112	2.126			0.390	0.379	hypothetical protein
str0113	-						0.394	hypothetical protein

str0124	-				0.427		2.931	hypothetical protein
str0254	-	0.407	0.337					hypothetical protein
str0260	scpA					3.440	3.049	segregation and condensation protein A
str0268	-					0.084	0.106	hypothetical protein
str0276	-			0.429		0.262	0.284	membrane protein
str0306	-	0.346	0.085					hypothetical protein
str0340	-		2.136			0.334	0.456	hypothetical protein
str0427	-				0.241		2.597	hypothetical protein
str0503	-		0.182		0.330			hypothetical protein, truncated
str0504	-	0.359	0.188					hypothetical protein, truncated
str0552	-				0.448	0.252		hypothetical protein
str0587	-		0.388					hypothetical protein
str0595	-						0.493	hypothetical protein
str0632	-	0.191	0.305			2.430	3.153	hypothetical protein
str0634	-					4.196	3.433	hypothetical protein
str0643	-					2.066		hypothetical protein
str0652	mip		3.069		7.075		0.147	macrophage infectivity potentiator-related protein, putative
str0657	-			2.053		0.401		hypothetical protein
str0661	-					2.585	3.081	hypothetical protein
str0698	-					5.230	5.492	transcriptional regulator
str0830	-						2.278	hypothetical protein
str0884	sthIR	9.108	11.074					DNA endonuclease, type III restriction and modification system
str0888	-	4.185	2.700				0.464	hypothetical protein
str0993	-		0.143					hypothetical protein
str1063	-	0.385				2.636	7.822	hypothetical protein
str1157	-		3.698			2.296		hypothetical protein
str1248	-				2.108	2.870		hypothetical protein
str1256	-		3.148	2.514		3.839	4.288	hypothetical protein
str1377	-	2.715		0.479		0.462	0.454	hypothetical protein
str1422	-					0.350	0.385	conserved hypothetical protein, putative transporter
str1425	pknB	2.344				0.477	0.407	protein kinase
str1458	-			0.422		0.332	0.425	hypothetical protein
str1459	-	5.252	6.147					GTP pyrophosphokinase, putative
str1466	-	10.249	9.188					hypothetical protein
str1551	-		0.458	0.409				hypothetical protein, citrulline cluster-linked gene
str1555	-	4.178					0.403	membrane protein
str1723	-	2.765	2.051					conserved hypothetical protein, Cof family
str1768	-		0.452					hypothetical protein
str1778	-					0.262	0.220	hypothetical protein
str1795	-		0.427					hypothetical protein
str1961	-	0.421	0.211					hypothetical protein
str1968	-	2.428	2.165				0.386	hypothetical protein
str1982	-	0.498			0.383	3.568	9.327	hypothetical protein
str1993	-		0.391			0.165	0.308	hypothetical protein
str1996	-		0.376			0.187	0.246	hypothetical protein
str2011	-		0.355	0.495		0.497		hypothetical protein
str2014	-				0.449	0.242		hypothetical protein
str2019	-			0.318		0.332	0.396	hypothetical protein
General function prediction only								
str0022	pcsB	0.384				0.289	0.389	glucan binding protein
str0059	cinA					0.309	0.358	competence damage-inducible protein A
str0066	-					0.491		hypothetical protein
str0136	-			0.449				MutT/nudix family protein, truncated
str0193	-					0.370		hypothetical protein
str0201	-		3.161	3.119		0.239	0.274	ABC transporter permease protein
str0202	-					0.305	0.318	ABC transporter ATP binding protein
str0231	recU						0.407	hypothetical protein
str0248	-					3.077	4.369	conserved hypothetical protein, putative hydrolase
str0249	-					3.037	4.158	conserved hypothetical protein, membrane protein
str0257	-					2.520	2.342	hypothetical protein
str0258	-					2.473	2.260	hypothetical protein
str0316	-	2.683				0.423	0.299	hypothetical protein
str0336	-	3.214	2.571			0.221	0.130	conserved hypothetical protein, xanthine/uracil permease family
str0364	-	0.419				3.548	5.144	acetoin utilization protein, truncated
str0375	recX					2.228	3.974	RecA regulator RecX
str0379	-						2.026	beta-phosphoglucomutase, putative
str0385	fabK		0.441			0.484		trans-2-enoyl-ACP reductase II
str0397	-					0.437	0.323	hypothetical protein
str0443	-	0.372				0.311	0.459	glucan binding protein
str0451	metG					0.436	0.377	methionine--tRNA ligase
str0455	-					0.449	0.209	methyltransferase, putative
str0462	-	2.792	3.062					hypothetical protein
str0471	-	5.751	10.869					membrane protein
str0474	-	3.095	2.856					hypothetical protein
str0548	-	2.629						hypothetical protein
str0554	hipO1	3.883	10.259	2.642	3.071		0.199	aminoacylase/N-acyl-L-amino acid amidohydrolase/hippurate hydrolase
str0575	hlyIII	0.295	0.209		0.337	0.360		similar to hemolysin III
str0597	-					0.300	0.357	oxidoreductase, truncated
str0601	-	0.480	0.289					conserved hypothetical protein, MutT/nudix family protein
str0617	-	2.972	2.280					hypothetical protein
str0619	era	4.007	4.342					GTP-binding protein Era
str0627	pplB					0.480	0.481	peptidyl-prolyl cis-trans isomerase
str0662	-						3.524	hypothetical protein
str0668	-						2.104	hypothetical protein
str0674	bioY1		0.230			0.325		biotin synthase
str0696	-	2.814	5.024					hypothetical protein
str0704	-						4.289	oxidoreductase, short chain dehydrogenase/reductase family
str0748	-	0.234	0.181					conserved hypothetical protein, DHH subfamily
str0808	-					2.137		hypothetical protein
str0812	-					4.652	5.750	conserved hypothetical protein, metallo-beta-lactamase superfamily
str0825	-					0.086	0.076	hypothetical protein
str0828	-		2.873			0.186	0.124	hypothetical protein
str0860	adcA		2.441		2.267	0.359	0.158	zinc ABC transporter substrate binding protein
str0893	-	2.372	3.156			2.587	2.218	GTP-binding protein
str0910	-						0.483	acetoin reductase, truncated
str0918	-						2.597	hypothetical protein
str0936	adhB						2.627	alcohol dehydrogenase, zinc-containing
str0937	-		0.402					hypothetical protein, truncated
str0938	-	0.238	0.371			2.175	2.075	hypothetical protein, truncated
str0942	-	4.679						phosphatase, putative
str0953	thiJ		0.474				2.419	4-methyl-5(B-hydroxyethyl)-thiazole monophosphate biosynthesis enzyme
str0983	thdF		0.350					tRNA modification GTPase
str0988	-	3.316				0.361	0.233	hypothetical protein
str0989	-	4.770	3.444		2.014	0.429	0.213	hypothetical protein
str1060	-					6.815	8.443	conserved hypothetical protein, putative phosphoesterase
str1062	-	0.191			0.410	7.261	17.703	transporter, putative
str1068	-					0.332	0.262	hypothetical protein
str1076	epsM	8.703	16.197					exopolysaccharide biosynthesis protein
str1081	epsH	3.152	3.704				0.424	exopolysaccharide biosynthesis protein, acetyltransferase
str1128	xylH					2.248	2.505	4-oxalocrotonate tautomerase
str1158	vicX		2.379					hypothetical protein
str1186	estA	3.136	2.254			0.292	0.424	esterase
str1188	-	2.528					0.472	ABC transporter ATP binding protein

str1190	-			0.237				ABC transporter substrate binding protein
str1225	-	4.028	3.927			0.414	0.260	oxidoreductase, short chain dehydrogenase/reductase family
str1226	elaC	3.889	4.113			0.357	0.290	ribonuclease Z
str1228	hflX					0.320	0.293	GTP-binding protein
str1232	hisK	2.409	2.753					hypothetical protein
str1257	-					4.204	3.773	hypothetical protein
str1281	nox	4.987	11.654	2.337	2.167	2.160		NADH oxidase (H2O-forming)
str1282	-		2.438		3.376		0.258	ABC transporter ATP binding protein
str1307	-	5.134	4.806		3.268	2.813		hydrolase, haloacid dehalogenase-like family
str1324	-						0.401	ABC transporter ATP binding protein, truncated
str1330	-		8.534	5.936	0.341		2.201	Na+-dependent transporter, putative
str1363	-				0.428			hypothetical protein
str1371	-	2.019		0.425	0.463	0.398		oxidoreductase, aldo/keto reductase family
str1425	pknB	2.344				0.477	0.407	protein kinase
str1433	-	2.088						conserved hypothetical protein, Cof family
str1480	-	4.781	4.477			0.253	0.304	polysaccharide/teichoic acid transporter, putative
str1504	obg	3.122	2.508					GTP-binding protein, GTP1/Obg family
str1517	-		2.907	2.198		3.778	6.485	permease, putative
str1524	-				0.484	0.401		hypothetical protein
str1547	-					0.405	0.320	hypothetical protein
str1605	-					4.415	2.414	hypothetical protein
str1615	-	0.333						hypothetical protein
str1616	-	0.430	0.424					hypothetical protein
str1641	hlyX					0.263	0.287	hemolysin, putative
str1665	-					0.259	0.267	hypothetical protein
str1666	-	2.367				0.253	0.236	ABC transporter ATP binding protein
str1723	-	2.765	2.051					conserved hypothetical protein, Cof family
str1775	-	0.442	0.338					glucan-binding protein
str1798	-		0.486					ribosome-associated GTPase
str1802	-		0.257			0.356	0.383	hypothetical protein
str1809	jag					0.475		conserved hypothetical protein, Jag protein
str1836	-	0.445	0.252		0.481			hypothetical protein
str1838	hipO3					0.365		aminoacylase/N-acyl-L-amino acid amidohydrolase/hippurate hydrolase
str1847	-	2.112		0.459	3.298		0.180	permease, putative
str1848	-						0.396	phenylalanyl-tRNA synthetase homolog
str1853	-					2.421	2.946	hypothetical protein
str1875	-					0.280	0.300	hypothetical protein
str1948	-	0.289	0.334					hypothetical protein
str1951	-			0.343				hypothetical protein
str1965	-	2.185						acetyltransferase, GNAT family
str2012	-		0.484	0.396		0.305	0.414	peptidase
str2013	-					0.207	0.366	protease, putative
str2016	guaB	3.169		0.253		0.207	0.290	inositol-5-monophosphate dehydrogenase
str2020	-	2.798		0.414				ABC transporter ATP-binding protein
Inorganic ion transport and metabolism								
str0061	spxA	0.240		2.700	0.237		2.339	transcriptional regulator Spx
str0264	trkH1	3.598	2.740					potassium Trk transporter membrane-spanning protein
str0265	trkA1	3.019	2.226			0.400	0.347	potassium Trk transporter NAD+ binding protein
str0288	cbiM	2.376	3.325					cobalt transport protein CbiM
str0289	cbiQ	2.396	3.741					cobalt ABC transporter permease protein
str0290	cbiO		3.344					cobalt ABC transporter ATP-binding protein
str0297	-	3.228	2.170		5.772		0.170	ABC transporter substrate binding protein
str0301	-	5.550	3.885		4.469		0.130	ABC transporter ATP binding protein
str0302	-	5.088	4.537		3.798	0.496	0.131	ABC transporter permease protein
str0308	-	0.369	0.098			0.230		ABC transporter ATP binding protein
str0310	-		0.271					ABC transporter permease protein
str0351	-					2.833		cation transporter P-type ATPase, truncated
str0498	-					0.365		Na/Pi cotransporter II-related protein, truncated
str0499	-		0.405		0.375	0.362		Na/Pi cotransporter II-related protein, truncated
str0608	feoB				2.582		0.377	ferrous ion transport protein B
str0677	mntH		2.372					manganese transport protein, NRAMP family
str0720	sodA				0.477		4.027	superoxide dismutase (Mn)
str0723	dpr	0.279			0.064	4.020	63.262	peroxide resistance protein,non-heme iron-containing ferritin
str0724	**fur**			**2.337**	**0.378**	**3.151**	**8.328**	**ferric transport regulator protein**
str0745	-	0.458	2.712	5.919				hypothetical protein
str0840	copB		0.390		0.372		2.580	cation transporting ATPase, copper transport
str0860	adcA		2.441		2.267	0.359	0.158	zinc ABC transporter substrate binding protein
str0874	phnA					0.305	0.247	conserved hypothetical protein, PhnA protein
str1001	pstS		2.125					phosphate ABC transporter substrate binding protein
str1002	pstC1		2.347					phosphate ABC transporter permease protein
str1003	pstC2		2.247					phosphate ABC transporter permease protein
str1022	-				16.904	3.182	0.188	hypothetical protein
str1023	-				9.424		0.174	hypothetical protein
str1024	-				14.007		0.170	hypothetical protein
str1025	fatB	10.846	9.884		24.748		0.212	ferrichrome ABC transporter, substrate-binding protein
str1026	fatA	10.406	10.717		32.109		0.211	ferrichrome ABC transporter, ATP-binding protein
str1027	fatC	15.104	13.797		37.808		0.159	ferrichrome ABC transporter, permease protein
str1028	fatD	11.262	8.874		27.298		0.174	ferrichrome ABC transporter, permease protein
str1056	-	7.257	4.731			0.189	0.213	cation efflux protein
str1116	-	2.063	2.806					chloride channel protein, truncated
str1150	pacL2			0.423				calcium transporter P-type ATPase
str1180	-		0.409	0.467		0.311	0.324	conserved hypothetical protein, voltage-gated chloride channel family
str1182	-					0.394		chloride channel protein
str1379	-					0.383		hypothetical protein
str1439	amiE	14.293	22.759		2.004	0.491	0.245	oligopeptide ABC transporter ATP-binding protein
str1440	amiD	18.279	24.126				0.280	oligopeptide ABC transporter membrane-binding protein
str1441	amiC	16.769	20.785		2.234		0.243	oligopeptide ABC transporter membrane-binding protein
str1525	-		6.754		0.305	0.299		hypothetical protein
str1569	corA1		0.325		0.415		2.772	cation transporter CorA family
str1584	copZ		17.137	10.478		9.747	19.606	copper chaperone - copper transport ATPase
str1585	copA		10.971	8.056		12.487	23.011	cation transporting ATPase, copper transport
str1596	ctpE	2.869	3.487				2.428	cation transporting ATPase
str1633	-				3.594			ABC transporter substrate binding protein
str1643	nha		2.034					Na+/H+ antiporter
str1749	corA2		2.192					cation transporter CorA family
str1830	-					3.070		formate-nitrate transporter, truncated
str2007	-		0.408	0.499				ABC transporter permease protein
str2008	-		0.414	0.452				ABC transporter ATP binding protein
Lipid metabolism								
str0028	plsX	0.494	0.230	0.465				fatty acid/phospholipid synthesis protein
str0029	acpP1	0.468	0.131	0.280				acyl carrier protein
str0197	uppS					0.417		undecaprenyl pyrophosphate synthetase
str0198	cdsA					0.475		phosphatidate cytidylyltransferase (CDP-diglyceride synthase)
str0269	-					0.109	0.128	conserved hypothetical protein, PAP2 family
str0384	acpP2					0.439		acyl carrier protein
str0391	accC					0.464		acetyl-CoA carboxylase
str0559	mvaK1	4.323				3.091	2.201	mevalonate kinase
str0560	mvaD					3.061	2.218	mevalonate pyrophosphate decarboxylase
str0561	mvaK2					3.280	2.177	phosphomevalonate kinase
str0576	mvaA	2.656						3-hydroxy-3-methylglutaryl-coenzyme A reductase
str0577	mvaS	2.472						hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase)
str0651	-				8.939		0.124	hypothetical protein, acyl-CoA dehydrogenase family

str0763	dltC	3.815	4.082					D-alanine--poly(phosphoribitol) ligase subunit 2
str0910	-						0.483	acetoin reductase, truncated
str1305	cls					2.864	2.885	cardiolipin synthetase
str1563	-	2.877	3.012					1-acyl-sn-glycerol-3-phosphate acyltransferase, putative
str1727	acpS	3.473	3.215					4'-phosphopantetheinyl transferase
Not in COGs								
str0003	-	0.388	0.302					hypothetical protein
str0005	pth	0.401	0.372					peptidyl-tRNA hydrolase
str0021	mreD					0.185	0.180	rod shape-determining protein MreD
str0030	purC		0.330		3.909		0.367	phosphoribosylaminoimidazole-succinocarboxamide synthase
str0034	purN				4.446		0.360	phosphoribosylglycinamide formyltransferase
str0035	purH				4.538		0.385	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
str0043	-				2.865		0.411	hypothetical protein
str0044	-				2.894		0.471	hypothetical protein
str0051	-						3.786	hypothetical protein
str0053	-	2.031		0.285		0.308	0.422	hypothetical protein
str0057	-		0.478					3-methyladenine DNA glycosylase I, truncated
str0073	rpsB		5.906				0.442	30S ribosomal protein S2
str0074	tsf	3.625	4.801					elongation factor Ts
str0075	qacE		0.493					small multidrug export related protein, putative
str0082	-	2.956				0.441	0.304	hypothetical protein
str0086	-	2.382				0.469	0.309	hypothetical protein
str0087	-	4.332					0.269	hypothetical protein
str0091	-			0.477				hypothetical protein
str0093	rplM						0.489	50S ribosomal protein L13
str0094	rpsI		2.072			0.473	0.440	30S ribosomal protein S9
str0097	-				0.223	0.194		hypothetical protein, putative bacteriocin
str0098	-				0.425	0.305		lantibiotic biosynthesis protein, truncated
str0109	-		0.404					hypothetical protein, truncated
str0111	-		0.108					hypothetical protein
str0116	dacB	0.450	0.323					D-alanyl-D-alanine-carboxypeptidase
str0135	-	2.112		0.397				hypothetical protein
str0146	dtd		0.448					D-tyrosyl-tRNA deacylase
str0147	-	0.262	0.105					1,6-alpha-glucanhydrolase (dextranase), truncated
str0148	-	0.474	0.238					1,6-alpha-glucanhydrolase (dextranase), truncated
str0149	-	0.487	0.129					1,6-alpha-glucanhydrolase (dextranase), truncated
str0151	def		0.419				2.113	peptide deformylase
str0153	msf	0.437	0.136	0.312				major facilitator superfamily transporter, efflux protein
str0154	rpsO		2.383					30S ribosomal protein S15
str0155	-					0.218	0.307	hypothetical protein
str0156	-					0.219	0.384	hypothetical protein
str0160	uppP		3.813				0.323	undecaprenyl pyrophosphate phosphatase
str0161	-					9.591	10.455	hypothetical protein
str0162	mecA		0.385			0.166	0.238	adaptor protein
str0182	-					0.360	0.396	positive transcriptional regulator MutR family
str0184	-					0.392	0.387	conserved hypothetical protein, peptide-efflux protein homolog
str0192	-					0.429	0.472	PTS glucose-specific enzyme IIABC components, truncated
str0195	-	2.283				0.368	0.425	hypothetical protein
str0208	-	0.435	0.244			0.186	0.282	hypothetical protein
str0209	-	0.359	0.295			0.167	0.243	hypothetical protein
str0210	-	0.476	0.347			0.293	0.376	hypothetical protein
str0216	-					0.310	0.324	hypothetical protein
str0232	-		0.350					hypothetical protein
str0241	pabC					0.488	0.486	aminodeoxychorismate lyase
str0261	scpB					3.983	3.390	segregation and condensation protein B
str0263	-					3.825	2.851	hypothetical protein
str0270	plcR		0.370	0.407		0.186	0.239	transcriptional regulator
str0280	ureI					0.480		urease accessory protein, putatuve transport protein
str0281	ureA					0.434		urea amidohydrolase (urease) gamma subunit
str0282	ureB					0.386	0.481	urea amidohydrolase (urease) beta subunit
str0287	ureD		2.735					urease accessory protein
str0299	-	3.449	2.671		6.639		0.133	succinyl-diaminopimelic descuccinlyasadipeptidase, truncated
str0304	-		0.232					hypothetical protein
str0319	-		0.411			0.217	0.232	hypothetical protein
str0323	-	0.240	0.253			0.468	0.381	hypothetical protein
str0325	-		0.461					hypothetical protein
str0328	-					6.101	6.636	hypothetical protein
str0331	manN		4.936	3.109	0.401		3.728	mannose PTS system component IID
str0332	manM		4.055	2.625	0.387		3.949	mannose PTS system component IIC
str0354	-						3.201	hypothetical protein
str0365	-	0.414				5.117	5.236	acetoin utilization protein, truncated
str0394	luxS						2.026	S-ribosylhomocysteinase
str0395	-	0.415	0.135			0.260	0.313	type I restriction modification system specificity subunit, truncated
str0402	-					2.077		PTS fructose-specific enzyme IIABC components, truncated
str0403	-	0.339		4.886				PTS fructose-specific enzyme IIABC components, truncated
str0404	-			2.441		2.146	2.194	PTS fructose-specific enzyme IIABC components, truncated
str0410	-						2.801	hypothetical protein
str0414	-	3.362	2.446					hypothetical protein
str0415	-	2.848	2.906					hypothetical protein, truncated
str0416	-	2.264						hypothetical protein, truncated
str0417	rplU		3.198					50S ribosomal protein L21
str0418	rpmA		2.073					50S ribosomal protein L27
str0435	-	4.257	2.850					hypothetical protein
str0439	frr					0.402	0.346	ribosome releasing factor
str0444	-					2.409		truncated IS1193 transposase
str0446	-					0.271	0.346	surface immunogenic protein, truncated
str0448	-	0.402	0.097					hypothetical protein
str0449	-		0.148					hypothetical protein
str0450	-		0.229					hypothetical protein
str0456	prtM	2.393	2.386			0.205	0.210	protease maturation protein precursor
str0458	-					3.508	5.120	cell wall protein precursor, similar to choline binding protein, truncated
str0459	-		4.958			4.163	5.933	cell wall protein precursor, similar to choline binding protein, truncated
str0460	-					4.452	4.599	cell wall protein precursor, similar to choline binding protein, truncated
str0468	-		4.953					hypothetical protein
str0472	-					0.330	0.396	hypothetical protein
str0473	-						2.556	hypothetical protein
str0479	atpB					0.442		ATP synthase subunit A
str0485	atpC		2.572					ATP synthase subunit epsilon
str0510	-			0.463		0.284	0.419	hypothetical protein
str0511	-					0.391		beta-glucoside-specific PTS system IIABC component, truncated
str0516	-	4.151						hypothetical protein
str0528	-						0.371	hypothetical protein
str0540	queA						0.490	S-adenosylmethionine:tRNA ribosyltransferase-isomerase
str0542	-		0.355			0.220	0.391	sensor histidine kinase, truncated
str0547	-		2.618					ABC transporter permease protein
str0565	-	2.978	2.726					hypothetical protein
str0571	-	4.847	4.055				0.438	hypothetical protein
str0574	-		2.431					hypothetical protein
str0578	thyA		0.435			4.113	4.995	thymidylate synthase
str0580	-		0.290	0.477				hypothetical protein
str0585	-		3.191	2.096			0.299	hypothetical protein
str0586	-	3.351	3.405				0.455	hypothetical protein
str0591	-		0.453	0.494		0.307	0.431	hypothetical protein

str0593	-					0.367	0.416	hypothetical protein
str0594	-					0.355	0.394	hypothetical protein
str0598	-					0.407	0.433	oxidoreductase, truncated
str0599	-					0.286	0.305	oxidoreductase, truncated
str0600	-	0.462	0.250					hypothetical protein
str0607	feoA				2.548		0.329	ferrous ion transport protein A
str0609	-				2.641		0.234	hypothetical protein
str0610	-				2.078		0.318	hypothetical protein
str0618	dgk		3.941				2.033	diacylglycerol kinase
str0622	pmrA		3.220					multi-drug resistance efflux pump
str0631	-	0.370	0.319			2.601	2.835	hypothetical protein
str0633	grk	0.379	0.404				2.260	glycerate kinase, putative
str0641	aroF					2.028		chorismate synthase
str0648	-		2.208			2.393	2.117	negative transcriptional regualtor-PBP synthesis, truncated
str0650	eetB				8.729		0.229	exfoliative exotoxin B, putative
str0654	-		3.379					hypothetical protein
str0656	-					0.301	0.480	hypothetical protein
str0660	-					0.317		hypothetical protein
str0664	-		6.512			2.067		hypothetical protein
str0667	lgt		2.068			2.509	2.357	prolipoprotein diacylglyceryl transferase
str0669	-					2.042	2.853	hypothetical protein
str0670	-	4.953	5.959			0.248	0.335	hypothetical protein
str0675	-	0.289	0.370			0.346		hypothetical protein
str0685	-	2.428	2.073					hypothetical protein
str0690	-		0.455				2.152	hypothetical protein
str0694	-		0.109					hypothetical protein
str0700	-		0.411			6.257	6.773	hypothetical protein
str0701	-	8.746	11.248					hypothetical protein
str0702	-					2.655	3.997	hypothetical protein
str0703	-						3.183	hypothetical protein
str0707	-	3.800	2.448			2.137		hypothetical protein
str0709	-	3.554	3.500			2.498		hypothetical protein
str0710	-		2.375			2.406		hypothetical protein
str0713	-		2.998					deoxyribonuclease
str0717	-		0.489					hypothetical protein
str0727	-					2.000	2.595	hypothetical protein
str0744	-					2.420		truncated IS1193 transposase
str0747	apbE	0.312	0.234					lipoprotein involved thiamine biosynthesis
str0756	-	7.194	6.972			0.482	0.290	hypothetical protein
str0757	-	2.948	2.397				0.294	hypothetical protein, RAS-related protein
str0760	dltX	2.725	2.524					component involved in D-alanylation of teichoic acids, putative
str0766	-					2.808		IS861, transposase (orf2), IS3 family, truncated
str0782	-	0.474	0.238					unknown protein, phage associated
str0784	-		0.222					unknown protein, phage associated
str0793	-	0.047	0.029			0.421	0.379	sensor histidine kinase (homolog to ciaH Spn), truncated
str0794	-	0.049	0.033					sensor histidine kinase (homolog to ciaH Spn), truncated
str0798	rpsT					0.399		30S ribosomal protein S20
str0819	-				2.017			hypothetical protein
str0820	-					3.105	3.277	truncated IS1193 transposase
str0821	-					2.288	2.600	truncated IS1193 transposase
str0838	cspB	0.368	0.378			0.383		cold shock protein B
str0861	-	0.241	0.234			0.346	0.340	hypothetical protein
str0862	-		0.037					hypothetical protein
str0868	-					0.291	0.395	hypothetical protein
str0906	ndk				0.474			nucleoside diphosphate kinase
str0912	-	0.459						hypothetical protein
str0913	-		0.277					hypothetical protein
str0914	-	0.227	0.199				2.544	hypothetical protein
str0922	-					0.357		hypothetical protein
str0930	-	0.394	0.405			4.664	4.872	hypothetical protein
str0934	-						2.313	ferrochelatase, truncated
str0935	-		2.258					ferrochelatase, truncated
str0951	-		3.544				0.381	hypothetical protein
str0952	-		5.151					hypothetical protein
str0969	-	0.244	0.097			2.367		amidase, truncated
str0978	-		4.003				3.600	hypothetical protein
str0980	-		5.759			3.619	3.744	hypothetical protein
str0981	-					5.512	7.344	transcriptional regulator, AraC family, truncated
str0982	-					3.155	3.586	transcriptional regulator, AraC family, truncated
str0992	-	0.467	0.224					hypothetical protein
str0998	-		2.652					hypothetical protein
str1013	malQ	0.267		4.462	0.221	2.197	9.940	4-alpha-glucanotransferase
str1020	tatA				24.584		0.181	Sec-independent protein translocase protein tatA, putative
str1021	tatC				21.304		0.186	Sec-independent protein translocase protein tatC
str1037	-	0.253	0.236				2.806	hypothetical protein
str1038	-	0.291	0.264				2.502	hypothetical protein
str1044	-		0.150					positive transcriptional regulator MutR family
str1047	-	0.475	0.102					hypothetical protein
str1052	-			3.818				hypothetical protein, truncated
str1053	-			4.722				hypothetical protein, truncated
str1078	epsK	7.424	11.246				0.481	exopolysaccharide gene claster protein
str1079	epsJ						0.461	exopolysaccharide polymerization protein
str1118	-		2.312					hypothetical protein, truncated
str1125	-	4.964	3.721				0.453	hypothetical protein
str1129	-		0.234					hypothetical protein
str1130	-	0.376	0.076			0.304		IS657, transposase, IS200 family, truncated
str1132	rplT		3.826	2.400		0.438	0.382	50S ribosomal protein L20
str1136	-	0.310	0.409					hypothetical protein
str1140	sipB					3.674	5.104	signal peptidase I
str1141	-		0.415					conserved hypothetical protein, TPR domain protein
str1151	-					3.248	3.214	hypothetical protein
str1153	-					2.863		hypothetical protein, acetyltransferase, truncated
str1165	endA						0.364	competence associated membrane nuclease
str1166	epuA					0.327	0.273	conserved hypothetical protein, competence associated
str1168	-		0.471			0.389	0.315	hypothetical protein
str1170	-					0.177	0.275	hypothetical protein
str1179	rplS	2.305	3.945			0.384	0.296	50S ribosomal protein L19
str1184	-	4.036		0.286		0.349	0.492	hypothetical protein
str1193	-						3.195	surface-associated protein cshA precursor, truncated
str1201	**leuC**				**0.382**	**0.374**		**isopropylmalate isomerase large subunit**
str1208	-	0.059	0.072		0.284	0.432		hypothetical protein
str1209	hstH	0.351	0.473					histone-like DNA-binding protein
str1227	-	2.967	2.821			0.438	0.374	hypothetical protein
str1233	-		2.045					hypothetical protein
str1239	-	0.102	0.089					hypothetical protein
str1240	-	0.269	0.115					hypothetical protein
str1241	-	0.226	0.152					hypothetical protein
str1250	-		2.377					hypothetical protein, GtrA family
str1253	-					4.442		hypothetical protein
str1283	-		6.255	6.327				hypothetical protein
str1285	-		3.911			0.278		hypothetical protein
str1286	-					0.327	0.342	hypothetical protein
str1291	-						2.074	hypothetical protein
str1313	hutH	13.155	5.569	0.423	10.061		0.149	histidine ammonia-lyase

str1314	-				17.738	2.746	0.155	hypothetical protein
str1315	hutU	8.369	4.237		6.335		0.174	urocanate hydratase
str1329	-						3.435	hypothetical protein
str1332	-	2.943	3.210					hypothetical protein
str1343	msrA1	0.129				44.088	18.265	bifunctional methionine sulfoxide reductase A/B protein
str1349	-			0.041				hypothetical protein
str1360	-		0.169	0.328	0.334	0.465		hypothetical protein
str1370	-				0.435	0.472		hypothetical protein
str1378	-	0.160				5.889	8.639	hypothetical protein
str1387	-				0.290			hypothetical protein
str1393	-		0.032					hypothetical protein
str1407	-					0.410	0.452	zinc metalloprotease ZmpB, truncated
str1408	-					0.334	0.350	zinc metalloprotease ZmpB, truncated
str1410	-					0.310	0.432	ABC transporter, putative ATP binding protein, truncated
str1412	-					0.211	0.226	ABC transporter, truncated
str1423	-			0.407		0.159	0.149	potassium channel protein, truncated
str1424	-	2.108		0.354		0.224	0.228	potassium channel protein, truncated
str1437	-	4.192	4.731				2.313	ABC transporter permease protein
str1450	oxlT	0.102	0.160		2.953		0.245	oxalate:formate antiporter
str1474	-		2.088			0.492		glycosyltransferase, putative teichoic acid biosynthesis protein
str1475	-	2.666	3.101			0.347	0.391	hypothetical protein
str1477	-	3.122	2.582			0.309	0.342	glycosyl transferase, truncated
str1487	-					0.278	0.359	hypothetical protein
str1499	-					0.225		hypothetical protein
str1500	-	3.018		0.284		0.228	0.491	hypothetical protein
str1503	-	2.966	2.693					hypothetical protein
str1509	-		0.137					hypothetical protein
str1510	-		0.405					hypothetical protein
str1515	-					2.723		hypothetical protein
str1518	-	0.343	0.211					hypothetical protein
str1523	-	0.456			0.463			hypothetical protein
str1528	-	7.255	21.065	2.904		0.407		acetyltransferase, GNAT family
str1542	amyL		0.492					cytoplasmic alpha-amylase
str1548	rpsP		2.700			0.488	0.463	30S ribosomal protein S16
str1552	-						2.127	ISSth2, transposase, IS5 family, truncated
str1564	-		0.440					peptidoglycan GlcNAc deacetylase, truncated
str1570	-		0.255	0.433				hypothetical protein
str1572	-						0.419	positive transcriptional regulator MutR family
str1573	-		0.298			0.399		macrolide-efflux protein, putative
str1575	-					0.435		hypothetical protein
str1595	-						3.144	hypothetical protein
str1602	-	0.275	0.244					hypothetical protein
str1604	ilvD1	0.388				0.366		dihydroxy-acid dehydratase
str1608	-	3.924	2.259				0.370	ABC transporter permease protein
str1611	-						2.412	hypothetical protein
str1626	gatA	2.758	3.480					glutamyl-tRNA amidotransferase subunit A
str1628	-				5.941		0.206	hypothetical protein
str1630	-				4.193		0.240	6-phospho-beta-glucosidase, truncated
str1631	-				4.214		0.272	6-phospho-beta-glucosidase, truncated
str1632	msrA2				5.599	2.340	0.418	methionine sulfoxide reductase A
str1635	codY		2.779	4.359			2.058	transcriptional repressor CodY
str1639	-		0.258					hypothetical protein
str1651	-						0.343	hypothetical protein
str1655	-	7.816						hypothetical protein
str1658	cah	0.040	0.022		0.211			carbonate dehydratase
str1659	-	0.039	0.049		0.337			hypothetical protein
str1662	-					0.458	0.404	plasmid mobilization protein, truncated
str1663	int3					0.188	0.167	integrase/recombinase plasmid associated, putative
str1664	-					0.294	0.273	hypothetical protein
str1668	cppA	4.255	3.649			0.355	0.294	C3-degrading proteinase
str1671	gla						0.327	glycerol uptake facilitator protein
str1672	pepXP					2.710	2.334	x-prolyl-dipeptidyl aminopeptidase
str1685	blpK		0.419		0.430		2.325	pore-forming peptide, putative bacteriocin
str1702	ftsL		0.475					cell division protein
str1703	mraW		0.436	0.403			0.470	S-adenosyl-methyltransferase MraW
str1718	-		0.185	0.134		0.237	0.414	hypothetical protein
str1719	-		0.196	0.237		0.351	0.360	hypothetical protein
str1735	scrB	2.252			0.460			sucrose-6-phosphate hydrolase
str1750	-		2.521					hypothetical protein
str1751	-		0.497					hypothetical protein
str1752	rpsR	7.072	15.726	2.224		0.419	0.280	30S ribosomal protein S18
str1755	-		0.424			0.352		hypothetical protein
str1756	-		0.286		0.414	0.366		hypothetical protein
str1763	-					0.435		hypothetical protein
str1773	-					0.495		hypothetical protein
str1776	gnlA					0.418		glutamine synthetase
str1791	rpsL	2.970	4.364				0.480	30S ribosomal protein S12
str1804	-		0.355	0.406				hypothetical protein
str1808	rpmH		4.900					50S ribosomal protein L34
str1816	-					0.269	0.420	oligopeptide ABC transporter substrate-binding protein, truncated
str1817	rplA	5.380	10.177			0.416	0.387	50S ribosomal protein L1
str1818	rplK		7.898			0.466	0.389	50S ribosomal protein L11
str1820	-					0.460		hypothetical protein
str1824	dut						0.477	deoxyuridine 5'-triphosphate nucleotidohydrolase
str1831	-	4.002				2.359		hypothetical protein
str1837	-		0.328		0.450	0.282		hypothetical protein
str1842	rrmA				0.273	0.276		rRNA (guanine-N1-)-methyltransferase
str1850	-					0.399	0.361	hypothetical protein
str1859	-		0.287					hypothetical protein
str1861	-		0.131					hypothetical protein
str1866	-	0.431	0.407			3.107	3.851	hypothetical protein
str1876	asp					0.244	0.298	alkaline-shock protein
str1883	-		0.150					alcohol-acetaldehyde dehydrogenase, truncated
str1886	-	0.254	0.067					trehalose-6-phosphate hydrolase, truncated
str1887	-	0.076	0.087					trehalose-6-phosphate hydrolase, truncated
str1888	-	0.086	0.042					trehalose-6-phosphate hydrolase, truncated
str1889	-	0.115	0.011					PTS trehalose-specific IIBC component, truncated
str1890	-	0.073	0.049					PTS trehalose-specific IIBC component, truncated
str1891	-	0.113	0.026					PTS trehalose-specific IIBC component, truncated
str1894	-	0.299	0.200			0.453		hypothetical protein
str1895	-	0.440	0.291			0.495		hypothetical protein
str1896	-	0.389	0.477					hypothetical protein
str1920	rpsH	3.832	4.871			0.393	0.348	30S ribosomal protein S8
str1924	rplN	4.578	6.740			0.383	0.330	50S ribosomal protein L14
str1926	rpmC	4.085	5.907			0.359	0.298	50S ribosomal protein L29
str1929	rplV	3.456	4.397			0.374	0.317	50S ribosomal protein L22
str1933	rplD	3.810	5.529			0.316	0.281	50S ribosomal protein L4
str1934	rplC	3.643	8.128			0.346	0.299	50S ribosomal protein L3
str1947	-						2.543	positive transcriptional regulator MutR family
str1950	-			0.309				positive transcriptional regulator MutR family, truncated
str1956	-	0.345	0.443		0.340	0.466		hypothetical protein
str1959	-	0.415	0.213			0.395		hypothetical protein
str1960	-	0.429	0.200			0.467		Holliday junction resolvase-like protein
str1962	-		0.326	0.490		0.362		hypothetical protein

str1964	-	2.630				0.450	0.434	hypothetical protein
str1970	-	2.788	2.948			0.489	0.465	hypothetical protein
str1977	-		0.182					hypothetical protein
str1983	-				0.288	3.222	11.174	hypothetical protein
str1984	-	0.388			0.320	3.457	10.811	hypothetical protein
str1986	-	0.285			0.306	3.783	12.374	hypothetical protein
str1987	-				0.401	4.621	11.524	hypothetical protein
str2004	-		0.353					hypothetical protein
str2005	-	0.323				0.186	0.318	hypothetical protein
str2021	-			0.353				ABC transporter permease protein
Nucleotide transport and metabolism								
str0023	prsA1	0.266	0.198			0.453	0.414	ribose-phosphate pyrophosphokinase
str0031	purL				3.864		0.360	phosphoribosylformylglycinamidine synthase II (FGAM synthetase)
str0032	purF				3.952		0.340	amidophosphoribosyltransferase
str0033	purM				4.276		0.310	phosphoribosylaminoimidazole synthetase
str0040	purD		0.487		2.729		0.342	phosphoribosylamine--glycine ligase
str0041	purE				2.749		0.404	phosphoribosylaminoimidazole carboxylase catalytic subunit
str0042	purK				2.668		0.432	phosphoribosylaminoimidazole carboxylase
str0045	purB1				2.331		0.468	adenylosuccinate lyase
str0134	pyrG					0.234	0.201	CTP synthetase
str0139	-		0.156				0.345	cyclo-nucleotide phosphodiesterase, truncated
str0140	-		0.094					cyclo-nucleotide phosphodiesterase, truncated
str0141	-		0.204					cyclo-nucleotide phosphodiesterase, truncated
str0142	-		0.185					cyclo-nucleotide phosphodiesterase, truncated
str0144	nrdI			0.476		0.430	0.344	hypothetical protein
str0256	-	0.418	0.410			3.158	2.451	putative deoxyribonucleotide triphosphate pyrophosphatase/unknown domain fusion protein
str0355	upp	3.369	2.379					uracil phosphoribosyltransferase
str0438	**pyrH**		**2.098**			**0.434**	**0.376**	**uridylate kinase**
str0489	tmk	0.495	0.293					thymidylate kinase
str0523	pyrR		2.246	3.346		0.352	0.439	pyrimidine regulatory protein PyrR
str0524	pyrP		2.868	3.722		0.411		uracil permease
str0525	pyrB		2.942	4.205				aspartate carbamoyltransferase catalytic subunit
str0526	carA		4.560	4.807				carbamoyl-phosphate synthase small subunit
str0527	carB		4.485	3.841				carbamoyl-phosphate synthase large subunit
str0551	-	5.149		0.261	0.438	0.110	0.250	phosphorylase, Pnp/Udp family, putative
str0566	pfs		2.104					5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
str0750	add			0.498				adenosine deaminase
str0751	tdk	2.655	2.009			0.403	0.376	thymidine kinase
str0791	fhs	0.426	0.297				0.283	formate-tetrahydrofolate ligase
str0801	-	0.460				3.223	3.360	pyrimidine-nucleoside phosphorylase, truncated
str0802	-	0.435				3.409	3.443	pyrimidine-nucleoside phosphorylase, truncated
str0803	-	0.352		2.548		3.565	3.567	pyrimidine-nucleoside phosphorylase, truncated
str0804	-	0.380				3.849	4.222	pyrimidine-nucleoside phosphorylase, truncated
str0805	-	0.337				8.001	5.826	deoxyribose-phosphate aldolase, truncated
str0806	-					8.323	6.657	deoxyribose-phosphate aldolase, truncated
str0807	cdd	0.305		2.571		7.450	5.934	cytidine deaminase
str0886	guaA	0.391	0.413			2.411		bifunctional GMP synthase/glutamine amidotransferase protein
str0941	-					0.279		phosphorylase, Pnp/Udp family
str0955	pyrDb		2.217	3.452				dihydroorotate dehydrogenase
str0967	pyrF		3.036	5.043				orotidine 5'-phosphate decarboxylase
str0968	pyrE	0.466		3.301				orotate phosphoribosyltransferase
str1054	pyrC		2.629	2.496				dihydroorotase
str1113	deoD	3.474	5.441					purine nucleoside phosphorylase (family 1)
str1114	gat		2.211					glutamine amidotransferase
str1117	punA		2.369					purine nucleoside phosphorylase
str1135	cmk	0.298	0.265					cytidylate kinase
str1207	pyrDa	5.938	2.484	0.418		0.424		dihydroorotate dehydrogenase
str1223	apt					0.389	0.333	adenine phosphoribosyltransferase
str1270	nrdE	2.072				0.279	0.162	ribonucleotide-diphosphate reductase alpha subunit
str1289	-						2.559	hypothetical protein
str1339	-				2.157			nucleobase:cation symporter for xanthine, truncated
str1340	-		0.376		2.475			nucleobase:cation symporter for xanthine, truncated
str1341	-		0.358					nucleobase:cation symporter for xanthine, truncated
str1698	-					3.891	2.690	hypothetical protein
str1844	-		0.293			0.473	0.399	cytidine/deoxycytidylate deaminase family protein, putative
str1913	adk	3.292	4.451			0.185	0.178	adenylate kinase
str1963	nrdD					0.365	0.396	anaerobic ribonucleoside triphosphate reductase
str2016	guaB	3.169		0.253		0.207	0.290	inositol-5-monophosphate dehydrogenase
Posttranslational modification, protein turnover, chaperones								
str0119	grpE					4.078	6.289	heat shock protein, chaperonin
str0120	dnaK					4.444	5.260	molecular chaperone DnaK
str0121	dnaJ		2.041			3.496	4.176	heat shock protein, chaperonin
str0132	tig					0.259	0.249	trigger factor
str0164	-						2.216	ABC transporter ATP binding protein
str0203	groES				0.361	3.005	8.328	co-chaperonin GroES
str0204	groEL				0.330	2.628	7.959	chaperonin GroEL
str0285	ureF		2.088					urease accessory protein
str0356	clpP				0.362		4.182	ATP-dependent Clp protease proteolytic subunit
str0515	ppiA	2.365		0.426			0.391	peptidyl-prolyl cis-trans isomerase
str0581	clpX		0.355		0.480			ATP-dependent protease ATP-binding subunit
str0602	clpE				0.297	2.655	8.936	ATP-dependent Clp protease
str0626	ssrA					0.389	0.369	SsrA-binding protein
str0627	pplB					0.480	0.481	peptidyl-prolyl cis-trans isomerase
str0715	htpX						2.510	heat shock protein HtpX
str0749	-		0.305	0.410		0.325		glutathione S-transferase family
str0827	-					0.115	0.089	hypothetical protein, coenzyme PQQ synthesis homologue
str0990	tpx				2.259		0.304	thiol peroxidase
str1185	-					0.304	0.438	proteinase
str1269	nrdH					0.257	0.146	glutaredoxin
str1417	trxB2						0.482	thioredoxin reductase
str1533	-	3.382					0.347	SPFH domain/Band 7 family protein
str1614	clpL	0.327		5.172	0.336	15.718	46.723	ATP-dependent proteinase ATP-binding subunit
str1640	pflA		0.209	0.255		2.276	2.408	pyruvate-formate lyase activating enzyme
str1650	trxB1						0.422	thioredoxin reductase
str1803	trxA1		0.262			0.385	0.454	thioredoxin
str1805	bta		0.403			0.200	0.332	bacteriocin transport accessory protein, putative
str1823	radA		2.259					DNA repair protein
str1846	-	6.934	3.622		2.188		0.279	transcription regulator, putative
str1849	trxA2					0.456	0.351	thioredoxin
str1885	pepO					0.243	0.321	endopeptidase O
str2024	htrA	0.319	0.196		0.361		2.174	exported serine protease
Secondary metabolites biosynthesis, transport and catabolism								
str0029	acpP1	0.468	0.131	0.280				acyl carrier protein
str0384	acpP2					0.439		acyl carrier protein
str0761	dltA	2.630	4.037					D-alanine--D-alanyl carrier protein ligase
str0763	dltC	3.815	4.082					D-alanine--poly(phosphoribitol) ligase subunit 2
str0910	-						0.483	acetoin reductase, truncated
str0924	aldB	2.994	4.938				2.428	alpha-acetolactate decarboxylase
str1516	-		0.469					hypothetical protein
str1616	-	0.430	0.424					hypothetical protein
str1634	entB	0.238		3.060			2.801	pyrazinamidase/nicotinamidase, putative

Signal transduction mechanisms								
str0112	-					0.413	0.318	hypothetical protein
str0145	relA			0.397		0.363	0.366	(p)ppGpp synthetase
str0152	-		0.389	0.437	0.497			transcriptional regulator, putative
str0159	-	5.305	5.739					amino acid (glutamine) ABC transporter substrate binding protein/permease protein
str0296	-	7.213	7.525		8.622		0.248	amino acid ABC transporter substrate binding protein, putative
str0317	rr01					0.297	0.291	response regulator (homolog to csrR/covR Spy)
str0318	hk01	2.784	2.527			0.220	0.208	sensor histidine kinase (homolog to csrS/covS Spy)
str0401	-	0.393		3.731				PTS fructose-specific enzyme IIABC components, truncated
str0437	-	4.150	2.810					sensor histidine kinase, uncoupled, truncated
str0543	rr02		0.328	0.388		0.179	0.239	response regulator
str0665	-	0.467			0.175		6.877	hypothetical protein
str0666	ptsK					2.021	2.221	HPr kinase/phosphorylase
str0729	typA	0.440		2.169				GTP-binding protein TypA/BipA (tyrosine phosphorylated protein A)
str0792	-	0.059	0.027					response regulator (homolog to ciaR Spn), truncated
str0795	-	0.049	0.041					sensor histidine kinase (homolog to ciaH Spn), truncated
str0877	-					0.492		amino acid (glutamine) ABC transporter, substrate binding protein
str0975	-						2.045	ABC transporter substrate binding protein, truncated
str1162	-				0.480		4.090	amino acid (glutamine) ABC transporter substrate-binding protein
str1335	hk07	6.277	3.575					sensor histidine kinase (homolog to HK01Spn & Sth)
str1380	-	0.117	0.215		0.363	5.809	16.007	response regulator (homolog to RR08 Spn) uncoupled, truncated
str1381	-	0.137	0.220			8.149	17.564	response regulator (homolog to RR08 Spn) uncoupled, truncated
str1420	rr08					0.491		response regulator (homolog to RR03 Spn)
str1421	hk08	2.623				0.332	0.411	sensor histidine kinase (homolog to HK03 Spn)
str1425	pknB	2.344				0.477	0.407	protein kinase
str1426	pppL					0.428	0.419	phosphoprotein phosphatase
str1492	-					0.277	0.450	amino acid (glutamine) ABC transporter substrate-binding protein
str1501	gnlP					0.488	0.407	glutamine ABC transporter permease protein
str1579	-	6.405	6.284		9.193		0.138	amino acid (glutamine) ABC transporter substrate binding protein
str1637	-	0.233	0.352		0.274	2.315	8.443	hypothetical protein
str1654	-	2.302	3.221				0.324	ABC transporter substrate-binding protein, amino acid
str1939	-		0.239					protein-tyrosine phosphatase
str1948	-	0.289	0.334					hypothetical protein
str2001	-					0.330	0.393	hypothetical protein
Transcription								
str0006	trcF	0.411	0.479					transcription repair coupling factor
str0065	-					0.415		transcriptional regulator, MarR family, truncated
str0101	-		0.301					hypothetical protein
str0118	hrcA					3.325	5.108	heat-inducible transcription repressor
str0133	**rpoE**	**0.432**	**0.364**			**0.371**	**0.362**	**DNA-directed RNA polymerase subunit delta**
str0145	relA			0.397		0.363	0.366	(p)ppGpp synthetase
str0185	adcR	3.105						zinc transport transcriptional repressor
str0215	nusG					0.359	0.384	transcription antitermination protein NusG
str0237	snf	2.074						ATP-dependent RNA helicase
str0242	**greA**		**0.418**	**0.452**				**transcription elongation factor GreA**
str0317	rr01					0.297	0.291	response regulator (homolog to csrR/covR Spy)
str0341	nusA		2.129			0.390	0.439	transcription elongation factor NusA
str0342	-		2.255			0.405	0.464	hypothetical protein
str0399	fruR	0.309		4.996				transcriptional repressor
str0452	-	2.684			4.401		0.471	transcriptional regulator, LysR family
str0520	-	2.505	3.134					transcriptional regulator, LysR family
str0543	rr02		0.328	0.388		0.179	0.239	response regulator
str0615	-		0.106			0.268		transcriptional antiterminator (BglG family), truncated
str0616	-		0.117			0.301		transcriptional antiterminator (BglG family), truncated
str0625	**rnr**					**0.495**	**0.401**	**exoribonuclease R**
str0630	ccpA					2.029	2.705	catabolite control protein
str0649	-		2.338			2.373	2.118	negative transcriptional regualtor-PBP synthesis, truncated
str0663	tex	4.884	5.514			2.289	2.116	transcriptional regulator, putative
str0665	-	0.467			0.175		6.877	hypothetical protein
str0728	glcK					2.163	2.694	glucose kinase
str0792	-	0.059	0.027					response regulator (homolog to ciaR Spn), truncated
str0813	dinG	4.221	4.125					ATP-dependent DNA helicase
str0887	-	2.987		0.442				transcription regulator GntR family
str0892	-		0.443					transcriptional regulator
str0895	-		5.840			4.662	2.523	transcriptional regulator
str0916	-	0.318	0.264					transcriptional regulator
str0931	-		0.499			4.193	3.259	transcriptional regulator, TetR family
str0950	fbp					0.493	0.410	fibronectin-binding protein-like protein A
str1173	birA						2.186	biotin--protein ligase
str1261	deaD					2.910	2.279	ATP-dependent RNA helicase
str1287	-					0.318		transcriptional regulator, putative
str1296	rncS	5.126	4.661					ribonuclease III
str1375	sthII	3.868						type II restriction-modification system restriction subunit
str1380	-	0.117	0.215		0.363	5.809	16.007	response regulator (homolog to RR08 Spn) uncoupled, truncated
str1381	-	0.137	0.220			8.149	17.564	response regulator (homolog to RR08 Spn) uncoupled, truncated
str1403	galR	0.436	0.449				2.234	galactose operon repressor
str1420	rr08					0.491		response regulator (homolog to RR03 Spn)
str1425	pknB	2.344				0.477	0.407	protein kinase
str1488	rpoD					0.299	0.350	RNA polymerase sigma factor
str1568	rheA		2.286			0.446	0.275	ATP-dependent RNA helicase
str1586	copY		8.458	5.665	0.367	9.972	27.158	negative transcriptional regulator - copper transport operon
str1600	-	0.059	0.052					transcriptional regulator, MerR family
str1699	rheB					2.799	2.783	ATP-dependent RNA helicase
str1733	scrK					3.499	3.700	fructokinase
str1736	-	2.283			0.369		2.074	sucrose regulon regulatory protein, truncated
str1737	-	2.025						sucrose regulon regulatory protein, truncated
str1867	rpoC		2.972					DNA-directed RNA polymerase beta' subunit
str1868	rpoB					0.386		DNA-directed RNA polymerase beta subunit
str1908	rpoA		4.270			0.257	0.247	DNA-directed RNA polymerase alpha subunit
str1948	-	0.289	0.334					hypothetical protein
str1995	-	0.485	0.368					transcriptional regulator, TetR/AcrR family
str2025	spoJ	0.385	0.172					chromosome segregation protein
Translation, ribosomal structure and biogenesis								
str_r03		0.441			0.451			
str_r05		2.272						
str_r09					0.491			
str_t01		2.086	2.214		0.445	0.164	0.368	
str_t02		3.073	2.942			0.262	0.405	
str_t03			3.073	2.547	0.422	0.251		
str_t04			2.721	2.311	0.461	0.274		
str_t05			4.430	3.142	0.316	0.181		
str_t06					0.363	0.290		
str_t07		5.799	4.349		0.450	0.389		
str_t08		2.441	2.813		0.479	0.342		
str_t09		3.733	5.603		0.321	0.346		
str_t10			8.052	8.718	0.108	0.177		
str_t11		4.250	2.489					
str_t13						0.386		
str_t14		3.593	3.108			0.354	0.428	
str_t16		5.766	2.777	0.482				
str_t17		5.379	5.675			0.337		
str_t18						0.413		

str_t23		4.354	3.029			0.297	0.401	
str_t27						0.472		
str_t30					0.413	0.290		
str_t44		3.059				0.256	0.470	
str_t46		3.998	3.338			0.317	0.350	
str_t52		2.143	2.062			0.458		
str_t55		2.407				0.413		
str_t58		8.853	3.793					
str_t60							4.028	
str0007	-		0.441				3.078	conserved hypothetical protein, S4 domain protein
str0081	pnpA	2.891				0.414	0.336	polynucleotide phosphorylase, (PNPase)
str0084	cysS	2.964	2.415			0.486	0.330	cysteinyl-tRNA synthetase
str0122	truA				0.449			tRNA pseudouridine synthase A
str0137	prmA	2.012						ribosomal protein L11 methyltransferase
str0181	nifR	2.388						possible transcriptional regulator
str0211	-					0.376	0.334	ribosomal large subunit pseudouridine synthase, RluD subfamily
str0220	leuS	2.564				0.406	0.355	leucyl-tRNA synthetase
str0247	spoU					2.124	2.504	rRNA methyltransferase
str0262	rluB					3.658	3.257	ribosomal large subunit pseudouridine synthase B
str0267	-		2.344			0.135	0.121	rRNA methyltransferase, TrmH family
str0329	serS		2.419					seryl-tRNA synthetase
str0343	-					0.430	0.494	hypothetical protein
str0345	rbfA	2.076	2.433			0.326	0.416	ribosome-binding factor A
str0370	-	0.381	0.416					ribosomal subunit interface protein, ribosomal protein S30AE family
str0419	-	0.145	0.089		0.142	0.318		acetyltransferase, GNAT family, truncated
str0420	-	0.130	0.124		0.142	0.265		acetyltransferase, GNAT family, truncated
str0451	metG					0.436	0.377	methionine--tRNA ligase
str0476	-	3.355	3.548					acetyltransferase, GNAT family
str0477	valS	4.339	4.178					valyl-tRNA synthetase
str0536	rplJ	15.589	31.716	2.034		0.439	0.408	50S ribosomal protein L10
str0537	rplL	7.282	17.898	2.458			0.472	50S ribosomal protein L7/L12
str0572	thrS		3.543					threonyl-tRNA synthetase
str0592	rpsA					0.222	0.240	30S ribosomal protein S1
str0653	-	2.150						tRNA methyltransferase, TrmA family
str0741	ileS	5.835	7.769		0.494	0.440		isoleucyl-tRNA synthetase
str0752	prfA					0.444	0.331	peptide chain release factor 1
str0753	hemK	3.406	2.810			0.441	0.281	protoporphyrinogen oxidase
str0754	-	3.017	2.856		2.036		0.304	hypothetical protein, Sua5/YciO/YrdC family protein
str0800	-					2.488	2.591	hypothetical protein
str0824	-		2.737	3.187				conserved hypothetical protein, translation initiation inhibitor protein
str0903	gid			0.485				glucose-inhibited division protein A
str1000	-	3.276	3.846					rRNA methyltransferase, putative
str1133	rpmI	3.274	6.171			0.497	0.380	50S ribosomal protein L35
str1134	infC	3.565	8.713	2.444		0.447	0.365	translation initiation factor IF-3
str1146	prfB					0.449		peptide chain release factor 2
str1199	-				0.434	0.375		hypothetical protein
str1261	deaD					2.910	2.279	ATP-dependent RNA helicase
str1427	sunL	2.231						RNA-binding protein
str1456	-	6.029	9.337					ribosomal large subunit pseudouridine synthase, RluD subfamily
str1490	rpsU		3.489				0.484	30S ribosomal protein S21
str1559	-						0.485	acetyltransferase, GNAT family
str1568	rheA		2.286			0.446	0.275	ATP-dependent RNA helicase
str1574	prfC					0.236	0.195	peptide chain release factor 3
str1625	gatB	3.793	3.714					aspartyl/glutamyl-tRNA amidotransferase subunit B
str1627	gatC	2.580	2.288				0.462	aspartyl/glutamyl-tRNA amidotransferase subunit C
str1699	rheB					2.799	2.783	ATP-dependent RNA helicase
str1754	rpsF	5.748	11.649	2.027		0.396	0.278	30S ribosomal protein S6
str1790	rpsG	2.913	6.445			0.427	0.460	30S ribosomal protein S7
str1799	ksgA		0.469			2.220		dimethyladenosine transferase
str1806	tgt					0.196	0.235	queuine tRNA-ribosyltransferase
str1811	rnpA	5.903	5.051			0.289	0.226	ribonuclease P
str1814	gltX	3.281	3.236				0.448	glutamyl-tRNA synthetase
str1844	-		0.293			0.473	0.399	cytidine/deoxycytidylate deaminase family protein, putative
str1870	tyrS	7.394	5.926			0.257	0.201	tyrosyl-tRNA synthetase
str1907	rplQ	3.559	5.900			0.249	0.243	50S ribosomal protein L17
str1909	rpsK	3.509	6.735			0.251	0.232	30S ribosomal protein S11
str1910	rpsM	3.323	5.709			0.261	0.250	30S ribosomal protein S13
str1911	rpmJ	4.027	5.309			0.333	0.265	50S ribosomal protein L36
str1912	infA	4.360	4.964			0.287	0.270	translation initiation factor IF-1
str1915	rplO	3.427	3.585			0.329	0.299	50S ribosomal protein L15
str1916	rpmD	3.227	5.594			0.418	0.378	50S ribosomal protein L30
str1917	rpsE	3.544	5.844			0.434	0.404	30S ribosomal protein S5
str1918	rplR	4.123	7.612			0.436	0.349	50S ribosomal protein L18
str1919	rplF	3.901	5.304			0.408	0.334	50S ribosomal protein L6
str1921	rpsN	4.034	4.218			0.364	0.292	30S ribosomal protein S14
str1922	rplE	3.603	4.271			0.374	0.335	50S ribosomal protein L5
str1923	rplX	3.702	5.194			0.393	0.342	50S ribosomal protein L24
str1925	rpsQ		4.542			0.384	0.332	30S ribosomal protein S17
str1927	rplP	3.904	7.083			0.388	0.331	50S ribosomal protein L16
str1928	rpsC		6.751			0.385	0.319	30S ribosomal protein S3
str1930	rpsS	4.411	7.034			0.349	0.309	30S ribosomal protein S19
str1931	rplB	3.957	7.273			0.321	0.272	50S ribosomal protein L2
str1932	rplW	3.491	5.162			0.291	0.277	50S ribosomal protein L23
str1935	rpsJ		5.358			0.333	0.253	30S ribosomal protein S10
str1958	rpmB					0.243	0.356	50S ribosomal protein L28
str1969	aspS	2.227				0.468	0.429	aspartyl-tRNA synthetase
str1971	hisS	2.057					0.484	histidyl-tRNA synthetase
str1974	rpmF	4.122	7.130			0.490	0.417	50S ribosomal protein L32
str1975	rpmGB	4.469	7.761			0.434	0.354	50S ribosomal protein L33
str1997	rpsD	2.668	5.930			0.374	0.289	30S ribosomal protein S4
str2003	trmU					0.460	0.374	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
str2018	trpS		0.309	0.470		0.164	0.219	tryptophanyl-tRNA synthetase

Supplementary Table 4. Significant (FDR≤0,05) differential expression of *L. bulgaricus* genes between parental mixed cultures and evolved mixed cultures, per functional category. Values represent ratios between the first and the second condition. Loci that showed a mutation in the comparative genome sequence analysis are in bold. P, parental culture; E_1, evolved culture that is used in all analyses; E_2, duplicate evolved culture.

	1st exponential phase			2nd exponential phase			
Locus ID	E_1 over P	E_2 over P	E_2 over E_1	E_1 over P	E_2 over P	E_2 over E_1	Product
Amino acid transport and metabolism							
LBUL_0089			2.050	5.289		0.476	Homoserine trans-succinylase
LBUL_0144				3.545		0.256	ABC-type polar amino acid transport system ATPase component
LBUL_0145				9.511		0.148	ABC-type amino acid transport system permease component
LBUL_0156		0.402					Phosphoribosylpyrophosphate synthetase
LBUL_0214	2.746	3.993		4.328		0.499	ABC-type amino acid transport system permease component
LBUL_0215				2.709		0.452	ABC-type amino acid transport system permease component
LBUL_0216				10.235	3.793	0.371	ABC-type polar amino acid transport system ATPase component
LBUL_0217	2.430	2.619					ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0233	2.017	3.790		2.840		0.440	ABC-type oligopeptide transport system periplasmic component
LBUL_0236				5.774		0.308	ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_0237				5.342		0.296	ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_0238	2.283			4.412		0.284	ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0239				2.962		0.269	ABC-type oligopeptide transport system ATPase component
LBUL_0242						2.235	Aminopeptidase C
LBUL_0261						0.420	ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0262		5.354	3.993				ABC-type polar amino acid transport system ATPase component
LBUL_0263			4.330				ABC-type amino acid transport system permease component
LBUL_0392		9.318	3.532				Aspartate/tyrosine/aromatic aminotransferase
LBUL_0431		6.584					Branched-chain amino acid permeases
LBUL_0452						2.369	Dipeptidase
LBUL_0458			2.931				ABC-type polar amino acid transport system ATPase component
LBUL_0459			2.764				ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0462		2.856					ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0686	4.777	5.129					Cysteine sulfinate desulfinase/cysteine desulfurase and related enzymes
LBUL_0737	0.098		5.181		0.393	0.293	Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_0915	0.366	0.351					Dipeptidyl aminopeptidases/acylaminoacyl-peptidases
LBUL_0931	0.335			2.196		0.319	Carbamoylphosphate synthase small subunit
LBUL_0932	0.363			2.327		0.259	Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0975				0.336			Carbamoylphosphate synthase small subunit
LBUL_1067					0.383		Phosphoglycerate dehydrogenase and related dehydrogenases
LBUL_1098		0.052					ABC-type proline/glycine betaine transport systems ATPase components
LBUL_1110	2.311						Asparagine synthetase A
LBUL_1126	3.915						Di- and tripeptidases
LBUL_1180					2.189		Aspartate/tyrosine/aromatic aminotransferase
LBUL_1213				2.140			ABC-type polar amino acid transport system ATPase component
LBUL_1214						0.450	ABC-type amino acid transport system permease component
LBUL_1219	2.143						Branched-chain amino acid aminotransferase/4-amino-4-deoxychorismate lyase
LBUL_1231	3.430						5 10-methylenetetrahydrofolate reductase
LBUL_1232	2.168						Methionine synthase II (cobalamin-independent)
LBUL_1235	0.264	0.356		0.220	0.046	0.210	Cysteine synthase
LBUL_1236	0.239	0.393		0.192	0.068	0.356	Cystathionine beta-lyases/cystathionine gamma-synthases
LBUL_1289	2.018	2.784		0.233	0.411		ABC-type dipeptide transport system periplasmic component
LBUL_1291	3.469	6.214		0.375	0.293		ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_1292	3.746	6.350		0.455	0.405		ABC-type oligopeptide transport system ATPase component
LBUL_1293	4.734	9.742	2.058		0.364		ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1324					0.337		Xaa-Pro aminopeptidase
LBUL_1353				6.616	2.465	0.373	Homoserine trans-succinylase
LBUL_1354				6.849		0.435	Cysteine synthase
LBUL_1368		0.373		0.364		2.597	Glutamine synthetase
LBUL_1474					2.003		Xaa-Pro aminopeptidase
LBUL_1575	0.065						Xaa-Pro aminopeptidase
LBUL_1619		0.418					Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase and related deacylases
LBUL_1622		0.148	0.132				Aspartate ammonia-lyase
LBUL_1646	3.652						Amino acid transporters
LBUL_1691	5.731			2.930			Selenocysteine lyase
LBUL_1880				3.432		0.404	Oligoendopeptidase F
LBUL_2020				3.814		0.137	ABC-type polar amino acid transport system ATPase component
LBUL_2034	2.723		0.256				Thiamine pyrophosphate-requiring enzymes [acetolactate synthase pyruvate dehydrogenase (cytochrome) glyoxylate carboligase phosphonopyruvate decarboxylase]
Cell Division and Chromosome Partitioning							
LBUL_0590		0.450		10.982	10.262		Integral membrane protein possibly involved in chromosome condensation
LBUL_1900				2.685			ATPases involved in chromosome partitioning
LBUL_2035		5.858	3.195	15.683		0.122	NAD/FAD-utilizing enzyme apparently involved in cell division
LBUL_0040	2.594				0.285		Predicted nucleoside-diphosphate-sugar epimerases
LBUL_0108				3.929		0.349	Lipopolysaccharide biosynthesis proteins LPS:glycosyltransferases
LBUL_0140					2.550	3.483	Cell wall-associated hydrolases (invasion-associated proteins)
LBUL_0154	0.261						Glycosyltransferases involved in cell wall biogenesis
LBUL_0206	6.230	5.443				0.497	Phosphoglycerol transferase and related proteins alkaline phosphatase superfamily
LBUL_0275	3.285						Large-conductance mechanosensitive channel
LBUL_0312	2.628	4.416					UDP-N-acetylmuramyl pentapeptide synthase
LBUL_0440	2.745		0.350				Glucosamine 6-phosphate synthetase contains amidotransferase and phosphosugar isomerase domains
LBUL_0498	2.682		0.400				UDP-glucose pyrophosphorylase
LBUL_0631	4.354						UDP-N-acetylmuramyl tripeptide synthase
LBUL_0664	4.779	5.243					Cell shape-determining protein
LBUL_0671	3.101						Cell division protein FtsI/penicillin-binding protein 2
LBUL_0672	5.761						UDP-N-acetylmuramyl pentapeptide phosphotransferase/UDP-N- acetylglucosamine-1-phosphate transferase
LBUL_0673	2.264						UDP-N-acetylmuramoylalanine-D-glutamate ligase
LBUL_0929	0.206	0.296		2.249		0.243	Lipoprotein signal peptidase
LBUL_0990	16.556		0.226				ABC-type transport system involved in lipoprotein release permease component
LBUL_1097	11.222						Periplasmic glycine betaine/choline-binding (lipo)protein of an ABC-type transport system (osmoprotectant binding protein)
LBUL_1356				2.215			Cyclopropane fatty acid synthase and related methyltransferases
LBUL_1600						2.181	Lipopolysaccharide biosynthesis proteins LPS:glycosyltransferases
LBUL_1664					2.111		UDP-glucose 4-epimerase
LBUL_1707	2.226						Phosphoglycerol transferase and related proteins alkaline phosphatase superfamily
LBUL_1800	9.029	7.276				0.390	Cytidylyltransferase
LBUL_1801	5.476	3.678			0.329	0.320	LPS biosynthesis protein
LBUL_1807	3.792	4.022			0.383	0.347	Glycosyltransferase
LBUL_1810	2.562						UDP-galactopyranose mutase
LBUL_1843				2.071		0.379	Glycosyltransferases probably involved in cell wall biogenesis
LBUL_1848	12.701		0.265	5.012		0.161	Glycosyltransferases involved in cell wall biogenesis
LBUL_1866	2.766	4.042					Cell wall-associated hydrolases (invasion-associated proteins)
LBUL_1982				2.078		0.454	Protein involved in D-alanine esterification of lipoteichoic acid and wall teichoic acid (D-alanine transfer protein)
Cell Motility and Secretion							
LBUL_0515	0.443						Negative regulator of genetic competence sporulation and motility

LBUL_0611		0.357			0.288		Type II secretory pathway component PulF
Coenzyme metabolism							
LBUL_0084	0.364	0.472			2.027		Lactate dehydrogenase and related dehydrogenases
LBUL_0208	2.797						Dihydroneopterin aldolase
LBUL_0209	5.203	2.985		2.489			GTP cyclohydrolase I
LBUL_0210	5.445	2.572	0.472	2.045			Folylpolyglutamate synthase
LBUL_0211	3.694						Dihydropteroate synthase and related enzymes
LBUL_0276				3.645		0.298	Nicotinic acid phosphoribosyltransferase
LBUL_0405	0.444	0.480					NAD synthase
LBUL_0829					2.410	4.522	Biotin-(acetyl-CoA carboxylase) ligase
LBUL_0917					2.596		Lactate dehydrogenase and related dehydrogenases
LBUL_1219	2.143						Branched-chain amino acid aminotransferase/4-amino-4-deoxychorismate lyase
LBUL_1455		2.839	2.577	2.497		0.294	S-adenosylmethionine synthetase
LBUL_1532		0.029					2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases
LBUL_1868		3.694	3.213	3.660		0.269	Lactate dehydrogenase and related dehydrogenases
LBUL_2034	2.723		0.256				Thiamine pyrophosphate-requiring enzymes [acetolactate synthase pyruvate dehydrogenase (cytochrome) glyoxylate carboligase phosphonopyruvate decarboxylase]
Defense mechanisms							
LBUL_0255		3.473					Lantibiotic modifying enzyme
LBUL_0256	7.110	6.384					ABC-type multidrug transport system ATPase and permease components
LBUL_0624				4.795	3.935		Glycopeptide antibiotics resistance protein
LBUL_0978	4.043	4.967					Na+-driven multidrug efflux pump
LBUL_0989	7.506		0.219				ABC-type antimicrobial peptide transport system ATPase component
LBUL_1203	51.057		0.081	5.936		0.063	ABC-type multidrug transport system ATPase and permease components
LBUL_1204	31.854	2.670	0.084	6.781		0.060	ABC-type multidrug transport system ATPase and permease components
LBUL_1300	7.319	7.140					ABC-type multidrug transport system ATPase and permease components
LBUL_1524	2.415					2.297	Beta-lactamase class C and other penicillin binding proteins
LBUL_1562			0.351				ABC-type multidrug transport system ATPase component
LBUL_1700			2.007				ABC-type antimicrobial peptide transport system ATPase component
LBUL_1701				2.056	2.047		ABC-type antimicrobial peptide transport system permease component
DNA Replication, Recombination and Repair							
LBUL_0001				2.794		0.392	ATPase involved in DNA replication initiation
LBUL_0002				2.593		0.407	DNA polymerase sliding clamp subunit (PCNA homolog)
LBUL_0008	3.711	6.394					Single-stranded DNA-binding protein
LBUL_0075				3.814		0.209	Exonuclease III
LBUL_0212	5.489		0.461				NTP pyrophosphohydrolases including oxidative damage repair enzymes
LBUL_0290		5.730					Serine/threonine protein kinase
LBUL_0313	**5.686**	**4.458**					**Superfamily II DNA and RNA helicases**
LBUL_0414	4.726						NAD-dependent DNA ligase (contains BRCT domain type II)
LBUL_0662	2.998	3.387					DNA repair proteins
LBUL_0683	2.008						NTP pyrophosphohydrolases including oxidative damage repair enzymes
LBUL_0702	0.037				0.282		DNA uptake protein and related DNA-binding proteins
LBUL_0912	0.327	0.371					Putative primosome component and related proteins
LBUL_0913	0.491	0.189					Predicted EndoIII-related endonuclease
LBUL_1132	3.674						Methylated DNA-protein cysteine methyltransferase
LBUL_1160		4.700					Transposase and inactivated derivatives
LBUL_1190	4.423						Topoisomerase IA
LBUL_1296		0.127					RecG-like helicase
LBUL_1362		11.096			7.420		Transposase and inactivated derivatives
LBUL_1489						2.613	Nucleotidyltransferase/DNA polymerase involved in DNA repair
LBUL_1566				0.109			NTP pyrophosphohydrolases including oxidative damage repair enzymes
LBUL_1653	33.725	13.943					Transposase and inactivated derivatives IS30 family
LBUL_1773				2.810			Transposase and inactivated derivatives IS30 family
LBUL_1842	3.264			3.094		0.349	Transposase and inactivated derivatives
Energy production and conversion							
LBUL_0084	0.364	0.472			2.027		Lactate dehydrogenase and related dehydrogenases
LBUL_0100	0.299	0.436					Malate/lactate dehydrogenases
LBUL_0618					3.071		Acetate kinase
LBUL_0644	2.075						F0F1-type ATP synthase epsilon subunit (mitochondrial delta subunit)
LBUL_0692	0.260	0.468					Pyruvate/2-oxoglutarate dehydrogenase complex dihydrolipoamide dehydrogenase (E3) component and related enzymes
LBUL_0716				5.560	10.851		Nitroreductase
LBUL_0726		0.314					Succinate dehydrogenase/fumarate reductase flavoprotein subunit
LBUL_0917					2.596		Lactate dehydrogenase and related dehydrogenases
LBUL_1096			0.178				NAD-dependent aldehyde dehydrogenases
LBUL_1532		0.029					2-polyprenylphin hydroxylase and related flavodoxin oxidoreductases
LBUL_1680	10.441	8.558		3.717		0.160	Na+/H+ antiporter
LBUL_1868		3.694	3.213	3.660		0.269	Lactate dehydrogenase and related dehydrogenases
LBUL_1873	4.102						Heme/copper-type cytochrome/quinol oxidases subunit 1
LBUL_1930	0.316	0.364		4.485		0.148	Fumarase
Function unknown							
LBUL_0063		**2.896**	**2.088**	**6.389**		**0.300**	**Uncharacterized protein conserved in bacteria**
LBUL_0081				5.404	2.738		Predicted membrane protein
LBUL_0082			2.244	2.961			Uncharacterized conserved protein
LBUL_0137	0.423	0.411					Uncharacterized conserved protein
LBUL_0205	4.296	5.265		2.710		0.364	Predicted membrane protein
LBUL_0270		0.142					Uncharacterized conserved protein
LBUL_0301	2.035	2.326				0.366	Uncharacterized protein conserved in bacteria
LBUL_0307		0.315					Uncharacterized protein conserved in bacteria
LBUL_0494	2.763						Predicted membrane protein
LBUL_0519	4.499						Uncharacterized protein conserved in bacteria
LBUL_0583				0.437			Uncharacterized protein conserved in bacteria
LBUL_0772	34.489	10.483					Predicted membrane protein
LBUL_0790		0.266					Uncharacterized protein conserved in bacteria
LBUL_0808						2.612	Uncharacterized conserved protein
LBUL_0921					2.081		Uncharacterized protein conserved in bacteria
LBUL_0934	0.473						Uncharacterized conserved protein
LBUL_0996					2.938		Uncharacterized phage-associated protein
LBUL_1127	5.139						Uncharacterized conserved protein
LBUL_1138					0.095	0.170	Uncharacterized protein conserved in bacteria
LBUL_1174						2.216	Uncharacterized protein conserved in bacteria
LBUL_1176				3.027			Uncharacterized conserved protein
LBUL_1245		3.680	2.882	2.842		0.305	Uncharacterized protein conserved in bacteria
LBUL_1263	0.400						Uncharacterized protein conserved in bacteria
LBUL_1264	0.317	0.222					Uncharacterized protein conserved in bacteria
LBUL_1298	0.339	0.248					Uncharacterized protein conserved in bacteria
LBUL_1442						2.368	Uncharacterized protein conserved in bacteria
LBUL_1468					3.055	3.464	Predicted membrane protein
LBUL_1470						2.350	Uncharacterized protein conserved in bacteria
LBUL_1483						2.174	Uncharacterized protein conserved in bacteria
LBUL_1485						2.006	Uncharacterized protein conserved in bacteria
LBUL_1559			0.346				Predicted membrane protein
LBUL_1798				3.992		0.332	Predicted integral membrane protein
LBUL_1876				3.575		0.207	Uncharacterized conserved protein
LBUL_1898	2.337	2.840					Uncharacterized protein conserved in bacteria
General function prediction only							
LBUL_0042	0.420			3.087			Uncharacterized protein 4-oxalocrotonate tautomerase homolog
LBUL_0078						2.109	Aldo/keto reductases related to diketogulonate reductase

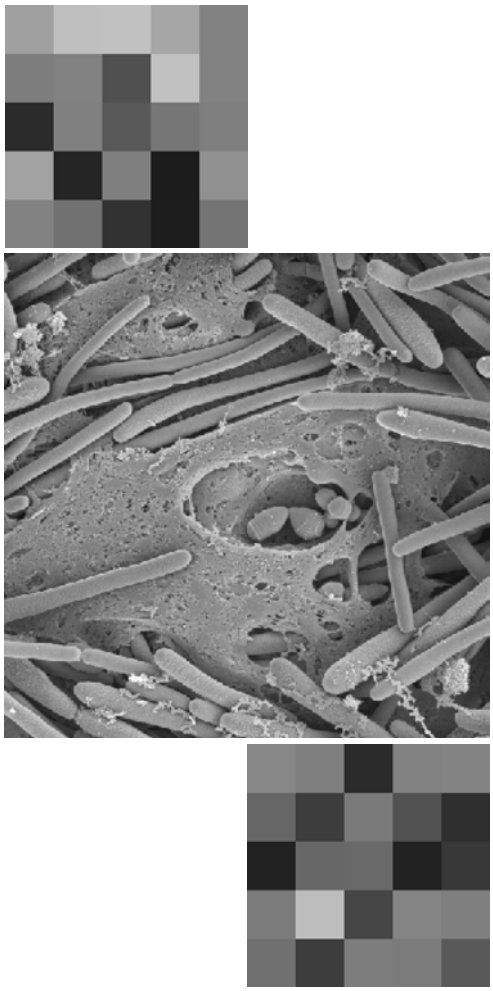
LBUL_0084	0.364	0.472			2.027		Lactate dehydrogenase and related dehydrogenases
LBUL_0123	0.432	0.173	0.401	4.022	6.914		Predicted flavin-nucleotide-binding protein structurally related to pyridoxine 5'-phosphate oxidase
LBUL_0212	5.489		0.461				NTP pyrophosphohydrolases including oxidative damage repair enzymes
LBUL_0223		0.333				2.729	Predicted flavin-nucleotide-binding protein structurally related to pyridoxine 5'-phosphate oxidase
LBUL_0225	5.504	16.478	2.994				Metal-dependent amidase/aminoacylase/carboxypeptidase
LBUL_0230	3.621	4.662		4.446		0.412	Predicted HD superfamily hydrolase
LBUL_0290		5.730					Serine/threonine protein kinase
LBUL_0393		4.651					Predicted membrane protein putative toxin regulator
LBUL_0445				2.201		0.463	ATPase components of ABC transporters with duplicated ATPase domains
LBUL_0450				2.314	3.756		Integral membrane protein interacts with FtsH
LBUL_0500	4.069					3.829	Hydrolases of the alpha/beta superfamily
LBUL_0516				0.489			Competence protein
LBUL_0536						0.310	Predicted HD superfamily hydrolase
LBUL_0572	3.002						Predicted hydrolases of the HAD superfamily
LBUL_0661		2.667					Predicted phosphatase/phosphohexomutase
LBUL_0683	2.008						NTP pyrophosphohydrolases including oxidative damage repair enzymes
LBUL_0703		0.284			0.495	0.343	Predicted hydrolase (metallo-beta-lactamase superfamily)
LBUL_0707	2.226						Predicted hydrolase of the metallo-beta-lactamase superfamily
LBUL_0749	3.646						Metal-dependent hydrolases of the beta-lactamase superfamily III
LBUL_0776	2.330	2.720					Predicted GTPases
LBUL_0814	3.102						Predicted solute binding protein
LBUL_0816			0.134				Predicted permease
LBUL_0818				0.439	4.045	9.218	3-oxoacyl-(acyl-carrier-protein)
LBUL_0821				0.397	3.656	9.216	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)
LBUL_0828				0.451	2.156	4.782	Enoyl-(acyl-carrier-protein)
LBUL_0917					2.596		Lactate dehydrogenase and related dehydrogenases
LBUL_0938	4.170	4.648					Predicted permease DMT superfamily
LBUL_0961	3.647			2.603			Metal-dependent amidase/aminoacylase/carboxypeptidase
LBUL_1122					0.385	0.289	Uncharacterized protein with an alpha/beta hydrolase fold
LBUL_1128	10.645	4.844					Predicted SAM-dependent methyltransferase
LBUL_1193	3.713						Predicted GTPases
LBUL_1255				0.135			Predicted O-methyltransferase
LBUL_1297	0.371	0.252					Predicted kinase related to dihydroxyacetone kinase
LBUL_1351	3.660						Predicted acyl esterases
LBUL_1500				0.437			ATPase components of ABC transporters with duplicated ATPase domains
LBUL_1566				0.109			NTP pyrophosphohydrolases including oxidative damage repair enzymes
LBUL_1606	2.334	2.352		2.077	2.428		Predicted kinase
LBUL_1624				0.467		2.997	Predicted solute binding protein
LBUL_1768					2.276		Aldo/keto reductases related to diketogulonate reductase
LBUL_1784				2.348		0.475	Superfamily I DNA and RNA helicases
LBUL_1802	11.995						Membrane protein involved in the export of O-antigen and teichoic acid
LBUL_1808	3.513	3.177					Predicted glycosyltransferases
LBUL_1834		5.979	4.151				Plasmid maintenance system antidote protein
LBUL_1841	6.380					0.197	Membrane protein involved in the export of O-antigen and teichoic acid
LBUL_1861		0.246					GTPases
LBUL_1868		3.694	3.213	3.660		0.269	Lactate dehydrogenase and related dehydrogenases
LBUL_1906	2.660	2.563		3.392			Predicted flavoprotein
LBUL_1907	2.648	2.774					Predicted flavoprotein
LBUL_1924					2.048	2.049	Uncharacterized C-terminal domain of topoisomerase IA
LBUL_1959		2.105					Predicted permeases
LBUL_1996		15.733	8.504	6.236		0.187	Uncharacterized ABC-type transport system periplasmic component/surface lipoprotein
LBUL_2001		5.362		6.031	2.702	0.448	Uncharacterized ABC-type transport system periplasmic component/surface lipoprotein
LBUL_2002				2.301			Uncharacterized ABC-type transport system periplasmic component/surface lipoprotein
LBUL_2003		2.396	2.538	2.429		0.483	ABC-type uncharacterized transport systems ATPase components
LBUL_2004	2.775	6.195	2.232				ABC-type uncharacterized transport system permease component
LBUL_2005		3.824	2.349				Uncharacterized ABC-type transport system permease component
LBUL_2021				2.784		0.294	Permeases
LBUL_2036		4.110	2.288	8.992		0.158	Predicted GTPase
Inorganic ion transport and metabolism							
LBUL_0087				3.686		0.476	ABC-type metal ion transport system periplasmic component/surface antigen
LBUL_0138				0.397	2.469	6.221	Na+/H+ antiporter NhaD and related arsenite permeases
LBUL_0176				2.926			ABC-type phosphate/phosphonate transport system periplasmic component
LBUL_0177	2.688	3.211		2.336		0.309	ABC-type phosphate/phosphonate transport system ATPase component
LBUL_0178	2.734	4.220		2.144		0.325	ABC-type phosphate/phosphonate transport system permease component
LBUL_0179	2.757	3.285					ABC-type phosphate/phosphonate transport system permease component
LBUL_0236				5.774		0.308	ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_0237				5.342		0.296	ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_0238	2.283			4.412		0.284	ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_0296	4.847	9.201					Cation transport ATPase
LBUL_0406					2.201		Cation transport ATPase
LBUL_0423	0.450	0.487					Carbonic anhydrase
LBUL_0607				5.841		0.174	ABC-type metal ion transport system periplasmic component/surface adhesin
LBUL_0763		0.480			0.222		ABC-type nitrate/sulfonate/bicarbonate transport system ATPase component
LBUL_0867				0.306	0.266		ABC-type phosphate transport system periplasmic component
LBUL_0882	2.673	2.441		10.530		0.138	ABC-type cobalt transport system ATPase component
LBUL_0883	3.908	5.111		9.960		0.151	ABC-type cobalt transport system ATPase component
LBUL_0884	3.386	3.956		14.394		0.153	ABC-type cobalt transport system permease component CbiQ and related transporters
LBUL_1157	2.600			0.110	3.842	34.985	Cation transport ATPase
LBUL_1291	3.469	6.214		0.375	0.293		ABC-type dipeptide/oligopeptide/nickel transport systems permease components
LBUL_1293	4.734	9.742	2.058		0.364		ABC-type dipeptide/oligopeptide/nickel transport system ATPase component
LBUL_1327	0.273	0.141					Arsenate reductase and related proteins glutaredoxin family
LBUL_1541	2.637		0.202				Ammonia permease
LBUL_1652				0.490	0.154	0.315	Cyanate permease
LBUL_1658	0.397			4.258		0.266	Nitrate/nitrite transporter
LBUL_1929	0.364			5.814		0.158	Di- and tricarboxylate transporters
Intracellular trafficking, secretion, and vesicular transport							
LBUL_0370	4.795	4.528				0.431	Preprotein translocase subunit SecY
LBUL_0559	2.751		0.388			2.565	Protease subunit of ATP-dependent Clp proteases
LBUL_0611		0.357			0.288		Type II secretory pathway component PulF
LBUL_0929	0.206	0.296		2.249		0.243	Lipoprotein signal peptidase
LBUL_1124						2.428	Signal peptidase I
LBUL_1282	2.641						Signal recognition particle GTPase
LBUL_1546		6.078		2.528		0.379	Preprotein translocase subunit SecE
LBUL_2037		6.673	3.829	3.895		0.330	Preprotein translocase subunit YidC
Lipid metabolism							
LBUL_0106				3.005		0.278	1-acyl-sn-glycerol-3-phosphate acyltransferase
LBUL_0109						0.240	1-acyl-sn-glycerol-3-phosphate acyltransferase
LBUL_0314					2.330		Phosphopantetheinyl transferase (holo-ACP synthase)
LBUL_0804	5.616	6.605		5.480		0.251	Acetyl-CoA acetyltransferase
LBUL_0805	7.406	5.610		3.672		0.331	Hydroxymethylglutaryl-CoA reductase
LBUL_0806				2.504		0.303	3-hydroxy-3-methylglutaryl CoA synthase
LBUL_0819				0.354	3.527	9.975	Acyl carrier protein
LBUL_0820				0.381	3.182	8.355	(acyl-carrier-protein) S-malonyltransferase
LBUL_0822				0.395	3.676	9.304	3-oxoacyl-(acyl-carrier-protein) synthase
LBUL_0823				0.330	4.053	12.291	Biotin carboxyl carrier protein
LBUL_0824				0.325	3.774	11.621	3-hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratases
LBUL_0825					3.852	6.046	Acetyl/propionyl-CoA carboxylase alpha subunit
LBUL_0826				0.396	3.283	8.291	Acetyl-CoA carboxylase beta subunit
LBUL_0827				0.315	2.278	7.225	Acetyl-CoA carboxylase alpha subunit
LBUL_0905	3.856						Mevalonate pyrophosphate decarboxylase
LBUL_1256						2.607	1-acyl-sn-glycerol-3-phosphate acyltransferase

LBUL_1295		0.400					Fatty acid/phospholipid biosynthesis enzyme
LBUL_1567		3.356					Biotin carboxylase
LBUL_1607		3.588	2.029	3.926	6.124		3-hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratases
LBUL_1800	9.029	7.276				0.390	Cytidylyltransferase
LBUL_1983						0.498	Acyl carrier protein
Not grouped							
LBUL_0882	2.673	2.441		10.530		0.138	
Nucleotide transport and metabolism							
LBUL_0079	0.438			6.856	3.571		Ribonucleotide reductase alpha subunit
LBUL_0156		0.402					Phosphoribosylpyrophosphate synthetase
LBUL_0172				14.884		0.179	Nucleoside 2-deoxyribosyltransferase
LBUL_0185			3.118	4.379		0.399	Oxygen-sensitive ribonucleoside-triphosphate reductase
LBUL_0246						0.240	IMP dehydrogenase/GMP reductase
LBUL_0249				3.068		0.309	Xanthine/uracil permeases
LBUL_0250		0.424		2.096		0.471	Adenine/guanine phosphoribosyltransferases and related PRPP-binding proteins
LBUL_0251				4.788	2.165	0.452	IMP dehydrogenase/GMP reductase
LBUL_0252				3.753	2.040		GMP synthase PP-ATPase domain/subunit
LBUL_0302						0.364	Adenine/guanine phosphoribosyltransferases and related PRPP-binding proteins
LBUL_0309		2.637		2.716			CTP synthase (UTP-ammonia lyase)
LBUL_0371	4.357	4.488				0.454	Adenylate kinase and related kinases
LBUL_0487	3.831						Xanthine/uracil permeases
LBUL_0685	4.722						Nucleoside phosphorylase
LBUL_0752	3.795						5'-nucleotidase/2' 3'-cyclic phosphodiesterase and related esterases
LBUL_0928	0.221	0.295				0.286	Formyltetrahydrofolate synthetase
LBUL_0931	0.335			2.196		0.319	Carbamoylphosphate synthase small subunit
LBUL_0932	0.363			2.327		0.259	Carbamoylphosphate synthase large subunit (split gene in MJ)
LBUL_0975				0.336			Carbamoylphosphate synthase small subunit
LBUL_1222		0.376					Adenine/guanine phosphoribosyltransferases and related PRPP-binding proteins
LBUL_1272						0.391	5'-nucleotidase/2' 3'-cyclic phosphodiesterase and related esterases
LBUL_1330	2.541					0.373	Phosphoribosylamine-glycine ligase
LBUL_1331		2.144			0.442	0.324	AICAR transformylase/IMP cyclohydrolase PurH (only IMP cyclohydrolase domain in Aful)
LBUL_1332						0.376	Folate-dependent phosphoribosylglycinamide formyltransferase PurN
LBUL_1333					0.460	0.323	Phosphoribosylaminoimidazole (AIR) synthetase
LBUL_1334						0.381	Glutamine phosphoribosylpyrophosphate amidotransferase
LBUL_1335						0.350	Phosphoribosylformylglycinamidine (FGAM) synthase synthetase domain
LBUL_1336						0.336	Phosphoribosylformylglycinamidine (FGAM) synthase glutamine amidotransferase domain
LBUL_1337						0.369	Phosphoribosylformylglycinamidine (FGAM) synthase PurS component
LBUL_1338						0.339	Phosphoribosylaminoimidazolesuccinocarboxamide (SAICAR) synthase
LBUL_1340		0.466					Phosphoribosylaminoimidazole carboxylase (NCAIR synthetase)
LBUL_1341		0.444					Phosphoribosylcarboxyaminoimidazole (NCAIR) mutase
LBUL_1358	4.960			26.832		0.101	Adenosine deaminase
LBUL_1421	2.846						Dihydroorotate dehydrogenase
LBUL_1422		4.326	2.696				Orotidine-5'-phosphate decarboxylase
LBUL_1423	3.271	12.977	3.967				Orotate phosphoribosyltransferase
LBUL_1479	0.402					2.722	Xanthosine triphosphate pyrophosphatase
LBUL_1753	2.651	2.028					Nucleoside 2-deoxyribosyltransferase
LBUL_1953		2.126					Aspartate carbamoyltransferase catalytic chain
LBUL_1954		2.777	2.413	3.906	3.693		Pyrimidine operon attenuation protein/uracil phosphoribosyltransferase
LBUL_1955				2.441		0.228	Dihydroorotate dehydrogenase
LBUL_2017				3.881		0.394	Deoxynucleoside kinases
LBUL_2018				3.529		0.390	Deoxynucleoside kinases
Posttranslational modification, protein turnover, chaperones							
LBUL_0191				0.454			Predicted metalloendopeptidase
LBUL_0243						4.685	Molecular chaperone (small heat shock protein)
LBUL_0339						0.416	ATPases with chaperone activity ATP-binding subunit
LBUL_0510				0.283		3.757	ATPases with chaperone activity ATP-binding subunit
LBUL_0515	0.443						Negative regulator of genetic competence sporulation and motility
LBUL_0559	2.751		0.388			2.565	Protease subunit of ATP-dependent Clp proteases
LBUL_0605	2.964						Peptide methionine sulfoxide reductase
LBUL_1004						3.536	Peptidyl-prolyl cis-trans isomerase (rotamase) - cyclophilin family
LBUL_1101						0.382	Zn-dependent protease with chaperone function
LBUL_1105	8.220	7.754		0.221	0.138		Subtilisin-like serine proteases
LBUL_1186						2.720	ATP-dependent protease HslVU (ClpYQ) ATPase subunit
LBUL_1226	4.349			0.248		5.327	DnaJ-class molecular chaperone with C-terminal Zn finger domain
LBUL_1227	5.090		0.347	0.236		7.233	Molecular chaperone
LBUL_1228	4.136		0.356	0.341		3.972	Molecular chaperone GrpE (heat shock protein)
LBUL_1424	2.606	2.953					Parvulin-like peptidyl-prolyl isomerase
LBUL_1466	2.848						Thioredoxin reductase
LBUL_1496			0.490	0.333		4.713	Chaperonin GroEL (HSP60 family)
LBUL_1497				0.249		7.597	Co-chaperonin GroES (HSP10)
LBUL_1689				3.317		0.367	ABC-type transport system involved in Fe-S cluster assembly permease component
LBUL_1692	5.694						ABC-type transport system involved in Fe-S cluster assembly permease component
LBUL_1693	8.340						ABC-type transport system involved in Fe-S cluster assembly ATPase component
LBUL_1704						0.407	tmRNA-binding protein
LBUL_1944				3.678			ATPases with chaperone activity ATP-binding subunit
Secondary metabolites biosynthesis, transport and catabolism							
LBUL_0279				3.426		0.498	Amidases related to nicotinamidase
LBUL_0819				0.354	3.527	9.975	Acyl carrier protein
LBUL_0821				0.397	3.656	9.216	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)
LBUL_0822				0.395	3.676	9.304	3-oxoacyl-(acyl-carrier-protein) synthase
LBUL_1983						0.498	Acyl carrier protein
Signal transduction mechanisms							
LBUL_0022				3.635			Predicted signal transduction protein with a C-terminal ATPase domain
LBUL_0088				3.876			LuxS protein involved in autoinducer AI2 synthesis
LBUL_0217	2.430	2.619					ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0261						0.420	ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0290		5.730					Serine/threonine protein kinase
LBUL_0316	2.365					2.508	CBS-domain-containing membrane protein
LBUL_0330				2.234		0.357	FOG: GGDEF domain
LBUL_0338				3.071		0.396	GAF domain-containing protein
LBUL_0430		2.452					cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases
LBUL_0459			2.764				ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0462		2.856					ABC-type amino acid transport/signal transduction systems periplasmic component/domain
LBUL_0515	0.443						Negative regulator of genetic competence sporulation and motility
LBUL_0594			0.341				FOG: GGDEF domain
LBUL_0696		3.743				0.480	Predicted membrane GTPase involved in stress response
LBUL_0885	3.372	3.604		7.817		0.164	Putative regulator of cell autolysis
LBUL_0933	0.476					0.325	ACT domain-containing protein
LBUL_1139		5.708		0.211	0.218		Response regulator containing CheY-like receiver domain and AraC-type DNA-binding domain
LBUL_1201	7.166		0.211				FOG: GGDEF domain
LBUL_1233	3.199						LuxS protein involved in autoinducer AI2 synthesis
LBUL_1781				0.239	0.222		Response regulator of the LytR/AlgR family
Transcription							
LBUL_0057					0.257		DNA-directed RNA polymerase specialized sigma subunit
LBUL_0099	7.210	3.536	0.490	3.225		0.300	Transcriptional regulator/sugar kinase
LBUL_0157	0.343	0.423					Transcriptional regulator
LBUL_0162	2.162						Predicted transcriptional regulators
LBUL_0204				2.988		0.473	Transcriptional regulator
LBUL_0290		5.730					Serine/threonine protein kinase

LBUL_0313	**5.686**	**4.458**					**Superfamily II DNA and RNA helicases**
LBUL_0343		**2.282**					**DNA-directed RNA polymerase beta' subunit/160 kD subunit**
LBUL_0376	2.123	2.329					DNA-directed RNA polymerase alpha subunit/40 kD subunit
LBUL_0399	0.287	0.272					Transcriptional regulators
LBUL_0566					2.142		Transcriptional regulator contains sigma factor-related N-terminal domain
LBUL_0587						6.759	Predicted transcriptional regulator
LBUL_0737	0.098		5.181		0.393	0.293	Transcriptional regulators containing a DNA-binding HTH domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs
LBUL_0780	3.660		0.488	3.624		0.261	Transcriptional regulator
LBUL_1099	0.199	0.057					Predicted transcriptional regulator
LBUL_1229	4.041		0.347	0.216		5.126	Transcriptional regulator of heat shock gene
LBUL_1243		2.637	2.397				Predicted nucleic-acid-binding protein implicated in transcription termination
LBUL_1244		2.804	2.433	2.501		0.377	Transcription elongation factor
LBUL_1287	0.026		7.986			6.466	dsRNA-specific ribonuclease
LBUL_1296		0.127					RecG-like helicase
LBUL_1381	2.408						Transcription elongation factor
LBUL_1535				2.277	2.267		Putative transcription activator
LBUL_1545		2.464					Transcription antiterminator
LBUL_1563			0.333				Predicted transcriptional regulators
LBUL_1705						0.466	Exoribonuclease R
LBUL_1758						0.273	Transcriptional regulator
LBUL_1781				0.239	0.222		Response regulator of the LytR/AlgR family
LBUL_1822	7.823	11.042					Predicted transcriptional regulators
LBUL_1901	4.881					0.376	Predicted transcriptional regulators
LBUL_1914		0.020					Transcriptional regulator
LBUL_1957		3.962	2.138	3.727		0.153	Cold shock proteins
Translation, Ribosomal Structure and Biogenesis							
LBUL_0007	4.632	7.086					Ribosomal protein S6
LBUL_0009	3.093	6.782	2.193	2.196		0.317	Ribosomal protein S18
LBUL_0219				2.812			Tyrosyl-tRNA synthetase
LBUL_0311						0.455	Ribosomal protein L31
LBUL_0313	**5.686**	**4.458**					**Superfamily II DNA and RNA helicases**
LBUL_0327		3.860				0.373	tRNA-dihydrouridine synthase
LBUL_0328		2.129					Lysyl-tRNA synthetase (class II)
LBUL_0346	2.331	3.063					Ribosomal protein S12
LBUL_0347	2.115	2.411					Ribosomal protein S7
LBUL_0348	**2.934**	**2.778**					**Translation elongation factors (GTPases)**
LBUL_0349		**7.554**	**2.183**				**Ribosomal protein S10**
LBUL_0350	3.828	6.602					Ribosomal protein L3
LBUL_0351	3.621	5.900					Ribosomal protein L4
LBUL_0352	4.279	7.543					Ribosomal protein L23
LBUL_0353	3.841	6.753					Ribosomal protein L2
LBUL_0354	3.614	6.839					Ribosomal protein S19
LBUL_0355	3.479	5.455					Ribosomal protein L22
LBUL_0356	3.611	5.516					Ribosomal protein S3
LBUL_0357	3.156	4.851					Ribosomal protein L16/L10E
LBUL_0358	4.060	5.199					Ribosomal protein L29
LBUL_0359	3.464	4.917					Ribosomal protein S17
LBUL_0360	3.500	4.955					Ribosomal protein L14
LBUL_0361	3.250	4.117					Ribosomal protein L24
LBUL_0362	3.741	4.938					Ribosomal protein L5
LBUL_0363	3.541	4.259					Ribosomal protein S14
LBUL_0364	3.480	4.219					Ribosomal protein S8
LBUL_0365	3.859	4.661					Ribosomal protein L6P/L9E
LBUL_0366	2.927	3.894					Ribosomal protein L18
LBUL_0367	4.469	4.699					Ribosomal protein S5
LBUL_0368	4.277	4.831				0.455	Ribosomal protein L30/L7E
LBUL_0369	3.215	4.109				0.472	Ribosomal protein L15
LBUL_0372	2.280	2.401					Translation initiation factor 1 (IF-1)
LBUL_0373		2.868					Ribosomal protein L36
LBUL_0374		2.506					Ribosomal protein S13
LBUL_0375		2.929					Ribosomal protein S11
LBUL_0377	2.235	2.971				0.458	Ribosomal protein L17
LBUL_0382		4.921					Ribosomal protein L13
LBUL_0383	2.962	4.013					Ribosomal protein S9
LBUL_0501	4.237	6.788					SAM-dependent methyltransferases related to tRNA (uracil-5-)-methyltransferase
LBUL_0654	2.200	2.166					Ribosomal protein S4 and related proteins
LBUL_0706	3.627	6.032		2.087		0.486	Ribosomal protein S15P/S13E
LBUL_0751		2.116					Ribosomal protein L32
LBUL_0812	2.812	3.094					Histidyl-tRNA synthetase
LBUL_0813	3.603	4.390					Aspartyl-tRNA synthetase
LBUL_0930	0.192	0.330		2.305		0.290	Pseudouridylate synthases 23S RNA-specific
LBUL_1166	2.353	2.414					Glycyl-tRNA synthetase beta subunit
LBUL_1167		2.127					Glycyl-tRNA synthetase alpha subunit
LBUL_1173	2.232						Ribosomal protein S21
LBUL_1242				2.023		0.449	Ribosomal protein HS6-type (S12/L30/L7a)
LBUL_1247		2.333					Prolyl-tRNA synthetase
LBUL_1253	2.346	2.948				0.408	Translation elongation factor Ts
LBUL_1254	4.643	6.418				0.426	Ribosomal protein S2
LBUL_1273	4.054	5.065					Ribosomal protein L19
LBUL_1281	5.445	7.161		2.014		0.473	Ribosomal protein S16
LBUL_1299		2.446					Ribosomal protein L28
LBUL_1325	4.116	5.567					Ribosomal protein L27
LBUL_1326	3.144	4.415					Ribosomal protein L21
LBUL_1396	3.887	10.574	2.720				Ribosomal protein L20
LBUL_1397	3.519	7.483	2.127				Ribosomal protein L35
LBUL_1398	3.489	7.312	2.096				Translation initiation factor 3 (IF-3)
LBUL_1401					2.164		Threonyl-tRNA synthetase
LBUL_1451					2.157		Leucyl-tRNA synthetase
LBUL_1486					2.114	2.011	Alanyl-tRNA synthetase
LBUL_1536	2.470	2.146				0.318	Ribosomal protein L7/L12
LBUL_1537	3.271	2.836					Ribosomal protein L10
LBUL_1547	3.308	5.421				0.463	Ribosomal protein L33
LBUL_1552		0.342					rRNA methylases
LBUL_1560	2.489	3.441					Glutamyl- and glutaminyl-tRNA synthetases
LBUL_1748		0.445					Seryl-tRNA synthetase
LBUL_1749				2.334			Acetyltransferases including N-acetylases of ribosomal proteins
LBUL_1778	3.483						Translation elongation factor P (EF-P)/translation initiation factor 5A (eIF-5A)
LBUL_2038		5.717	3.172	4.106		0.324	RNase P protein component

Chapter 6

A genome-scale metabolic model of mixed culture growth of *S. thermophilus* and *L. bulgaricus*



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ABSTRACT

The interactions between microorganisms are of key importance for the performance of mixed culture fermentations such as the yoghurt fermentation, which is carried out by a consortium of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. These two species stimulate each other's growth by the exchange of growth factors associated with purine and amino acid metabolism (5, 7, 9). However, a detailed view on the regulatory responses and metabolic basis of these stimulatory interactions remains to be established. To advance this insight, mapping the metabolism of the two separate species can be instrumental. We used genome-scale metabolic modeling to identify the reactions occurring in both bacteria. Moreover, carbon and amino acid fluxes in both bacteria were calculated and included in the models. Finally, we demonstrate the possibility to couple such models in order to identify reactions associated with interactions between consortium members. In this case nutritional exchange was indeed predicted to be based on formic acid, folic acid and proteolysis. In addition, the experimental observation of an increased biomass yield on lactose was confirmed in the model. It is anticipated that such models will aid the development or improvement of other mixed culture fermentations in the near future.

INTRODUCTION

Microorganisms are almost uniquely found in ecosystems consisting of multiple species. The interplay with other microorganisms is of key importance for their behavior and performance (13, 17). A well-known example in the field of food fermentations is the yoghurt fermentation. In this process (bovine) milk is transformed into yoghurt, through the combined action of the two lactic acid bacteria (LAB) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These two species stimulate each other's growth by the exchange of growth factors associated with purine and amino acid (AA) metabolism (5, 7, 9, 23), in a process also referred to as protocooperation. A number of recent post-genomic studies identified several genetic and physiological responses associated with co-cultivation (11, 12, 26).

Previously, kinetic models of the yoghurt fermentation were constructed to quantify interactions between *S. thermophilus* and *L. bulgaricus* in yoghurt (6, 27). Although these models proved to be very useful for the quantification of interactions in mixed cultures, they are highly specific to the conditions used for the assessment of the kinetic parameters and they have little predictive value for other

conditions. These black-box models also do not allow the identification of the underlying mechanisms behind the mutualistic interactions between *S. thermophilus* and *L. bulgaricus*. Genome-scale metabolic models, on the other hand, may provide an attractive alternative as they enable the prediction of the behavior of a microorganism in a complex and changing environment, especially when fed with experimental fermentation data used to calculate fluxes (30, 32). However, the construction of metabolic models that take into account the effects of competition and the exchange of metabolites between members of a microbial community is still challenging (2, 35).

Here we report the construction of genome-scale metabolic models of *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365 and their validation using experimental fermentation data. Furthermore, we comment on the possibility to couple both models in order to identify the nature of the interactions between both bacteria in yoghurt. Preliminary results suggest interactions based on the exchange of formic acid, folic acid (provided by *S. thermophilus*) and proteolysis (executed by *L. bulgaricus*), which is in accordance with literature findings and the data reported in chapter 4. Although determining the metabolite fluxes in the mixed culture model presented is not finalized yet and the model needs to be validated with additional experimental data, it is anticipated that such genome-scale metabolic models are valuable for the prediction of mixed culture growth in dynamic environments

MATERIALS AND METHODS

Strains and culture conditions

S. thermophilus CNRZ1066 (3), *L. bulgaricus* ATCC BAA-365 (16) and two co-adapted strains by an experimental evolution study, *S. thermophilus* NIZO3938 and *L. bulgaricus* NIZO3939 (24), were maintained as frozen M17 broth (Difco) and MRS broth (Difco) cultures with 22% glycerol (Scharlau) at -80 °C. Pre-cultures and 275 mL final cultures were prepared as described in (26). Colony-forming units (CFUs) were determined by selective plating (M17 agar supplemented with 1% (w/v) glucose, aerobic at 42°C for *S. thermophilus* and MRS agar, anaerobic at 42°C for *L. bulgaricus*) using the faster plating method developed before (25).

Optical density, dry weight and metabolite measurements

The optical density at 600 nm (OD_{600}) of yoghurt cultures was determined in duplicate by mixing one volume of culture was mixed with 9 volumes of a 0.2%

(w/v) NaEDTA / 0.2% (w/v) NaOH solution. The cell dry weight of cultures was derived from OD_{600} values after calibration as described in (21).

Sugar (lactose, glucose, galactose), acid (lactic acid, acetic acid, formic acid, citric acid and succinic acid) and ethanol content of the medium were determined using HPLC as described before (24). The AA content was determined as described in (26).

Calculation of fluxes

Fluxes of metabolites (mmol gDW^{-1}) were calculated as follows. The average increase in biomass (g h^{-1}) was calculated by linear regression of the growth curve. The average production (or consumption) of substrates and metabolites (mmol h^{-1}) was calculated in the same way. The specific production of metabolites per g dry weight was calculated by dividing the metabolite production by the biomass production.

Exopolysaccharide isolation

Exopolysaccharides (EPS) were extracted from 275 mL cultures grown for 24 h at 42°C as described before (26). The composition of EPS was determined as in (15).

Model development

Two genome-scale metabolic models were constructed on the basis of the genomes of *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365 (see Table 1). First drafts of the models were generated using the AUTOGRAPH method (18). In short, the genome of each organism was compared with the genomes for which a curated genome-scale metabolic model was already available, i.e. for *Lactobacillus plantarum* WCFS1 (30), *Bacillus subtilis* ATCC 23857 (19), *S. thermophilus* LMG18311 (20) and *Lactococcus lactis* MG1363 (Teusink and Notebaart, unpublished results). For orthologous genes, the corresponding gene-protein-reaction associations were copied to the draft models. For the *S. thermophilus* model, the model of strain LMG13811 was used with priority; for *L. bulgaricus*, *L. plantarum* was used as primary source. The other models were used as supplements for reactions that were not present in the *S. thermophilus* LMG18311 and *L. plantarum* models.

The draft models were manually curated as described before (30, 32). All gene-protein-reaction associations were checked manually for consistency and by comparing with other annotation resources such as the ERGO bioinformatics suite and KEGG pathways. In case a gene encoded an enzyme with a specific metabolic function, it was included in the model. Here after, the models were analyzed for

potential gaps in the network by testing their ability to produce biomass components *in silico*. Corresponding gaps in the models of *S. thermophilus* LMG18311 and *L. plantarum* were used for insertion of non-gene associated reactions. Such non-gene associated reactions are inferred to be present based on physiological or biochemical data without having identified the gene that codes for the corresponding enzyme (note that also non-enzymatic reactions fall into this category). The genomes of the organisms studied have gone through a process of extensive reductive evolution (16, 34) leading to potential of cross-feeding between the two species. Therefore, it is difficult to determine *a priori* whether a postulated metabolic gap is genuine. We therefore only allowed non-gene associated reactions if they were present in at least one of the already curated models. Other gaps were considered real and potentially filled by cross-feeding (see below).

Table 1. Numbers of genes, reactions and metabolites included in the curated models of *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365.

	S. thermophilus	*L. bulgaricus*
Genes	1968	1933
Included in model	464 (23%)	500 (26%)
Reactions	511	423
Non-gene associated	65 (13%)	61 (14%)
Metabolites	549	492
Extracellular	76	70

For growth in milk, new reactions that had not previously been included in any genome-scale metabolic model of LAB had to be included in the models. These involved proteolysis of casein and uptake and degradation of the resulting peptides. Casein was defined in terms of the average AA composition as published in (29). It was assumed that all AA of casein were accessible as peptides or free AA through proteolysis. For ATP-driven peptide uptake, an average peptide length of 9 AA was assumed, based on the finding that the substrate-binding subunit of OppA has the highest affinity for peptides consisting of 9 AA (8). In addition, it was assumed that peptides taken up by the cell were completely hydrolyzed into the free AA by peptidolysis. Reversible AA transporters were defined to accommodate efflux of excess AA where needed.

Modeling the consortium

Once the models of the individual strains allowed growth *in silico* using casein (*L. bulgaricus*) or casein-derived peptides (*S. thermophilus*) as a nitrogen source, the two models were combined into one model. In the combined model all extracellular metabolites were assumed to be available to both organisms provided that the required transport activity was defined (see Figure 1). Extracellular metabolites were allowed to be exchanged with the environment through so-called exchange reactions in order to prevent accumulation. For example, lactose was allowed to go into the system, whereas galactose could leave the system. In this way, appropriate sources and sinks for substrates and products were created, allowing e.g. steady state growth. If no such exchange reactions were defined for external metabolites, they had to be balanced by the organisms themselves (e.g. compounds Y and Z in Figure 1).

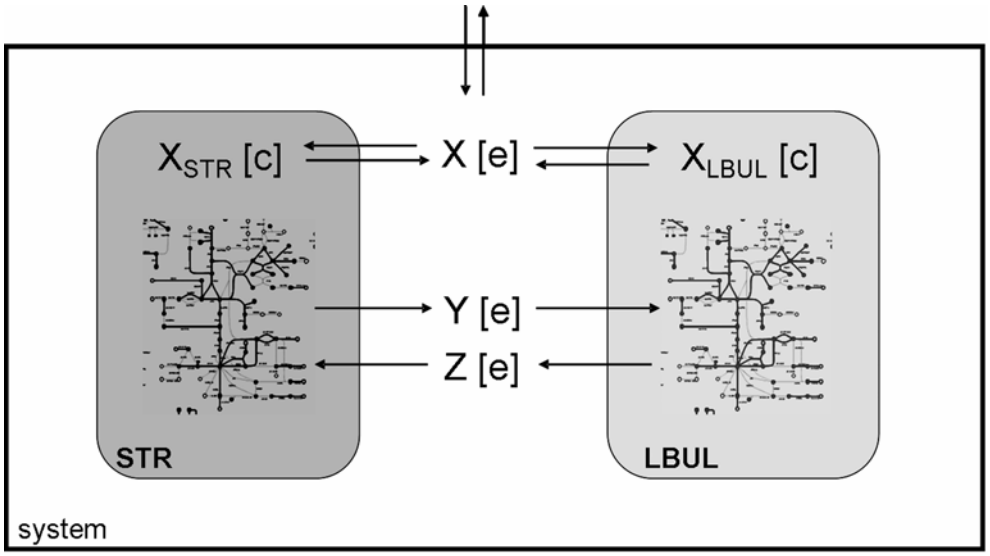

Figure 1. Principle of multi-species modeling of the yoghurt consortium. All external metabolites (denoted by [e]) are shared by both organisms (but only if the appropriate transporters are present). Exchange reactions, represented by the arrows at the top of the figure, define which compounds can enter or leave the system, and hence, can net be consumed or taken up by the consortium (in the figure, only X can be net produced or consumed: Y and Z can only be exchanged between the two organisms). Once internalized, conversion of X[c] ([c] for cytosol) becomes organism specific. STR, *S. thermophilus*; LBUL, *L. bulgaricus*.

In such a combined multi-species model, essential interactions between the organisms can be predicted by deletion analysis. For that both species are grown *in silico* in a medium containing the minimal set of compounds that both require for growth. Subsequently, reactions in one organism are deleted one by one and growth in the other organism is maximized using Flux Balance Analysis (FBA). If the growth rate is zero, the reaction in the one organism apparently provides a necessary compound for the other organism. When doing this for the yoghurt consortium (in a medium containing lactose, milk protein and a minimal set of vitamins), *S. thermophilus* genes essential for growth of *L. bulgaricus* are all involved in folic acid or formic acid production. Proteolysis by *L. bulgaricus* was the only essential reaction for *S. thermophilus* growth.

Genome-scale metabolic models are used for interpretation of fermentation data (32) and even prediction of yields and fluxes (30), using constraint-based modeling approaches. This is not trivial in a multi-species model. The most important complication is the fact that a single objective function that is optimized with FBA in mono-cultures is biologically not meaningful in a mixed culture. For instance, optimizing growth rate cannot be used. The rate of which of the two (or more) should be chosen? In order to circumvent this, it is possible to assume a fixed ratio for growth of both species (28). There is no principle argument for this constraint in general, and also in our case growth rates were not equal (see Figure 2). In fact, growth in yoghurt cultures is often partly sequential (6). Therefore alternative ways were explored to use constraint-based modeling for mixed cultures: (i) fixing the growth rate ratio to 1 (as done before (22)) and (ii) by calculating the maximal growth of one species as a function of the other, and *vice versa*. In both cases, it is important to realize that the fluxes within the organisms are usually expressed as specific rates (mmol h^{-1} gDW^{-1}). A major complication is the normalization with respect to the biomass of the two organisms. In this study we have solved this issue by constraining essential input (or output) fluxes through linkage to the growth rate. That means, uptake rate and growth rate are coupled through the yield. Experimentally, it was found that lactose consumption for the evolved *S. thermophilus* (STR) mono-culture was 170 mmol/gDW, and for the evolved *L. bulgaricus* (LBUL) mono-culture it was 140 mmol/gDW (see Table 2). Therefore, we used the constraints:

$$10^{-4} \leq \frac{r_{lactose,STR}}{\mu_{STR}} \leq 170$$

$$10^{-4} \leq \frac{r_{lactose,LBUL}}{\mu_{LBUL}} \leq 140 \qquad \text{(Eq 1)}$$

Where $r_{lactose}$ is the uptake rate of lactose in mmol gDW^{-1}. Note that because in the biomass equation we define components in terms of mmol gDW^{-1} as well (e.g. biomass of STR contains x mmol protein gDW^{-1}), the growth rate μ is dimensionless. One could multiply the uptake rate by the actual growth rate (in h^{-1}) to get an uptake rate in the usual units mmol h^{-1} gDW^{-1}, but the current notation emphasizes the fact that this unit refers to yields (see (31) for more details). The constraints of Eq 1 ensure that lactose can only be taken up if there is also growth. Without this constraint, fluxes could run through the network of these organisms without there being any biomass to catalyze it. In this way we can also model interactions between the two organisms. For *L. bulgaricus* to grow better, it requires formate produced by *S. thermophilus*, but for this, it has to allow S. *thermophilus* to grow as well (and utilize lactose). This constraint needs to be applied to all inputs or outputs in the network relevant for the interaction:

$$10^{-4} \leq \frac{r_{proteolysis,LBUL}}{\mu_{LBUL}} \leq 40$$
$$10^{-4} \leq \frac{r_{formate,STR}}{\mu_{STR}} \leq 6 \qquad \text{(Eq2)}$$

The constraint on formate production comes from the formate yield in the mono-culture of *S. thermophilus* (see Table 2). The constraint on proteolysis in *L. bulgaricus* is based on the assumptions that (i) all casein-derived peptides are taken up completely, (ii) excess AA that cannot be incorporated into biomass or metabolized by the network, are exported into the medium (see Figure 3), and (iii) the uptake of peptides is in excess of what is needed for growth (growth is assumed to be limited by formate availability), and the extent of excess is estimated by fitting proteolysis to the average rate of export of AA.

Table 2. Overview of formed biomass and primary metabolism metabolites in 24 h. The values of the metabolites are in mM. Par, parental strains CNRZ1066 and ATCC BAA-365; Ev, evolved strains NIZO3938 and NIZO3939. Note that negative values represent consumption.

	Average production per h						Average production per g biomass					
	S. thermophilus		L. bulgaricus		Mixed		S. thermophilus		L. bulgaricus		Mixed	
	Par	Ev	Par	Ev	Par	Ev	Par	Ev	Par	Ev	Par	Ev
Biomass (mg/L)	8.19	11.79	11.64	11.85	45.81	73.26						
EPS (mg/L)	33.26	49.22	60.94	56.27	63.46	163.08	4061.10	4174.73	5235.54	4748.90	1385.35	2226.09
lactose	-2.13	-2.01	-1.75	-1.67	-2.81	-4.16	-259.77	-170.59	-150.39	-140.66	-61.44	-56.73
glucose	0.75	0.00	0.10	0.30	0.00	0.00	92.17	0.00	8.30	25.51	0.00	0.00
galactose	1.44	1.51	0.97	1.21	2.39	3.17	175.43	127.81	83.08	101.81	52.13	43.27
citric acid	-0.07	-0.03	-0.06	-0.03	-0.10	-0.12	-8.24	-2.86	-4.99	-2.17	-2.27	-1.59
lactic acid	1.97	3.53	1.69	1.87	5.02	6.78	241.09	299.58	145.15	158.13	109.67	92.54
formic acid	0.06	0.07	0.05	0.06	0.11	0.13	7.23	6.01	4.20	5.30	2.46	1.79
succinate	0.00	0.00	0.07	0.09	0.02	0.05	0.00	0.00	5.95	7.29	0.50	0.70
ethanol	-0.01	-0.02	-0.01	-0.01	-0.01	-0.03	-1.81	-1.43	-0.43	-0.89	-0.22	-0.34
acetic acid	0.02	0.01	0.05	0.07	0.03	0.07	2.22	1.25	4.04	5.77	0.73	0.95

RESULTS

Fermentations

Mono and mixed cultures were sampled every 1.5 h for 12 h and at the end of fermentation, after 24 h, for measurement of optical density, CFUs and metabolite profiling (see Table 2 and Figures 2 and 3). The OD_{600} was recalculated to dry weight. Growth of the mixed culture was similar to what was described before (14, 26). After the lag phase, there were two exponential phases separated by a transition phase. The mono-cultures, however, showed only one exponential phase. The *L. bulgaricus* mono-culture showed improved growth compared to previous studies (24, 26), which may be caused by mixing the culture every time prior to sampling (1) or the fact that another milk batch was used.

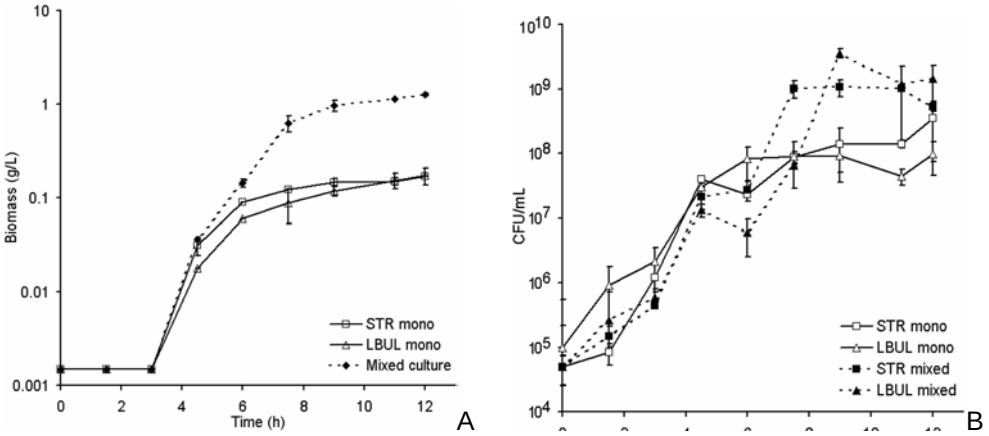

Figure 2. Growth of the evolved strains *S. thermophilus* NIZO3938 and *L. bulgaricus* NIZO3939 in mono and mixed culture in milk. A, Biomass production per culture in g L^{-1}. B, CFU mL^{-1} per strain per culture. STR, *S. thermophilus*; LBUL, *L. bulgaricus*. Error bars indicate standard deviations of triplicate measurements.

In accordance with previous results, the amount of produced biomass and EPS and the quantity of consumed lactose consumed per h was higher in the mixed cultures compared to the mono-cultures (24). Moreover, the evolved strains – which were used because they showed an improved protocooperation in mixed culture compared to a mixed culture of both parental strains in a previous study (24) – displayed a higher production/consumption rate than the parental strains. The yield of biomass on lactose increased from 3.8 g to 5.9 g per mol of lactose for the parental and the evolved *S. thermophilus*, respectively. For *L. bulgaricus* this was an increase from 6.6 g to 7.1 g per mol lactose and for the mixed culture from

16.3 g to 17.6 g per mol of lactose for the parental and the evolved cultures, respectively. That means that the yield of biomass produced per lactose increased significantly in both mixed cultures compared to their corresponding mono-cultures. Moreover, the yield of the evolved strains was higher than that of the parental strains. For the flux calculations during modeling, the measurements of the evolved strains were used.

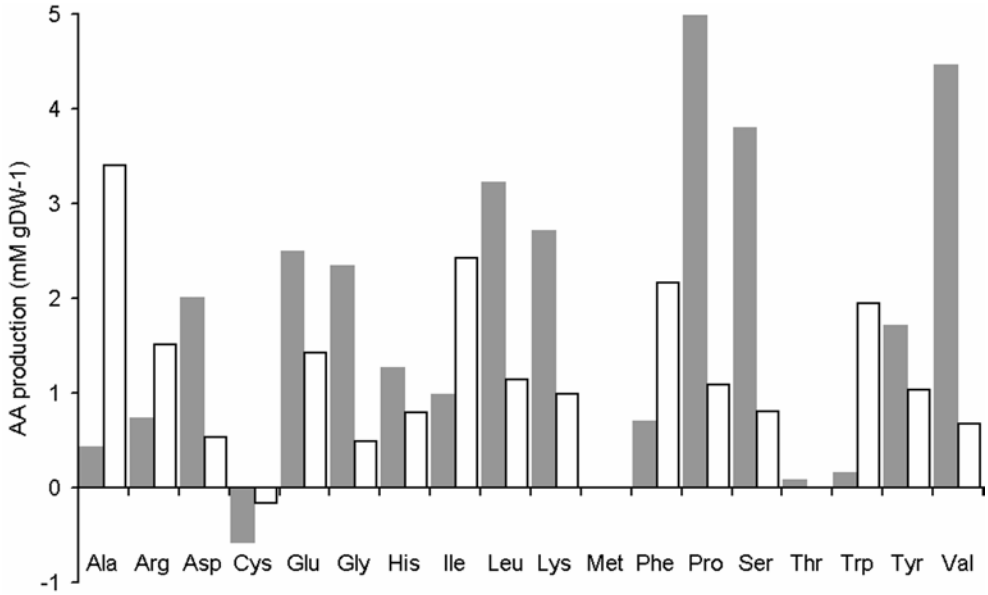

Figure 3. Measured amino acid production per g DW (grey) versus model prediction (white) of the evolved *L. bulgaricus* mono-culture. The production per g DW was calculated by dividing linear regression values of biomass and AA production. Model prediction was based on a presumed limitation of the growth rate by proteolysis of casein. The model prediction suggests that actual proteolysis is in 3-fold excess over biomass requirement.

Modeling the mono-cultures

When comparing the models of *S. thermophilus* and *L. bulgaricus*, the most obvious differences are in AA metabolism. Whereas *S. thermophilus* is almost completely prototrophic except for histidine and cysteine (20), *L. bulgaricus* requires all AA for growth (34) which is in agreement with the absence of complete pathways of many AA biosynthetic pathways. As a consequence all AA (except the one that limits protein synthesis and, hence, growth) are taken up in excess in the form of peptides by *L. bulgaricus* and excreted into the medium. Indeed, we found significant AA accumulation in the *L. bulgaricus* cultures, especially in that containing the evolved strain (see Figure 3).

The modeling of growth of *L. bulgaricus* in milk was complicated by the fact that the carbon balance was significantly off. Despite the fact the balances were based on samples at 10 different time-points throughout the fermentation, the carbon input to output ratio was in the order of 0.6-0.7. This may be due to the low rate of galactose export compared to lactose import. Of the 40.3 mM consumed lactose (i.e. 40.3 mM galactose) in the evolved *L. bulgaricus*, 26.2 mM was secreted as galactose and 3.7 mM ended up in EPS (the ratio of glucose to galactose in *L. bulgaricus* EPS is 1), leaving 10.3 mM of untraceable galactose. The carbon balance problem was circumvented by constraining only the input and leaving the output unconstraint, unless we had good evidence that a specific product was not formed (such as ethanol and acetate, see Table 2). Another unexpected finding is the formation of small amounts of formic acid by *L. bulgaricus*. According to the model and current insights, *L. bulgaricus* either is strictly dependent on *S thermophilus* for formic acid (6, 23) or at least there is a strong stimulation of formic acid supplementation (26). Apparently, there are alternative routes in *L. bulgaricus* for formic acid production that remain to be identified.

Initial modeling and experiments suggested that proteolysis is not limiting growth of *L. bulgaricus*. When formic acid and folic acid were supplied in excess and proteolysis was tuned to match a growth rate of 1 (thereby simulation a proteolysis-limited condition), most predicted AA production yields were lower than what was measured experimentally (on average the model yields were only 30% of the experimental yields, see figure 3). However, the relative distribution of measured and predicted amino acid production profiles are similar with few exceptions (there was more ala, arg, ile, phe and trp produced *in silico* than experimentally). The higher amino acid production than what is needed for growth strongly suggests that growth is not limited by amino acid acquisition but by other factors, notably products of *S. thermophilus*, such as formate and folic acid. The experimentally found production of formate by *L. bulgaricus* was ignored based on previous results (26), and for modeling purposes. *In silico*, formate was used as a proxy for any positive interaction that *S. thermophilus* has on *L. bulgaricus*. Formate consumption was set to limit growth of *L. bulgaricus* in mono-culture, the uptake limit of which was 0.06 mmol gDW^{-1} to reach a growth rate of 1. Under this constraint, we adjusted the proteolysis rate such that the average AA production rate matched the measured one. This rate was 40 mmol gDW^{-1} in the AA composition of casein. Under these conditions (formate 0.06; proteolysis 40; lactose 140 mmol gDW^{-1}) we subsequently performed a Flux Variability Analysis (FVA) to determine the flexibility in the network to reach the growth yield of 1. Since

growth was not energy limited, lactose consumption could be uncoupled from growth as discussed in (32). The minimum lactose uptake rate needed to support growth under these conditions was 78 mmol gDW^{-1}. Without EPS production as a constraint, minimal lactose uptake was only 24 mmol gDW^{-1}. This observation will be relevant in future efforts for extended modeling of the consortium.

S. thermophilus is mostly prototrophic for AA. Nevertheless, AA analysis revealed that the essential AA histidine and cysteine were not consumed to appreciable extent to support the observed growth. This observation points to (short) peptides as additional source of AA in the milk medium used. The carbon balance for *S thermophilus* did not show major gaps as 90-95% of the lactose could be accounted for in terms of metabolic end products (galactose and lactic acid being the major products, see Table 2). Using the measured data as constraints (lactose uptake 170 mmol gDW^{-1}, no limitation in his and cys), growth yields were up to 9 times higher than expected: clearly, lactose is not limiting growth. The flux distributions pointed at very high rates of urea assimilation as a nitrogen source for AA production. We therefore assumed that nitrogen (in the form of either ammonia or urea) is limiting growth in the evolved *S. thermophilus*. An uptake rate of 3 mmol gDW^{-1} urea was fitted to obtain the reference growth yield of 1. Using FVA, the minimal lactose uptake rate needed to support growth and EPS production was 96 mmol gDW^{-1}. This was only 26 mmol gDW^{-1} without EPS, showing the degree of uncoupling of growth and lactose consumption under these conditions.

Modeling the mixed culture

We constrained growth in the models of the mono-cultures of *L. bulgaricus* and *S. thermophilus* by formate and urea, respectively. Improved growth was therefore expected in the consortium as *L. bulgaricus* would provide additional nitrogen to *S. thermophilus* in the form of amino acids. In turn, *S. thermophilus* would provide formate to *L. bulgaricus*. Indeed, when we constrained growth of both species to be equal, we found a substantial increase in growth of both species (maximal yield of 1.23) giving a 2.5 fold increase in total biomass production. This increase is reaching the limit of fully coupled growth and energy limitation. The experimentally found lactose uptake yield was only 57 mmol gDW^{-1} lactose, which is close to 2.5 times 25 mmol gDW^{-1} lactose that would be expected to be the minimum lactose uptake needed for a growth yield of 2.5). That is, if EPS production is neglected. When EPS production is taken into account, biomass production is actually higher than the theoretical limit suggested by the model. That may be caused by

energetically beneficial interactions that are not yet included in the model such as uptake of fatty acids by *L. bulgaricus* upon lipolytic action by *S. thermophilus*. *S. thermophilus* could economize on lactose consumption by consuming amino acids secreted by *L. bulgaricus*. We found that in the optimal flux distribution, all peptides released by proteolysis by *L. bulgaricus* were also taken up by *L. bulgaricus*. In this scenario, *S. thermophilus* profits from proteolysis exclusively via the AA excreted by *L. bulgaricus*. These AA were, however, partly still accumulating in the medium. *In silico*, *L. bulgaricus* profits from proteolysis and amino acid secretion, as AA export results in proton extrusion which corresponds to the generation of $^1/_3$ ATP per exported AA. Since uptake of 1 amino acid via the Opp transport system costs on average $^1/_9$ ATP (8), the model predicts that the operation of this pathway results in energy generation.

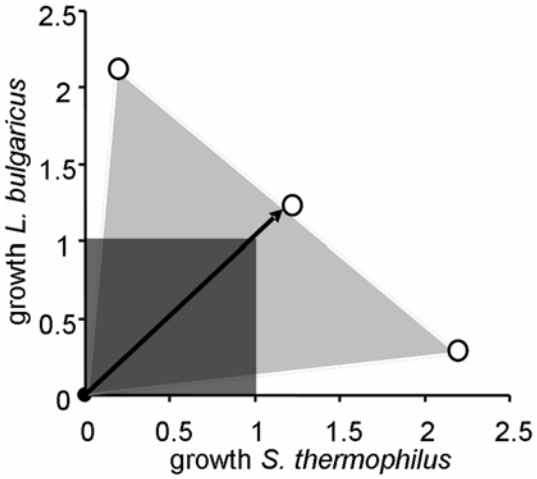

Figure 4: Expansion of growth rate potential in mixed culture compared to mono-culture. Dark grey square area is the growth rate range in mono-culture, light grey triangle area the range in the mixed culture. The arrow points to the result obtained if the growth ratio is fixed at 1.

When we used the second modeling technique, i.e. maximizing one species as a function of the other, we found that the maximal yield of *S. thermophilus* was 2.2, with the yield for *L. bulgaricus* being 0.7. Alternatively, the maximal yield of *L. bulgaricus* was 2.1, with the yield for *S. thermophilus* maximally being 0.3. This is a substantial increase in biomass yield which is in close agreement with the total biomass produced when growth rates were fixed to equal rates (see Figure 4). The increase in yield was confirmed experimentally (see Table 2) and in literature (6, 11, 26). However, in the current optimization scenario's, peptide uptake was

equally divided between *S. thermophilus* and *L. bulgaricus* when growth for *S. thermophilus* was maximized. Moreover, since growth of *L bulgaricus* was relatively low (0.3), not all casein could be proteolysed (see constraints Eq. 2) suggesting - much to our surprise - that already at very low growth rates of *L. bulgaricus*, the competition for lactose outweighs the benefit of peptide or amino acid provision to *S. thermophilus*. As expected, *S. thermophilus* peptide uptake was zero when growth of *L. bulgaricus* was maximized.

DISCUSSION AND FUTURE PROSPECTS

We have developed two functional genome-scale metabolic models of *S. thermophilus* and *L. bulgaricus*. Based on an *in silico* simulation of knock-outs and *in silico* growth, we were able to confirm metabolites that are exchanged between the two bacteria in milk. These were formic acid, folic acid (provided by *S. thermophilus*) and AA (released by proteolysis by *L. bulgaricus*). These interactions resulted in a total yield of the mixed culture that was increased 2.3-2.5 fold compared to the mono-cultures. That means that the interaction between the two organisms, despite lower uptake of lactose per g DW (57 mmol gDW^{-1} instead of 140 and 170 for *L. bulgaricus* and *S. thermophilus*, respectively), resulted in a much larger possible range of growth rates (see Figure 4). Indeed, the amount of lactose consumed per g of biomass was 2.5-3.0 fold lower in our experimental data. The lower uptake of lactose can partly be explained by lower EPS production compared to the biomass production (2.2 g per g biomass in the evolved mixed culture versus 4.2 g and 4.7 g for the *S. thermophilus* and *L. bulgaricus* mono-cultures, respectively, see Table 2). That is striking, because genes coding for EPS biosynthesis were shown to be higher expressed in mixed culture in a previous study (26). The lower uptake of lactose per g produced biomass can also partly be explained by less spilling of AA into the medium. The AA secreted by *L. bulgaricus* were utilized by *S. thermophilus*, reducing the need for lactose for *de novo* AA production. The modeling exercise also showed the extent of uncoupling that is possible in milk between ATP production through glycolysis and growth. Although some improvement is seen in the evolved mono-cultures compared to the parental ones (see Table 2), the real improvement in lactose yield is caused by the provision of essential growth components by the other species in mixed cultures. The nature of the limitation is still speculative at the moment due to lack of required data sets, but the explanation provided by the model seems insensitive to the exact

molecules being exchanged, and formate appears a reasonable proxy for that purpose.

By calculating fluxes using fermentation data, we were able to nearly close the carbon balance of the *S. thermophilus* model, but were not successful in closing that of *L. bulgaricus*. The consumed galactose moiety from lactose was much higher (25%) than the secreted galactose, either as galactose monomers or as EPS. With few exceptions (4), *L. bulgaricus* was reported to be unable to utilize galactose due to the absence of a *galT* gene (10, 33). Indeed, blasting the *galT* gene of *L. plantarum* WCFS1 against the *L. bulgaricus* ATCC BAA-365 genome showed that this gene is not present. We have not been able to find an explanation for the galactose gap so far. The measured AA secretion by *L. bulgaricus* did not correspond to that predicted by the model, i.e. on average it was 3 fold higher. Moreover, the ratio between the experimentally measured and the predicted AA secretion differed for a number of AA (i.e. ala, arg, ile, phe and trp). This may have two explanations: (i) *L. bulgaricus* also grows on free peptides in milk that have a different composition than casein or (ii) the AA content of *L. bulgaricus* biomass is significantly different from that of *L. plantarum*, of which the AA content was taken as that of *L. bulgaricus* (30). Explanation (i) is also supported by the growth of *S. thermophilus* in mono-culture. As this organism is non-proteolytic, auxotroph for histidine, which is present as free AA in a negligible quantity in milk (24 µM), *S. thermophilus* probably has another source of histidine, such as small peptides present in milk.

The current mixed culture model is not finished yet, but it allows the yoghurt consortium to grown *in silico* and predicts the type of mutualistic interactions of which we know that they do occur (11, 23, 26). This model fulfills the requirements for a novel class of predictive microbial growth models (35): because it is whole-genome based, it takes into account the interactions between both consortium members and has therefore a much higher molecular resolution than previous models (6, 27). Furthermore, the model can be updated with new information of reactions catalyzed by enzymes in both species and with experimental data, improving the accuracy of the model. At this moment, we propose the inclusion of other fermentation data such as the production of volatile components (the quantity of produced acetaldehyde, diacetyl and acetone by these bacteria is significant (24)) and the quantity of folic acid, produced by *S. thermophilus* and consumed by *L. bulgaricus* (7), that is available in the medium. Further improvements may deal with the discrepancies between (i) measured and predicted AA secretion by *L. bulgaricus* and (ii) the content of casein and the free AA in milk after fermentation. Therefore, the precise AA content of *L. bulgaricus* and *S. thermophilus* should be

experimentally determined. Moreover, the presence and AA content of peptides initially present in milk should be studied. Finally, the gap in the carbon balance of *L. bulgaricus* should be fixed, possibly by the identification of other means of galactose metabolism (i.e. a currently unknown galactose utilization pathway) or by measuring more potential end products of primary metabolism. Once these improvements of the mono-culture models and the mixed culture model provide a reliable representation of the fermentation, it should be possible to model the different growth phases of the fermentations, providing predictions of the extent and the nature of the interactions between *S. thermophilus* and *L. bulgaricus*.

It is expected that these models, when elaborated as described above, will be suitable also to predict the performance of this culture in other dairy or non dairy substrates. Furthermore, as sequencing and genome-scale modeling rapidly develop, we anticipate that multi-strain and community genome-scale models will find wider application in research on microbial ecosystems.

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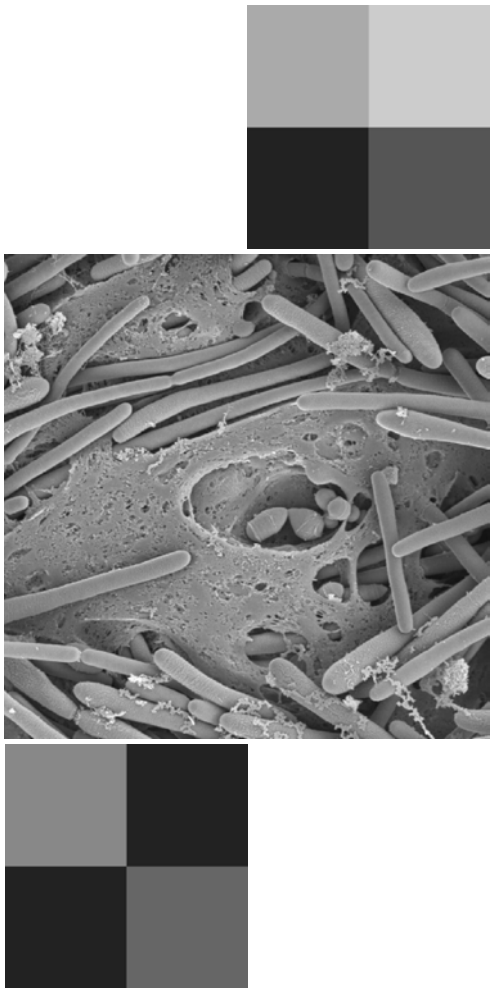
Chapter 7

Summary, discussion and future perspectives



INTRODUCTION AND SUMMARY

Most microbial ecosystems consist of multiple species. The interactions between consortium members in these systems are at the base of the performances of the individual bacteria. Consequently, in fermentations the interactions between microbes are very important for the culture's performance and the quality of the fermented foods. There is a strong need in industry to improve the efficiency of existing industrial fermentations or to develop more sustainable processes. In addition, the current market trends demand a wider range of healthy fermented foods (20) and the application of strains marketed as probiotics. These trends underpin the importance of characterizing the interactions between microorganisms, not only quantitatively, but also qualitatively by unraveling the underlying molecular mechanisms. This also includes a higher predictability of (the interactions involved in) the final microbial composition of fermentations (42). Moreover, there is considerable interest in the interactions that structure microbial communities from an ecological (29, 36) and evolutionary (36, 37) perspective. Therefore, unraveling the interactions between the microbes in a fermentation is highly relevant from an industrial and a scientific point of view.

A well-studied fermentation is the one carried out by the lactic acid bacteria (LAB) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The combined action of these two bacteria turns milk into yoghurt. It has been recognized in the first half of the 20th century that these two LAB stimulate each other in growth and acid production when co-cultivated (49). This mutual stimulation is also referred to as protocooperation. The basis behind this protocooperation has partly been unraveled in the last six decades. It was shown that *S. thermophilus* stimulates *L. bulgaricus* by supplying formic acid (16), folic acid (9, 59) and CO_2 (18), all involved in purine metabolism. *L. bulgaricus*, in turn, provides *S. thermophilus* with peptides and amino acids (AA) released from milk casein with its exoprotease PrtB (8). With the classical microbiological methods that were available until the 1980's, it was not possible to further unravel the molecular basis behind these interactions. However, the availability of genome sequences of these bacteria and of ~omics tools the last decade meant a breakthrough in interaction research (26, 38). A number of post-genomic studies of the yoghurt fermentation revealed the involvement of sulfur AA, branched-chain AA (BCAA) specifically in the protocooperation between *S. thermophilus* and *L. bulgaricus* (33, 34). The research described in this thesis aimed at further unraveling the interactions between these two species using an integrated approach of classical and novel techniques and methodologies. As these novel

techniques are based on ~omics approaches, two sequenced strains, namely *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365, were used in all studies. Chapter 1 gave a general introduction to the subject and briefly discussed the novel techniques that were used in this thesis research, i.e. transcription profiling, high-throughput screening and next-generation sequencing. Also experimental evolution and genome-scale metabolic modeling were briefly introduced. Chapter 2 summarized the status of mixed culture research in general and of the yoghurt consortium in more detail. In addition, it described the current trends in the use of ~omics in mixed culture research. Chapter 3 described a tool for rapid assessment of viable counts of microorganisms in (mixed) cultures. This method was used in the research of Chapters 4, 5 and 6. In Chapter 4, a combination of transcriptomics and screening was used to identify the genetic and physiological basis behind the interactions between the two yoghurt bacteria grown in milk (see also the section below). It confirmed that interactions between the yoghurt bacteria are based on the exchange of formic acid, folic acid and AA. Supply of sulfur AA and BCAA through proteolysis was not sufficient to sustain the increased growth in mixed culture, leading to a higher expression of sulfur AA and BCAA production genes in both species. Moreover, it was shown that exopolysaccharide (EPS) synthesis genes were higher expressed in both species in mixed culture and that *L. bulgaricus* lowered the expression of long-chain fatty acids (LCFA) biosynthesis upon co-culture, possibly due to lipolytic action of *S. thermophilus*. In Chapter 5, the two sequenced strains of *S. thermophilus* and *L. bulgaricus* that that had no history of co-cultivation were co-evolved for ~1000 generations in milk. A detailed analysis of fermentation profiles (e.g. acidification rate), gene expression, and mutations further elucidated the molecular basis behind their protocooperation. Moreover, it was shown that as little as ~1000 generations of co-evolution is sufficient to adapt a relatively slow growing and acidifying mixed culture into one that is comparable to a commercial starter in terms of acidification rate and EPS production. The development of a genome-based mixed culture model of the yoghurt fermentation was discussed in Chapter 6. Although the model is currently still in development, various predictions could be made about the interactions based on the genome content of the two species. Indeed, the predicted interactions included AA metabolism (i.e. proteolysis) and purine metabolism (i.e. formic acid and folic acid), which was already experimentally confirmed in Chapter 4. Using flux balance analysis (FBA), it was possible to determine the effects of the protocooperation on biomass yields on lactose. The biomass yields were found to be significantly improved in the mixed culture compared to the mono-cultures. The general discussion below will

elaborate on the findings in this thesis, place these in a wider context and discuss the future perspectives of this thesis research.

IMPROVED UNDERSTANDING OF THE YOGHURT CONSORTIUM USING ~OMICS TECHNOLOGIES AND MODELING

We have successfully applied mixed culture gene expression studies and genome-based metabolic modeling on the yoghurt consortium in order to identify genes and pathways that play a role in the interactions during, or are affected by, co-culture growth. In Chapter 4, the effect of adding specific components, expected to influence the interactions, on the growth and acidification of mono-cultures and mixed cultures were studied. The results were combined with results of transcription profiling studies of both bacteria during the fermentation. By using this combined approach we were able to identify (i) the effects of the single compounds on the performance of both species in milk, and (ii) which pathways were differently expressed in both species in mixed cultures compared to the mono-cultures. This confirmed the involvement of purine and AA in the interactions as was also found in a recent transcriptome study on *S. thermophilus* by Hervé-Jimenez *et al*. (33). Moreover, a role for LCFA metabolism and peptidolysis was found in the interactions, and the genes for iron metabolism in *S. thermophilus* and EPS biosynthesis in both species were shown to be affected by the interactions (see Figure 1 and below).

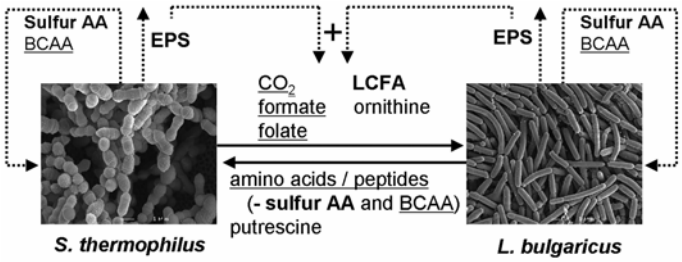


Figure 1. Schematic representation of the mutualistic interactions between *S. thermophilus* and *L. bulgaricus* in yoghurt. Solid arrows indicate interactions; dotted arrows indicate pathways that are affected by the interactions. Pathways that were for the first time shown to be regulated at the transcriptome level upon co-culture are indicated in bold. Pathways that were confirmed in our study to be regulated at the transcriptome level upon co-culture are underlined. EPS is hypothesized to promote the exchange of both bacteria. There was no evidence at the transcriptome level for the exchange of putrescine and ornithine. AA, amino acids; BCAA, branched-chain AA; EPS, exopolysaccharides; LCFA, long-chain fatty acids.

Long-chain fatty acids acquisition by *L. bulgaricus*. It was shown that *L. bulgaricus* lowered the expression of LCFA biosynthesis genes (i.e. those coding for decarboxylases, hydrolases, oxidoreductases and acyltransferases) in mixed culture. The last step in the phosphatidic acid synthesis from acyl-carrier proteins containing the fatty acid chains was higher expressed in mixed than in mono-culture. Although LCFA such as lauric acid and oleic acid had previously been reported to stimulate the growth of *L. bulgaricus* (47), no attempts have been made to link this to the protocooperation between the yoghurt bacteria. In an earlier study, several genes involved in fatty acid production (*fabH*, *accC* and *fabI*) were higher expressed in *L. bulgaricus* as an adaptation to an acidic (pH 4.2) environment (23). However, these genes (*LBUL_0822*, *LBUL_0829* and *LBUL_0828* in *L. bulgaricus* ATCC BAA-365, respectively) were 3-10-fold lower expressed in mixed culture compared to mono-culture despite the lower pH. This suggests that the acyl-carrier proteins were loaded with fatty acid chains from another source, for instance fatty acids liberated from milk fat by the lipolytic action of *S. thermophilus* (53). It is not clear how these fatty acids are taken up by *L. bulgaricus*, but evidently this species is capable of utilizing fatty acids from the medium (47). Possibly, this role is executed by the so far uncharacterized *LBUL_0063* identified in Chapter 5. The *S. thermophilus* genome encodes multiple lipolytic enzymes (3, 25, 39) that also play a role in the flavor formation (48).

Branched-chain and sulfur amino acids. It was stated in Chapter 4 that the proteolytic activity of *L. bulgaricus* does not supply a sufficient amount of BCAA leading to a higher expression of *de novo* synthesis of this pathway in both species. This is notably the case for the BCAA transaminase (*LBUL_1219* and *str0590*) responsible for the formation of valine, leucine and isoleucine from 3-methyl-2oxobutanoate, 4-methyl-2-oxopentanoate and (S)-3-methyl-2-oxopentanoate, respectively. This is most likely due to the high abundance of valine, leucine and isoleucine in the total proteomes of *S. thermophilus* (33) and *L. bulgaricus* (GenomeAtlas database (30)). Moreover, the pathways for sulfur AA biosynthesis were upregulated in both species when grown in a mixed culture. This may be due to the low amount of sulfur AA in casein (cysteine 0.35% and methionine 2.9%) (58) that becomes available during proteolysis of milk compared to that of the proteome of *S. thermophilus* and *L. bulgaricus* (cysteine ~1% and methionine ~2%). Indeed, the measured levels of free cysteine and methionine were very low. In addition, it was previously reported based on a transcription profiling approach with a *S. thermophilus* mono-culture that sulfur AA limitation is likely to occur, particularly in the later stages of fermentation, and that this also occurs in mixed culture (34).

Exopolysaccharide biosynthesis. EPS is mainly synthesized in the later phases of fermentation (4). Although there has not been assigned an evident function for EPS in yoghurt, it is possible that EPS plays a role in acid resistance (45) or in facilitating exchange of metabolites by allowing stable close proximities or even physical contact between the two species, which is likely also the function of EPS in kefir (6, 7). In addition, the combination of different EPS-producing strains of *S. thermophilus* was shown to stimulate total EPS production in mixed culture (14, 24). However, these observations had not yet been linked to gene expression. Interestingly, in Chapter 4 it was shown that the expression of EPS biosynthesis genes in both species was increased during growth in mixed culture. Furthermore, this was confirmed in a transcription profiling study of mixed culture biofilms, suggesting that this effect is generic. Basically, there were two reasons to choose for biofilm growth to confirm this effect: (i) when grown in biofilms, there is a forced physical contact between *S. thermophilus* and *L. bulgaricus* strengthening potential interactions; (ii) biofilms are relatively well accessible for analytical techniques such as staining and microscopy compared to liquid cultures (see also Chapter 4). Indeed, the mixed culture biofilm showed a stronger staining by Wheat-Germ Agglutin, a lectin that is commonly used for staining polysaccharides, than the mono-culture biofilm (31). In fact, the EPS concentrations after 24 h culturing at 42°C increased from 0.80±0.02 g/L in a *S. thermophilus* mono-culture and 1.18±0.12 g/L in a *L. bulgaricus* mono-culture to 1.46±0.04 g/L in the mixed culture. In the cultures with the evolved strains from Chapter 5 this was 1.35±0.26 g/L, 1.52±0.03 g/L and 3.91±0.48 g/L, respectively. This all underpins the importance of EPS for the bacteria, especially in mixed culture.

Mixed culture metabolic modeling. In Chapter 6, the development of a mixed culture metabolic model, which was based on the genome content of both species, was presented. It confirmed that the interactions were based on the exchange of formic acid, folic acid and on AA metabolism. By measuring the biomass and EPS production and by determining the fluxes of lactose and fermentation products, it was possible to calculate the yield of biomass on lactose. This was substantially higher (2.3-2.9 fold) in the mixed culture than in the corresponding mono-cultures, which can be explained by a lower EPS production per g of total biomass and by the benefits of the interactions. In the model, these benefits included less spilling of AA. That means that *S. thermophilus* consumed a part of the AA that were released by proteolysis and peptidolysis by *L. bulgaricus*, i.e. *L. bulgaricus* consumed all the released peptides and secreted all AA, except the one that was limiting growth. Subsequently, *S. thermophilus* could consume these AA in stead of synthesizing them itself, saving energy and thus lactose.

However, in the mixed culture transcriptome study (Chapter 4), it was shown that *S. thermophilus* in mixed culture mainly upregulates peptide transporters and peptidases in stead of AA transporters. In addition, the carbon balance of *L. bulgaricus* was not closed: around 25% of the consumed galactose moiety in lactose could not be found as secreted galactose monomers or in EPS. Due to the absence of a *galT* gene (10, 32).*L. bulgaricus* can not utilize galactose (27, 60). These two discrepancies should still be dealt with in order to make the mixed culture model sufficiently predictive.

In conclusion, there is strong evidence from various studies (physiological, transcription profiling, experimental evolution and genome-scale metabolic modeling) that the interactions between *S. thermophilus* and *L. bulgaricus* are based on the exchange of various growth factors provided by *S. thermophilus*, proteolysis (executed by *L. bulgaricus*) and lipolysis (executed by *S. thermophilus*). The growth factors provided by *S. thermophilus* are related to purine and AA biosynthesis: formic acid, folic acid and CO_2 (the latter is not discussed here, see Chapter 4). In addition, there is a potential role for EPS in facilitating these nutritional exchanges (see Figure 1). There was no evidence for the exchange of ornithine and putrescine as suggested by Van de Guchte *et al.* (62), but it can not be excluded if any benefits resulting from their exchange do not lead to evident differential gene expression or increased growth.

EXPERIMENTAL EVOLUTION IMPROVES KEY CHARACTERISTICS OF YOGHURT PRODUCTION REPRODUCIBLY

In Chapter 5, experimental evolution has proven to be a useful methodology for the optimization of mixed culture fermentations. For the development of novel industrial starters, or for the improvement of existing ones, it is definitely worthwhile to investigate the possibilities of experimental evolution. In this thesis research, a relatively slow growing and acidifying mixed culture (i.e. *S. thermophilus* CNRZ1066 and *L. bulgaricus* ATCC BAA-365) was turned into one that can meet with commercial starters in the course of ~1000 generations of co-culture. The acidification rate increased step-wise (see Figure 1 in Chapter 5) caused by the accumulation of rare beneficial mutations (21, 35). The acidification rate of the evolved mixed culture was closer to that of the commercial starter yoghurt culture I-St than to its own parental mixed culture. Moreover, the efficiency of biomass production increased from 0.12 g/mol consumed lactose in the parental culture to 0.22 g/mol in the evolved culture (this is 0.30 g/mol for the commercial culture I-St)

during exponential growth. That indicates that either the metabolism of the evolved organisms compared to their parental strains had become more efficient, or that the interactions between the two species improved making the evolved mixed culture more efficient than the original mixed culture. The flux balance analysis in Chapter 6 and the improved stimulation of parental strains by evolved strains suggest that both instances occurred.

The amount of produced EPS in 24 h more than doubled from 1.46 g/L by the parental mixed culture to 3.91 g/L by the evolved mixed culture (see also above). This was also reflected in the culture's viscosity, which also increased by a factor of 2.6-2.8. The viscosity of the evolved culture was comparable to – or even higher than – that of the commercial starter composed of *S. thermophilus* Sts and *L. bulgaricus* Ib. This higher production of EPS was related to the higher expression of EPS biosynthesis genes, notably in *S. thermophilus*. Interestingly, the increase in EPS production, visualized in biofilms, also occurred step-wise (see Figure 2), indicating that the higher production is not solely due to the higher quantity of cells in the evolved culture. Unfortunately, no mutation was found that clearly indicates the cause of this higher EPS production in evolved strains compared to their parental strains.

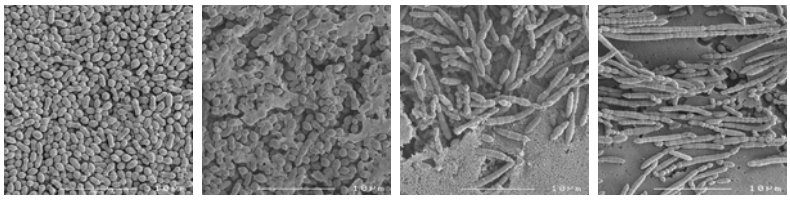


Figure 2. SEM images of *S. thermophilus* cells grown as mono-culture biofilms in the course of evolution. Single colony isolates were selected from the evolving culture and grown on anopore as mono-culture before imaging. The increasing extracellular matrix is assumed to be EPS (see Chapter 4).

It appears to be not a coincidence that specifically these improvements (faster acidification, more growth and more EPS production) occur during experimental evolution with the strict growth regime discussed in Chapter 5. A second culture of *S. thermophilus* and *L. bulgaricus* that had undergone the same process also showed a higher acidification rate and a higher viscosity. It was shown that this second evolved *S. thermophilus* had mutations compared to the parental strains in three of the same sites. Gene expression studies showed upregulation of the same pathways as the strains from the other evolved culture. This is by itself not very

surprising as the optimization strategies of comparable cultures in the same ecological conditions are likely to be similar (55, 63). Interestingly, the gene expression study showed that in particular pathways involved in folic acid (*L. bulgaricus*), sulfur AA, BCAA, LCFA (*L. bulgaricus*) and EPS were differentially expressed between the evolved and parental strains. This indicates the fine-tuning of interactions in order to maximize growth in both species.

Novel types of mixed cultures show similar improvements. The experimental evolution procedures (Chapter 5) were also applied to uncommon species combinations containing the probiotic *Lactobacillus plantarum*. Previously, it had been shown that growth of some *L. plantarum* strains was stimulated by *L. bulgaricus*, but there was no stimulation of yoghurt bacteria by *L. plantarum* (12). All possible two-species and three-species combinations of *S. thermophilus*, *L. bulgaricus* and five different *L. plantarum* strains were prepared (see Table 1). The two strains that probably have a higher number of IS elements (61) were assumed to have a potentially higher mutation rate and a correspondingly faster adaptation.

The step-wise increase in acidification rate reported for the two evolved yoghurt cultures was also evident in all five evolved cultures consisting of *S. thermophilus* and *L. plantarum* and the five three-species cultures (see Figure 3). It did not occur in any of the evolved cultures consisting of *L. bulgaricus* and *L. plantarum*. This implies that the step-wise increases in acidification rates were caused by mutations in *S. thermophilus* (13).

Table 1. The five *L. plantarum* strains that were co-evolved for ~1000 generations in milk with *S. thermophilus* CNRZ1066, *L. bulgaricus* ATCC BAA-365, or both. The rationale behind their use is stated at the right. The strains were obtained from NIZO food research, the Netherlands.

L. plantarum strain	Rationale
WCFS1	Reference strain with sequenced genome
LMG9208 (NIZO 2806)	Relative good growth in milk
BLL(EI31) (NIZO 2830)	Relative good growth in milk
SECT4645 (NIZO 2776)	High number of IS elements (61)
NCTH19-1 (NIZO 2484)	High number of IS elements (61)

As expected, all five *L. plantarum* strains remained stable in co-cultures with *L. bulgaricus*. In the cultures containing *S. thermophilus* and *L. plantarum*, only the strains SECT4645 and NCTH19-1 were outcompeted within five transfers. That was due to their slow growth rate compared to the other three *L. plantarum* strains. When serial transfers were applied every other day, *L. plantarum* SECT4645 and NCTH19-1 stayed at a constant level in combination with *S. thermophilus*. In all three-species cultures, *L. plantarum* was present for at least 800 generations.

However, in the course of evolution, the amount of viable *L. plantarum* cells decreased in all five cultures. Strains WCFS1, LMG9208 and NCTH19-1 were below the detection limit (~10^3/mL) after ~800 generations. This was associated with the step-wise increase in acidification rate, indicating that the rapid (lactic) acid production by *S. thermophilus* and *L. bulgaricus* inhibited growth of *L. plantarum*. Indeed, a pH below 6 and the presence of lactic acid impede optimal growth of *L. plantarum* (28), leading to fewer cells at each transfer despite any stimulatory effects of the yoghurt bacteria.

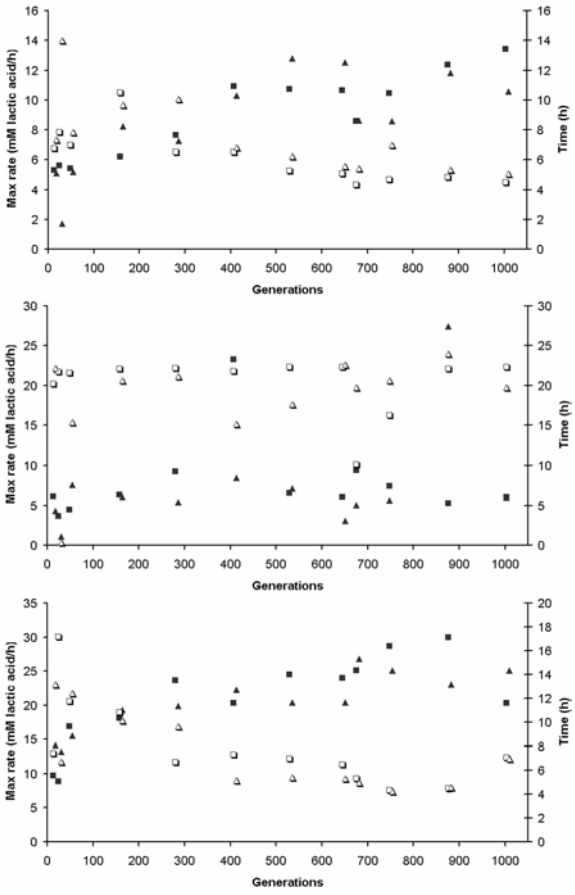


Figure 3. Maximal acidification rate (Δlactic acid/Δt) (■,▲) and the time necessary to reach this maximal rate (□,Δ) of three different cultures in duplicate in the course of evolution. Squares and rectangles represent two duplicate evolving cultures. Acidification was measured in 10 mL milk at 37°C as described before (56). Top, a mixed culture of *S. thermophilus* and *L. plantarum*; middle, a mixed culture of *L. bulgaricus* and *L. plantarum*; bottom, a three-species mixed culture.

Above-mentioned results signify two main conclusions. (i) The high reproducibility of experimental evolution of these mixed cultures indicates that experimental evolution procedures can very well be applied to adapt other unfamiliar strain combinations to each other and to their environment. The highly

reproducible outcome of experimental evolution had also been found for mono-cultures of *Escherichia coli* cells grown under a strict regime (63), supporting this conclusion. (ii) Although interactions between yoghurt bacteria also promote growth of other (probiotic) species, the interactions within new species combinations do not necessarily strengthen to form a well-performing stable mixed culture. That means that it may be necessary to rationally introduce nutritional dependencies in advance in order to stably include probiotics at a reasonable level (10^5-10^8 per gram of product (44)) in existing relatively optimal fermentations such as the yoghurt fermentation (10, 32) (see below). However, this is the case for *L. plantarum* and may not necessarily apply to other newly included species that already display mutually synergistic interactions. Moreover, the addition of probiotic species or strains in suboptimal systems, such as a combination with either *S. thermophilus* or *L. bulgaricus*, is more likely to succeed as there is much more room for improvement and the development of interdependencies (22, 51).

As discussed, in order to include probiotics in existing optimized mixed culture fermentations, rationally engineering of interactions may be necessary. Therefore, it is not only from a scientific but also from an industrial point of view relevant to identify the exact modes of interaction and their underlying molecular mechanisms in the current mixed cultures. Once the engineering of interactions between an endogenous species and the probiotic species has been performed, experimental evolution can be applied to optimize these new interactions and stabilize the new mixed culture. The high reproducibility of experimental evolution indicates that the outcome can – at least in part – be predicted based on the new (environmental) conditions the microorganisms find themselves in (15). The structure of the environment and the conditions used for the fermentations are therefore important considerations when applying experimental evolution (22, 51, 54).

EVALUATION OF THE USED MODERN TECHNOLOGIES

Transcription analysis with mixed culture microarrays.

The face of transcription analysis has changed rapidly the past decade with the use of whole-genome analysis tools such as microarrays. Currently, there are many studies where microarrays containing probes targeting different species are used. Examples include the use of metatranscriptomics in the field of metagenomics such as in environmental microbial communities and fermented food products (46). Microarrays have also been used before to differentiate between different *Bifidobacterium* species in the intestine (2) and to study gene

expression of one species in a mixed culture (33, 43). The use of microarrays targeting the whole genomes of both species in a food fermentation is novel. It is not trivial as the probes in the microarrays should be selective (see Chapter 4): the target gene in one of the species may be very similar to its orthologue in the other species, especially when closely related species are used. Moreover, the efficacy of the applied extraction method is essential for the quality of the extracted RNA (52). That is, one has to ensure that the method works equally efficient for both bacteria in order to circumvent a bias in the normalization of gene expression data if the hybridized cDNA of one species is overrepresented compared to that of the other species. This problem can be avoided by using next-generation sequencing of cDNA in stead of microarrays as discussed in (40), but this method is still in development.

Next-generation sequencing.
In Chapter 5, Illumina Solexa sequencing was used to identify mutations in the evolved strains of *S. thermophilus* and *L. bulgaricus* compared to their parental strains. The alignment of data is not trivial. Issues regarding local low coverage and repeatedly occurring sequences have been discussed in Chapter 1. Moreover, the choice of cut-offs is and the alignment method(s) applied are important factors in the reliability of the results (17, 57). We applied different alignment methods and filtered their results with a stringent cut-off for the quality score (see Chapter 5), providing the possibility to find substitutions as well as (one base and larger) insertions or deletions. Solexa sequencing has indeed proven to be a good method for the detection of mutations.

Mixed culture metabolic modeling.
Considering the rapid increase in papers discussing genome-scale metabolic modeling (no Pubmed hits before 2002, 25 in 2008 and 25 until July 1, 2009), there is a great and growing interest in this topic. That is not an odd development as such models provide a solid interpretative framework for experimental data related to gene content and metabolic state of the microorganism (19). Coupling two of these models in order to identify possible modes of interaction and their effects on both bacteria is a novel application. It is expected that the inadequacies of the current mixed culture model discussed above and in Chapter 6 can be settled in the near future.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This thesis research has provided novel insights in the protocooperation between *S. thermophilus* and *L. bulgaricus*, in particular in the molecular base behind these interactions (see Figure 1). It has not been shown before that EPS biosynthesis genes were induced in mixed culture in both species and that EPS production increased in the course of mixed culture experimental evolution. This strongly suggests that EPS has a function in the interactions, but the details of this function remain to be elucidated. Similarly, a novel finding is that genes coding for LCFA production in *L. bulgaricus* were lower expressed in mixed culture despite the higher use of LCFA to sustain a higher growth rate. It was suggested that lipolysis executed by *S. thermophilus* provides here a novel mode of interaction, but hard experimental evidence to confirm that still has to come. The gene *LBUL_0063* is a good target to start with.

The extensive list of interactions based on nutritional exchanges indicates the complexity that can occur in a relatively simple (in terms of members involved) microbial community such as the yoghurt consortium. However, the interactions in this system are indicative for the types of interaction that can be expected in other mixed culture ecosystems. Experimental evolution (Chapter 5) and mixed culture modeling (Chapter 6) showed the extent of the benefits that microorganisms can achieve by cross-feeding in terms of increased growth rate and efficiency of metabolism. Indeed, there are numerous examples of cross-feeding interactions in the microbial world (5). In conclusion, nutritional interdependencies like in the yoghurt consortium are ubiquitous and likely structure and stabilize many different ecosystems (1, 11, 50). The post genomic approaches applied in this thesis have increased our understanding of this classic mixed culture fermentation process. Suggested further research might start with improving the annotation of the genomes, i.e. 32% of the COGs in *S. thermophilus* CNRZ1066 are not or poorly annotated (3); in *L. bulgaricus* ATCC BAA-365 this is 38% (41). The galactose sink and the production of formic acid by *L. bulgaricus* discussed in Chapter 6 clearly indicate that there are metabolic pathways operational for which the corresponding genes and proteins remain to be identified. Therefore, the mixed culture model will be elaborated in the near future as discussed.

How can we apply the used methods in future research? The rapid plating method discussed in Chapter 3 can be implemented easily for any type of microbiological work that requires the assessment of large numbers of colonies. For this, there is no need for special equipment. Furthermore, it reduces the use of consumables and the concomitant production of waste by a factor 100 compared to conventional

plating, which is becoming increasingly important in this era of environmental problems. The mixed culture microarrays can be used to evaluate the molecular responses of both yoghurt consortium members to various environmental factors. For example, the responses to supplementation of fatty acids to a yoghurt culture growing in fat-free milk may confirm the suggestion of lipolysis playing a role in the interactions. Moreover, by using transcription profiling of other strains, it will be possible to identify whether responses associated with co-culture are similar in other strain combinations.

Currently, most industrial mixed culture starters are selected by screening combinations of strains for desired properties. Given the results in this thesis, it is anticipated that directed experimental evolution of mixed cultures containing interesting strains or species, such as probiotics, can allow the production of industrially relevant starters that would not be selected otherwise. In addition, the new insights in the yoghurt consortium interactions provide targets for the rational development or optimization of stable mixed cultures, e.g. by introducing similar mutual dependencies in other mixed cultures. In the near future, the work described in this thesis will (i) improve research techniques in other mixed culture studies such as in kefir or cheese, (ii) lead a further increase in knowledge on the yoghurt consortium by providing novel targets for additional research, and (iii) facilitate (food) industry to develop new or improved fermented products with desired properties.

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Nederlandse samenvatting

Melkzuurbacteriën zijn een groep Grampositieve bacteriën die suikers zoals lactose fermenteren en daarbij hoofdzakelijk melkzuur produceren. Het is een groep van industrieel belangrijke micro-organismen, die worden toegepast voor de productie van veel gefermenteerd voedsel. Dit omvat zowel voedingsmiddelen van plantaardige origine (b.v. zuurkool en wijn) als van dierlijke origine (b.v. worst en zuivelproducten zoals yoghurt). De huidige tendensen in de voedselmarkt, zoals de toename van duurzame en gezondheidsbevorderende producten, vragen efficiëntere en meer verschillende fermentaties. De meeste fermentaties worden uitgevoerd door combinaties van verschillende soorten micro-organismen. De interactie tussen consortiumleden zijn belangrijk voor de prestaties van de individuele micro-organismen binnen een microbieel ecosysteem, en daarmee ook van de gehele fermentatie. Deze microbiële interacties zijn vaak slecht begrepen. *Streptococcus thermophilus* en *Lactobacillus delbrueckii* subsp. *bulgaricus* zijn twee melkzuurbacteriesoorten die middels fermentatie (koeien-) melk in yoghurt omzetten. Deze twee bacteriën stimuleren elkaars groei en zuurproductie. Zij produceren exopolysacchariden, belangrijk voor de textuur van yoghurt, en kenmerkende aromaverbindingen zoals acetaldehyde en diacetyl. De moleculaire basis van de mutualistische interactie tussen deze twee bacteriën was nog niet goed bekend.

In dit promotieonderzoek werd een combinatie gebruikt van screening, mengcultuur transcriptoomanalyse, genoomwijde metabolische modellering, experimentele evolutie en next-generation sequencen. Dit werd gedaan om de moleculaire basis van de interactie tussen *S. thermophilus* en *L. bulgaricus* in melk te ontrafelen. De resultaten toonden aan dat de interactie hoofdzakelijk was gebaseerd op de uitwisseling van metabolieten. Bovendien werd getoond welke genen/pathways werden beïnvloed door de interacties. Er was bewijs gevonden dat *S. thermophilus L. bulgaricus* mierenzuur, foliumzuur (allebei betrokken bij met purinemetabolisme), lange-keten vetzuren (door de actie van lipolytische enzymen om melkvet af te breken) en CO_2 verschafte. De proteolyse door de exoprotease van *L. bulgaricus* verschafte beide soorten peptiden, die door de cel kunnen worden opgenomen en vervolgens tot aminozuren worden afgebroken door intracellulaire peptidases. Dit zorgde echter niet voor voldoende beschikbaarheid van valine, leucine, isoleucine en zwavelhoudende aminozuren, wat bleek uit een hogere expressie van biosynthese genen voor deze aminozuren in beide soorten in mengcultuur. Daarnaast waren ook de biosynthesegenen voor exopolysacchariden hoger tot expressie gebracht in gemengde cultuur, wat tot

meer productie van exopolysacchariden en een hogere viscositeit van de yoghurt leidde.

Een genoomwijd metabolisch model van de mengcultuur bevestigde dat de interacties tussen de yoghurtbacteriën waren gebaseerd op het purine en het aminozuur metabolisme. Daarnaast werd dit model gebruikt om aan te tonen dat de interactie een significant voordeel aan beide bacteriën opleverde, d.w.z. hun opbrengst van biomassa op lactose was ongeveer 50% hoger in mengcultuur.

Experimentele evolutie onthulde dat het mogelijk is om een nieuwe combinatie van *S. thermophilus* en *L. bulgaricus* stammen aan elkaar aan te passen. Het bleek dat hun wederzijdse stimulatie versterkte door hun interactie te optimaliseren middels het fine-tunen van pathways die betrokken zijn bij de interactie. Slechts ~1000 generaties samen kweken volstond om de relatief slecht groeiende mengcultuur te veranderen in één die vergelijkbaar was met een commerciële cultuur in zeer belangrijke kenmerken zoals verzuringsnelheid en viscositeit.

Het betere begrip van de interacties die ten grondslag liggen aan de yoghurtfermentatie geven ons doelwitten voor de rationele optimalisering van bestaande mengcultuurfermentaties en de rationele ontwikkeling van nieuwe industrieel relevante mengculturen, zoals die met probiotica. Bovendien zijn de resultaten in het bijzonder interessant voor het onderzoeksgebied van microbiële ecologie aangezien zij tonen hoe de wederzijdse interacties evolueren en de microbiële samenstelling van dit ecosysteem structureren.

List of publications

Genomics and high-throughput screening approaches for optimal flavour production in dairy fermentation. 2008. Margreet I. Pastink[#], Sander Sieuwerts[#], Frank A.M. de Bok, Patrick W.M. Janssen, Bas Teusink, Johan E.T. van Hylckama Vlieg, Jeroen Hugenholtz. *International Dairy Journal* 18:781-789. [#]These authors contributed equally to this work

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There are many other people that I worked with or at least enjoyed their presence at work and/or outside work hours. It would simply double the size of my thesis if I was to write a short note of them all separately. I will therefore only briefly mention some highlights; the people involved will know it is about them. I really enjoyed the C-015 team and in particular the collaborations I had with some of them. I think the thesis research would not be so complete without your contributions. That also counts for the 'computer guys' BT, DM, MW and SvH that helped me with the more advanced bioinformatics. Moreover, I think that working wouldn't have been so pleasant without the people in the Kluyver lab (not to be confused with Kluyver Centre) and the office. We have had some good laughs, especially at the Friday afternoons. Similarly, I enjoyed the great discussions we sometimes started when I actually came for one small question. Though I saw the basement-people

on a less regular basis, I definitely feel that they've been an enrichment to my stay at Nizo as well.

As a PhD student sometimes you get a bit (well… a bit) fed-up with all the practical work and you want to do something else. Fortunately, I had three excellent students KZ, ES and ET coming to the rescue. They continued the practical work giving me time to write and attend conferences and courses. Thank you! Similarly, it was good to spend some time with other people during teaching, for instance (KH, SP, JS, LB and MG). And I am sure that I would have thrown my computer, a centrifuge or something else expensive through the window if I did not cool down talking to BvdB or MP. As I sill have to work at Nizo for two more years, I am very grateful for their excellent listening skills.

Not so much involved in my work and currently far away, there were some people important in my personal life. AC, GF and MdV, you were worthwhile to spend the scarce free time with these last four years. I must say that *I still miss you; Ancora lo manco; ik mis je nog*.

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Well, that's it for so far. If you still would like to be mentioned in particular, please fill in the following line: Dear . . . . . , you are the best and therefore I want to thank you for . . . . . .

Sander

Curriculum Vitae

Sander Sieuwerts was born at March 8, 1982 in Zoetermeer, the same city as he lived the first 23 years of his life. There he followed primary and secondary school as most other kids. At secondary school Sander followed preparatory scientific education (VWO) with mainly science subjects. Although he had always been a person that likes nature very much, it was there that he decided to study biology and focus on molecular biology. In 2000, Sander started his MSc (doctoraal) studies at Leiden University, which he managed to finish in March 2005. During these studies he performed three internships in plant physiology (TNO-TPW, Leiden), fungal genetics (DSM-DFS-GEN, Delft) and plant genetics (Leiden University). Although plants stayed important in his personal life, Sander considered microorganisms more fun to work with. He was excited when he was offered the position of PhD student in project C-015 (Biodiversity and mixed cultures) at WCFS (the former TI Food and Nutrition). In March 2005, he started working for four years at NIZO food research under supervision of Johan van Hylckama Vlieg as primary supervisor and Willem de Vos as promotor. The results of this fundamental yet applied research are described in this thesis. After a short but very welcome holiday, Sander started working as a postdoc for the Kluyver Centre for Industrial fermentation on a research topic closely related to his thesis research: interactions between (lactic acid) bacteria and yeasts.

Overview of completed training activities

Discipline specific activities

- Principles of ~omics data analysis, EPS/NBIC, 2005
- Food fermentation, VLAG, 2008
- Stralingshygiëne, VLAG/Larenstein, 2005
- SRS/BLAST/ClustalW, CMBI, 2006
- Protein sequence analysis, CMBI, 2006
- Applied genomics of industrial microorganisms (incl. poster presentation), BODL/Kluyver, 2006
- Genetics and physiology of food associated microorganisms, VLAG, 2007
- NIZO dairy conference (incl. poster presentation and oral presentation), Arnhem, 2007
- Kluyver centre symposium 08 (incl. poster presentation), Egmond aan Zee, 2008
- 9th International symposium on lactic acid bacteria (incl. poster presentation), Egmond aan Zee, 2008
- Kluyver centre symposium fall 08 (incl. oral presentation), Wageningen, 2008
- Kluyver centre symposium 09 (incl. poster presentation), Egmond aan Zee, 2009
- International dairy federation Science and technology week (incl. oral presentation), Rennes, France, 2009

General courses

- PhD scientific writing, CENTA, 2005
- PhD presentation skills, CENTA, 2006
- Assisting in undergraduate microbial physiology practical, 2007/8
- MSc course Didactiek en communicatie (ECS-20806), ECS, 2008

Other activities

- Preparation of PhD thesis research proposal, 2005
- PhD VLAG week, VLAG, 2006
- WE-days WCFS/TIFN (Programme 3 - Microbial functionality and safety), 2005-8
- Organisation of Programme 3 WCFS Fall WE-days, Koudum, 2007
- Project meetings Biodiversity and mixed cultures, 2005-9
- Bio-IT meetings WCFS/TIFN, 2005-8
- Schoolpracticum (ECS-31306), ECS, 2008

Analysis of Molecular Interactions between Yoghurt Bacteria by an Integrated Genomics Approach

Sander Sieuwerts