Lactobacillus sakei metabolism and diversity

Lactobacillus sakei metabolisme og diversitet

Philosophiae Doctor (PhD) Thesis

Anette McLeod

Department of Chemistry, Biotechnology and Food Science
Norwegian University of Life Sciences

Ås 2010

Thesis number 2010:21
ISSN 1503-1667
ISBN 978-82-575-0932-3

TABLE OF CONTENTS

ABSTRACT

Lactic acid bacteria are associated with food fermentation, acidification and preservation. *Lactobacillus sakei* is an industrially important species mainly due to its ability to ferment and preserve meat. It is used as starter culture for industrial meat fermentation and has potential as a biopreservative to extend storage life and ensure microbial safety of meat and fish products. The work in this thesis aims at increasing the understanding of the primary metabolism of various *L. sakei* food isolates, and at defining the diversity existing among these.

Growth characteristics on various media, carbohydrate-fermentation abilities and acidification properties tested in a meat model, were demonstrated to vary between strains. By genetic fingerprint techniques, a distinction between two genetic groups consistent with the two *L. sakei* subspecies, *sakei* and *carnosus*, was observed, with the majority of strains belonging to the latter. Microarray-based comparative genome hybridization using an array mainly based on the sequenced *L. sakei* strain 23K was introduced for clustering the strains. The same division into two genetic groups was observed, and a detailed view of the gene content between various test strains compared to the 23K strain was obtained. By pulsed field gel electrophoresis genome sizes were estimated to vary from 1.880 to 2.175 Mb, and the 23K genome was among the smallest. Consequently, a large part of the 23K genome belongs to a common gene pool of the species. The majority of genes important for adaption to meat products, the ability to utilize meat components, and robustness during meat processing and storage were conserved, indicative of the role these genes play in niche specialization within the species. Proteomic analysis was used to study the primary metabolism in different strains when grown on ribose compared with glucose, the main sugars available for *L. sakei* in meat and fish. Increased expression was observed for proteins directly involved in ribose catabolism and the phosphoketolase pathway, as well as pyruvate and glycerol/glycerolipid metabolism. Simultanously, enzymes involved in the glycolytic pathway were less expressed. These findings were confirmed at the level of gene expression using microarrays, and it was also obvious that ribose catabolism is tightly linked with catabolism of nucleosides. Moreover, enzymes important in the regulation of carbon metabolism and in sugar transport were induced. A global regulation mechanism seems to permit a fine tuning of the expression of enzymes that control efficient exploitation of available carbon sources.

SAMMENDRAG

Melkesyrebakterier er forbundet med fermentering, syrning og konservering av mat. *Lactobacillus sakei* er en industrielt viktig art hovedsaklig på grunn av evnen den har til å fermentere og konservere kjøtt. Den brukes som starterkultur for industriell kjøttfermentering og har potensiale for å forlenge holdbarhet og ivareta mikrobiell trygghet for kjøtt- og fiskeprodukter. Målet for arbeidet i denne avhandlingen var å øke forståelsen omkring primærmetabolismen til forskjellige *L. sakei* stammer isolert fra mat, og i tillegg studere mangfoldet som eksisterer blant disse.

Vekstegenskaper i forskjellige medier, karbohydrat-fermenteringsevner og evne til syreproduksjon testet i en kjøttmodell ble vist å variere mellom stammene. Ved å bruke genetiske fingerprintteknikker kunne to genetiske grupper skjelnes fra hverandre. Grupperingen oppnådd i dette arbeidet var forenlig med de to *L. sakei* underartene, *sakei* og *carnosus*, med flesteparten av stammene tilhørende den sistnevnte. Mikroarray-basert komparativ genom hybridisering ved bruk av et array hovedsaklig basert på den sekvenserte stammen *L. sakei* 23K, ble også benyttet for å gruppere stammene. Dette gav den samme inndelingen som ved bruk av genetisk fingerprintmetodikk, og gav også et detaljert bilde av geninnholdet mellom teststammene sammenliknet med 23K stammen. Ved pulsfelt gelelektroforese ble genomstørrelsene beregnet til å variere fra 1.880 til 2.175 Mb, og 23K genomet var blant de minste. Følgelig hører en stor del av 23K genomet til en felles genpool for arten. De fleste av genene som er viktige for tilpasning til et liv i kjøttprodukter, som evnen til å utnytte komponenter fra kjøtt og til å overleve kjøttprosessering og lagring var bevart, noe som antyder betydningen disse genene har i nisjespesialisering. Proteomanalyse ble benyttet for å studere primærmetabolismen i de forskjellige stammene etter vekst på ribose sammenliknet med glukose, hovedsukrene som er tilgjengelig for *L. sakei* i kjøtt og fisk. Økt produksjon ble observert for proteiner som er direkte involvert i ribosenedbrytning og fosfoketolaseveien, og i pyruvat- and glycerol/glycerolipid-metabolisme. Samtidig var enzymer involvert i den glykolytiske veien mindre uttrykt. Disse funnene ble bekreftet på genekspresjonsnivå ved å bruke mikroarray. Transkripsjonsstudiene viste også at riboseutnyttelse er tett knyttet opp mot utnyttelse av nukleosider. Dessuten var enzymer som er viktige innen regulering av karbonmetabolisme og i sukkertransport indusert. Resultatene tyder på tilstedeværelse av en global regulatorisk mekanisme som finjusterer uttrykket av gener som koder for enzymer som kontrollerer effektiv utnyttelse av tilgjengelige karbonkilder.

ACKNOWLEDGEMENTS

The work with this thesis was carried out at Nofima Mat AS (former Matforsk AS), as part of the project “Molecular microbiology of Gram-positive food bacteria; functional genomics and applications”, with financial support by Grant 159058/I10 from the Norwegian Research Council.

First of all, I wish to express my gratitude to my two supervisors at Nofima Mat, Dr. Lars Axelsson and Dr. Kristine Naterstad. Thank you both for giving me excellent guidance throughout the PhD period. Your doors have always been open, something that I have really appreciated. I would also like to thank Prof. Ingolf F. Nes, as my formal supervisor at the Norwegian University of Life Sciences, and all my co-authors for your contribution and collaboration.

My colleagues at Nofima Mat, especially the microbiologists, thank you for always being helpful and for providing an inspiring and interesting working environment! It has been great working with you all. Special thanks to Tone Mari, Elin and Hanne for all the great moments we have had while sharing office and lunch breaks together.

Finally, I would like to thank all my family and friends for their encouragement. Thanks to my mother, sister and brother for your excitement for my work even though you did not fully understand what I was researching. It has been a great inspiration for me throughout the PhD period to know that my father was so very proud when I started the work. I wish he could have been here to call me Dr. McLeod. He would have loved that. Most of all, thanks to my perfect husband Roddy for all your love and support, and our amazing children Maia, Ella and Liam for brightening every single day. I love you so much, all of you!

Ås, May 2010

Anette McLeod

LIST OF PAPERS

Paper I

McLeod A, Nyquist OL, Snipen L, Naterstad K, Axelsson L. **Diversity of *Lactobacillus sakei* strains investigated by phenotypic and genotypic methods.** *Syst Appl Microbiol* 2008, **31**(5):393-403.

Paper II

McLeod A, Zagorec M, Champomier-Vergès MC, Naterstad K, Axelsson, L. **Primary metabolism in *Lactobacillus sakei* food isolates by proteomic analysis.** *BMC Microbiol* 2010, **10**(120).

Paper III

Nyquist OL, McLeod A, Brede DA, Snipen L, Aakra A, Nes IF. **Comparative genomics of *Lactobacillus sakei* with emphasis on strains from meat.** (Manuscript)

Paper IV

McLeod A, Snipen L, Naterstad K, Axelsson L. **Global transcriptome response in *Lactobacillus sakei* during growth on ribose.** (Manuscript)

1. INTRODUCTION

Throughout history lactic acid bacteria (LAB) have been associated with food fermentation, acidification and preservation. Still, LAB play a prominent role performing the main bioconversions in fermented dairy products, meats, and vegetables, and they are critical for the production of wine, coffee, silage, cocoa, sourdough, and numerous indigenous food fermentations. Thus, LAB is an industrially important group of microorganisms. Their contribution to preservation is mainly due to the production of lactic acid, which acidifies the foodstuff and thereby suppresses growth and survival of undesirable spoilage bacteria and human pathogens [1]. They also successfully compete with less favourable bacteria and thereby prevent these from using certain molecules necessary for their development. Moreover, some LAB produce antibacterial substances including bacteriocins which provide an additional hurdle [2]. The acidification as well as the lactic acid and other metabolic compounds produced by LAB contribute to product characteristics such as texture, aroma and color [3,4]. It has also become clear that LAB, besides their significance in food preservation and manufacture, are important transient or permanent inhabitants of the human gastrointestinal (GI) tract, where they may display several effects that are beneficial for human well-being [5]. Understanding the metabolic mechanisms underlying the growth performance of strains used for food fermentations is important in order to achieve high-quality, healthy and safe products. *Lactobacillus sakei* is a LAB important in food microbiology mainly due to its ability to ferment and preserve meat. The focus of this study was to compare various strains of this species and to investigate the primary metabolism, focusing on utilization of glucose and ribose, the main carbon sources available for growth of *L. sakei* on meat and fish. In the following chapter, a short status of the field will be given.

2. BACKGROUND

2.1 The *Lactobacillus* genus

LAB constitute a group of Gram-positive bacteria with low G+C content defined as acid tolerant, generally non-sporulating, non-respiring cocci or rods, which produce lactic acid as the major end product of carbohydrate fermentation [1]. A variety of industrially important genera, including *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus* and *Lactobacillus* species, are included in the group. The largest genus of LAB is the last mentioned *Lactobacillus*, which currently includes more than 150 species (http://www.bacterio.cict.fr). The natural habitat of lactobacilli ranges from diary, meat and plant material fermentations to being members of the normal microbiota of the GI tract and oral cavity of humans and animals, some with probiotic effects [6,7]. An inherent level of phenotypic variability exists between *Lactobacillus* species, and closely related species can have different morphology and metabolism, hence the taxonomy of this genus is difficult [1]. As a result, phenotypic tests such as carbohydrate-fermentation assays, usually give variable affiliations [8,9]. Furthermore, a drawback in using physiological, biochemical or related methods is that the similarities between the results obtained for strains belonging to different species hinder differentiation between the taxa [1]. To overcome this uncertainty, modern bacterial classification emphasizes molecular systematics. For routine use, soluble protein patterns, PCR-based genetic fingerprinting methods as well as sequencing genes that have not undergone extensive horizontal gene transfer, such as the 16S rRNA gene, are some of the most promising tools [1].

Among the lactobacilli, there are both aero-tolerant and anaerobic species and strains, but all are classically regarded as strictly fermentative. Two main carbohydrate fermentation pathways can be distinguished among LAB, and traditionally, lactobacilli have been divided into three groups based on their fermentation characteristics: (I) obligate homofermentative lactobacilli that use glycolysis (i.e. Embden-Meyerhof-Parnas pathway) for hexose fermentation leading essentially to lactic acid, thus termed homolactic fermentation; (II) facultative heterofermentative lactobacilli that use glycolysis for hexose fermentation and an inducible phosphoketolase pathway for pentose fermentation (pentoses act as inducers), thus they are homofermentative with regard to hexoses and heterofermentative with regard to pentoses; and (III) obligate heterofermentative lactobacilli that use the phosphoketolase pathway which leads to production of end products like acetate, ethanol and CO_2, in addition

to lactic acid, thus termed heterolactic fermentation [7,10]. However, in a subset of *Lactobacillus* species, the presence of heme and/or menaquione may stimulate aerobic respiration, leading to increased biomass without acidification [11]. In general, lactobacilli are the most acid-tolerant of the LAB [12]. Potential health effects of LAB have gained increased interest in recent decades. Consequently, published genome sequences of lactobacilli largely concern species that are associated with humans and claimed to be probiotic. Reflecting the importance of the group, public databases contain 20 complete *Lactobacillus* genomes, and about 60 genome sequencing projects are ongoing (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). Comparative analyses of the *Lactobacillus* genomes have revealed wide phylogenetic and phenotypic diversities of the different species [13-15]. The number of predicted protein-coding genes ranges from ~1725 to ~3000 between *Lactobacillus* genomes, and comparative genomics has suggested that evolusion of LAB has been driven by gain of functions by horizontal gene transfer from other LAB genomes and loss of dispensable ancestral functions [14,16]. For example, large differences were shown in genome size and gene content between the versatile *Lactobacillus* (*L.*) *plantarum* [17], which occurs in a variety of ecological niches, and the more specialized *L. acidophilus* [18] or *L. johnsonii* [19] typically occurring in the human GI tract. Niche-specific genomic adaption features has been described to be reflected in some of the *Lactobacillus* genomes [20,21]. The genome sequence of *L. sakei* [22] shows a highly specialized *Lactobacillus* which is more of a "classical" LAB as its main contribution to human quality of life is in food preservation.

2.2 *Lactobacillus sakei*

L. sakei is a facultative heterofermentative species naturally associated with the meat environment. It got its name from a Japanese alcoholic beverage made by fermenting rice (saké) which was the product where it was first isolated in the 1930s [23]. The species was not considered of particular interest until the mid 1980s, when strains of *L. sakei* and its close relative *L. curvatus* were shown to dominate spontaneous meat fermentations, the flora in vacuum-packed meat and processed meat products stored at low temperatures. Before modern classification tools for bacteria were applied, these meat strains were difficult to classify and designated as "atypical lactobacilli" [24]. Early research on *L. sakei* focused on its capabilities as a starter culture, and it became an industrially important species widely used for the manufacture of fermented sausages [22,25,26]. Although its main habitat as we know it, is meat, it has also been isolated from fermented vegetal products [27,28] and a range of fish

products [29-31], and it has great potential as a protective culture and biopreservative to extend storage life and ensure microbial safety of nonfermented meat and fish products [32-36]. Some strains have been reported as part of the spoilage flora on vacuum packed meat and fish stored at low temperatures producing various off-flavours and ropy slime [37-39]. The species has been observed as a transient inhabitant of the human GI tract [40-42], and it was recently published that mutants with modified colony morphology which had acquired modified response to various stresses that can be encountered in the GI tract, are able to colonize the intestinal environment in axenic mice [43,44]. The estimated amount of the species in human fecal samples (10^6 cfu/g of feces) that has been reported does not allow to state whether its presence is strictly food-related or not [40].

L. sakei is a robust species that resists a variety of adverse conditions, such as low temperature and pH, high salt concentration, ethanol, smoke, radiation and low water activity [24]. The 1990s saw a great effort in research on bacteriocins, small antibacterial peptides that inhibit growth of undesirable bacteria such as *Listeria monocytogenes* [2]. Various strains of *L. sakei* produce potent bacteriocins, in particular the antilisterial sakacin A and P [36,45-47]. This property is often favoured when selecting strains for biopreservation. The complete genome sequence of *L. sakei* strain 23K was published in 2005 by Chaillou et al. [22], revealing a specialized metabolic repertoire which reflects adaption to meat products, differentiating it from other LAB. The 23K strain in not a bacteriocin producer, although the genome contains genes related to the production of several bacteriocins and immunity genes that may provide an innate resistance towards bacteriocins produced by closely related bacteria [22,48]. *L. sakei* strains are known to display a range of phenotypic differences, and currently, the species is divided into two subspecies, *L. sakei* subsp. *sakei* and *L. sakei* subsp. *carnosus*, mainly based on results from numerical analyses of total cell soluble protein content and randomly amplified polymorphic DNA (RAPD) patterns [49-51].

2.2.1 General genome features

The 1884 kb genome of *L. sakei* 23K was published in 2005 and shown to contain 1883 protein/peptide coding genes [22]. After re-annotation and sequence updates in 2009, the NCBI genome database lists 1879 protein coding genes. The G+C content is 41.3%, and coding density 86%. One prophage remnant and 12 insertion sequences (IS) were identified in two regions with a high A+T content, suggested to be hotspots for genome evolution. Despite the modest genome size, seven rRNA (16S-23S-5S) operons were identified. As the number of rRNA genes has been suggested to correlate with the rate at which phylogenetically diverse

bacteria respond to resource availability, this high copy number may reflect the ability of *L. sakei* to rapidly adapt to changing environmental conditions [52,53]. Within the species, a 25% variation in genome size ranging from 1,815 kb to 2,310 kb has been reported [54], and consequently, as the 23K genome is among the smallest, the full range of existing features and potential within the species is yet to be unravelled.

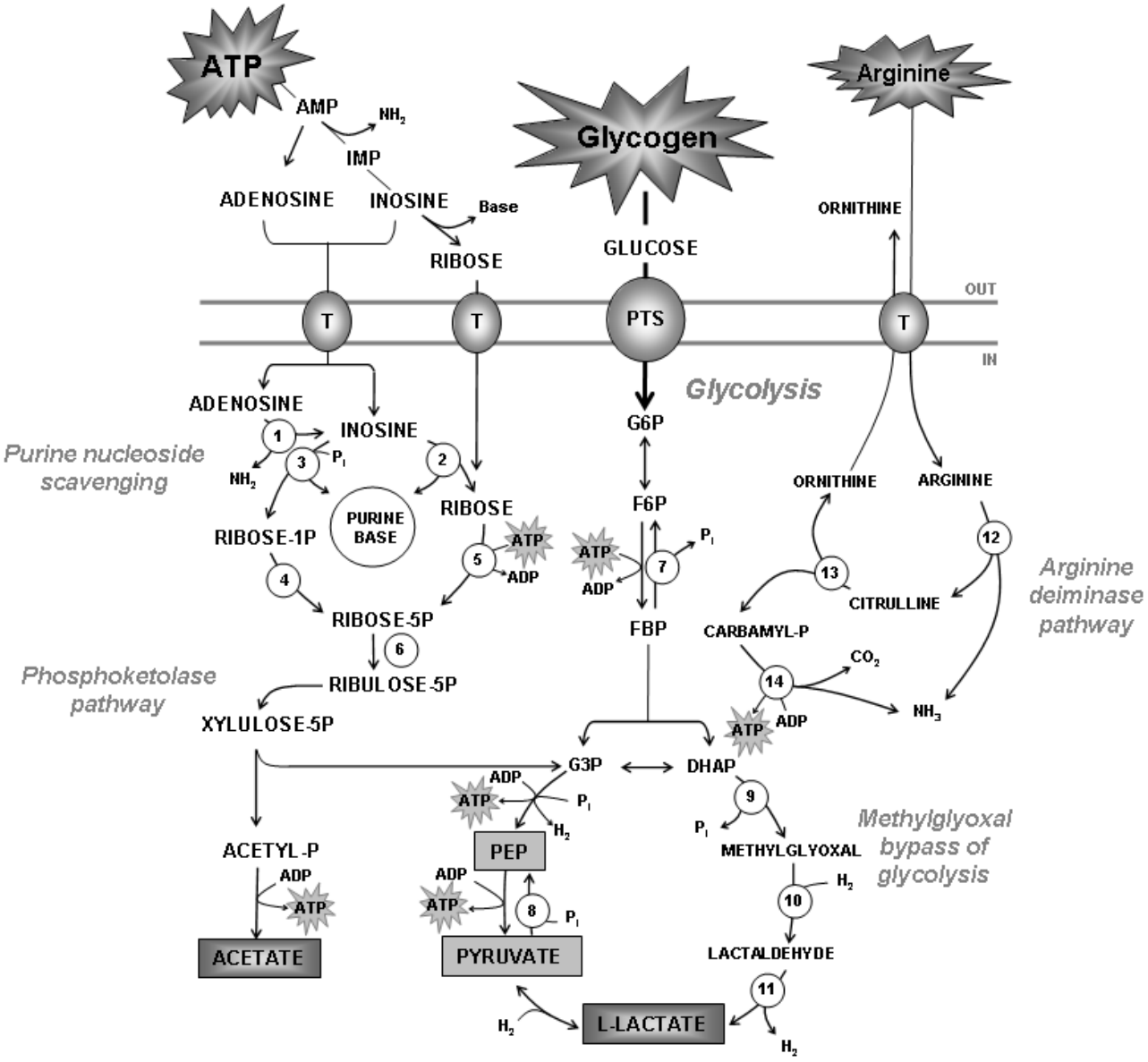
2.2.2 Metabolism and utilization of various carbon sources

L. sakei is auxotrophic for all amino acids, except aspartic and glutamic acids [29], consistent with life in the protein-rich meat environment, where during ageing, proteolytic enzymes are released from lysosomes and amino acids are liberated from meat proteins without bacterial activity. Although raw meat provides nutrients for growth, it contains limited amounts of carbohydrates. Reflecting this low diversity of carbohydrates, only six phosphoenolpyruvate (PEP)-dependent carbohydrate phosphotransferase systems (PTSs) are present in the 23K genome, and these are transporters of glucose/mannose, N-acetylglucosamine, fructose, sucrose, trehalose and cellobiose [22]. The 23K genome encodes β-galactosidase responsible for lactose catabolism, and some strains of the species have been reported to harbour phospho-β-galactosidase and a plasmid encoded lactose-specific PTS for lactose utilization [55,56]. Catabolic pathways for melibiose, gluconate, arabinose, glycerol, malate, citrate and ribose are also present in the 23K genome [22]. However, within the *L. sakei* species, a relatively wide variation of options with respect to carbohydrate fermentation has been reported to exist based on carbohydrate-fermentation abilities [9,57,58]. From the few sugars found in meat and fish, *L. sakei* can utilize mainly glucose from glycogen, and ribose released by ATP hydrolysis, a utilization biased in favour of glucose [22,59,60]. Being facultative heterofermentative, the bacterium uses glycolysis for glucose fermentation and an inducible phosphoketolase pathway for ribose fermentation [59]. The presence of a gene encoding methylglyoxal synthase (MgsA) in the 23K genome is uncommon among LAB and a unique feature among lactobacilli sequenced so far [22]. The methylglyoxal pathway represents an energetically unfavourable bypass to the glycolysis, which functions in the regulation of carbon metabolism and creates flexibility in the glycolytic process [61]. As suggested by Chaillou et al. [22], this may reflect a requirement to deal with glucose starvation and to modulate carbon flux during co-metabolism of alternative carbon sources. Sugars are rapidly exhausted in meat and, accordingly, *L. sakei* seems well suited to derive energy from other compounds which are abundant in meat. It has a well-developed potential for amino acid catabolism and can catabolize arginine, threonine and aspartate [22]. Arginine is abundant in

meat, and an arginine deiminase (ADI) pathway has been shown to enhance survival under anaerobiosis and during stationary phase [62,63]. Identification of a second putative ADI pathway in the 23K genome further support the importance of arginine catabolism [22]. Interestingly, the 23K genome contains an abundance of catabolic genes involved in exogenous nucleoside scavenging, and the ability to catabolize the nucleosides inosine and adenosine for energy has been demonstrated [22]. Catabolism of nucleosides as carbon and energy source is not common among lactobacilli [15,64], though efficient mechanisms exist in bacteria such as *Escherichia coli* [65] and *Bacillus subtilis* [66-68]. *L. sakei* 23K energy production pathways contributing to its adaption to meat is shown in Figure 1.

2.2.3 Meat adaption

Low temperatures (refrigeration; 4°C) and high salt concentrations (curing; 3-9% NaCl) are two of the most common preservative conditions employed in meat and fish processing [69]. *L. sakei* is well equipped to resist these conditions. It has more cold stress proteins than most other lactobacilli, and has the ability to efficiently accumulate osmo- and cryoprotective solutes, which could play a role in its acclimation to cold and salt. Under high salt concentrations, it is suggested to use extracellular sodium to drive substrate cotransport and export, as several Na^+/H^+ antiporters and an ATP-dependent Na^+ efflux pump was found in the 23K genome [22]. Meat surfaces are exposed to oxygen, and the ability to handle oxidative stress is important for the bacterium to be competitive. In general, LAB tolerate oxygen but grow best under nearly anaerobic conditions. *L. sakei* appears to be highly competent in coping with changing redox and oxygen levels, which typically fluctuate during meat processing, and it deals effectively with toxic byproducts. Genes encoding a heme-dependent catalase, a superoxide dismutase and a NADH oxidase is present in the 23K genome, as well as more than 30 putative oxidoreductases [22]. The genome of strain 23K also showed the presence of putative cytochromes and a mutated cytochrome P450 gene [22], the latter gene being intact in some other *L. sakei* strains [54]. This indicates that some strains may even be capable of some form of respiration, similar to what has been observed for *Lactococcus lactis* [70]. The bacterium is devoid of heme biosynthesis machinery and therefore would have to take up heme from meat. LAB are generally considered to have no iron requirement [71,72], and it was shown for *L. sakei* that iron is dispensable [73]. However the genome contains several putative iron transport systems, iron-dependent transcriptional regulators, and a heme-dependent catalase [74,75].



Figur 1. *L. sakei* 23K energy production pathways contributing to its adaption to meat. Glycogen is the main source of glucose whereas ribose is released by ATP hydrolysis. Inosine and IMP are the most abundant intermediates of ATP breakdown. Enzymes involved in nucleoside scavenging include (1) adenosine deaminase, (2) three inosine-preferring hydrolases involved in the release of the ribose moiety, and (3) three nucleoside phosphorylases producing ribose-1-phosphate. Subsequent metabolism of ribose-derived molecules for energy production include (4) phosphopentomutase, (5) two ribokinases and (6) ribose-5-phosphate isomerase. The elements of the *L. sakei* glycolytic pathway that may facilitate energy production regulation includes (7) fructose-1,6-bisphosphatase; (8) pyruvate phosphodikinase involved in the anaplerotic conversion of pyruvate to PEP (phosphoenolpyruvate) and (9) a possible methylglyoxal bypass with methylglyoxal synthase which may subsequently be reduced by a putative oxidoreductase (10) to lactaldehyde and by an aldehyde dehydrogenase (11) to lactate. The four glycolytic steps from glyceraldehyde-3-phosphate (G3P) to PEP are shown as one. The duplicated arginine deiminase pathway (ADI) pathways involved in catabolism of the secondary energy source arginine: (12) arginine and peptidyl-arginine deaminases, (13) ornithine transcarbamoylases and (14) carbamate kinases. [2H]: NADH reducing equivalent; Pi: phosphate; G6P: glucose-6-P, F6P: fructose-6-P; FBP: fructose-1,6-bisphosphate; DHAP: dihydroxyacetone phosphate; PTS: phosphotransferase system; IMP: inosine 5-P; AMP: adenosine 5-P. (revised from Chaillou et al. [22])

Moreover, iron sources present in the meat environment were shown to highly benefit *L. sakei* by sustaining a long term survival [73]. Although the bacterium seem to lack the common proteins involved in adhesion to intestinal mucus, genes encoding proteins believed to be involved in biofilm formation and cellular auto- and co-aggregation, mechanisms that allow *L. sakei* to proliferate and colonize meat surfaces, are present in the 23K genome [22]. To summarize, *L. sakei* harbours various mechanisms that allow it to thrive on meat surfaces, and that thereby may contribute to out-compete other, less desirable microorganisms in the same niche.

2.3 Primary metabolism in lactic acid bacteria (LAB)

The main goal of LAB metabolism is efficient fermentation of carbohydrates coupled to substrate level phosphorylation in order to generate energy in form of ATP needed for cell growth and maintenance [1].

2.3.1 Glycolysis and the phosphoketolase pathway in LAB

Sugars are a primary source of energy for LAB, and glucose is the preferred hexose sugar for most LAB. Mainly two metabolic pathways are employed by LAB in glucose fermentation [1]; glycolysis (i.e. Embden-Meyerhof-Parnas pathway) and the phosphoketolase pathway (Figure 2). Glucose fermentation through glycolysis leads to the production of lactate as the main end product (homolactic fermentation). The pathway consists of ten biochemical reactions (Figure 2A), where the first five reactions can be described as the energy-investment phase and the last five as the energy-generation phase. In the energy-investment phase, ATP is used to phosphorylate six-carbon sugars, before these are cleaved into two moles of triose-phosphate. Firstly, glucose is phosphorylated by the PTS (see section 2.4.1), concomitant with transport, or alternatively by the enzyme glucokinase after transport by a permease. Glucose-6-phosphate is formed and then further rearranged into fructose-6-phosphate by glucose phosphate isomerase. By the action of phosphofructokinase, another phosphate group is added to yield fructose-1,6-bisphosphate. The six-carbon sugar is cleaved by aldolase into three-carbon molecules yielding dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, which are in equilibrium with each other through the enzyme triose-phosphate isomerase. Furthermore, the energy-generation phase is characterized by the gain of two moles of pyruvate and two moles of ATP. The intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid. Triose phosphate is

converted to 1,3-bisphosphoglycerate and NADH, and further to 3-phosphoglycerate and ATP with the aid of glyceraldehyde-3-phosphate dehydrogenase and phosphoglucokinase. The remaining steps of the glycolysis involve the conversion of 3-phosphoglycerate to 2-phosphoglycerate by phosphoglyceromutase, the dehydration of 2-phosphoglycerate to PEP by enolase and conversion of PEP to pyruvate and ATP by pyruvate kinase. Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+ [1].

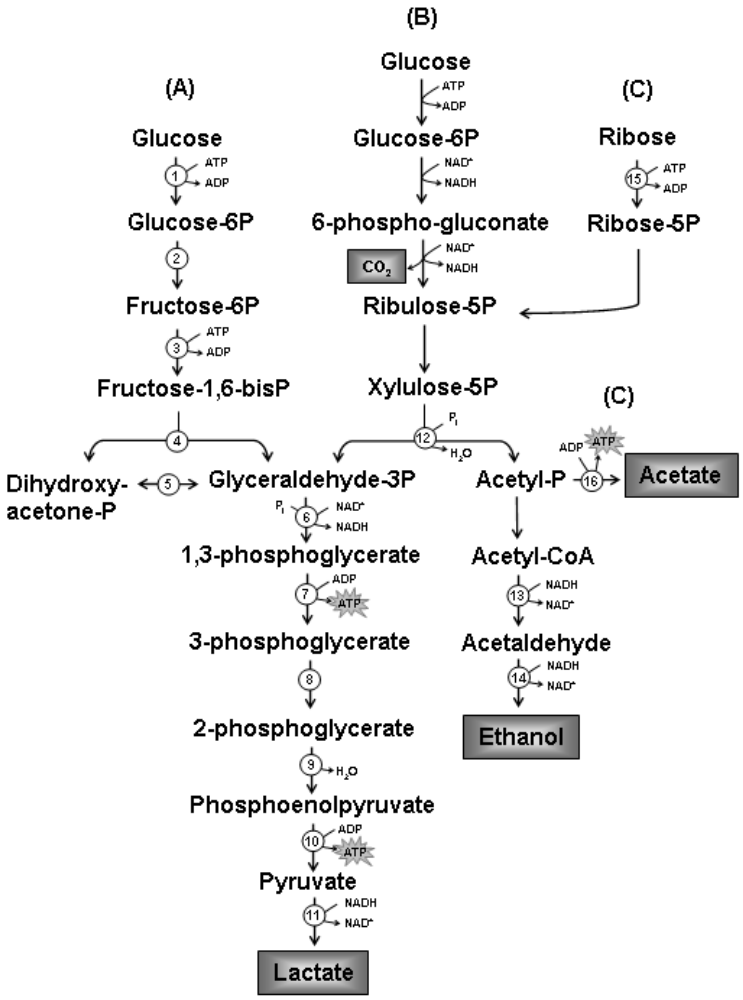


Figure 2. The major fermentation pathways of glucose and ribose. (A) Homolactic fermentation of glucose (glycolysis, Embden-Meyerhof-Parnas pathway); (B) Heterolactic fermentation of glucose (phosphoketolase pathway); (C) Heterolactic fermentation of ribose (phosphoketolase pathway). (1) Glucokinase; (2) Phosphoglucoisomerase; (3) Phosphofructokinase; (4) Fructose-1,6-bisphosphate aldolase; (5) Triosephosphate isomerase; (6) Glyceradehyde-3-phosphate dehydrogenase; (7) Phosphoglycerate kinase; (8) Phosphoglycerate mutase; (9) Enolase; (10) Pyruvate kinase; (11) Lactate dehydrogenase; (12) Phosphoketolase; (13) Acetaldehyde dehydrogenase; (14) Alcohol dehydrogenase; (15) Ribokinase; (16) Acetate kinase. P, phosphate.

The fermentation of glucose through the phosphoketolase pathway leads to the production of other end products in addition to lactate and is called heterolactic fermentation (Figure 2B). One mole glucose-6-phosphate is initially dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of CO_2 and ribulose-5-phosphate. Ribulose-5-phosphate is converted into xylulose-5-phosphate, which is further cleaved into one mole glyceraldehyde-3-phosphate and one mole acetyl-phosphate. Glyceraldehyde-3-phosphate is metabolized to lactate in the same manner as for the glycolytic pathway, whereas acetyl-phosphate is reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. In theory, end-products (including ATP) are produced in equimolar quantities from the catabolism of one mole of glucose. Hexoses other than glucose, such as fructose, mannose, and galactose are also fermented by many LAB, and these will enter the pathway at the level of glucose-6-phosphate or fructose-6-phosphate after isomerization and/or phosphorylation.

Many LAB are able to ferment pentoses, such as ribose, arabinose, xylulose and ribulose, and the phosphoketolase pathway is optimized for the fermentation of pentoses. After transport by specific permeases, they are phosphorylated and converted to ribulose-5-phosphate or xylulose-5-phosphate by epimerases or isomerases (Figure 2C). These compounds are metabolized in the lower part of the phosphoketolase pathway, though heterolactic fermentation of pentoses results in different end products compared to glucose fermented by the same pathway. This is because the dehydrogenation steps in the upper part of the pathway are not present. The reduction of acetyl-phosphate to ethanol becomes redundant, and acetyl-phosphate is instead converted to acetate and ATP by the enzyme acetate kinase. The total yield is thus one mole each of lactate and acetate, and two moles of ATP [1].

2.3.2 Glucose and ribose utilization in *L. sakei*

As mentioned above, *L. sakei* utilizes mainly glucose and ribose for its growth on meat, with glucose being the preferred sugar. A few studies have been performed regarding utilization of these two sugars in *L. sakei*. Glucose was shown to be transported and phosphorylated by the mannose-specific PTS as well as by one or more additional non-PTS permeases [59]. The central glycolytic operon, also called the *gap* operon (*cggR-gap-pgk-tpi-eno*), has been identified and characterized in *L. sakei* [76]. It encodes the four glycolytic enzymes: glyceraldehyde-3-phosphate dehydrogenase (GapA), phosphglycerate kinase (Pgk), triose-phosphate isomerase (TpiA) and enolase (Eno), as well as the central glycolytic genes

regulator (CggR). Genome sequences of several lactobacilli have shown that such operon organization of the glycolytic genes is conserved among lactobacilli, although some species lack the *cggR* or *eno* genes. [14,18,19,77,78].

The *ptsHI* operon encoding the general enzymes of the PTS, the histidine protein (HPr) and enzyme I (EI), has been characterized [79], and a unique *rbsUDKR* operon responsible for ribose utilization was described. The operon encodes a ribose transporter (RbsU), a D-ribose pyranase (RbsD), a ribokinase (RbsK), and the ribose operon transcriptional regulator (RbsR) [22,60,80]. RbsR was shown to function as a local repressor on *rbsUDK*, and as a *ptsI* mutant increased transport and phosphorylation of ribose, the PTS was suggested to negatively control ribose utilization [79]. Moreover, regulation by carbon catabolite repression (CCR) mediated by catabolite control protein A (CcpA) [81,82] has been suggested, as a putative catabolite-responsive element (*cre*) site, the binding site of CcpA, was found preceding the *rbs* operon [83].

2.3.3 Pyruvate metabolism

Pyruvate is important in both glycolysis and in the phosphoketolase pathway, where it is converted into lactate by a NAD-dependent lactate dehydrogenase, which regenerates NAD^+ and maintains the redox balance. However, alternative ways of pyruvate metabolism can be used, producing various end products such as lactate, acetate, formate, acetaldehyde, ethanol, diacetyl, acetoin, and 2,3-butanediol, and the fermentation is thus termed mixed acid production (Figure 3) [1]. Different species may use different pathways, dependent on the growth conditions and enzymatic capacity. A shift in pyruvate metabolism can benefit the bacteria by generating ATP, or by gaining NAD^+ for maintaining the redox balance. Under aerobic conditions, pyruvate oxidase induced by oxygen or hydrogen peroxide, can convert pyruvate into acetyl-phosphate and CO_2. Pyruvate formate lyase or the pyruvate dehydrogenase enzyme complex can form acetyl-phosphate and CO_2 through acetyl-CoA, and the pyruvate formate lyase can also result in the formation of formate. By converting acetyl-phosphate to acetate by an acetate kinase, the bacteria can gain ATP. Acetyl-CoA can be reduced to acetaldehyde and further to ethanol, thus providing alternative pathways for regenerating NAD^+ in addition to the reduction of diacetyl and acetoin/2,3-butanediol.

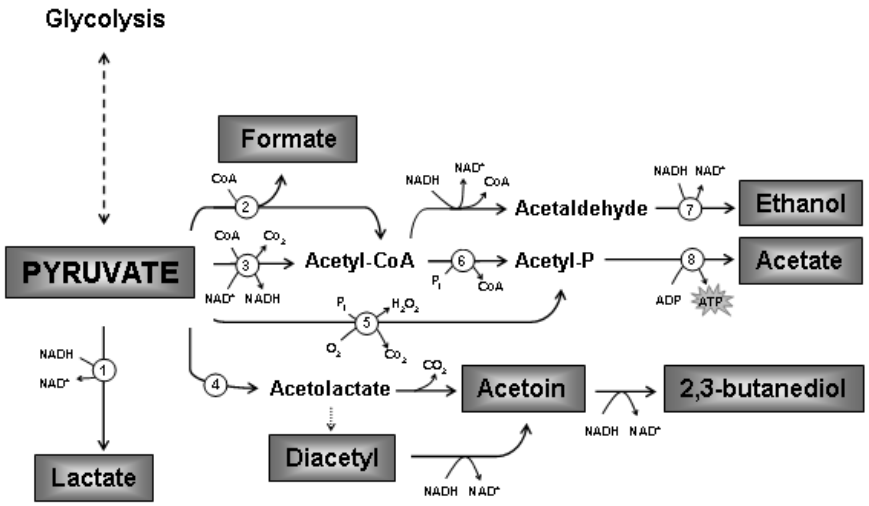


Figure 3. Pathways for alternative fates of pyruvate in LAB. Important metabolites are framed and selected enzymatic reactions are included: (1) Lactate dehydrogenase; (2) Pyruvate formate lyase; (3) Pyruvate dehydrogenase complex; (4) Acetolactate synthase; (5) Pyruvate oxidase; (6) Phosphotransacetylase; (7) Alcohol dehydrogenase; (8) Acetate kinase (8). P, phosphate.

2.3.4 Nucleoside catabolism

In bacteria, catabolism of nucleosides, when favourable carbon and energy sources are not available, may be accomplished by nucleoside transporters and of inducible catabolic enzymes for purine and pyrimidine nucleosides and for pentose phosphates [84]. Nucleoside phosphorylases catalyse the cleavage of ribonucleosides and deoxyribonucleosides to the free base plus ribose-1-phosphate or deoxyribose-1-phosphate, respectively. The bases serve anabolic (reutilization in nucleotide synthesis) or catabolic (use as nitrogen sources) functions. The ribose-1-phosphate formed from ribonucleosides can be converted to ribose-5-phosphate by phosphopentomutase and then further catabolized or converted to phosphoribosylpyrophosphate (PRPP) which is utilized in the synthesis of nucleotides. Deoxyribose-1-phosphate, formed from deoxyribonucleosides, is also a substrate for phosphopentomutase and is converted to deoxyribose-5-phosphate. Deoxyribose-5-phosphate cannot be rescued for deoxynucleotide synthesis and is degraded by deoxyriboaldolase to acetaldehyde and glyceraldehyde-3-phosphate (Figure 4). Glyceraldehyde-3-phosphate can further be catabolized to lactate through glycolysis, while acetaldehyde may be converted into acetyl-CoA [64,84].

Bacteria such as *Escherichia coli* efficiently utilize nucleosides as carbon and energy sources by degrading the pentose moiety of the nucleoside. The amino group in cytidine and adenosine may in addition be utilized as nitrogen source [65]. Many LAB have both cytidine and adenosine deaminase but the contribution of deamination of these nucleosides has been reported as difficult to estimate as the bacteria usually require many amino acids. When nucleosides have been tested as sole energy source in lactococci, no growth was observed in the absence of glucose. Therefore, the main function of the salvage pathways in LAB has been described to be for rescuing nucleobases or nucleosides for nucleotide synthesis [64]. The enzymes responsible for degradation of almost all nucleosides in *E. coli* are specified by the *deo*-operon [65], whereas in *Bacillus subtilis* and many of the low G+C Gram-positive bacteria including LAB, two separate gene clusters are present instead of this large genetic unit [64,66-68].

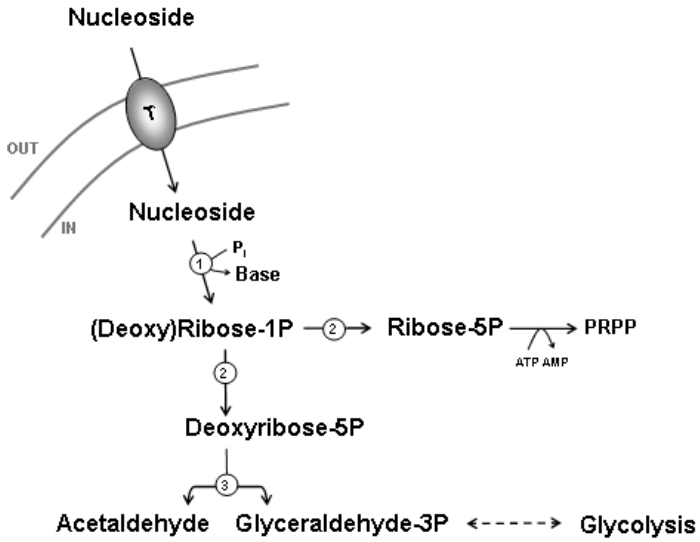


Figure 4. Salvage pathway of external nucleosides in organisms with nucleoside phosphorylases. Nucleosides are taken up directly by (T) transporters and cleaved to a nucleobase and a ribose or deoxyribose moiety by (1) purine or pyrimidine phosphorylases. Ribose-1-phosphate is converted to ribose-5-phosphate by (2) phosphopentomutase and salvaged into PRPP used in nucleotide formation. The deoxyribose-1-phosphate enters glycolysis after conversion to deoxyribose-5-phosphate by (2) phosphopentomutase to acetaldehyde and glyceraldehyde-3-phosphate by (3) deoxyribose-phosphate aldolase. P, phosphate.

2.4. Carbohydrate uptake and regulation of the primary metabolism

2.4.1 Carbohydrate uptake and the phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTSs)

The first step in the metabolism of a substrate is the transport into the cell. In bacteria, carbohydrates are transported by various mechanisms involving permeases, membrane transport proteins, which use chemical energy or an electrochemical gradient. Carbohydrates translocated by permeases are subsequently phosphorylated by ATP-dependent kinases [85]. Another pathway for carbohydrate utilization involves phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTSs), the most efficient and therefore the commonly preferred mode of transport mechanism [86,87]. The PTS is involved in transporting many carbohydrates into bacteria, including glucose, mannose, fructose and cellobiose, referred to as PTS-sugars. The PTS is a multicomponent system consisting of the general energy coupling proteins enzyme I (EI) and histidine protein (HPr), whereas enzymes II (EIIs) are the various substrate specific permeases of the PTS, composed of independent or linked EIIA, B, C and sometimes D domains. The phosphoryl group on the glycolytic intermediate PEP is eventually transferred to the imported carbohydrate via these proteins in a phosphorylation cascade. EI transfers the phosphoryl group from PEP to a conserved histidine residue (His15) of HPr. From HPr the phosphoryl group is transferred to EIIA and then EIIB which phosphorylates the incoming substrate during its translocation by the membrane-spanning EIIC (and D) [82,88]. The transfer of the phosphoryl group to the substrate once it has been imported through the membrane transporter prevents the transporter from recognizing the substrate again, and it maintains a concentration gradient that favors further import of the substrate through the transporter. A carbohydrate such as glucose will thus be phosphorylated as it passes through the plasma membrane, forming glucose-6-phosphate. The benefit of transforming it into glucose-6-phosphate is that it will not leak out of the cell, therefore providing a one-way concentration gradient of glucose. The carbohydrate specificity of the EIIs may not be absolute. For example the glucose PTS in most LAB also recognizes mannose and is therefore designated mannose PTS [89].

As a generalization, the PTS is linked to glycolysis in most bacteria. PEP holds a key position in a cycle where sugar transport is directly coupled to the subsequent metabolism, which in turn provides the PEP needed for a new cycle to begin. PEP can either donate the phosphoryl group to EI, and initiate the PTS cycle, or it can be used by pyruvate kinase to form ATP. Fructose-1,6-bisphosphate (FBP) acts as an activator and inorganic phosphate (Pi)

as inhibitor of pyruvate kinase. When glycolyzing conditions are optimal, when FBP level is high and Pi level is low, the pyruvate kinase is most active and the PEP concentration is low. As a result of limiting sugar concentration the glycolytic rate and FBP levels decrease, while the Pi level increases. Consequently, pyruvate kinase activity decreases and the concentration of PEP increases.

Carbon metabolism regulation is complex and it is an interplay between components that have roles in several contexts, connecting transport of solutes, transcriptional control, and catabolism [1,90]. In a certain environment, a bacterium needs only a subset of the enzymes encoded by the genome to propagate, and therefore the gene expression is regulated differentially. For instance, if a particular substrate is absent, the genes encoding enzymes needed for its uptake and metabolism are often repressed. Most carbon sources act as inducers for transcription of genes needed for transport and catabolism of that particular substrate, however, when provided with a mixture of substrates, this is not sufficient as a regulatory response. Several mechanisms exist for regulation of catabolic genes in Gram-positive bacteria.

2.4.2 Carbon catabolite repression (CCR)

By global transcriptional control, bacteria are able to preferentially utilize a mixture of substrates in a strict hierarchical manner in order to yield maximum profit for growth. Only enzymes necessary for utilizing the preferred substrate are synthesized, and genes necessary for catabolizing the less preferred substrates are thus repressed. In case of carbon source utilization in Gram-positive bacteria of low G+C content, this is achieved via carbon catabolite repression (CCR) [81,82,91]. During CCR, the transcription of catabolic genes is controlled by three main components: the catabolite control protein A (CcpA), a transcriptional regulator of the LacI-GalR family with both repressor and activator activities; HPr; and conserved DNA target sites termed catabolite-responsive elements (*cre*) to which CcpA may bind [81,82,91]. The HPr protein has diverse regulatory functions in carbon metabolism dependent on its phosphorylation state. The specificity of HPr binding to its different interaction partners is controlled by catalytic phosphorylation of the conserved histidine residue (His15) and the regulatory phosphorylation of a conserved serine residue (Ser46). The balance between the different forms, HPr, P-His-HPr, and P-Ser-HPr reflects the metabolic state of the cell.

Formation of P-Ser-HPr is regulated by the bifunctional enzyme HPr kinase/phosphatase (HPrK/P), which phosphorylates HPr in response to high throughput

through glycolysis, thereby high intracellular concentrations of glycolytic intermediates such as FBP, glucose-6-phosphate or fructose-1-phosphate, whereas it dephosphorylates P-Ser-HPr when the concentration of glycolytic intermediates drop [82,89,92]. This phosphorylation allows HPr to bind to CcpA and convert it into its DNA-binding-competent conformation. The *cre* sites to which it binds have the proposed consensus target sequence of 14-bp TGWNANCGNTNWCA (W: A/T, N: A/T/G/C) [93]. The *cre* site location in relation to the promoter of a gene affects whether the gene/operon is repressed or activated. Binding of CcpA to a *cre* site within the promoter or in the coding region disables initiation of transcription or leads to abortion of transcription, respectively, and repression occurs. Binding to a *cre* site upstream to the promoter region is characteristic of activation of transcription [82].

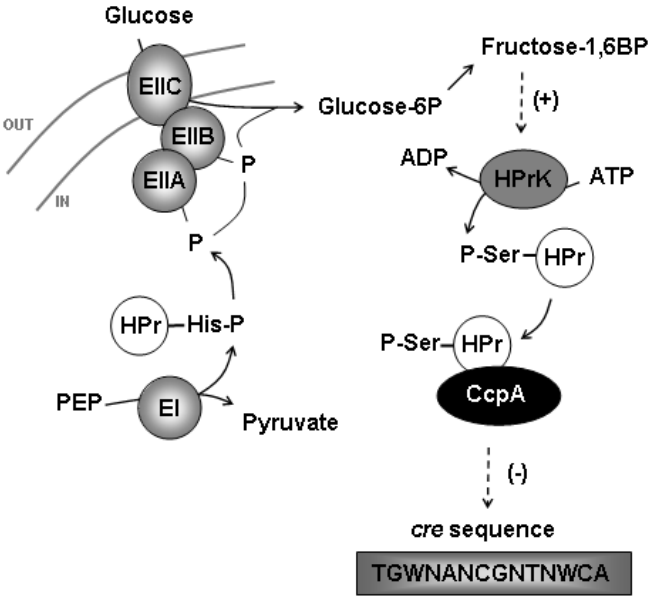


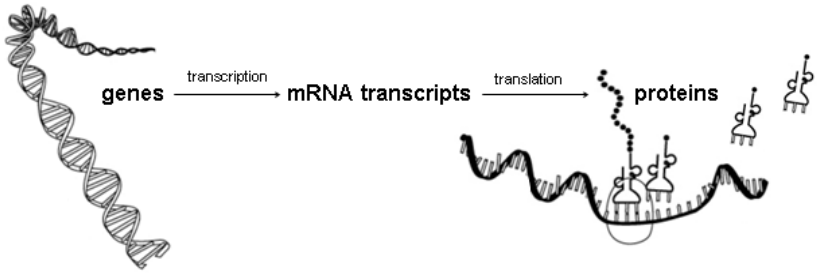
Figure 5. Schematic representation of CcpA-mediated carbon catabolite repression (CCR) pathway. Shown on the left-hand side is PTS-mediated glucose uptake in the model organism *Bacillus subtilis*. The phospho-carrier protein HPr is phosphorylated at the catalytic histidine residue by enzyme EI at the expense of phosphoenolpyruvate (PEP). The phosphoryl group is then transferred to EIIA, which is part of the multidomain complex EIIABC in *B. subtilis*. From EIIA, the phosphoryl group is transferred to EIIB, a soluble domain attached to the integral membrane transporter domain EIIC. The glucose molecule is transported into the cell and at the same time phosphorylated by EIIB, yielding glucose-6-phosphate in the cell. Shown on the right-hand side is transcriptional regulator-mediated CCR. Fructose-1,6-bisphosphate produced from glucose-6-phosphate in glycolysis activates HPrK that phosphorylates HPr at the regulatory-site serine at the expense of ATP or PPi. Binding of HPr-Ser-P to CcpA results in a complex that binds to *cre* sequence and inhibits the transcription of target genes. The HPr molecule in HPr-mediated signal transduction is the same as the HPr involved in glucose uptake (revised from Warner & Lolkema [94]).

P-Ser-HPr has also been reported to participate in inducer exclusion. It can inhibit the activity of several non-PTS permeases, thereby preventing the entry of the inducer for the corresponding catabolic operon [95-98]. Under conditions of low intracellular glucose concentrations, His15 is phosphorylated to give P-His-HPr with catalytic function in the PTS. In addition, P-His-HPr and several P-EIIBs also phosphorylate three different types of non-PTS proteins and regulate their activities. In the first type, an EIIA domain, a phosphoryl acceptor of P-His-HPr within the PTS phosphorylation cascade, or an HPr domain is fused to the target protein. These PTS proteins are not active in sugar transport, but they regulate fusion protein activity in response to their phosphorylation state [89,99]. The second type of proteins phosphorylated by P-His-HPr and P-EIIBs contains two PTS regulation domains (PRDs). A PRD domain seems to have evolved to control the RNA binding activity of transcription antiterminators and the DNA binding function of transcription activators in response to phosphorylation by PTS proteins [88,100]. PTS-controlled transcription activators also contain EIIA and EIIB domains [101]. The third type of non-PTS proteins phosphorylated by P-His-HPr is the glycerol kinase (GlpK) from low G+C Gram-positive bacteria, which becomes phosphorylated at a conserved histidyl residue [102,103]. Phosphorylation of GlpK is used to regulate glycerol uptake and metabolism in response to the presence or absence of a PTS substrate. When a rapidly metabolizable PTS sugar like glucose is present, the GlpK is dephosphorylated and less active. This regulatory mechanism can also be considered a form of PTS-mediated inducer exclusion as the presence of a PTS substrate prevents the GlpK activity.

2.5 Genomics, proteomics, transcriptomics

The suffix -ome as used in molecular biology refers to a totality of a sort, and is used to address objects such as the genome, proteome or transcriptome, studied by genomics, proteomics or transcriptomics, respectively. The word genome, formed from the two words gene and chromosome [104], is defined as the entirety of an organism's hereditary information, including both the genes and the non-coding sequences of the DNA [105]. When global properties of genomes of related organisms are studied, this is referred to as genomics, which is distinguished from genetics which generally studies the properties of single genes or groups of genes. The proteome is the entire set of proteins expressed by a genome, cell, tissue or organism. More specifically, it is the set of expressed proteins in a given type of cells or an

organism at a given time under defined conditions. The word proteome is a blend of protein and genome. While the genome of a cell is static, the proteome will vary with time and requirements, or with stresses that the cell or organism undergoes. It reflects the state of the cell and how it functions [106]. The transcriptome is the complete set of RNA transcripts produced by a genome, including mRNA, rRNA, tRNA, and non-coding RNA. It can be applied to the total set of transcripts in an organism, or to the specific subset of transcripts present in a particular cell type. Unlike the genome, which is quite fixed for a given cell line (excluding mutations), the transcriptome vary with external environmental conditions. Because it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being expressed at a given time, and the study of the transcriptome, transcriptomics, is also referred to as gene expression profiling [105].



2.5.1 Proteomic analyses

Proteomics has largely been practiced through the separation of proteins by two dimensional gel electrophoresis (2-DE), where mixtures of proteins are separated according to two independent properties in two discrete steps; isoelectric focusing (IEF), which resolves proteins on the basis of charge by their isoelectric point (pI) in the first dimension, then according to molecular weight (MW) by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension [107]. This highly sensitive technique, introduced more than 30 years ago, received renewed interest by the expanding genome sequencing projects. By 2-DE, a large amount of the proteins synthesized within a bacterial cell can be separated and visualized. The identification of large numbers of protein spots has been accommodated by developments in mass spectrometric techniques such as matrix assisted desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) [108,109] that rely on genome sequencing data. As a large number of proteins can be visualised simultaneously, 2-DE with subsequent identification of the protein spots, can be used in a

differential display format. Thus, complex biological systems may be studied in their entirety and complex relationships between proteins in the functioning cell can be discovered.

Two dimensional gel electrophoresis (2-DE)

Firstly, the proteins in the sample must be completely disaggregated, denatured and solubilised. Sample solubilisation is usually performed in a buffer containing: chaotropic agents to disrupt hydrogen and hydrophobic bonds in both proteins and water; nonionic and/or zwitterionic detergents to disrupt hydrophobic interactions; reducing agents to disrupt disulfide bonds and to maintain all proteins in their fully reduced state; carrier ampholytes that enhance sample solubility and produce a more uniform conductivity across the pH gradient during IEF. The original method for the first dimension IEF depended on carrier ampholyte-generated pH gradients in polyacrylamide tube gels [107]. However, a limitation of this method is unstable pH gradients, which reduce the reproducibility of the first dimension separation. An important improvement was the introduction of immobilized pH gradient (IPG) gel strips with fixed positions of the molecules generating the pH gradient [110]. Solubilised proteins are introduced into an IPG gel strip. The hydrated gel strip is subjected to a strong electric field and a protein will migrate to the position in the gradient where the net charge is zero. A protein with positive net charge will migrate towards the cathode, and a protein with a negative net charge will migrate towards the anode.

After IEF, the strips are equilibrated before polymerization on top of the second dimension SDS-PAGE gel. The proteins are unfolded when a reducing agent is added to disrupt disulphide bonds, and in a second step, an alkylating sulfhydryl reagent is added to prevent re-oxidation [111]. SDS disrupts hydrogen bonds, blocks hydrophobic interactions, partially unfolds the proteins, and all the proteins will be covered with negative charges. In the second dimension, an electric field is again applied, but at a 90 degree angle from the first field, causing the negatively charged proteins to move through the gel matrix depending on their molecular weight; larger proteins being retained higher in the gel and smaller proteins being able to reach lower regions of the gel.

After electrophoresis, the proteins on the gels can be visualized by staining, and several methods are available. A good method should have low cost, high sensitivity, high linear dynamic range, as well as being compatible with MS [112]. The most common gel stains are silver and Coomassie brilliant blue (CBB). The latter dye is low cost, easy to use and compatible with MS. Silver staining is 10-100 times more sensitive, but MS compatibility is an issue. This method was introduced as an adaption of photo development to protein

straining for 2-DE [113]. Either silver nitrate is used in combination with formaldehyde developer in alkaline carbonate buffer or using an ammonia-silver complex in combination with formaldehyde developer in citrate buffer. Silver ions are bound to the proteins in the gel and reduced to metallic silver which give a black/brown color. The development reaction of silver staining has to be stopped, requiring the user to decide when the gels are optimally stained based on visual inspection, making reproducibility a challenge. A number of fluorescent stains are available, such as SYPRO Ruby, Larva Purple, Flamingo and Krypton [114], and cyanine-based dyes (Cy2, Cy3 and Cy5) can be used in difference gel electrophoresis (DIGE) [115,116].

Image and data analysis

The 2-DE gels are scanned and transformed into digital images before being imported into an analysis program. Several commercial software packages are available for image processing and statistical analysis [117]. A set of images are aligned so that information can be matched across all the gel images, the protein spots are detected, background noise and artefacts are eliminated, and the detected protein spots are quantified. Applying the same spot outline across the image series adresses the problem of missing values and reduces variance in spot volume across biological or technical replicates [118,119]. The statistical approaches used in the data analysis often include multivariate analysis tools such as principal component analysis (PCA) to visualize the main variation in the data set, and classical analysis of variance (ANOVA) in which the purpose is to test for significant changes between means. As multiple responses are common in experiments, alternatives of classical ANOVA is often required. Classical multivariate ANOVA (MANOVA) is often used in cases where more than one dependent variable exists, and where the dependent variables cannot be combined. However this method does not perform optimal in cases with several highly correlated responses. 50-50 MANOVA is a method which can handle this problem [120,121].

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

Matrix-assisted laser desorption/ionization (MALDI) is an ionization technique used in mass spectrometry, which allows the analysis of biomolecules. The sample is embodied into a solid matrix on a target plate and allowed to co-crystallize. This matrix protects the sample from being destroyed by direct laser beam and it facilitates vaporization and ionization. The laser is fired at the crystals and the sample is ionized and transferred from the condensed phase to gas phase. The vaporized and ionized sample molecules are transferred elecrostatically into a

mass analyzer where they are separated from the matrix ions and detected based on their mass-to-charge (m/z) ratios [108]. Time-of-flight (TOF) is a mass analyzer which uses the differences in transit time through a drift region to separate ions of different masses [109]. The MALDI-TOF is used successfully for identification of proteins and peptides, and the most common identification technique is peptide mass fingerprinting (PMF) [122]. The unknown protein is cleaved into short peptides by the enzyme Trypsin, and the absolute masses of the peptides are accurately measured with the mass spectrometer. A list of molecular weights, often called a peak list, is created for database searching. The measured peptide masses are matched against sequence databases which contain protein sequence information. Software programs theoretically cut the proteins into peptides with the same enzyme used in the chemical cleavage and calculate the absolute masses of the peptides from each protein. A comparison is made between the peak list of measured peptide masses and all the masses from the calculated peptides. The results are statistically analyzed to find the best match. A disadvantage of MALDI-TOF MS is that the protein sequence is not obtained; though by tandem mass spectrometry (MS/MS) with multiple steps of mass analysis, protein sequence data can be produced. The MS/MS contains two analyzers, e.g. TOF/TOF [123]. However, both molecular weight, experimental and theoretical pI values, the number of peptide matches, as well as sequence coverage are used to evaluate the database search results, and the protein identification is greatly facilitated in organisms for which the genomes has been sequenced [123].

2.5.2 Microarray technology

The development of DNA microarrays [124] has in the past decade led to a shift from studies of expression of individual genes to the analysis of thousands of genes in one experiment. A DNA microarray consists of an arrayed series of thousands of microscopic spots attached to predetermined positions on a solid support such as a microscope slide, where each spot contains one or more single-stranded DNA oligonucleotide fragment (probe) of a specific DNA sequence [125]. This probe can be a short section of a gene or other DNA element and is used to hybridize a DNA or cDNA sample under high-stringency conditions. Target sequences can be labeled in different ways [126], but the two fluorophores Cy3 and Cy5 are widely used. DNA from two samples are labeled with the different fluorophores and co-hybridized to the microarray slide. After non-specific bound targets are washed away, the signal intensity of each fluorophore is in principle proportional to the amount of target sequence. The number of targets bound to their respective probes is quantified by scanning

the microarrays and measuring the fluorescence emitted by the hybridized labeled targets when excited by the light from the lasers. Microarrays have many applications. Gene expression profiling is the most common application in microbiology, followed by comparative genome hybridizations (CGH) [127].

Gene expression/transcription profiling

In a gene expression/transcription profiling experiment, the expression levels of thousands of genes are simultaneously monitored to study for example the effects of certain treatments, different growth phases, or wild type versus mutant, on gene expression. In this technique the RNA in a tissue or organism is isolated and converted to labeled cDNA, which is hybridized to the fragments on the array. If the probes on the microarray represent the complete genome sequence of a microorganism, the global gene expression profile, or transcriptome, can then be obtained. The transcriptome is a dynamic entity that reflects the organism's ongoing response to its environment, and important steps therefore include knowledge about growth conditions and growth rate, as well as accurate sample preparations. Especially the rapid mRNA turnover has a large impact on transcript levels. Moreover, control of RNA quality, measurement of amount of cDNA produced and labelling efficiency are important in quality insurance of the final results. To repeat the experiment is crucial, and to chose an appropriate statistical analysis is important [128,129].

Comparative genome hybridization (CGH)

Microarray-based CGH is now a commonly used tool in comparative genomics, and whole-genome DNA microarrays are used in microbiology to assess genomic diversity between e.g. different bacterial strains [130-132]. The purpose of such analysis is to detect highly divergent or absent genes in a test strain compared to a reference strain. This field of application requires the labeling of chromosomal DNA that is to be hybridized with the array. If genes are present in both strains, the corresponding probe will yield a signal from both fluorophores, whereas when the gene is absent in one of the strains, the signal from the fluorophore with which it was labeled, will be missing. CGH has the advantage of being a fast way to gain information about differences in the genome of different bacterial strains. The disadvantage of the method is that knowledge can only be gained about which genes are divergent in a test strain compared to the reference strain, and no knowledge about the genes unique for the test strains [133]. However, it is possible to add more genomes to one microarray slide, and the

use of pan-genome arrays will allow better characterization of new strains and can provide insights into phylogenetic relationships between strains [134].

Microarray data analysis

Before statistical analysis of the hybridization data, image analysis is performed which includes localization of the spots, manual filtering to ensure that the spots are properly located, and spots should be excluded on the basis of slide or morphology abnormalities. Normalization is then performed to adjust the microarray data for effects that arise from variation in the technology [135]. This corrects for systematic biases resulting from different amounts of RNA/DNA used for labeling, different incorporation efficiencies of the Cy3 and Cy5 dyes, and different detection efficiencies of the dyes. Different kinds of replication are distinguished. The same probe should be spotted multiple times on each array, which provides a backup in case a spot cannot be evaluated due to technical artefacts. The RNA/DNA that has been prepared from one biological experiment should be labelled and hybridized several times, and a dye switch/swap should be included as there are gene-specific dye effects. Finally, biological replicates should be included [127,136]. Different statistical methods have been developed to analyze microarray data. For gene expression, microarray results are challenging due to the complex datasets consisting of a large number of regulated genes compared to relatively few experiments. Mixed model approaches, which accommodate for both fixed and random effects from the microarray experiment, are often used. Effects modelled as fixed effects are typically those we are interesting in finding, e.g. effects of certain treatments. Random effects are for example the effect of the array used and variation between replicate spots, which are different types of noise [137,138]. The methods are used to test whether genes are expressed differentially at a certain significance level by providing p-value. The p-values are further adjusted to control the false discovery rate (FDR) [139]. Concerning microarray data from CGH experiments, different approaches exist for the predictions of presence and divergence/absence of genes. The common analysis of CGH data focuses on the log-ratios, which can be described as $\log_2(T_i/R_i)$ where T_i is the signal intensity of the test strain and R_i similar for the reference strain, for gene i. To rank the genes by log-ratios, one approach is to choose a cutoff log-ratio, and then classify the genes into divergent or present either manually or by statistics calculated from the log-ratio distribution [140]. However, as this approach is a paradigm inherited from expression studies, a recently developed method was proposed which computes a predicted sequence identity between each probe and the test strain genome based on the array signals and sequence identity between

probes and the reference genome. An identity score is chosen which is used for discriminating present from divergent, i.e. if the score 0.7 is chosen, probes with more than 70% identity with the genome are assumed to give hybridization signals, and are thus defined as present [141].

2.5.3 Genetic fingerprinting

High-resolution genotypic techniques such as randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) are reportedly two of the most discriminatory techniques applied in strain typing [142,143]. The techniques are often used to generate species-specific electrophoretic profiles e.g. when investigating genetic diversity between *Lactobacillus* species, and are also used to investigate the presence of distinct sub-groups within species [49,50,144-146]. RAPD is a type of PCR reaction where low-stringency hybridization conditions with a single random oligonucleotide primer are used. The segments of DNA that are amplified are random, and the discriminatory power of this analysis is affected by the primer used. The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. Firstly restriction enzymes are used to cut genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments are then amplified using primers complementary to the adaptor and part of the restriction site fragments. RAPD has lower discriminatory power than AFLP, but gives the fastest typing results with the least hands-on time. Both methods can be performed with the ease of direct computational analysis if an automatic sequencer analyzes the gels. Computer-assisted analysis of the electrophoretic profiles is a useful tool.

Another genetic fingerprinting technique applied in strain typing is pulsed field gel electrophoresis (PFGE), which is essentially the comparison of large genomic DNA fragments after digestion with a restriction enzyme. The procedure for this technique is relatively similar to performing a standard gel electrophoresis, though instead of constantly running the voltage in one direction, the voltage is periodically switched among three directions; one that runs through the central axis of the gel and two that run at an angle of 120 degrees either side, allowing separation of larger pieces of DNA than conventional agarose gel electrophoresis. In addition to its application in strain typing, this technique is used to estimate bacterial chromosome size [38,147].

3. OBJECTIVES OF THIS STUDY

The main objectives of the work presented in this thesis were to study the diversity among various food isolates, and to increase the knowledge of the primary metabolism in *L. sakei*.

This work includes the following tasks:

- Characterize the phenotypic and genotypic diversity among various *L. sakei* food isolates, and create a base for selecting interesting strains for further studies.
- Compare the gene content of various *L. sakei* strains to the sequenced *L. sakei* strain 23K, and identify the common gene pool invariant among the *L. sakei* species, as well as define regions of the 23K chromosome where variation is prominent and contribute to genetic diversity in the species.
- Identify differentially expressed proteins in various *L. sakei* food isolates during growth on ribose compared with glucose.
- Examine the global transcriptome response of different *L. sakei* isolates during growth on ribose compared with glucose, and identify differentially expressed genes.
- Predict the frequency of *cre* sites presumed to be involved in CCR, and to define if a global regulation mechanism is acting during ribose catabolism in *L. sakei*.

4. MAIN RESULTS AND DISCUSSIONS

LAB play an important role in production, preservation, and improvement of food, and *L. sakei* is among the important food-born LAB frequently isolated from fresh or fermented meat. The importance of this species in the meat industry has significantly increased in the last years due to its technological properties optimal for fermentation of sausages [22,148] and its potential use as bioprotective cultures for biopreservation of meat and fish [32-35]. Moreover, the fact that the bacterium was detected in human feces [40] and a recent publication reports mutant strains able to colonize the GI tract of axenic mice [43,44], will likely increase the interest of this species even more in the years to come. Knowledge of metabolic mechanisms underlying the growth performance of strains to be used for food fermentations is both of a fundamental as well as of applied importance. Investigations of the primary metabolism of this species, and defining differences between various isolates in traits important for the use in food were objectives in this work. Phenotypic, genotypic and genomic diversity presented among various *L. sakei* food isolates were investigated in Papers I and III. The results in these papers gave us a detailed view of the gene content of various strains compared to the sequenced *L. sakei* strain 23K, confirmed the existence of the two genetic subgroups within the species, and revealed phenotypic heterogeneity. A base for selecting interesting strains for further studies of the metabolism of the species was also provided. In Papers II and IV, proteomic and transcriptomic analyses were used to study the primary metabolism of selected strains after growth on ribose compared to glucose. The methods proved to be very useful for unraveling details of the catabolic pathways involved.

Paper I describes the investigation of ten food isolates of *L. sakei* by phenotypic and genotypic methods. The growth characteristics from various complex media, carbohydrate-fermentation abilities, as well as acidification properties tested in a meat model were demonstrated to vary between isolates. The results agree with previous studies in reporting phenotypic heterogeneity within the species [29]. A commercial starter culture strain, LS 25, showed the fastest growth rates on all the media, including media with glucose and ribose as carbohydrate sources, and together with a strain isolated from fish, MF1053, it fermented the highest number of carbohydrates. Together with another starter culture strain (MF1328) and a protective culture strain (MF1058), it also showed fast acid production in a meat model system [149], at the same time as being able to compete with the indigenous microbiota of the meat batter. The variation in growth rates and acidification properties implicates that some strains are more suited as starter or protective cultures than others. Fast growth on the

available carbon sources, the ability to produce sufficient amounts of lactic acid and to dominate the fermentation process are important traits for strains to be used as a starter or a protective culture in food [24]. The ability to ferment a variety of carbohydrates could be advantegous on some substrates, although the bacterium mainly utilizes glucose and ribose for growth on meat [59,60]. The division of *L. sakei* into two subspecies has mainly been based on results from numerical analyses of total cell soluble protein content and randomly amplified polymorphic DNA (RAPD) patterns [49-51]. We analyzed the ten strains by RAPD and introduced the application of amplified fragment length polymorphism (AFLP) for clustering the strains. A distinction between two genetic groups was obvious, consistent with the species division into *L. sakei* subsp. *sakei* and *L. sakei* subsp. *carnosus* [50,51], with the majority of the strains belonging to the latter [54]. With the genome sequence of *L. sakei* strain 23K available [22], an oligonucleotide set representing the 23K genes and in addition other sequenced *L. sakei* genes, has been constructed (http://migale.jouy.inra.fr/sakei/Supplement.html/). By applying DNA microarray technology, whole-genome comparisons to the sequenced strain were performed. We introduced the microarray-based comparative genome hybridization (CGH) technique for clustering the strains and showed how the same division into two genetic groups was obtained.

Microarray-based CGH was conducted for a larger set of *L. sakei* strains including nine of the strains studied in Paper I, and in Paper III a detailed view, where the gene content of 18 strains was compared with the gene content of the 23K strain, are shown. By using pulsed field gel electrophoresis (PFGE), genome sizes were estimated to vary from 1.880 to 2.175 Mb, of which the 23K genome was among the smallest. Consequently, a large part of the 23K genome belongs to a common gene pool invariant among the species. The majority of genes suggested to be important in adaption to meat products, the ability to flexibly use meat components, and robustness during meat processing and storage [22], were conserved, indicative of the role these genes play in niche specialization within the species. All the strains carried remnants of or complete bacteriocin operons, strongly indicating that bacteriocin-associated genes are, or have been, important in the evolution of the species, and as previously suggested may give the strains a competitive advantage in its environment [22,48]. The genome sequence of strain 23K is currently the only complete genome available of the species, and it is among the smallest of this species [54]. Consequently, we know little about the genomic divergence among strains with larger genomes. Chromosome sizes within the species have been reported to be up to 2,310 kb, and PCR detection of variable genes in a flexible gene pool has indicated that a wide genomic variation exists between strains [54].

Within the *Lactobacillales*, wide phylogenetic and functional diversity has been described, which is mediated by extensive gene loss and horizontal gene transfer of genes from other LAB genomes [14]. A considerable part of the genomic divergence was shown to correspond to five distinct regions in the 23K genome. The regions showed lower G+C content than that of the rest of the genome, which could be indicative of horizontally transferred genes [150,151]. In addition, foreign DNA fragments from integrative elements such as phages and plasmids and their associated transposons were represented. Clustering of the strains in Paper III correlated in general with the clustering obtained in Paper I, although minor differences were observed. In the two papers, different data analysis methods of the CGH results were used, which resulted in more of the genes with uncertain status in Paper I being defined as present in Paper III. Finally, the genetic groups (Papers I and III) did not correlate with clustering based on carbohydrate-fermenting abilities (Paper I) or with chromosomal sizes (Papers I and III).

For the purpose of investigating the primary metabolism of the species, we used a proteomic approach to compare growth on ribose with glucose for the ten strains described in Paper I. A correlation between glucose and ribose metabolism in *L. sakei* has been been suggested, which could be advantegous in increasing its competitiveness with the other microbial flora on meat [29,59,60]. Indeed, increased growth rates similar to those when the bacterium grows in a defined medium [152] with glucose only (DMLG), was observed when adding small amounts of glucose to the ribose medium (DMLRg). In Paper II, differentially expressed proteins caused by the change of carbon source from glucose (in DMLG) to ribose (in DMLRg) were identified. In correlation with previous observations [60,80], enzymes directly involved in ribose catabolism including a ribokinase encoded from the *rbsK* gene, as well as the xylulose-5-phosphate phosphoketolase (Xpk) important in the phosphoketolase pathway, were over-expressed on ribose in all the strains, while glycolytic enzymes showed a lower expression. The starter culture strain LS 25 and the protective culture strain MF1058, showed a lower expression of several glycolytic enzymes as well as the L-lactate dehydrogenase, than the other strains, and we suggested that these strains might have the ability to down-regulate the glycolytic pathway more efficiently than the rest of the strains when grown on ribose. Enzymes involved in pyruvate and glycerol/glycerolipid metabolism showed an over-expression on ribose in all the strains when comparing to growth on glucose. The MF1053 strain showed a high expression of stress related proteins growing on both carbon sources compared to the other strains. The overall 2-DE protein expression pattern was similar for the different strains, although distinct differences were seen between the two

subspecies in the migration pattern of the glyceraldehyde-3-phosphate dehydrogenase (GapA) isoforms, as also previously reported [54]. In addition, in accordance with previous observations, a variation of about 20% in the number of spots in the 2-DE gels was observed between strains [54].

For further investigation of the primary metabolism of *L. sakei*, we selected the three strains that we found most interesting from the proteomic analysis in Paper II: the sequenced strain 23K, the commercial starter culture strain LS 25, and MF1053 isolated from fish. In Paper IV we used the same microarray as for the CGH analysis in Papers I and III to examine the global transcriptome response during growth on ribose compared with glucose in these three strains. The increased expression observed for proteins directly involved in ribose catabolism and the phosphoketolase pathway, as well as glycerol/glycerolipid metabolism and alternative fates of pyruvate (Paper II), were confirmed at the level of gene expression. Also the simultanous down-regulation of the glycolytic pathway (Paper II) was obvious, and genes involved in metabolism of glucose were repressed. Several genes involved in transport and metabolism of various carbon sources were up-regulated, as was the gene encoding the methylglyoxal synthase, an enzyme uncommon among LAB and so far unique for *L. sakei* among lactobacilli. A possible glycolytic methylglyoxal bypass, which is energetically unfavourable [61], was previously suggested to reflect a requirement to deal with glucose starvation and to modulate carbon flux during co-metabolism of alternative carbon sources [22]. However, as the methylglyoxal produced is toxic to the cell [153], and the important detoxification process was not obvious, it is not clear if this bypass is actually functional in *L. sakei*. The transcriptome data showed that ribose catabolism is closely linked with catabolism of nucleosides. Two gene clusters involved in nucleoside catabolism including *lsa0254* encoding a second ribokinase, were strongly induced. By CGH it was shown in Paper III that the *lsa0254* gene was present in all the strains investigated, whereas the *rbsK* gene was divergent in some strains. The ribokinase encoded by *lsa0254* could function as the main ribokinase in some strains. Interestingly, *deoR* encoding the deoxyribonucleoside synthesis operon transcriptional regulator, DeoR, with sigma (σ) factor activity was the only strongly up-regulated transcriptional regulator gene in all three strains. We can only speculate whether this regulator could function as activator of transcription of some of the regulated genes. In the meat environment, ribose as well as nucleosides, are products of the degradation of the organic materials DNA, RNA and ATP. The simultaneous catabolism of ribose and nucleosides allows the bacterium to access the different substrates simultaneously as carbon and energy source. Moreover, *hprK* encoding the HPr kinase/phosphatase (HPrK/P), which

plays a major role in the regulation of carbon metabolism and sugar transport, was strongly up-regulated, as was genes encoding the general PTS enzyme I, and the mannose-specific enzyme II complex (EII^{man}) which encode glucose uptake. We predicted the frequency of *cre* sites [93] presumed to be involved in CCR [89] during ribose catabolism, and these were frequently found in proximity to the promoter of several genes and operons affected by the change of carbon source. It seems likely that a global CcpA-mediated CCR mechanism permit a fine tuning of the expression of enzymes that control the efficient exploitation of available carbon sources in *L. sakei*. Moreover, the EII^{man} is possibly indirectly involved in this regulation during growth on ribose, as observed in other lactobacilli [154-156].

5. CONCLUSIONS AND FUTURE WORK

The ability of *L. sakei* to ferment meat and fish is related to the capacity of the bacterium to utilize the available carbohydrates and other components for growth. It also needs to successfully compete with the microbial flora present, and to survive the challenges it is exposed to. The importance of a strains metabolic repertoire and its ability to cope with food processing and storage conditions stimulates research aiming at understanding its mechanisms. Moreover, to define diversity within the species is important, with the ultimate goal to be able to select proper strains for the purpose needed, or possibly improve strains. The work presented in this thesis has provided new knowledge about the primary metabolism in *L. sakei* as well as described differences between various strains of the species.

A large selection of methods was used to study the diversity of the species, including phenotypic, genotypic, genomic, proteomic and transcriptomic approaches, and the results may contribute to a framework for selection of strains with optimal properties for its specific applications. It is obvious from the data obtained that the proteomic and transcriptomic approaches efficiently identified proteins and genes differentially expressed between growth on the two carbohydrates which *L. sakei* utilizes from meat and fish. We were able to demonstrate on both protein and gene expression level how *L. sakei* ferments glucose through glycolysis and shifts to use the phosphoketolase pathway for catabolism of ribose. Despite the basic similarity in the strains metabolic routes during fermentation of the two different carbohydrates, there were also interesting differences, especially in the alternative fates of pyruvate, which consequently may lead to variation in the end products between strains. Further studies, including experiments to measure the metabolites, intermediates, as well as end-products in these pathways from various strains, are needed to exploit the information provided in this work. Some discrepancy was seen between the proteome and transcriptome results. Whereas strain LS 25 on the protein level seemed to have the ability to down-regulate the glycolytic pathway more efficiently than other strains during growth on ribose, this was not confirmed by the transcriptome data. Also the higher expression of stress related proteins in MF1053 during growth on both carbohydrates compared to the other strains could not be confirmed by transcriptomics. Further experiments are needed to look into this discrepancy. Real time quantitative PCR could be useful to validate the expression levels for selected genes. The ribose uptake and catabolic machinery was highly regulated at the transcription level, and a global regulatory mechanism was revealed. The involvement of CcpA and EII^{man} in the regulation of expression of enzymes that control exploitation of available carbon

sources could be further investigated through mutants affected in their phosphorylation sites, or knock-out mutants. CcpA binding to putative *cre* sites could identify target genes which are subject to CCR.

Simultanous ribose and nucleoside catabolism seem to benefit *L. sakei*. Further investigations of the DeoR transcriptional regulator with σ factor activity seem to be important to reveal more about the nucleoside catabolism. Experiments including e.g. knock-out mutants of the *deoR* gene or over-expression of its gene product could increase the knowledge about the function of this regulator, and promoter binding experiments could identify target genes which it regulates. A co-regulation between genes involved in both ribose and nucleoside catabolism exists in other bacterial species [157,158], and further experiments could reveal if similar mechanisms of co-control exists in *L. sakei*. Both ribokinase genes (*rbsK* and *lsa0254*) were present in most of the *L. sakei* strains that were investigated, whereas in some strains the *rbsK* gene seemed divergent. Knocking out *lsa0254* could reveal the importance of this ribokinase gene. Comparing the growth on ribose for various strains which harbour different combinations of the ribokinase genes, e.g. '*rbsK* and *lsa0254*' versus 'only *lsa054*' versus '*rbsK* and *lsa0254* knock-out', could reveal if one is more preferred than the other for growth on ribose. Furthermore, the induced *mgsA* gene leads us to speculate whether the possible methylglyoxal bypass pathway is functional during growth on ribose, and if the pathway could benefit some strains under certain conditions. Comparing the transcriptome response after growth on ribose compared to glucose between strains which do not possess the *mgsA* gene to strains that does, might answer these questions.

Application of whole-genome microarrays to study *L. sakei* is in its infancy. Because being among the genetically smallest ones, the 23K strain seems to be a suitable model organism, and the microarray based on this strain is useful for studying the core features of the *L. sakei* species. However, more sequences of larger genomes will hopefully be available in the close future, as a wider genomic representation is needed to unravel the existing features and potential within the *L. sakei* species.

6. REFERENCES

1. Axelsson L: **Lactic acid bacteria: classification and physiology.** In *Lactic acid bacteria: microbiological and functional aspects.* Third revised and expanded edition. Edited by Salminen S, von Wright A, Ouwehand A. New York, USA: Marcel Dekker, Inc./CRC Press; 2004: 1-66
2. Eijsink VG, Axelsson L, Diep DB, Havarstein LS, Holo H, Nes IF: **Production of class II bacteriocins by lactic acid bacteria; an example of biological warfare and communication.** *Antonie Van Leeuwenhoek* 2002, **81:**639-654.
3. Marshall VM: **Lactic acid bacteria: starters for flavour.** *FEMS Microbiol Rev* 1987, **46:**327-336.
4. Olson NF: **The impact of lactic acid bacteria on cheese flavor.** *FEMS Microbiol Rev* 1990, **87:**131-147.
5. Ouwehand AC, Salminen S, Isolauri E: **Probiotics: an overview of beneficial effects.** *Antonie Van Leeuwenhoek* 2002, **82:**279-289.
6. Vaughan EE, de Vries MC, Zoetendal EG, Ben-Amor K, Akkermans AD, de Vos WM: **The intestinal LABs.** *Antonie Van Leeuwenhoek* 2002, **82:**341-352.
7. Hammes WP, Vogel RF: **The genus *Lactobacillus*.** In *The genera of lactic acid bacteria. Volume* 2. Edited by Wood BJB, Hilzapfel WH. Glasgow, UK: Blackie Academic, Professional; 1995: 19-54
8. Boyd MA, Antonio MA, Hillier SL: **Comparison of API 50 CH strips to whole-chromosomal DNA probes for identification of *Lactobacillus* species.** *J Clin Microbiol* 2005, **43:**5309-5311.
9. Nigatu A: **Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate *Lactobacillus plantarum, Lact. fermentum, Lact. rhamnosus, Lact. sake, Lact. parabuchneri, Lact. gallinarum, Lact. casei, Weissella* minor and related taxa isolated from kocho and tef.** *J Appl Microbiol* 2000, **89:**969-978.
10. Kleerebezem M, Hols P, Bernard E, Rolain T, Zhou M, Siezen RJ, Bron PA: **The extracellular biology of the lactobacilli.** *FEMS Microbiol Rev* 2010, **34:**199-230.
11. Brooijmans R, Smit B, Santos F, van Riel J, de Vos WM, Hugenholtz J: **Heme and menaquinone induced electron transport in lactic acid bacteria.** *Microb Cell Fact* 2009, **8:**28.
12. Kashket ER: **Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance.** *FEMS Microbiol Rev* 1987, **46:**233-244.

13. Claesson MJ, van Sinderen D, O'Toole PW: **The genus *Lactobacillus* - a genomic basis for understanding its diversity.** *FEMS Microbiol Lett* 2007, **269:**22-28.
14. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, Pavlov A, Pavlova N, Karamychev V, Polouchine N, et al: **Comparative genomics of the lactic acid bacteria.** *Proc Natl Acad Sci U S A* 2006, **103:**15611-15616.
15. Canchaya C, Claesson MJ, Fitzgerald GF, van Sinderen D, O'Toole PW: **Diversity of the genus *Lactobacillus* revealed by comparative genomics of five species.** *Microbiology* 2006, **152:**3185-3196.
16. Pfeiler EA, Klaenhammer TR: **The genomics of lactic acid bacteria.** *Trends Microbiol* 2007, **15:**546-553.
17. Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R, Tarchini R, Peters SA, Sandbrink HM, Fiers MW, et al: **Complete genome sequence of *Lactobacillus plantarum* WCFS1.** *Proc Natl Acad Sci U S A* 2003, **100:**1990-1995.
18. Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, Souther N, Dobson A, Duong T, Callanan M, et al: **Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM.** *Proc Natl Acad Sci U S A* 2005, **102:**3906-3912.
19. Pridmore RD, Berger B, Desiere F, Vilanova D, Barretto C, Pittet AC, Zwahlen MC, Rouvet M, Altermann E, Barrangou R, et al: **The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533.** *Proc Natl Acad Sci U S A* 2004, **101:**2512-2517.
20. Cai H, Thompson R, Budinich MF, Broadbent JR, Steele JL: **Genome Sequence and Comparative Genome Analysis of *Lactobacillus casei*: Insights into Their Niche-Associated Evolution.** *Genome Biol Evol* 2009, **2009:**239-257.
21. O'Sullivan O, O'Callaghan J, Sangrador-Vegas A, McAuliffe O, Slattery L, Kaleta P, Callanan M, Fitzgerald GF, Ross RP, Beresford T: **Comparative genomics of lactic acid bacteria reveals a niche-specific gene set.** *BMC Microbiol* 2009, **9:**50.
22. Chaillou S, Champomier-Verges MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V, Beaufils S, Darbon-Rongere E, Bossy R, Loux V, Zagorec M: **The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K.** *Nat Biotechnol* 2005, **23:**1527-1533.
23. Katagiri H, Kitahara K, Fukami K: **The characteristics of the lactic acid bacteria isolated from moto, yeast mashes for sake manufacture. Part IV. Classification of**

the lactic acid bacteria. *Bulletin of Agricultural and Chemical Society of Japan* 1934, **10:**156–157.

24. Axelsson L, Ahrné S: **Lactic acid bacteria.** In *Applied microbial systematics.* Edited by Priest FG, Goodfellow M. Dordrechet, The Netherlands: Kluwer Academic Press; 2000: 365-386
25. Hammes WP, Bantleon A, Min S: **Lactic acid bacteria in meat fermentation.** *FEMS Microbiol Rev* 1990, **87:**165-174.
26. Hammes WP, Hertel C: **New developments in meat starter cultures.** *Meat Science* 1998, **49:**125-138.
27. Vogel RF, Lohmann M, Nguyen M, Weller AN, Hammes WP: **Molecular characterization of *Lactobacillus curvatus* and *Lact. sake* isolated from sauerkraut and their application in sausage fermentations.** *J Appl Bacteriol* 1993, **74:**295-300.
28. Klaenhammer T, Altermann E, Arigoni F, Bolotin A, Breidt F, Broadbent J, Cano R, Chaillou S, Deutscher J, Gasson M, et al: **Discovering lactic acid bacteria by genomics.** *Antonie Van Leeuwenhoek* 2002, **82:**29-58.
29. Champomier-Verges MC, Chaillou S, Cornet M, Zagorec M: **Erratum to "*Lactobacillus sakei*: recent developments and future prospects".** *Res Microbiol* 2002, **153:**115-123.
30. Leroi F, Joffraud JJ, Chevalier F, Cardinal M: **Study of the microbial ecology of cold-smoked salmon during storage at 8 degrees C.** *Int J Food Microbiol* 1998, **39:**111-121.
31. Lyhs U, Bjorkroth J, Korkeala H: **Characterisation of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping.** *Int J Food Microbiol* 1999, **52:**77-84.
32. Katikou P, Georgantelis D, Paleologos EK, Ambrosiadis I, Kontominas MG: **Relation of biogenic amines' formation with microbiological and sensory attributes in *Lactobacillus*-inoculated vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets.** *J Agric Food Chem* 2006, **54:**4277-4283.
33. Vermeiren L, Devlieghere F, Debevere J: **Evaluation of meat born lactic acid bacteria as protective cultures for biopreservation of cooked meat products.** *Int J Food Microbiol* 2004, **96:**149-164.

34. Bredholt S, Nesbakken T, Holck A: **Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat.** *Int J Food Microbiol* 1999, **53:**43-52.

35. Bredholt S, Nesbakken T, Holck A: **Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats.** *Int J Food Microbiol* 2001, **66:**191-196.

36. Katla T, Moretro T, Sveen I, Aasen IM, Axelsson L, Rorvik LM, Naterstad K: **Inhibition of *Listeria monocytogenes* in chicken cold cuts by addition of sakacin P and sakacin P-producing *Lactobacillus sakei*.** *J Appl Microbiol* 2002, **93:**191-196.

37. Nilsson L, Gram L, Huss HH: **Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora.** *J Food Prot* 1999, **62:**336-342.

38. Bjorkroth J, Ridell J, Korkeala H: **Characterization of *Lactobacillus sake* strains associating with production of ropy slime by randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) patterns.** *Int J Food Microbiol* 1996, **31:**59-68.

39. Bjorkroth J, Korkeala H: **rRNA gene restriction patterns as a characterization tool for *Lactobacillus sake* strains producing ropy slime.** *Int J Food Microbiol* 1996, **30:**293-302.

40. Dal Bello F, Walter J, Hammes WP, Hertel C: **Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition.** *Microb Ecol* 2003, **45:**455-463.

41. Heilig HG, Zoetendal EG, Vaughan EE, Marteau P, Akkermans AD, de Vos WM: **Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA.** *Appl Environ Microbiol* 2002, **68:**114-123.

42. Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP: **Detection of *Lactobacillus, Pediococcus, Leuconostoc,* and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis.** *Appl Environ Microbiol* 2001, **67:**2578-2585.

43. Chiaramonte F, Anglade P, Baraige F, Gratadoux JJ, Langella P, Champomier-Verges MC, Zagorec M: **Analysis of *Lactobacillus sakei* mutants selected after adaptation to the gastrointestinal tract of axenic mice.** *Appl Environ Microbiol* 2010.

44. Chiaramonte F, Blugeon S, Chaillou S, Langella P, Zagorec M: **Behavior of the meat-borne bacterium *Lactobacillus sakei* during its transit through the gastrointestinal tracts of axenic and conventional mice.** *Appl Environ Microbiol* 2009, **75:**4498-4505.

45. Eijsink VG, Brurberg MB, Middelhoven PH, Nes IF: **Induction of bacteriocin production in *Lactobacillus sake* by a secreted peptide.** *J Bacteriol* 1996, **178:**2232-2237.

46. Axelsson L, Holck A: **The genes involved in production of and immunity to sakacin A, a bacteriocin from *Lactobacillus sake* Lb706.** *J Bacteriol* 1995, **177:**2125-2137.

47. Holck A, Axelsson L, Birkeland SE, Aukrust T, Blom H: **Purification and amino acid sequence of sakacin A, a bacteriocin from *Lactobacillus sake* Lb706.** *J Gen Microbiol* 1992, **138:**2715-2720.

48. Moretro T, Naterstad K, Wang E, Aasen IM, Chaillou S, Zagorec M, Axelsson L: **Sakacin P non-producing *Lactobacillus sakei* strains contain homologues of the sakacin P gene cluster.** *Res Microbiol* 2005, **156:**949-960.

49. Berthier F, Ehrlich SD: **Genetic diversity within *Lactobacillus sakei* and *Lactobacillus curvatus* and design of PCR primers for its detection using randomly amplified polymorphic DNA.** *Int J Syst Bacteriol* 1999, **49:**997-1007.

50. Torriani S, Van Reenen GA, Klein G, Reuter G, Dellaglio F, Dicks LM: ***Lactobacillus curvatus* subsp. *curvatus* subsp. nov. and *Lactobacillus curvatus* subsp. *melibiosus* subsp. nov. and *Lactobacillus sake* subsp. *sake* subsp. nov. and *Lactobacillus sake* subsp. *carnosus* subsp. nov., new subspecies of *Lactobacillus curvatus* Abo-Elnaga and Kandler 1965 and *Lactobacillus sake* Katagiri, Kitahara, and Fukami 1934 (Klein et al. 1996, emended descriptions), respectively.** *Int J Syst Bacteriol* 1996, **46:**1158-1163.

51. Klein G, Dicks LMT, Pack A, Hack B, Zimmerman K, Dellaglio F, Reuter G: **Emended description of *Lactobacillus sake* (Katahiri, Katahara and Fukami) and *Lactobacillus curvatus* (Abo-Elnega Kandler): Numerical classification revealed by protein fingerprinting and identification based on biochemical patterns and DNA–DNA hybridizations.** *Int J Syst Bacteriol* 1996, **46:**367-376.

52. Condon C, Liveris D, Squires C, Schwartz I, Squires CL: **rRNA operon multiplicity in *Escherichia coli* and the physiological implications of *rrn* inactivation.** *J Bacteriol* 1995, **177:**4152-4156.

53. Klappenbach JA, Dunbar JM, Schmidt TM: **rRNA operon copy number reflects ecological strategies of bacteria.** *Appl Environ Microbiol* 2000, **66:**1328-1333.

54. Chaillou S, Daty M, Baraige F, Dudez AM, Anglade P, Jones R, Alpert CA, Champomier-Verges MC, Zagorec M: **Intraspecies genomic diversity and natural population structure of the meat-borne lactic acid bacterium *Lactobacillus sakei*.** *Appl Environ Microbiol* 2009, **75:**970-980.

55. Obst M, Hehn R, Vogel RF, Hammes WP: **Lactose metabolism in *Lactobacillus curvatus* and *Lactobacillus sake*.** *FEMS Microbiol Lett* 1992, **97:**209-214.

56. Garriga M, Hugas M, Aymerich T, Monfort JM: **Bacteriocinogenic activity of lactobacilli from fermented sausages.** *J Appl Bacteriol* 1993, **75:**142-148.

57. Koort J, Vandamme P, Schillinger U, Holzapfel W, Bjorkroth J: ***Lactobacillus curvatus* subsp. *melibiosus* is a later synonym of *Lactobacillus sakei* subsp. *carnosus*.** *Int J Syst Evol Microbiol* 2004, **54:**1621-1626.

58. McLeod A, Nyquist OL, Snipen L, Naterstad K, Axelsson L: **Diversity of *Lactobacillus sakei* strains investigated by phenotypic and genotypic methods.** *Syst Appl Microbiol* 2008, **31:**393-403.

59. Lauret R, Morel-Deville F, Berthier F, Champomier-Verges M, Postma P, Ehrlich SD, Zagorec M: **Carbohydrate utilization in *Lactobacillus sake*.** *Appl Environ Microbiol* 1996, **62:**1922-1927.

60. Stentz R, Cornet M, Chaillou S, Zagorec M: **Adaption of *Lactobacillus sakei* to meat: a new regulatory mechanism of ribose utilization?** *INRA, EDP Sciences* 2001, **81:**131-138.

61. Yomano LP, York SW, Shanmugam KT, Ingram LO: **Deletion of methylglyoxal synthase gene (*mgsA*) increased sugar co-metabolism in ethanol-producing *Escherichia coli*.** *Biotechnol Lett* 2009, **31:**1389-1398.

62. Zuniga M, Champomier-Verges M, Zagorec M, Perez-Martinez G: **Structural and functional analysis of the gene cluster encoding the enzymes of the arginine deiminase pathway of *Lactobacillus sake*.** *J Bacteriol* 1998, **180:**4154-4159.

63. Champomier Verges MC, Zuniga M, Morel-Deville F, Perez-Martinez G, Zagorec M, Ehrlich SD: **Relationships between arginine degradation, pH and survival in *Lactobacillus sakei*.** *FEMS Microbiol Lett* 1999, **180:**297-304.

64. Kilstrup M, Hammer K, Ruhdal Jensen P, Martinussen J: **Nucleotide metabolism and its control in lactic acid bacteria.** *FEMS Microbiol Rev* 2005, **29:**555-590.

65. Hammer-Jespersen K: **Nucleoside catabolism.** In *Metabolism of nucleotides, nucleosides and nucleobases in microorganisms.* Edited by Munch-Petersen A. Copenhagen: Academic press Inc; 1983: 203-258

66. Schuch R, Garibian A, Saxild HH, Piggot PJ, Nygaard P: **Nucleosides as a carbon source in *Bacillus subtilis*: characterization of the *drm-pupG* operon.** *Microbiology* 1999, **145:**2957-2966.

67. Zeng X, Saxild HH: **Identification and characterization of a DeoR-specific operator sequence essential for induction of *dra-nupC-pdp* operon expression in *Bacillus subtilis*.** *J Bacteriol* 1999, **181:**1719-1727.

68. Zeng X, Saxild HH, Switzer RL: **Purification and characterization of the DeoR repressor of *Bacillus subtilis*.** *J Bacteriol* 2000, **182:**1916-1922.

69. Leistner L: **Basic aspects of food preservation by hurdle technology.** *Int J Food Microbiol* 2000, **55:**181-186.

70. Duwat P, Sourice S, Cesselin B, Lamberet G, Vido K, Gaudu P, Le Loir Y, Violet F, Loubiere P, Gruss A: **Respiration capacity of the fermenting bacterium *Lactococcus lactis* and its positive effects on growth and survival.** *J Bacteriol* 2001, **183:**4509-4516.

71. Weinberg ED: **The *Lactobacillus* anomaly: total iron abstinence.** *Perspect Biol Med* 1997, **40:**578-583.

72. Bruyneel B, Vanderwoestyne M, Verstraete W: **Lactic acid bacteria - Microorganisms able to grow in the absence of iron and copper.** *Biotechnol Lett* 1989, **11:**401-406.

73. Duhutrel P, Bordat C, Wu TD, Zagorec M, Guerquin-Kern JL, Champomier-Verges MC: **Iron sources used by the nonpathogenic lactic acid bacterium *Lactobacillus sakei* as revealed by electron energy loss spectroscopy and secondary-ion mass spectrometry.** *Appl Environ Microbiol* 2010, **76:**560-565.

74. Knauf HJ, Vogel RF, Hammes WP: **Cloning, sequence, and phenotypic expression of *katA*, which encodes the catalase of *Lactobacillus sake* LTH677.** *Appl Environ Microbiol* 1992, **58:**832-839.

75. Hertel C, Schmidt G, Fischer M, Oellers K, Hammes WP: **Oxygen-dependent regulation of the expression of the catalase gene *katA* of *Lactobacillus sakei* LTH677.** *Appl Environ Microbiol* 1998, **64:**1359-1365.

76. Naterstad K, Rud I, Kvam I, Axelsson L: **Characterisation of the *gap* operon from *Lactobacillus plantarum* and *Lactobacillus sakei*.** *Curr Microbiol* 2007, **54:**180-185.

77. Callanan M, Kaleta P, O'Callaghan J, O'Sullivan O, Jordan K, McAuliffe O, Sangrador-Vegas A, Slattery L, Fitzgerald GF, Beresford T, Ross RP: **Genome sequence of *Lactobacillus helveticus*, an organism distinguished by selective gene loss and insertion sequence element expansion.** *J Bacteriol* 2008, **190:**727-735.

78. van de Guchte M, Penaud S, Grimaldi C, Barbe V, Bryson K, Nicolas P, Robert C, Oztas S, Mangenot S, Couloux A, et al: **The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution.** *Proc Natl Acad Sci U S A* 2006, **103:**9274-9279.

79. Stentz R, Lauret R, Ehrlich SD, Morel-Deville F, Zagorec M: **Molecular cloning and analysis of the *ptsHI* operon in *Lactobacillus sake*.** *Appl Environ Microbiol* 1997, **63:**2111-2116.

80. Stentz R, Zagorec M: **Ribose utilization in *Lactobacillus sakei*: analysis of the regulation of the *rbs* operon and putative involvement of a new transporter.** *J Mol Microbiol Biotechnol* 1999, **1:**165-173.

81. Stulke J, Hillen W: **Carbon catabolite repression in bacteria.** *Curr Opin Microbiol* 1999, **2:**195-201.

82. Titgemeyer F, Hillen W: **Global control of sugar metabolism: a gram-positive solution.** *Antonie Van Leeuwenhoek* 2002, **82:**59-71.

83. Rodionov DA, Mironov AA, Gelfand MS: **Transcriptional regulation of pentose utilisation systems in the *Bacillus/Clostridium* group of bacteria.** *FEMS Microbiol Lett* 2001, **205:**305-314.

84. Tozzi MG, Camici M, Mascia L, Sgarrella F, Ipata PL: **Pentose phosphates in nucleoside interconversion and catabolism.** *Febs J* 2006, **273:**1089-1101.

85. Poolman B: **Transporters and their roles in LAB cell physiology.** *Antonie Van Leeuwenhoek* 2002, **82:**147-164.

86. Saier MH, Jr., Reizer J: **The bacterial phosphotransferase system: new frontiers 30 years later.** *Mol Microbiol* 1994, **13:**755-764.

87. Erni B: **Coupling of energy to glucose transport by the bacterial phosphotransferase system.** *Res Microbiol* 1990, **141:**360-364.

88. Postma PW, Lengeler JW, Jacobson GR: **Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria.** *Microbiol Rev* 1993, **57:**543-594.

89. Deutscher J, Francke C, Postma PW: **How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria.** *Microbiol Mol Biol Rev* 2006, **70:**939-1031.

90. Reizer J, Saier MH, Jr., Deutscher J, Grenier F, Thompson J, Hengstenberg W: **The phosphoenolpyruvate:sugar phosphotransferase system in gram-positive bacteria: properties, mechanism, and regulation.** *Crit Rev Microbiol* 1988, **15:**297-338.

91. Muscariello L, Marasco R, De Felice M, Sacco M: **The functional *ccpA* gene is required for carbon catabolite repression in *Lactobacillus plantarum*.** *Appl Environ Microbiol* 2001, **67:**2903-2907.

92. Schumacher MA, Allen GS, Diel M, Seidel G, Hillen W, Brennan RG: **Structural basis for allosteric control of the transcription regulator CcpA by the phosphoprotein HPr-Ser46-P.** *Cell* 2004, **118:**731-741.

93. Weickert MJ, Chambliss GH: **Site-directed mutagenesis of a catabolite repression operator sequence in *Bacillus subtilis*.** *Proc Natl Acad Sci U S A* 1990, **87:**6238-6242.

94. Warner JB, Lolkema JS: **CcpA-dependent carbon catabolite repression in bacteria.** *Microbiol Mol Biol Rev* 2003, **67:**475-490.

95. Ye JJ, Saier MH, Jr.: **Allosteric regulation of the glucose:H+ symporter of *Lactobacillus brevis*: cooperative binding of glucose and HPr(ser-P).** *J Bacteriol* 1995, **177:**1900-1902.

96. Ye JJ, Saier MH, Jr.: **Cooperative binding of lactose and the phosphorylated phosphocarrier protein HPr(Ser-P) to the lactose/H+ symport permease of *Lactobacillus brevis*.** *Proc Natl Acad Sci U S A* 1995, **92:**417-421.

97. Dossonnet V, Monedero V, Zagorec M, Galinier A, Perez-Martinez G, Deutscher J: **Phosphorylation of HPr by the bifunctional HPr Kinase/P-ser-HPr phosphatase from *Lactobacillus casei* controls catabolite repression and inducer exclusion but not inducer expulsion.** *J Bacteriol* 2000, **182:**2582-2590.

98. Viana R, Monedero V, Dossonnet V, Vadeboncoeur C, Perez-Martinez G, Deutscher J: **Enzyme I and HPr from *Lactobacillus casei*: their role in sugar transport, carbon catabolite repression and inducer exclusion.** *Mol Microbiol* 2000, **36:**570-584.

99. Reizer J, Schneider B, Reizer A, Saier MH, Jr.: **A hybrid response regulator possessing a PEP-dependent phosphorylation domain.** *Microbiology* 1999, **145:**987-989.

100. Stulke J, Arnaud M, Rapoport G, Martin-Verstraete I: **PRD-a protein domain involved in PTS-dependent induction and carbon catabolite repression of catabolic operons in bacteria.** *Mol Microbiol* 1998, **28:**865-874.

101. Greenberg DB, Stulke J, Saier MH, Jr.: **Domain analysis of transcriptional regulators bearing PTS regulatory domains.** *Res Microbiol* 2002, **153:**519-526.

102. Deutscher J, Bauer B, Sauerwald H: **Regulation of glycerol metabolism in *Enterococcus faecalis* by phosphoenolpyruvate-dependent phosphorylation of glycerol kinase catalyzed by enzyme I and HPr of the phosphotransferase system.** *J Bacteriol* 1993, **175:**3730-3733.

103. Charrier V, Buckley E, Parsonage D, Galinier A, Darbon E, Jaquinod M, Forest E, Deutscher J, Claiborne A: **Cloning and sequencing of two enterococcal *glpK* genes and regulation of the encoded glycerol kinases by phosphoenolpyruvate-dependent, phosphotransferase system-catalyzed phosphorylation of a single histidyl residue.** *J Biol Chem* 1997, **272:**14166-14174.

104. Winkler H: *Verbreitung und ursache der parthenogenesis im pflanzen - und tierreiche.* Jena, Germany: Verlag von Gustav Fischer; 1920.

105. Brown TA: *Genomes 2.* 2nd edn. Oxford, UK: BIOS Scientific Publishers Ltd; 2002.

106. James P: **Protein identification in the post-genome era: the rapid rise of proteomics.** In *Quarterly reviews of biophysics. Volume* 30: Cambridge University Press; 1997: 279-331

107. O'Farrell PH: **High resolution two-dimensional electrophoresis of proteins.** *J Biol Chem* 1975, **250:**4007-4021.

108. Hillenkamp F, Karas M, Beavis RC, Chait BT: **Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers.** *Anal Chem* 1991, **63:**1193A-1203A.

109. Guilhaus M, Selby D, Mlynski V: **Orthogonal acceleration time-of-flight mass spectrometry.** *Mass Spectrom Rev* 2000, **19:**65-107.

110. Bjellqvist B, Pasquali C, Ravier F, Sanchez JC, Hochstrasser D: **A nonlinear wide-range immobilized pH gradient for two-dimensional electrophoresis and its definition in a relevant pH scale.** *Electrophoresis* 1993, **14:**1357-1365.

111. Gorg A, Obermaier C, Boguth G, Weiss W: **Recent developments in two-dimensional gel electrophoresis with immobilized pH gradients: wide pH gradients up to pH 12, longer separation distances and simplified procedures.** *Electrophoresis* 1999, **20:**712-717.

112. Gorg A, Weiss W, Dunn MJ: **Current two-dimensional electrophoresis technology for proteomics.** *Proteomics* 2004, **4:**3665-3685.

113. Merril CR, Switzer RC, Van Keuren ML: **Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain.** *Proc Natl Acad Sci U S A* 1979, **76:**4335-4339.

114. Ball MS, Karuso P: **Mass spectral compatibility of four proteomics stains.** *J Proteome Res* 2007, **6:**4313-4320.

115. Unlu M, Morgan ME, Minden JS: **Difference gel electrophoresis: a single gel method for detecting changes in protein extracts.** *Electrophoresis* 1997, **18:**2071-2077.

116. Alban A, David SO, Bjorkesten L, Andersson C, Sloge E, Lewis S, Currie I: **A novel experimental design for comparative two-dimensional gel analysis: two-dimensional difference gel electrophoresis incorporating a pooled internal standard.** *Proteomics* 2003, **3:**36-44.

117. Marengo E, Robotti E, Antonucci F, Cecconi D, Campostrini N, Righetti PG: **Numerical approaches for quantitative analysis of two-dimensional maps: a review of commercial software and home-made systems.** *Proteomics* 2005, **5:**654-666.

118. Faergestad EM, Rye M, Walczak B, Gidskehaug L, Wold JP, Grove H, Jia X, Hollung K, Indahl UG, Westad F, et al: **Pixel-based analysis of multiple images for the identification of changes: a novel approach applied to unravel proteome patters of 2-D electrophoresis gel images.** *Proteomics* 2007, **7:**3450-3461.

119. Karp NA, Feret R, Rubtsov DV, Lilley KS: **Comparison of DIGE and post-stained gel electrophoresis with both traditional and SameSpots analysis for quantitative proteomics.** *Proteomics* 2008, **8:**948-960.

120. Langsrud Ø: **50-50 multivariate analysis of variance for collinear responses.** *The statistician* 2002, **51:**305-317.

121. Langsrud Ø, Jørgensen R, Ofstad R, Næs T: **Analyzing designed experiments with multiple responses.** *J Appl Stat* 2007, **34:**1275-1296.

122. Yates JR, 3rd, Speicher S, Griffin PR, Hunkapiller T: **Peptide mass maps: a highly informative approach to protein identification.** *Anal Biochem* 1993, **214:**397-408.

123. Suckau D, Resemann A, Schuerenberg M, Hufnagel P, Franzen J, Holle A: **A novel MALDI LIFT-TOF/TOF mass spectrometer for proteomics.** *Anal Bioanal Chem* 2003, **376:**952-965.

124. Wodicka L, Dong H, Mittmann M, Ho MH, Lockhart DJ: **Genome-wide expression monitoring in *Saccharomyces cerevisiae*.** *Nat Biotechnol* 1997, **15:**1359-1367.

125. Harrington CA, Rosenow C, Retief J: **Monitoring gene expression using DNA microarrays.** *Curr Opin Microbiol* 2000, **3:**285-291.

126. Lockhart DJ, Winzeler EA: **Genomics, gene expression and DNA arrays.** *Nature* 2000, **405:**827-836.

127. Ehrenreich A: **DNA microarray technology for the microbiologist: an overview.** *Appl Microbiol Biotechnol* 2006, **73:**255-273.

128. Rhodius VA, LaRossa RA: **Uses and pitfalls of microarrays for studying transcriptional regulation.** *Curr Opin Microbiol* 2003, **6:**114-119.

129. Conway T, Schoolnik GK: **Microarray expression profiling: capturing a genome-wide portrait of the transcriptome.** *Mol Microbiol* 2003, **47:**879-889.

130. Siezen RJ, Tzeneva VA, Castioni A, Wels M, Phan HT, Rademaker JL, Starrenburg MJ, Kleerebezem M, Molenaar D, van Hylckama Vlieg JE: **Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches.** *Environ Microbiol* 2009.

131. Molenaar D, Bringel F, Schuren FH, de Vos WM, Siezen RJ, Kleerebezem M: **Exploring *Lactobacillus plantarum* genome diversity by using microarrays.** *J Bacteriol* 2005, **187:**6119-6127.

132. Aakra A, Nyquist OL, Snipen L, Reiersen TS, Nes IF: **Survey of genomic diversity among *Enterococcus faecalis* strains by microarray-based comparative genomic hybridization.** *Appl Environ Microbiol* 2007, **73:**2207-2217.

133. Dorrell N, Hinchliffe SJ, Wren BW: **Comparative phylogenomics of pathogenic bacteria by microarray analysis.** *Curr Opin Microbiol* 2005, **8:**620-626.

134. Willenbrock H, Hallin PF, Wassenaar TM, Ussery DW: **Characterization of probiotic *Escherichia coli* isolates with a novel pan-genome microarray.** *Genome Biol* 2007, **8:**R267.

135. Smyth GK, Speed T: **Normalization of cDNA microarray data.** *Methods* 2003, **31:**265-273.

136. Lee ML, Kuo FC, Whitmore GA, Sklar J: **Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridizations.** *Proc Natl Acad Sci U S A* 2000, **97:**9834-9839.

137. Smyth GK: **Linear models and empirical bayes methods for assessing differential expression in microarray experiments.** *Stat Appl Genet Mol Biol* 2004, **3:**Article3.

138. Wernisch L, Kendall SL, Soneji S, Wietzorrek A, Parish T, Hinds J, Butcher PD, Stoker NG: **Analysis of whole-genome microarray replicates using mixed models.** *Bioinformatics* 2003, **19:**53-61.

139. Pawitan Y, Michiels S, Koscielny S, Gusnanto A, Ploner A: **False discovery rate, sensitivity and sample size for microarray studies.** *Bioinformatics* 2005, **21:**3017-3024.

140. Snipen L, Repsilber D, Nyquist L, Ziegler A, Aakra A, Aastveit A: **Detection of divergent genes in microbial aCGH experiments.** *BMC Bioinformatics* 2006, **7:**181.

141. Snipen L, Nyquist OL, Solheim M, Aakra A, Nes IF: **Improved analysis of bacterial CGH data beyond the log-ratio paradigm.** *BMC Bioinformatics* 2009, **10:**91.

142. Savelkoul PH, Aarts HJ, de Haas J, Dijkshoorn L, Duim B, Otsen M, Rademaker JL, Schouls L, Lenstra JA: **Amplified-fragment length polymorphism analysis: the state of an art.** *J Clin Microbiol* 1999, **37:**3083-3091.

143. Speijer H, Savelkoul PH, Bonten MJ, Stobberingh EE, Tjhie JH: **Application of different genotyping methods for *Pseudomonas aeruginosa* in a setting of endemicity in an intensive care unit.** *J Clin Microbiol* 1999, **37:**3654-3661.

144. Aymerich T, Martin B, Garriga M, Vidal-Carou MC, Bover-Cid S, Hugas M: **Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages.** *J Appl Microbiol* 2006, **100:**40-49.

145. Nigatu A, Ahrne S, Molin G: **Randomly amplified polymorphic DNA (RAPD) profiles for the distinction of *Lactobacillus* species.** *Antonie Van Leeuwenhoek* 2001, **79:**1-6.

146. Torriani S, Clementi F, Vancanneyt M, Hoste B, Dellaglio F, Kersters K: **Differentiation of *Lactobacillus plantarum, L. pentosus* and *L. paraplantarum* species by RAPD-PCR and AFLP.** *Syst Appl Microbiol* 2001, **24:**554-560.

147. Dudez AM, Chaillou S, Hissler L, Stentz R, Champomier-Verges MC, Alpert CA, Zagorec M: **Physical and genetic map of the *Lactobacillus sakei* 23K chromosome.** *Microbiology* 2002, **148:**421-431.

148. Cocconcelli PS: **Starter cultures: bacteria.** In *Handbook of fermented meat and poultry.* Edited by Toldra F: Blackwell publishing; 2007: 137-145

149. Hagen BF, Naes H, Holck AL: **Meat starters have individual requirements for Mn^{2+}.** *Meat Science* 2000, **55:**161-168.

150. Lawrence JG, Ochman H: **Amelioration of bacterial genomes: rates of change and exchange.** *J Mol Evol* 1997, **44:**383-397.

151. Daubin V, Lerat E, Perriere G: **The source of laterally transferred genes in bacterial genomes.** *Genome Biol* 2003, **4:**R57.

152. Moretro T, Hagen BF, Axelsson L: **A new, completely defined medium for meat lactobacilli.** *J Appl Microbiol* 1998, **85:**715-722.

153. Totemeyer S, Booth NA, Nichols WW, Dunbar B, Booth IR: **From famine to feast: the role of methylglyoxal production in *Escherichia coli*.** *Mol Microbiol* 1998, **27:**553-562.

154. Monedero V, Maze A, Boel G, Zuniga M, Beaufils S, Hartke A, Deutscher J: **The phosphotransferase system of *Lactobacillus casei*: regulation of carbon metabolism and connection to cold shock response.** *J Mol Microbiol Biotechnol* 2007, **12:**20-32.

155. Chaillou S, Postma PW, Pouwels PH: **Contribution of the phosphoenolpyruvate:mannose phosphotransferase system to carbon catabolite repression in *Lactobacillus pentosus*.** *Microbiology* 2001, **147:**671-679.

156. Veyrat A, Monedero V, Perez-Martinez G: **Glucose transport by the phosphoenolpyruvate:mannose phosphotransferase system in *Lactobacillus casei* ATCC 393 and its role in carbon catabolite repression.** *Microbiology* 1994, **140:**1141-1149.

157. Brinkrolf K, Ploger S, Solle S, Brune I, Nentwich SS, Huser AT, Kalinowski J, Puhler A, Tauch A: **The LacI/GalR family transcriptional regulator UriR negatively controls uridine utilization of *Corynebacterium glutamicum* by binding to catabolite-responsive element (*cre*)-like sequences.** *Microbiology* 2008, **154:**1068-1081.

158. Nentwich SS, Brinkrolf K, Gaigalat L, Huser AT, Rey DA, Mohrbach T, Marin K, Puhler A, Tauch A, Kalinowski J: **Characterization of the LacI-type transcriptional repressor RbsR controlling ribose transport in *Corynebacterium glutamicum* ATCC 13032.** *Microbiology* 2009, **155:**150-164.

Paper I

Available online at www.sciencedirect.com
ScienceDirect
Systematic and Applied Microbiology 31 (2008) 393–403
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

Diversity of *Lactobacillus sakei* strains investigated by phenotypic and genotypic methods

Anette McLeod[a,b], O. Ludvig Nyquist[b], Lars Snipen[b], Kristine Naterstad[a], Lars Axelsson[a,*]

[a]*Matforsk AS, Nofima Food, Osloveien 1, N-1430 Ås, Norway*
[b]*Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway*

Received 1 April 2008

Abstract

The diversity of 10 strains of *Lactobacillus sakei*, a commercially important species of lactobacilli, was characterized by studying food isolates. Growth characteristics varied among the strains when examined after growth in a complex medium and a defined medium with either glucose or ribose. A commercial starter culture strain showed the fastest growth rates and high biomass formation on all media, while two of the strains hardly grew on ribose. Based on acidification properties in a meat model, some of the strains had the ability to compete with the indigenous microbiota of the meat batter in addition to being fast acid producers. Carbohydrate-fermentation abilities revealed a relatively wide variation, clustering the strains into two phenotypic groups. The isolates were analyzed using different genetic fingerprinting techniques, demonstrating a distinction between two genetic groups, a grouping consistent with previous studies dealing with *L. sakei* strains. Comparative genome hybridization (CGH) was introduced for clustering the strains and the same division into two genetic groups was observed. Chromosomal sizes of the strains were estimated by pulsed field gel electrophoresis (PFGE) and were found to vary from 1884 to 2175 kb. The genetic groups did not correlate with the clustering obtained with carbohydrate-fermenting abilities or with chromosomal sizes.

Keywords: *Lactobacillus sakei*; Meat; Fermentation; Diversity; AFLP; RAPD; CGH; PFGE

Introduction

Lactobacillus sakei is a member of the lactic acid bacteria (LAB) group. It differs from many LAB in being tolerant to various adverse conditions such as low temperature, high salt concentrations and varying oxygen levels [12,13]. Although the organism can be isolated from diverse habitats, such as plant fermentations [52] and fermented fish products [32,36], its major habitat is meat [24]. The bacterium is one of the commercially important species of lactobacilli often used in starter cultures for industrial production of fermented sausage [22,23], and it has a great potential as a biopreservative culture in meat and fish products [10,11,26,51]. The primary metabolism, essentially glycolysis, resulting in production of lactic acid is an important property both in a starter and a protective culture. The acidification caused by lactic acid contributes to food preservation and affects the quality of

*Corresponding author. Tel.: +4764970288; fax: +4764970333.
E-mail address: lars.axelsson@nofima.no (L. Axelsson).

0723-2020/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2008.06.002

the product [3,22,31]. Many strains also produce different antibacterial substances including bacteriocins, which contribute to biopreservation [5,17,38]. Recently, the complete genome sequence of the plasmid-cured sausage isolate *L. sakei* 23K [9] was published, and the 1.88-Mb chromosome encoding 1883 predicted genes was explored, revealing a specialised metabolic repertoire that reflect the adaptation to meat products [12].

Early classification of species of *Lactobacillus* relied on phenotypic properties, and for *L. sakei*, phenotypic differentiation between strains and other species was mostly based on the type of lactic acid isomer produced, the type of sugar fermentation pattern and whether ammonia was produced from arginine. In particular, strains of *L. curvatus* and *L. sakei* might be difficult to differentiate [8,13,29,48]. Studies based on biochemical and physiological features have reported a wide phenotypic heterogeneity within strains of *L. sakei*. Phenotypic techniques such as the API 50 CH assay, where fermentation of 49 carbohydrates is tested, has often been used as a tool for discriminating between species of *Lactobacillus*, though the use of genetic methods has been shown to be more reliable [4,39,40]. High-resolution genotypic techniques such as randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) are often used to generate species-specific electrophoretic profiles when investigating genetic diversity between *Lactobacillus* species [7,8,40,48,49]. On the basis of phenotypic and genetic properties, *L. sakei* has been divided into two sub-groups based mainly on results from numerical analyses of whole-cell protein and RAPD patterns [8,29,48]. The sub-groups are in several publications described as sub-species: *L. sakei* subsp. *sakei* and *L. sakei* subsp. *carnosus* [13,30,48]. Analyses of *Eco*RI and *Hind*III ribotypes and 16S rRNA genes, on the other hand, did not allow a clear separation between sub-groups of *L. sakei* [30]. Identification of *Lactobacillus* species is an important step in the development of new and interesting cultures, but also the identification at an intraspecies level may be important. Although more accurate than other typing techniques, the RAPD and AFLP provide limited information about the complete genome. Previous DNA–DNA hybridization experiments have revealed close relatedness between *L. sakei* strains at the genomic level ($\geqslant$75% identity) [29,48], but only after the completion of a genome sequence is it possible to perform genome-wide comparisons of strains within the species. Microarray-based comparative genome hybridization (CGH), also named genomotyping, is a powerful tool for estimating whole-genomic diversity [15,18]. By allowing hybridization of genomic DNA from a test strain to a microarray representing the 23K genome, a measurement of the similarity and divergence between two closely related *L. sakei* strains can be obtained.

The purpose of this work was to study the diversity of *L. sakei*, analyzing 10 strains of different origins from meat and fermented meat products, sake and fermented fish. Some of the strains are commercially used starter or protective cultures, and some are bacteriocin producers. Based on growth characteristics, pH- and fermentation profiles, we investigated phenotypic variation between the strains. We compared different genetic fingerprint analyses and determined the sizes of the chromosomes by pulsed field gel electrophoresis (PFGE), CGH using an open-reading frame (ORF)-specific *L. sakei* microarray based on the sequenced strain 23K was introduced for clustering the strains, and the clustering from the molecular techniques was compared. This work provides new insights into the diversity among isolates of the *L. sakei* species, and we introduce the application of AFLP and microarray-based CGH for the separation of the species into two genetic groups.

Materials and methods

Bacterial strains, media and growth

The bacterial strains used in this study are listed in Table 1. Strains were maintained at −80 °C in MRS broth (Oxoid, Hampshire, UK) supplemented with 20% glycerol. Cells were cultivated in Honeycomb microplates (Labsystems, Helsinki, Finland) in wells with 400 μl medium at 30 °C in a Bioscreen C (Labsystems). Growth was monitored by optical density at 600 nm (OD_{600}) every 30 min, after 10 s agitation, for 70 h. Cells from overnight cultures grown in the complex medium MRS were washed twice with sterile physiological saline (0.9% sodium chloride) before inoculation to OD_{600} 0.05 in MRS or in the completely defined medium DML [37], supplemented with either 0.5% glucose (DMLG) or 0.5% ribose (DMLR). All conditions were examined in triplicate and performed twice. The average maxOD_{600} from the two independent experiments was calculated. The average specific growth rate (μ) from the two experiments was calculated for the interval OD_{600} 0.2–0.4.

Acidification properties in a meat model

A recipe for traditional Norwegian salami was used to prepare a meat batter containing (% w/w) beef (37.85), pork (37.85), pork back fat (20.00), sodium chloride (3.19), sodium nitrite (0.0096), dextrose (0.70), spices (0.30) and ascorbic acid (0.05). The *L. sakei* strains were added to 150 g aliquots of this batter at a concentration of 10^6 cfu g^{-1}, and pH was recorded as described by Hagen et al. [21]. Duplicate samples were incubated at 24 °C for 72 h. Mean and standard deviations were

Table 1. Strains used in this study

Bacterial strain	Source of isolation, characteristics	Reference, source
L. sakei 23K	Sausage	[9,16]
L. sakei MF1053	Fermented fish, sakacin P producer	Matforsk
L. sakei LS 25	Commercial starter culture for fermented meat/salami sausage	[21]
L. sakei Lb790x	Meat	[6]
L. sakei LTH673	Fermented dry sausage, sakacin P producer	[17,46]
L. sakei MF1328	Fermented sausage	Matforsk
L. sakei MF1058 (TH1)	Vakuum-packed cooked meat, protective culture	[10,11]
L. sakei CCUG 31331[a] (R 14 b/a)	Fermented sausage, type strain for *L. sakei* subsp. *carnosus*	[30,49]
L. sakei DSM 20017[b] (ATCC 15521[c])	Sake, alcoholic beverage made by fermenting rice, typestrain for *L. sakei* subsp. *sakei*	[49]
L. sakei Lb16 (Lb1048[d], CCUG 42687[a])	Minced meat, sakacin P producer	[2,37]

[a]CCUG, Culture Collection, University of Gothenburg, Sweden.
[b]DSM, Deutsche Samlung von Microorganismen und Zellkulturen, Braunschweig, Germany.
[c]ATCC, American Type Culture Collection, Manassas, VA, USA.
[d]Designation used in the strain collection at Federal Institute for Meat research, Kulmbach, Germany.

calculated. Growth of the added cultures was assayed by plating appropriate dilutions of meat batter homogenized in peptone water on MRS agar (Oxoid). The plates were visually inspected after incubation at 30 °C for 2 days and the bacterial colonies were compared to the appearance of colonies from pure cultures of the added strains.

API 50 CH fermentation assay

Fermentation of carbohydrates for the *L. sakei* strains was determined using API 50 CH assay (BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Changes in color from purple were monitored after 24 h and verified after 48 h. Fermentation of each of the 49 carbohydrates in the carbohydrate medium were interpreted as: positive (+), complete change to yellow; weakly positive (w), change to green; and negative (−), no change at all. Esculine hydrolysis (revealed by a change to a darker color) was interpreted as positive (+) while no change was negative (−). Strains were tested in duplicate to determine the test reproducibility. Fermentation profiles were analyzed using BioNumerics (Applied Maths, Gent, Belgium) with Pearson correlation and the unweighted pair group method with arithmetic mean (UPGMA).

Genomic DNA extraction

Genomic DNAs from the *L. sakei* strains were prepared by using the DNeasy tissue kit (Qiagen Nordic, Solna, Sweden) according to the manufacturer's recommendations, and DNA for microarray-based CGH hybridizations was extracted using ADVAMAX beads (Edge BioSystems, Gaithersburg, MD, USA) as described by Aakra et al. [1].

16S rRNA sequencing and species determination

The universal primers 5′-AGAGTTTGATCYTGGCTCAG-3′ and 5′-GTAAGGTTCTTCGCGT-3′ (*E. coli* positions 7–26 and 968–983, respectively) were used for the specific amplification of a 972-bp DNA fragment of the 16S rRNA gene, followed by sequencing using a BigDye v3.1 terminator cycle sequencing kit, the primer 5′-GTAAGGTTCTTCGCGT-3′ and the sequencing device ABI PRISM 3100 (Applied BioSystems, Foster City, CA, USA). Sequences were confirmed using the Ribosomal Database Project II (http://rdp.cme.msu.edu/) to determine the closest known relatives of the partial 16S rRNA gene sequence obtained. PCR reactions were performed as described by Urso et al. [50] to exclude the possibility that the obtained sequence could be from the closely related *L. curvatus*.

RAPD and AFLP analyses

The *L. sakei* strains were analyzed by RAPD using a single HEX (2,5,2′,4′,5′,7′- hexachloro-6-carboxyfluorescein)-labelled 9-bp primer 5′-ACGCGCCCT-3′ as described by Johansson et al. [25], and AFLP as described by Katla et al. [27]. The AFEC4 primer used in the AFLP was 5′ end-labeled using 6-carboxyfluorescein (FAM). For both analyses, the fragment profiles were separated by capillary gel electrophoresis on the ABI PRISM 3100 Genetic Analyzer using the Genescan-500-ROX (Applied Biosystems) as internal lane standard, and fragment profiles were analyzed using BioNumerics (Applied Maths) with Pearson correlation and UPGMA. To ensure reproducibility of the analyses, independent DNA isolates of the same strain were tested twice in duplicates.

Microarray-based CGH

A *Lactobacillus sakei* Array Ready Oligo Set™ (Operon Biotechnologies GmbH, Cologne, Germany) was used in this study. On the array, each ORF is represented as 70-base oligonucleotides. A description

of the oligoset can be found at http://migale.jouy.inra.fr/sakei/Supplement.html/. All nine strains (Table 1) were each cohybridized with the 23K reference strain in CGH experiments performed as described by Nyquist et al. [41]. In order to discriminate between present and divergent genes, ROTMIX scores were calculated for every ORF as described by Snipen et al. [44]. This score is described as an improvement of the standard procedures of using simple log-ratio cutoff for classifying genes. The score can be seen as a posterior probability of divergence, i.e. a gene is divergent if the ROTMIX score is close to 1.0 and present if close to 0.0. If this score is ~0.5 the status of the gene is uncertain. A cutoff for the posterior probability of 0.55 was chosen, thus all genes with a ROTMIX score between 0.45 and 0.55 will be left unclassified. Sequence similarity searches for all probe sequences against the genome sequence of *L. sakei* 23K were performed using NCBI blastn (www.ncbi.nlm.gov/BLAST/). A dendrogram was created using the UPGMA algorithm (MATLAB®, The MathWorks, Inc., Natick, MA, USA) where the distance between two strains (experiments) was calculated as the number of probes with different classification, leaving out all unclassified probes. The 23K strain was included, but these data were based on BLAST sequence identity only, not CGH results.

Microarray accession number

The microarray data have been deposited in the Array Express database (http://www.ebi.ac.uk/arrayexpress/). The accesssions assigned are A-MEXP-1166 for the array design and E-MEXP-1639 for the experiment.

PFGE analysis

Colonies of *L. sakei* cells from MRS agar (Oxoid) grown at 30 °C for 1–2 days were suspended in 300 µl of a buffer containing 6 mM Tris/HCl (pH 8.0), 1 M NaCl, 100 mM EDTA, 1% sarcosyl (pH 7.6), 10 mg ml^{-1} lysozyme (Sigma-Aldrich, Oslo Norway) and 20 U ml^{-1} mutanolysin (Sigma-Aldrich). After incubation at 37 °C for 10 min, the bacterial suspension was added to an equal volume of 2% low-melting-point agarose before casting into plugs which were incubated overnight at 37 °C. Plugs were washed in 100 mM EDTA, 1% sarcosyl (pH 8.0) before incubation overnight in the same buffer containing 20 mg ml^{-1} Proteinase K (Sigma-Aldrich). Plugs were washed in $TE_{(10+1)}$ buffer (10 mM Tris/HCl, pH 8.0, 1 mM EDTA) and incubated for 2 h at 37 °C in $TE_{(10+1)}$ buffer added 20 mM Pefabloc (Sigma-Aldrich). A washing step in $TE_{(10+1)}$ buffer and incubation for 15 min at 50 °C was then performed four times before storing the plugs in $TE_{(10+100)}$ buffer (10 mM Tris/HCl, pH 8.0, 100 mM EDTA) at 4 °C until use. For restriction enzyme digestion the plugs were first dialyzed against sterile water, then equilibrated for 3 h in 500 µl of restriction enzyme-specific buffer at 4 °C. About 5 U of the restriction enzyme I-*Ceu*I (New England BioLabs, Ipswich, MA, USA) was added and the plugs were incubated at 4 °C overnight and then for 4 h at 37 °C. After digestion, the plugs were rinsed for 3 h in $TE_{(10+100)}$ buffer and then submitted to PFGE on a CHEF-DR II apparatus (Bio-Rad Laboratories). PFGE was performed in 1% SeaKem Gold Agarose (Cambrex, East Rutherford, NJ, USA) in 0.5-TBE buffer [42] at 14 °C. A first migration was carried out at 150 V with a first switch time of 70 s for 15 h, and a second switch time of 99 s for 11 h. To separate the largest fragments, a second migration was performed at 200 V for 48 h with a switch time of 120 s. The molecular mass markers used were yeast chromosome PFG marker (NO345S; New England BioLabs) and Lambda ladder PFG marker (NO340S; New England BioLabs). After migration, gels were stained in ethidium bromide and photographed under UV light.

Results

The strains investigated were identified as *L. sakei* by the sequence of a 972-bp DNA fragment of the 16S rRNA gene, and results from PCR reactions performed as described by Urso et al. [50] confirmed the status of the strains as being *L. sakei*.

Growth and acidification properties

As shown in Table 2, differences in growth characteristics between the *L. sakei* strains were observed after growth in MRS, DMLG and DMLR. From the Bioscreen C experiments performed, all 10 strains reached an average $maxOD_{600}$ in DMLG close to their $maxOD_{600}$ in MRS. Strains LS 25, MF1328, MF1058 and Lb16 reached a $maxOD_{600}$ in DMLR slightly lower than in the two other media, while the rest of the strains showed approximately half $maxOD_{600}$ values. Also when comparing specific growth rate (μ) values (Table 2), variation between the strains was observed. When grown in DMLG, Lb790x, MF1328, MF1058 and Lb16 obtained μ values slightly lower than in MRS, and MF1053, LTH673, CCUG 31331 and DSM 20017 showed even lower values. All strains showed low μ values when grown in DMLR compared to the other media. LS 25 obtained the highest μ value of the strains when grown in DMLR. Comparable μ values in DMLR for strains CCUG 31331 and DSM 20017 could not be obtained, since these strains did not reach an OD_{600} above 0.4.

Table 2. Growth characteristics of *L. sakei* strains grown in MRS, DML supplemented with 0.5% glucose (DMLG) or ribose (DMLR) at 30 °C

Bacterial strain	MRS		DMLG		DMLR	
	μ (h^{-1})	$MaxOD_{600}$	μ (h^{-1})	$MaxOD_{600}$	μ (h^{-1})	$MaxOD_{600}$
L. sakei 23K	0.240	1.481	0.236	1.246	0.104	0.449
L. sakei MF1053	0.300	1.515	0.171	1.428	0.105	0.677
L. sakei LS 25	0.402	1.677	0.394	1.544	0.159	1.327
L. sakei Lb790x	0.300	1.684	0.255	1.469	0.101	0.760
L. sakei LTH673	0.304	1.547	0.153	1.288	0.070	0.693
L. sakei MF1328	0.369	1.360	0.313	1.395	0.104	1.120
L. sakei MF1058	0.293	1.577	0.261	1.434	0.105	1.085
L. sakei CCUG 31331	0.327	1.284	0.200	1.397	–	0.297
L. sakei DSM 20017	0.242	1.488	0.182	1.388	–	0.321
L. sakei Lb16	0.300	1.556	0.264	1.519	0.119	1.233

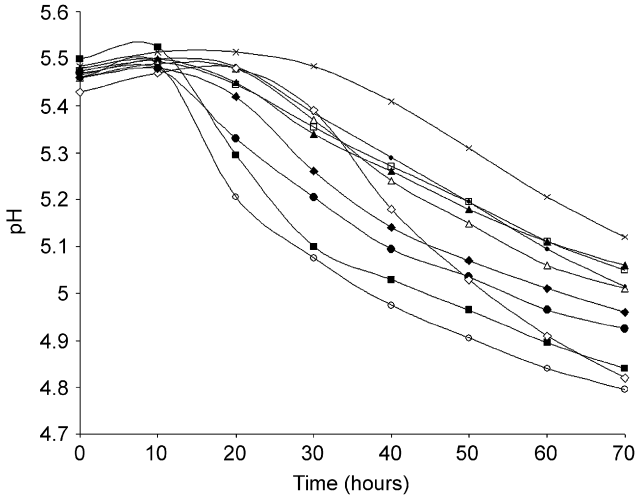


Fig. 1. Acid production in a meat model by *L. sakei* strains (●) MF1328, (●) LTH673, (△) 23K, (□) CCUG 31331, (×) Lb790x, (○) MF1058, (♦) Lb16, (■) LS 25, (▲) MF1053 and (◇) DSM 20017, measured by recording the pH. Data points are shown with 10-h intervals. Determinations were carried out twice with a standard deviation of ±0.05. All the meat batters were added *L. sakei* at a level of 10^6 cfu g^{-1}.

From the meat model system based on minced meat fermentation, pH profiles of the 10 *L. sakei* strains were obtained (Fig. 1). LS 25 and MF1058 performed the fastest acidification. Within 30 h, these strains acidified the meat model to a pH level of 5.1. A slower acidification was observed for MF1328, Lb16 and DSM 20017 reaching pH 5.1 after 40–50 h of incubation. For the remaining strains, more than 55 h were needed to reach pH 5.1. When growth of the added cultures was assayed using visual inspection of the plates, it was observed that the *L. sakei* culture dominated in the samples with the fast acid producers LS 25, MF1058 and MF1328. For the remaining strains, the background microbiota from the meat batter and the added cultures occurred in equal number (results not shown). The lag phases (Fig. 1), which are longer when the background microbiota is prominent, also reflect these two groups.

Fermentation properties

The carbohydrate-fermentation pattern of the *L. sakei* strains determined using the API 50 CH assay and CHL medium are summarized in Fig. 2. The reproducibility of the fermentation tests was 100%. Nine carbohydrates were fermented by all of the strains within 24–48 h. In addition, 12 carbohydrates were fermented by at least one of the strains investigated. MF1053 and LS 25 showed the abilities to ferment 18 carbohydrates, while 23K, Lb790x and MF1328 were only able to ferment 11. Clustering based on fermentation profiles showed separation of the strains into two phenotypic groups, defined at a similarity level of 74%. One was composed of Lb16/LTH673 and MF1053, defined at 83%. The remaining strains formed the other group, which subdivided into two groups at 78%. One group was composed of 23K/MF1328 and Lb790x defined at 88%, and the other group of MF1328, which separated at 82%, and LS 25 and CCUG 31331/DSM 20017 defined at 87%.

RAPD and AFLP analyses

The *L. sakei* strains were subjected to RAPD and AFLP analyses, and clustering based on the fragment profiles are shown in Fig. 3. All profiles of a single isolate yielded similarity levels of ⩾87% for the RAPD analysis and ⩾94% for the AFLP analysis when computed and clustered by UPGMA, a level which can be defined as a cutoff value. Similarity levels above the cutoff value can therefore not distinguish between strains. The RAPD analysis resulted in roughly 10 major bands per pattern (results not shown), and clustering showed a clear separation of strains DSM

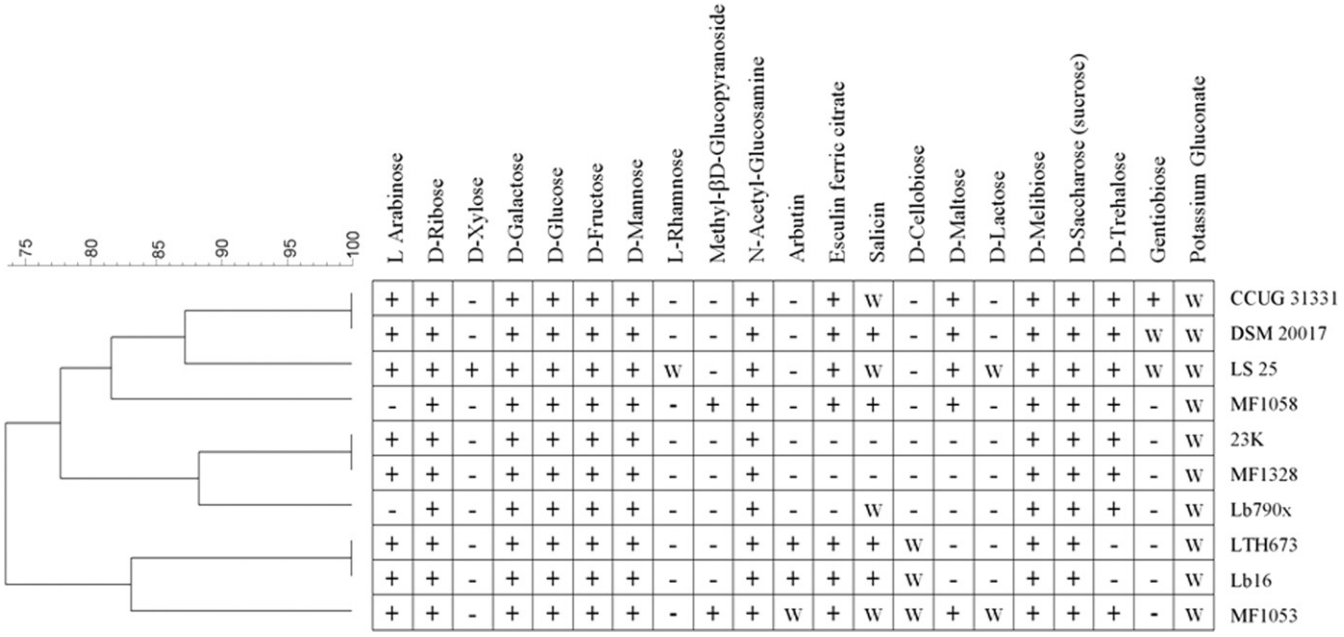


Fig. 2. API 50 CH fermentation of *L. sakei* strains studied. Only carbohydrates where at least one strain had positive result are shown. Dendrogram created with BioNumerics software is based on fermentation of the 49 carbohydrates, and scale above the dendrogram shows the percent similarity level in the cluster analysis. Fermentation was interpreted as positive (+), weakly positive (w) and negative (−).

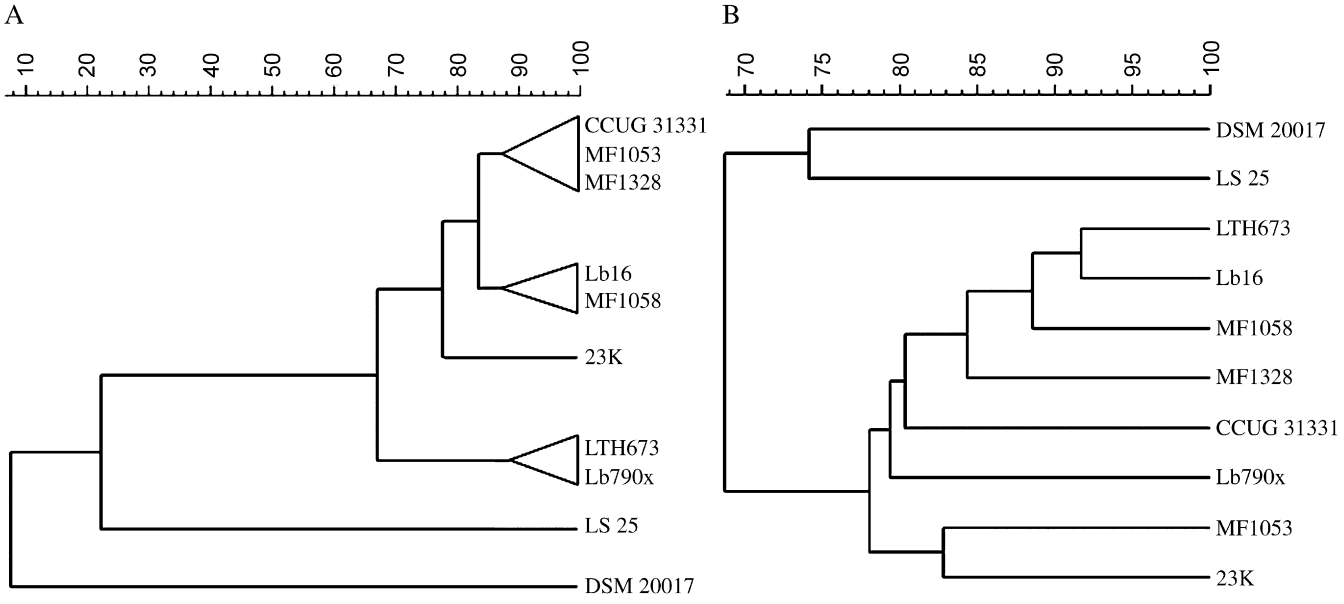


Fig. 3. Dendrograms created with BioNumerics software based on RAPD (A) and AFLP (B) analyses of 10 *L. sakei* strains. Scale above the dendrograms shows the percent similarity level in the cluster analysis.

20017 and LS 25 from the remaining strains at a low similarity level. The remaining strains clustered into two groups. Lb790x and LTH673 comprised one group, and the strains in the second group were divided into 23K, MF1058/Lb16 and CCUG 31331/MF1053/MF1328. Based on fragment profiles from the AFLP analysis, which resulted in above 100 bands per pattern (results not shown), two distinct genetic groups were obtained, defined at a similarity level of 68%. One group comprised DSM 20017 and LS 25, while the other group comprised two sub-groups. One of these sub-groups was composed of 23K and MF1053, while the other sub-group could be subdivided into several groups defined at different similarity levels, containing the six

strains LTH673, Lb16, MF1058, MF1328, CCUG 31331 and Lb790x.

Microarray-based CGH

The microarray used in this study represents the ORFs in the sequenced strain *L. sakei* 23K, which comprise only chromosomal genes, since this strain is plasmid cured [9]. ROTMIX scores for each ORF were calculated from CGH results, and a posterior cutoff at 0.55 gave 1244 probes classified as present or absent/divergent. Based on number of ORFs with different classification, the dendrogram created shows percent similarity between the strains, which represents genomic diversity (Fig. 4). The strains clustered into two genetic groups defined at a similarity level of 88%, where one group comprised the two strains DSM 20017 and LS 25, and the other group contained the remaining strains. 23K and Lb790x separated at similarity levels between 93% and 94%, and MF1328 at 95%. Two minor groups separated at 96%, one was composed of MF1053 and CCUG 31331, and the other one of MF1058, LTH673 and Lb16.

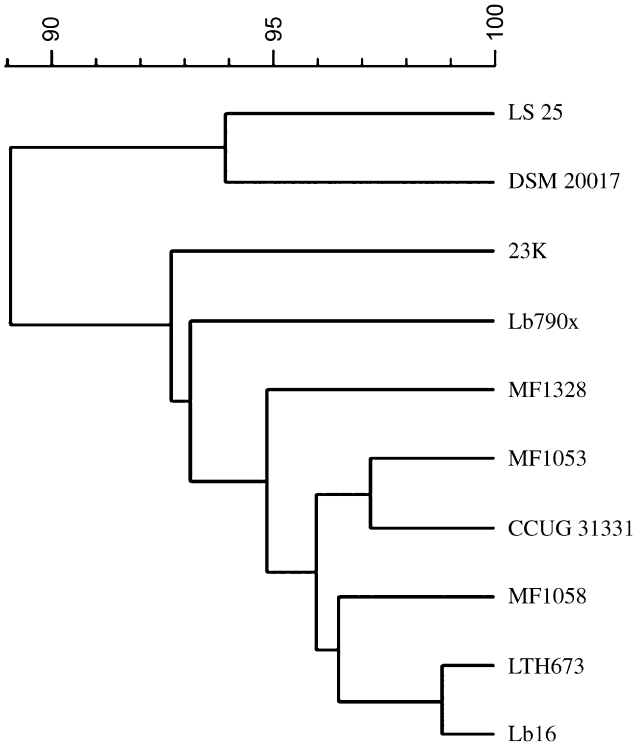


Fig. 4. Dendrogram based on 1244 classified ORFs when the posterior cutoff = 0.55. Scales above the dendrogram show percent similarity between 10 *L. sakei* strains based on number of ORFs with different classification. Strain 23K is included based on sequence identity to the ORFs and not based on CGH results.

Chromosome sizes and *rrn* operons as revealed by PFGE

When chromosomal DNAs from the *L. sakei* strains were digested with the intron-encoded endonuclease I-*Ceu*I followed by analysis with PFGE, a pattern of seven bands was identified for all strains. Table 3 summarizes the size of the I-*Ceu*I restriction fragments obtained. The recognition site of I-*Ceu*I is located in the 23S rRNA gene in bacteria, and the number of bands indicates the number of *rrn* operons [35,47]. The presence of seven *rrn* operons in the sequenced strain *L. sakei* 23K has previously been described by Dudez et al. [16], and the sequences of the *rrnA-G* have been described by Chaillou et al. [12], two of which are continuous, *rrnAB*. The smallest fragment (5 kb) corresponds to the size of a classical bacterial *rrn* operon. Thus, the chromosomal sizes of the *L. sakei* strains investigated were estimated to vary from 1884 to 2175 kb as shown in Table 3.

Table 3. Fragment sizes of restriction enzyme digests with I-*Ceu*I of the chromosomes of *L. sakei* strains

Strain	Fragment size (kb) obtained with I-*Ceu*I							
	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6	Fragment 7	Estimated genome size (kb)
23K	1104	331	175	135	98	36	5	1884
Lb790x	1120	345	170	135	98	34	5	1907
Lb16	1120	340	178	135	106	34	5	1918
DSM 20017	1093	380	175	135	103	34	5	1925
MF1058	1173	345	170	136	103	34	5	1970
MF1053	1145	361	200	175	130	34	5	2045
LTH673	1295	340	178	135	98	36	5	2085
MF1328	1283	361	190	140	103	35	5	2117
LS 25	1283	380	186	135	110	34	5	2133
CCUG 31331	1350	382	170	136	98	34	5	2175

Discussion

In this study, we have investigated the diversity within *L. sakei* by studying food isolates. We were also interested in whether it is possible to identify variation between strains in interesting characteristics that might indicate how to select potential new strains to be used as starters and biopreservative cultures in meat and fish products. Our results agree with previous studies in reporting a wide phenotypic heterogeneity within strains of *L. sakei* and the presence of two genetic groups, named *L. sakei* subsp. *sakei* and *L. sakei* subsp. *carnosus* [8,13,29,30,48]. Based on growth characteristics, pH- and carbohydrate-fermentation profiles we investigated variation in phenotype, we compared the use of three different molecular techniques, RAPD, AFLP and microarray-based CGH to cluster the strains genetically, and we estimated chromosomal sizes by PFGE.

The ability of a starter culture to produce sufficient amounts of lactic acid is important to ensure safety and texture of the final products, and selected strains should be able to rapidly dominate the fermentation process. Growth characteristics (Table 2) varied among the strains when examined after growth in MRS, DMLG and DMLR, though the general pattern showed similar growth in MRS and DMLG, while all strains grew less well in DMLR. The commercial starter culture strain LS 25 deviated from the other strains by showing the fastest growth rates and high biomass formation in all media, while strains DSM 20017 and CCUG 31331 hardly grew on ribose. In contrast, ribose was fermented by all strains in the API 50 CH fermentation assay. The API 50 CHL medium in the assay is based on meat extract which contains small amounts of glucose. From growth experiments in DML when 0.02% glucose was added in addition to 0.5% ribose, values similar to those obtained from DMLG was observed (results not shown). The small amount of glucose added clearly provides the growth needed for ribose-related genes to be initiated and for ribose to be efficiently metabolized by the bacterium. This indicates a strong correlation between glucose and ribose metabolism in *L. sakei*, as also suggested by Champomier et al. [13], a mechanism that needs more investigation to be fully understood. From the strains investigated, LS 25, MF1058 and MF1328 were fast acid producers and showed good abilities as starter cultures based on acidification properties in the meat model (Fig. 1) and based on their ability to compete with the indigenous microbiota of the meat batter. LS 25 and MF1328 are used in the industry as starter cultures for fermented sausage, while MF1058 has been shown to be suitable as a protective culture in vacuum packed fresh meat [10,11]. The others did not have the same ability to compete with the indigenous microbiota. Although carbohydrate-fermentation abilities revealed by the API 50 CH assay (Fig. 2) demonstrate a relatively wide phenotypic variation between the strains, no correlation could be demonstrated between metabolic capacity and fast acid production. LS 25 showed the ability to ferment 18 carbohydrates while MF1328 and MF1058 only fermented 11 and 14 carbohydrates, respectively. Fast growth on media such as MRS and DMLG containing glucose seems to be more important as an indicator for fast acid production in meat batter. The use of bacteriocin producers as starter cultures or protective cultures may offer considerable food safety advantages. Of the strains investigated, MF1053, LTH673 and Lb16 are sakacin P producers. Sakacin P is an amphiphilic peptide interacting with the cell membrane of susceptible strains [46]. However, the bacteriocin activity levels in meat can be low for these types of molecules due to the specific conditions in the food environment [33,34], and it did not give the strains advantages when competing with the indigenous microbiota of the meat batter in our experiments. Since functionality depends on the type of product, pH, temperature, the technology of fermentation, and the ingredients and raw materials used, the performance of a culture must be seen in context of the application [19,20,33,34].

Molecular techniques with high discriminatory power are essential to distinguish between bacterial isolates. We analyzed the *L. sakei* isolates by RAPD and AFLP (Fig. 3), reportedly two of the most discriminatory techniques applied in strain typing [43,45]. RAPD uses low-stringency hybridization conditions with a single random oligonucleotide primer, and the AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The discriminatory power of the RAPD analysis with the primer used in this study, appeared to be less than of the AFLP analysis, shown in the large difference in number of bands per pattern between the techniques, and in a higher cutoff value for strain differentiation, which was $\geqslant$87% for RAPD and $\geqslant$94% for AFLP. Although earlier studies have shown the application of RAPD to discriminate between the two genetic groups within *L. sakei* [8,29,48] the results from this study separated DSM 20017 and LS 25 from the remaining strains, but the method did not define the two strains as one genetic group. The AFLP method appeared more specific with the potential to detect more genomic variations than RAPD. From the AFLP clustering, a clear distinction between the two genetic groups was demonstrated, in agreement with previous studies [8,13,29,30,48]. LS 25 and the type strain DSM 20017 comprised one group, which may be described as *L. sakei* subsp. *sakei*, and the remaining strains including the type strain CCUG 31331, comprised the second group, which may be described as *L. sakei* subsp. *carnosus*. We were interested in whether the microarray would cluster the strains in a manner comparable to

those of the two fingerprinting techniques. In terms of species delineation, we found an excellent agreement of the separation into the two genetic groups as shown by AFLP. Similar global clustering profiles were obtained from AFLP and CGH (Fig. 4), but although MF1328, CCUG 31331, Lb790x, 23K and MF1053 clustered together at a high similarity level from both methods, MF1053 and 23K occurred closer genetically in the AFLP results, while MF1053 was closer to CCUG 31331 in the CGH results. For both methods strains MF1058, LTH673 and Lb16 clustered together at an even higher similarity level than the remaining strains. The DNA microarrays provide a method for comparison of the *L. sakei* strains that is more gene specific and that can reveal genomic differences between strains that the RAPD and AFLP may not identify. It provides a whole-genome fingerprint of *L. sakei* isolates. It should be mentioned that this is a study with a limited number of strains. To get a more complete picture of the species diversity at the genomic level, many more strains have to be investigated using these methods. Clustering based on carbohydrate-fermentation patterns divided the strains into two phenotypic groups (Fig. 2), but no correlation with the two genetic groups could be demonstrated. LS 25 and MF1053 showed the highest metabolic capacity of the strains, but they clustered in different phenotypic groups, and the type strains DSM 20017 and CCUG 31331, representing different genetic groups, clustered in the same phenotypic group based on API profiles. Also no correlation could be demonstrated between fast acid production and the two genetic groups.

At present, only one complete genome sequence from the species *L. sakei* is available. Our PFGE analysis showed that the plasmid-cured sequenced strain 23K with its 1884 kb had the smallest genome of the strains investigated, and the type strain CCUG 31331 had the largest genome of 2175 kb. For clustering the strains in this study by the microarray-based CGH approach, we assume that the 23K genome sequence is representative of the species as a whole, but further studies are needed to confirm this. The composition of the ORF-specific *L. sakei* microarray based on the sequenced strain 23K makes a one-way comparison to discover the genes deleted or divergent in the test strain compared to the sequenced strain, but it is unable to detect genes specific to the test strains that are not present in the reference strain. Sequencing starting from interesting genes may be performed, which enable the development of more comprehensive microarrays in the future. Inclusion of multiple strain-specific sequences enable a two-way comparison to the reference strain that in addition to identifying deleted or divergent genes in the test strain, also detects the presence of genes specific to the test strain that are absent in the reference strain. Further CGH studies are needed and the use of an expanded set of strains from each genetic group is relevant to further elucidate the value of this. The technique may identify strain-specific factors contributing to phenotypic variability among isolates. No correlation between genome sizes and other phenotypic or genotypic characteristics could be observed. Seven *rrn* clusters were observed to be present in all strains. In general, organisms with multiple *rrn* operons are capable of achieving faster doubling times than those with just one or two. The high copy number of *rrn* clusters in *L. sakei* might reflect rapid adaption to changing environmental conditions as was demonstrated for *Escherichia coli* [14] and soil bacteria [28]. Experiments of the diversity of strains, including comparative genomics, transcriptomics and proteomics, are being carried out. Hopefully this may generate a framework leading to the selection of potential new starters and biopreservative cultures with increased diversity, stability and industrial performance. It will permit rapid, high-throughput screening of strains with interesting functional properties with the potential to improve both the hygienic status of the food and their competitiveness in food fermentations.

Acknowledgments

This work was supported by Grant 159058/I10 from the Norwegian Research Council. The authors would like to thank Brit Oppegård Pedersen and Birgitta Baardsen for technical assistance, and Hanne Guldsten for her contribution during the microarray-based CGH experiment.

References

[1] A. Aakra, O.L. Nyquist, L. Snipen, T.S. Reiersen, I.F. Nes, Survey of genomic diversity among *Enterococcus faecalis* strains by microarray-based comparative genomic hybridization, Appl. Environ. Microbiol. 73 (2007) 2207–2217.

[2] I.M. Aasen, T. Møretrø, T. Katla, L. Axelsson, I. Storrø, Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687, Appl. Microbiol. Biotechnol. 53 (2000) 159–166.

[3] J.C. Acton, R.L. Dick, E.L. Norris, Utilization of various carbohydrates in fermented sausages, J. Food Sci. 42 (1997) 174–178.

[4] C. Andrighetto, P. De Dea, A. Lombardi, E. Neviani, L. Rossetti, G. Giraffa, Molecular identification and cluster analysis of homofermentative thermophilic lactobacilli isolated from dairy products, Res. Microbiol. 149 (1998) 631–643.

[5] L. Axelsson, A. Holck, The genes involved in production of and immunity to sakacin A, a bacteriocin from *Lactobacillus sake* Lb706, J. Bacteriol. 177 (1995) 2125–2137.

[6] L. Axelsson, G. Lindstad, K. Naterstad, Development of an inducible gene expression system for *Lactobacillus sakei*, Lett. Appl. Microbiol. 37 (2003) 115–120.

[7] T. Aymerich, B. Martin, M. Garriga, M.C. Vidal-Carou, S. Bover-Cid, M. Hugas, Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages, J. Appl. Microbiol. 100 (2006) 40–49.

[8] F. Berthier, S.D. Ehrlich, Genetic diversity within *Lactobacillus sakei* and *Lactobacillus curvatus* and design of PCR primers for its detection using randomly amplified polymorphic DNA, Int. J. Syst. Bacteriol. 49 (1999) 997–1007.

[9] F. Berthier, M. Zagorec, M.C. Champomier-Verges, S.D. Ehrlich, F. Morel-Deville, Efficient transformation of *Lactobacillus sake* by electroporation, Microbiology 142 (1996) 1273–1279.

[10] S. Bredholt, T. Nesbakken, A. Holck, Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat, Int. J. Food Microbiol. 53 (1999) 43–52.

[11] S. Bredholt, T. Nesbakken, A. Holck, Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats, Int. J. Food Microbiol. 66 (2001) 191–196.

[12] S. Chaillou, M.C. Champomier-Verges, M. Cornet, A.M. Crutz-Le Coq, A.M. Dudez, V. Martin, S. Beaufils, E. Darbon-Rongere, R. Bossy, V. Loux, M. Zagorec, The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K, Nat. Biotechnol. 23 (2005) 1527–1533.

[13] M.C. Champomier-Verges, S. Chaillou, M. Cornet, M. Zagorec, Erratum to "*Lactobacillus sakei*: decent developments and future prospects," Res. Microbiol. 153 (2002) 115–123 (Research in Microbiology 152 (2001) 839).

[14] C. Condon, D. Liveris, C. Squires, I. Schwartz, C.L. Squires, rRNA operon multiplicity in *Escherichia coli* and the physiological implications of *rrn* inactivation, J. Bacteriol. 177 (1995) 4152–4156.

[15] N. Dorrell, S.J. Hinchliffe, B.W. Wren, Comparative phylogenomics of pathogenic bacteria by microarray analysis, Curr. Opin. Microbiol. 8 (2005) 620–626.

[16] A.M. Dudez, S. Chaillou, L. Hissler, R. Stentz, M.C. Champomier-Verges, C.A. Alpert, M. Zagorec, Physical and genetic map of the *Lactobacillus sakei* 23K chromosome, Microbiology 148 (2002) 421–431.

[17] V.G. Eijsink, M.B. Brurberg, P.H. Middelhoven, I.F. Nes, Induction of bacteriocin production in *Lactobacillus sake* by a secreted peptide, J. Bacteriol. 178 (1996) 2232–2237.

[18] J.R. Fitzgerald, J.M. Musser, Evolutionary genomics of pathogenic bacteria, Trends Microbiol. 9 (2001) 547–553.

[19] M. Garriga, M. Hugas, T. Aymerich, J.M. Monfort, Bacteriocinogenic activity of lactobacilli from fermented sausages, J. Appl. Bacteriol. 75 (1993) 142–148.

[20] M. Garriga, M. Hugas, P. Gou, M.T. Aymerich, J. Arnau, J.M. Monfort, Technological and sensorial evaluation of *Lactobacillus* strains as starter cultures in fermented sausages, Int. J. Food Microbiol. 32 (1996) 173–183.

[21] B.F. Hagen, H. Naes, A.L. Holck, Meat starters have individual requirements for Mn2+, Meat Sci. 55 (2000) 161–168.

[22] W.P. Hammes, A. Bantleon, S. Min, Lactic acid bacteria in meat fermentation, FEMS Microbiol. Rev. 87 (1990) 165–174.

[23] W.P. Hammes, C. Hertel, New developments in meat starter cultures, Meat Sci. 49 (1998) 125–138.

[24] M. Hugas, M. Garriga, T. Aymerich, J.M. Monfort, Biochemical characterization of lactobacilli from dry fermented sausages, Int. J. Food Microbiol. 18 (1993) 107–113.

[25] M.L. Johansson, M. Quednau, G. Molin, S. Ahrne, Randomly amplified polymorphic DNA (RAPD) for rapid typing of *Lactobacillus plantarum* strains, Lett. Appl. Microbiol. 21 (1995) 155–159.

[26] T. Katla, T. Moretro, I. Sveen, I.M. Aasen, L. Axelsson, L.M. Rorvik, K. Naterstad, Inhibition of *Listeria monocytogenes* in chicken cold cuts by addition of sakacin P and sakacin P-producing *Lactobacillus sakei*, J. Appl. Microbiol. 93 (2002) 191–196.

[27] T. Katla, K. Naterstad, M. Vancanneyt, J. Swings, L. Axelsson, Differences in susceptibility of *Listeria monocytogenes* strains to sakacin P, sakacin A, pediocin PA-1, and nisin, Appl. Environ. Microbiol. 69 (2003) 4431–4437.

[28] J.A. Klappenbach, J.M. Dunbar, T.M. Schmidt, rRNA operon copy number reflects ecological strategies of bacteria, Appl. Environ. Microbiol. 66 (2000) 1328–1333.

[29] G. Klein, L.M.T. Dicks, A. Pack, B. Hack, K. Zimmerman, F. Dellagio, G. Reuter, Emended description of *Lactobacillus sake* (Katahiri, Katahara and Fukami) and *Lactobacillus curvatus* (Abo-Elnega Kandler): Numerical classification revealed by protein fingerprinting and identification based on biochemical patterns and DNA–DNA hybridizations, Int. J. Syst. Bacteriol. 46 (1996) 367–376.

[30] J. Koort, P. Vandamme, U. Schillinger, W. Holzapfel, J. Bjorkroth, *Lactobacillus curvatus* subsp. *melibiosus* is a later synonym of *Lactobacillus sakei* subsp. *carnosus*, Int. J. Syst. Evol. Microbiol. 54 (2004) 1621–1626.

[31] L. Leistner, Basic aspects of food preservation by hurdle technology, Int. J. Food Microbiol. 55 (2000) 181–186.

[32] F. Leroi, J.J. Joffraud, F. Chevalier, M. Cardinal, Study of the microbial ecology of cold-smoked salmon during storage at 8 °C, Int. J. Food Microbiol. 39 (1998) 111–121.

[33] F. Leroy, L. de Vuyst, The presence of salt and a curing agent reduces bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation, Appl. Environ. Microbiol. 65 (1999) 5350–5356.

[34] F. Leroy, L. de Vuyst, Temperature and pH conditions that prevail during fermentation of sausages are optimal for production of the antilisterial bacteriocin sakacin K, Appl. Environ. Microbiol. 65 (1999) 974–981.

[35] S.L. Liu, A. Hessel, K.E. Sanderson, Genomic mapping with I-*Ceu* I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia*

coli, and other bacteria, Proc. Natl. Acad. Sci. USA 90 (1993) 6874–6878.
[36] U. Lyhs, J. Bjorkroth, H. Korkeala, Characterisation of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping, Int. J. Food Microbiol. 52 (1999) 77–84.
[37] T. Møretrø, B.F. Hagen, L. Axelsson, A new, completely defined medium for meat lactobacilli, J. Appl. Microbiol. 85 (1998) 715–722.
[38] C.I. Mortvedt, J. Nissen-Meyer, K. Sletten, I.F. Nes, Purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus sake* L45, Appl. Environ. Microbiol. 57 (1991) 1829–1834.
[39] A. Nigatu, Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate *Lactobacillus plantarum*, *Lact. fermentum*, *Lact. rhamnosus*, *Lact. sake*, *Lact. parabuchneri*, *Lact. gallinarum*, *Lact. casei*, *Weissella minor* and related taxa isolated from kocho and tef, J. Appl. Microbiol. 89 (2000) 969–978.
[40] A. Nigatu, S. Ahrne, G. Molin, Randomly amplified polymorphic DNA (RAPD) profiles for the distinction of *Lactobacillus* species, Antonie van Leeuwenhoek 79 (2001) 1–6.
[41] O.L. Nyquist, A. McLeod, Å. Aakra, L. Snipen, I.F. Nes, Comparative genomics of *Lactobacillus sakei*, 2008, submitted for publication.
[42] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.
[43] P.H. Savelkoul, H.J. Aarts, J. de Haas, L. Dijkshoorn, B. Duim, M. Otsen, J.L. Rademaker, L. Schouls, J.A. Lenstra, Amplified-fragment length polymorphism analysis: the state of an art, J. Clin. Microbiol. 37 (1999) 3083–3091.
[44] L. Snipen, D. Repsilber, L. Nyquist, A. Ziegler, A. Aakra, A. Aastveit, Detection of divergent genes in microbial aCGH experiments, BMC Bioinformatics 7 (2006) 181.
[45] H. Speijer, P.H. Savelkoul, M.J. Bonten, E.E. Stobberingh, J.H. Tjhie, Application of different genotyping methods for *Pseudomonas aeruginosa* in a setting of endemicity in an intensive care unit, J. Clin. Microbiol. 37 (1999) 3654–3661.
[46] P.S. Tichaczek, R.F. Vogel, W.P. Hammes, Cloning and sequencing of sakP encoding sakacin P, the bacteriocin produced by *Lactobacillus sake* LTH 673, Microbiology 140 (1994) 361–367.
[47] T. Toda, M. Itaya, I-*Ceu*I recognition sites in the *rrn* operons of the *Bacillus subtilis* 168 chromosome: inherent landmarks for genome analysis, Microbiology 141 (1995) 1937–1945.
[48] S. Torriani, G.A. Van Reenen, G. Klein, G. Reuter, F. Dellaglio, L.M. Dicks, *Lactobacillus curvatus* subsp. *curvatus* subsp. nov. and *Lactobacillus curvatus* subsp. *melibiosus* subsp. nov. and *Lactobacillus sake* subsp. *sake* subsp. nov. and *Lactobacillus sake* subsp. *carnosus* subsp. nov., new subspecies of *Lactobacillus curvatus* Abo-Elnaga and Kandler 1965 and *Lactobacillus sake* Katagiri, Kitahara, and Fukami 1934 (Klein et al. 1996, emended descriptions), respectively, Int. J. Syst. Bacteriol. 46 (1996) 1158–1163.
[49] S. Torriani, F. Clementi, M. Vancanneyt, B. Hoste, F. Dellaglio, K. Kersters, Differentiation of *Lactobacillus plantarum*, *L. pentosus* and *L. paraplantarum* species by RAPD-PCR and AFLP, Syst. Appl. Microbiol. 24 (2001) 554–560.
[50] R. Urso, G. Comi, L. Cocolin, Ecology of lactic acid bacteria in Italian fermented sausages: isolation, identification and molecular characterization, Syst. Appl. Microbiol. 29 (2006) 671–680.
[51] L. Vermeiren, F. Devlieghere, J. Debevere, Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products, Int. J. Food Microbiol. 96 (2004) 149–164.
[52] R.F. Vogel, M. Lohmann, M. Nguyen, A.N. Weller, W.P. Hammes, Molecular characterization of *Lactobacillus curvatus* and *Lact. sake* isolated from sauerkraut and their application in sausage fermentations, J. Appl. Bacteriol. 74 (1993) 295–300.

Paper II

BMC Microbiology

RESEARCH ARTICLE Open Access

Primary metabolism in *Lactobacillus sakei* food isolates by proteomic analysis

Anette McLeod*[1,2], Monique Zagorec[3], Marie-Christine Champomier-Vergès[3], Kristine Naterstad[1] and Lars Axelsson[1]

Abstract

Background: *Lactobacillus sakei* is an important food-associated lactic acid bacterium commonly used as starter culture for industrial meat fermentation, and with great potential as a biopreservative in meat and fish products. Understanding the metabolic mechanisms underlying the growth performance of a strain to be used for food fermentations is important for obtaining high-quality and safe products. Proteomic analysis was used to study the primary metabolism in ten food isolates after growth on glucose and ribose, the main sugars available for *L. sakei* in meat and fish.

Results: Proteins, the expression of which varied depending on the carbon source were identified, such as a ribokinase and a D-ribose pyranase directly involved in ribose catabolism, and enzymes involved in the phosphoketolase and glycolytic pathways. Expression of enzymes involved in pyruvate and glycerol/glycerolipid metabolism were also affected by the change of carbon source. Interestingly, a commercial starter culture and a protective culture strain down-regulated the glycolytic pathway more efficiently than the rest of the strains when grown on ribose. The overall two-dimensional gel electrophoresis (2-DE) protein expression pattern was similar for the different strains, though distinct differences were seen between the two subspecies (*sakei* and *carnosus*), and a variation of about 20% in the number of spots in the 2-DE gels was observed between strains. A strain isolated from fermented fish showed a higher expression of stress related proteins growing on both carbon sources.

Conclusions: It is obvious from the data obtained in this study that the proteomic approach efficiently identifies differentially expressed proteins caused by the change of carbon source. Despite the basic similarity in the strains metabolic routes when they ferment glucose and ribose, there were also interesting differences. From the application point of view, an understanding of regulatory mechanisms, actions of catabolic enzymes and proteins, and preference of carbon source is of great importance.

Background

Lactobacillus sakei is an important food-associated lactic acid bacterium (LAB). Although initially characterized from rice wine [1] and isolated from plant fermentations [2,3] and fermented fish [4,5], its main habitat is meat [6]. It is widely used as starter culture in the production of fermented meat products [7], and is regarded as a potential meat and fish biopreservative [8-10]. *L. sakei* resists harsh conditions which often prevail during preservation, such as high salt concentration, low water activity, low temperature and pH [11]. An important property of the bacterium is the production of lactic acid that acidifies the product and both inhibits growth of spoilage bacteria and food pathogens, and confers taste and texture to the fermented products. The species has also been observed as a transient inhabitant of the human gastrointestinal tract [12-15]. Sequence analysis of the *L. sakei* 23K genome has provided valuable information, showing a specialized metabolic repertoire that reflects adaptation to meat products [16].

Among the few sugars available in meat and fish, *L. sakei* utilizes glucose and ribose for growth. The two sugars are fermented through different metabolic pathways: sugar hexose fermentation is homolactic and proceeds via the glycolytic pathway leading to lactate, whereas pentoses are fermented through the heterolactic phosphoketolase pathway ending with lactate and other end products such as acetate [17,18]. A correlation between

* Correspondence: anette.mcleod@nofima.no
[1] Nofima Mat AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Osloveien 1, NO-1430 Ås, Norway
Full list of author information is available at the end of the article

glucose and ribose metabolism has been suggested for *L. sakei*, and this metabolism could be advantageous in competition with the other microbial flora found on meat [17,19]. With regard to glucose metabolism, the central glycolytic operon, also called the *gap* operon (*cggR-gap-pgk-tpi-eno*), encodes enzymes that catalyse steps of the glycolysis and the putative central glycolytic gene regulator (CggR) [20]. Glucose is transported and phosphorylated by the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) encoded by the *ptsHI* operon, and by one or more additional non-PTS permeases [18]. A unique *L. sakei rbsUDKR* (LSA0200-0203) gene cluster responsible for ribose catabolism has been described, which encodes a ribose transporter (RbsU), a D-ribose pyranase (RbsD), a ribokinase (RbsK) and the ribose operon transcriptional regulator (RbsR) [16,17,21]. RbsR was shown to function as a local repressor on *rbsUDK*, and as a *ptsI* mutant increased transport and phosphorylation of ribose, the PTS was suggested to negatively control ribose utilization [16,17,21,22]. Moreover, regulation by carbon catabolite repression (CCR) mediated by catabolite control protein A (CcpA) has been suggested, as a putative catabolite responsive element (*cre*) site, the binding site of CcpA, was found preceding *rbsD* [23-25].

It has been proposed that the species can be divided into two subspecies described as *L. sakei* subsp. *sakei* and *L. sakei* subsp. *carnosus* based on results from numerical analyses of total cell soluble protein content and randomly amplified polymorphic DNA (RAPD) patterns [26-28]. *L. sakei* species display a large genomic diversity with more than 25% variation in genome size between isolates [29]. In a previous study, we investigated the diversity of ten *L. sakei* strains by phenotypic and genotypic methods, and could report a wide phenotypic heterogeneity and the presence of two genetic groups which coincide with the subspecies [30]. The growth rates of the strains on glucose and ribose varied, indicating different abilities to metabolize the two sugars. Acidification properties in a meat model also showed differences between the strains, possibly reflecting that some are more suited as starter or protective cultures than others [30]. In this study, we used a proteomic approach to compare the same ten strains, which are isolates from meat and fermented meat products, saké, and fermented fish [30]. We investigated their metabolic routes when growing in a defined medium [31] supplemented with glucose and ribose. Two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry (MS) allowed identification of proteins, the expression of which varied depending on the carbon source used for growth. Previous studies used 2-DE to obtain an overview of global changes in the *L. sakei* proteome as function of uracil deprivation [32], anaerobiosis [33], adaption to cold temperatures and addition of NaCl [34], and high hydrostatic pressure [35]. However, studies on the global protein expression patterns during growth of this bacterium on various carbohydrates have not been reported, and importantly, studies to detect specific differences between strains of *L. sakei* are needed. Our aim in this study was to gain further knowledge about the primary metabolism in *L. sakei*, and to look at strain diversity in this regard.

Methods

Bacterial strains, media and growth conditions

The bacterial strains included in this work are listed in Table 1. The organisms were maintained at -80°C in MRS broth [36] (Oxoid) supplemented with 20% glycerol. The complex medium MRS (Oxoid) was used for *L. sakei* propagation, and a completely defined medium (DML) [31], supplemented with either 0.5% glucose (DMLG), 0.5% ribose (DMLR) or 0.5% ribose + 0.02% glucose (DMLRg), was used for liquid cultures. Optical density at 600 nm (OD_{600}) was monitored on an Ultrospec 3000 UV/Visible Spectrophotometer (Pharmacia Biotech). Cells were grown at 30°C in MRS to early exponential phase (OD_{600} = 0.2-0.5), before inoculation (about 10^4 times diluted) in DML. Under these conditions the cultures were in exponential phase after an overnight incubation. The subcultures were used to inoculate to an initial concentration of 0.07 OD_{600} in fresh DML medium. To monitor the growth rate, flasks containing the cell cultures were stirred moderately to keep bacteria in suspension. For 2-DE analysis samples were prepared from DMLG and DMLRg cultures. Samples were extracted from two independent 100 ml cultures grown to mid-exponential phase (OD_{600} = 0.5-0.6).

Extraction of soluble proteins

Proteins were prepared as described by Marceau *et al.* [32] with the following modifications: Cultures of 100 ml were centrifuged at 2800 × *g* at 4°C and washed twice in 0.01 M Tris-HCl buffer, pH 7.5 for 15 min. Bacterial pellets were resuspended in 0.5 ml of the same buffer and 500 mg glass beads were added (acid-washed <106 microns; Sigma-Aldrich). Cells were mechanically disrupted with an FP120 FastPrep cell disruptor (BIO101, Thermo Savant) by four 30 s cycles of homogenization at speed 6.5 with 1 min intervals in ice. Unbroken cells and large cellular debris were removed by centrifugation at 20 800 × *g* for 30 min at 4°C. Protein concentrations of the supernatant (cytosolic fraction) were measured using the colorimetric assay *RC DC* Protein Assay (Bio-Rad), using bovine serum albumin (BSA) as standard protein, according to the manufacturer's instructions. The supernatants were stored in aliquots at -80°C.

Table 1: Strains used in this study.

Bacterial strain	Source	Reference
L. sakei 23K	Sausage	[66,67]
L. sakei MF1053	Fermented fish (Norwegian "Rakfisk")	[30]
L. sakei LS 25	Commercial starter culture for salami sausage	[68]
L. sakei Lb790x	Meat	[69]
L. sakei LTH673	Fermented sausage	[70,71]
L. sakei MF1328	Fermented sausage	[30]
L. sakei MF1058 (TH1)	Vakuum-packed cooked meat, protective culture	[9,10]
L. sakei CCUG 31331[a] (DSM 15831[b], R 14 b/a)	Fermented sausage, type strain for *L. sakei* subsp. *carnosus*	[27,72]
L. sakei DSM 20017[b] (ATCC 15521[c])	Sake, alcoholic beverage made by fermenting rice, type strain for *L. sakei* subsp. *Sakei*	[27]
L. sakei Lb16 (Lb1048[d], CCUG 42687[a])	Minced meat	[31,73]

[a] CCUG, Culture Collection, University of Gothenburg, Sweden.
[b] DSM, Deutsche Samlung von Microorganismen und Zellkulturen, Braunschweig, Germany.
[c] ATCC, American Type Culture Collection, Manassas, VA, USA.
[d] Designation used in the strain collection at Federal Institute for Meat research, Kulmbach, Germany.

Two-dimensional gel electrophoresis conditions

Aliquots of the *L. sakei* cytosolic fraction corresponding to 50 μg (analytical gel) or 200 μg (preparative gel) of protein were diluted by adding a rehydration buffer (6 M urea (Merck), 2 M thiourea (Merck), 4% 3- [(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS; Sigma-Aldrich), 0.5% immobilized pH gradient (IPG) buffer pH 4-7 (GE Healthcare Bio-Sciences), and 2.5% dithiothreitol (DTT; Bio-Rad)) to a final volume of 380 μl. This solution was used to rehydrate 18-cm pH 4-7 linear IPG strips (GE Healthcare BioSciences). Strips were passively rehydrated at room temperature for 12-16 h under mineral oil, before isoelectric focusing (IEF) was performed in an Ettan IPGphor II unit (GE Healthcare Bio-Sciences, Uppsala, Sweeden) as follows: 200 V for 1 h, 500 V for 1 h, 1000 V for 1 h, from 1000 to 8000 V in 30 min, and finally 8000 V for 6 h. The strips were incubated at room temperature for 15 min in equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% (v/l) glycerol (Merck) and 2% (w/v) sodium dodecyl sulfate (SDS; Shelton Scientific)) supplemented with 1% (w/v) DTT, followed by 15 min in equilibration buffer containing 2.5% (w/v) iodoacetamide (Merck). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% acrylamide gels was carried out with an Ettan DALT II system (GE Healthcare Bio-Sciences, Uppsala, Sweeden). Proteins were resolved at 20°C at a current of 2.5 mA/gel for 45 min and then at 25 mA/gel until the tracking dye had migrated to the bottom of the gel. Analytical gels were silver stained as described by Blum *et al.* [37] and preparative gels according to Shevchenko *et al.* [38]. For the final analysis, three 2-DE gels were run from each strain from each of the two independent bacterial cultures.

Image and statistical analysis

Digitized 2-DE images (16-bit greyscale, 300 dpi) of the stained gels were acquired with an office scanner (Epson Perfection 4990 Photo, Epson) and imported into Progenesis SameSpots software v.3.1 (Nonlinear Dynamics). For each strain, five glucose images and five ribose images were aligned using one selected glucose image as a reference [39]. Spots were detected simultaneously across the images leading to one spot map, an approach which addresses the problems of missing values and reduces variance in spot volume across biological or technical replicates by applying the same spot outline across the image series [39,40]. The spot pattern was manually edited, gel artefacts were removed, and images were grouped glucose vs. ribose. An automatic analysis (spot detection, background subtraction, normalisation, and matching) was performed by the software, creating one way ANOVA p-values and q-values as measures of statistical significance, and fold change based on spot normalized volumes of the two groups. Whereas the p-value is a measure of significance in terms of false positive rate, the q-value (or FDR adjusted p-value) is a measure in terms of the false discovery rate (FDR) [41]. Spot normalized volumes were in addition imported into 50-50 MANOVA

http://www.langsrud.com/stat/ffmanova.htm for statistical analysis. Rotation tests were performed with 9999 simulations for spot normalized volumes, producing q-values. Differential protein expression was considered to be significant at the level of $q < 0.05$ from both the SameSpots software and rotation tests, and the expression patterns were checked visually to observe how the spot intensity differed. For strain comparison, a representative image from the sequenced strain *L. sakei* 23K was used as a reference. Selected images from each of the other strains from both carbon sources were compared to detect distinct strain differences.

Protein identification

The protein spots of interest presenting a change in volume depending on carbon source used for growth were excised from preparative gels from the sequenced strain 23K. To confirm the identity of the same spots in other strains, we also excised the spots from strains MF1053 and LS 25. Spots presenting distinct strain differences were excised from strain 23K and MF1053. Samples were prepared for matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS analysis according to the method of Jensen *et al*. [42] with modifications described previously [43]. For purification of digested proteins columns were prepared by packing a plunge of C18 material (3 M Empore C18 extraction disc, Varian) into a gel loader tip (20 μl, Eppendorf). An Ultraflex MALDI-TOF/TOF mass spectrometer with the LIFT module (Bruker Daltonics, GmbH, Bremen, Germany) was used for protein identification. Peptide calibration standard I (Bruker Daltonics) was used for external calibration. The software FlexAnalysis 2.4 (Bruker Daltonics) was used to create peak lists using median baseline subtraction with 0.8 in flatness and smoothing by the Savitzky-Golay filter of 0.2 *m/z* in width. BioTools 3.1 (Bruker Daltonics) was used for interpretation of MS and MS/MS spectra. Proteins were identified by peptide mass fingerprinting (PMF) using the database search program MASCOT http://www.matrixscience.com/, searching against the NCBInr database http://www.ncbi.nih.gov/ with the following settings: Other firmicutes, MS tolerance of 50 ppm and MS/MS tolerance of 0.5 Da, maximum missed cleavage sites was 1, Carbamidomethyl (C) and Oxidation (M) were set as fixed and variable modification, respectively. The number of peptide matches, sequence coverage, pI and MW were used to evaluate the database search results.

Results and Discussion

In this study, we used proteomics to compare ten *L. sakei* food isolates regarding their metabolic routes when growing on glucose and ribose.

Growth of L. sakei strains on glucose and ribose

The ten strains investigated showed faster growth rates when utilizing glucose as the sole carbon source (DMLG; glucose 0.5%) compared with ribose (DMLR; ribose 0.5%), a finding in agreement with previous observations [16-18,30], confirming that glucose is the preferred carbon source in *L. sakei*. Preliminary 2-DE analysis of strains 23K, MF1053 and LS 25 resulted in gels with large differences in protein spot resolution (results not shown). Gels of samples issued from bacteria grown on ribose as the sole carbon source were of poor quality. Cell proteolysis due to slow growth and prolonged incubation time may result in protein degradation and solubilization defect, as has previously been proposed [44]. Previous studies suggested a regulation of ribose utilization by the PTS and co-metabolism of these two sugars that are present in meat [17,19,21]. Since the addition of small amounts of glucose has been described to enhance growth on ribose [45], we used DMLRg (ribose 0.5%, glucose 0.02%) for further experiments. This indeed resulted in faster growth rates and a better spot resolution of the resulting 2-DE gels that were comparable to the gels from bacterial samples grown in DMLG (results not shown). Thus further experiments were performed by growing bacteria in DMLG and DMLRg to study the glucose and ribose metabolisms, respectively.

Protein patterns of the ten L. sakei strains

After growth on glucose (in DMLG) and ribose (in DMLRg) an average of approximately 400 spots was observed after 2-DE in the pI range investigated. A variation of about 20% in the number of spots was detected between the strains, as previously observed within the species [29,35]. The overall protein expression pattern was similar for the different strains grown on both carbon sources (data not shown), though distinct differences in the 40-kDa region of the 2-DE gels were observed (Figure 1). These differences were identified as resulting from two different migration profiles of four isoforms (different pI) of the glyceraldehyde-3-phosphate dehydrogenase (GapA) protein. The isoforms displayed a size variation, previously described by Chaillou *et al.* [29] to differentiate two *L. sakei* subgroups. Grouping of our ten strains based on the GapA isoforms migration profile was identical to the two genetic clusters previously obtained from rapidly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microarray-based comparative genome hybridization (CGH) analyses [30]. If those grouping methods reflect the subspecies division of *L. sakei*, eight of our strains including the sequenced strain 23K and the type strain CCUG 31331 belong to *L. sakei* subsp. *carnosus*, while the type strain DSM 20017 and the commercial starter culture strain LS 25 belong to *L. sakei* subsp. *sakei*.

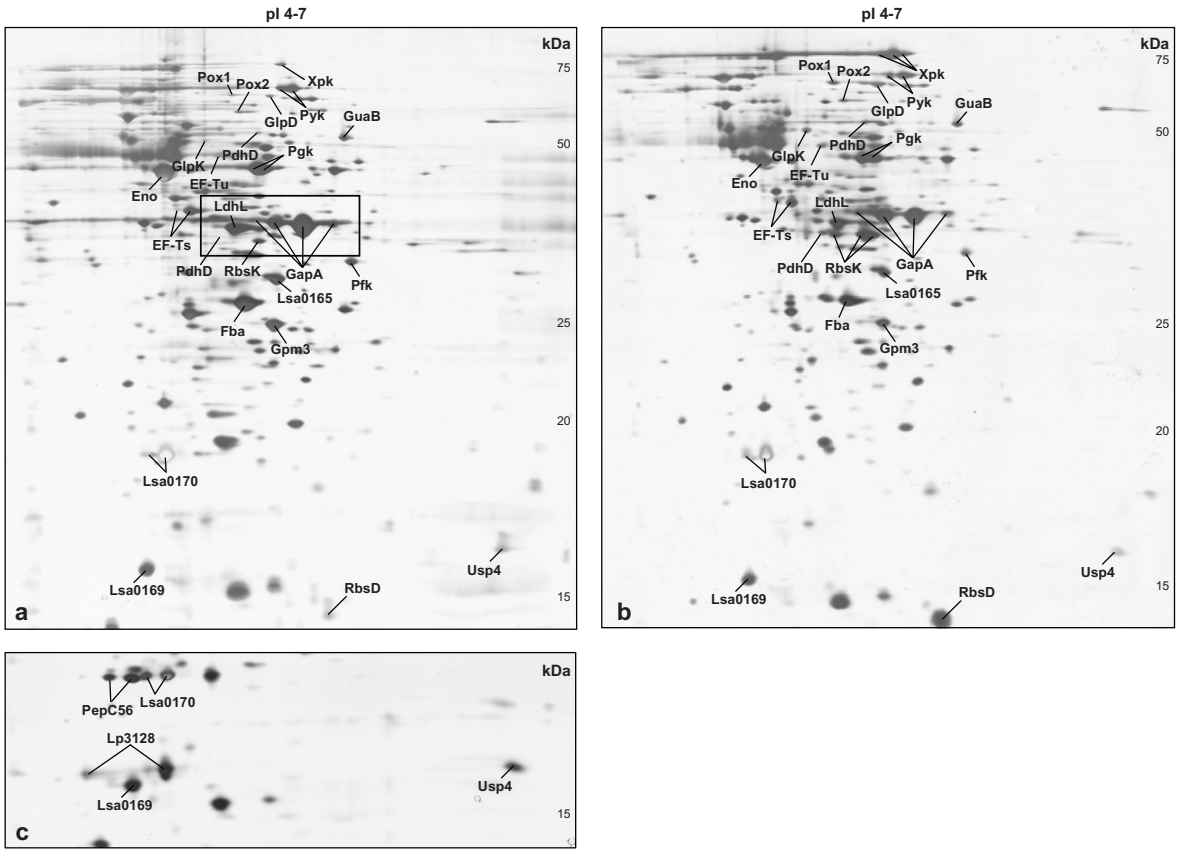


Figure 1 Silver-stained 2-DE gels images of *Lactobacillus sakei* strain 23K grown in a completely defined medium supplemented with glucose (a) or ribose (b), and the lower part of a 2-DE gel image from *L. sakei* strain MF1053 grown on glucose (c). Protein (50 μg) was loaded, and 2-DE was performed using a pH range of 4-7 in the first dimension and SDS-PAGE (12.5%) in the second dimension. Protein size (kDa) is shown on the right side of each gel image. Spots listed in Additional files 1 and 2, Tables S2 and S3 are indicated. The black rectangle (a) shows the region of the GapA isoforms which differ among the strains.

Comparison of protein patterns obtained from cells grown on glucose or ribose revealed, for all the strains, differences in the expression profiles. The spots presenting a volume change depending on the carbon source used for growth and identified by MALDI-TOF MS are shown in Figure 1ab in representative 2-DE gel images. All the proteins could be identified against *L. sakei* 23K proteins, as shown in Additional file 1, Table S2. Data obtained for a few spots gave less statistically significant results (q = 0.05-0.1) due to co-migration of proteins which made quantification measurements unreliable. However, visual inspection of these protein spots in the 2-DE gels confirmed a modification in their volume. Nine proteins displayed a different level of expression in all tested strains, whereas 11 proteins varied in at least one of the strains (Additional file 1). Moreover, when compared to the other strains we observed that *L. sakei* MF1053 over-expressed a set of seven proteins after growth on both carbon sources, as shown in Additional file 2, Table S3. The proteins could be identified against *L. sakei* 23K proteins, except for two proteins which identified against proteins from other *L. sakei* strains and were similar to proteins from *Lactobacillus plantarum* and *Lactobacillus buchneri* (Additional file 2). The presence of several isoforms with different pIs was also noticed for several proteins (Additional files 1 and 2). Many proteins are modified after synthesis by different types of post-translational modifications (PTM) which may control the protein activity, and the most common PTM accounted for pI differences is phosphorylation [46].

Proteins differentially expressed between growth on glucose and ribose

In total, ten proteins were up-regulated in all or most of the strains after growth on ribose. Among those, three are directly involved in ribose catabolism: RbsD, the D-

ribose pyranase, RbsK, the ribokinase, and Xpk, the putative phosphoketolase. This is in accordance with finding by Stentz *et al*. [17] who observed the induction of the *rbsUDKR* operon transcription and an increase of phosphoketolase and ribokinase activity after growth on ribose. The two pyruvate oxidases and two of the four components of the pyruvate dehydrogenase complex (PDC) were also detected as up-regulated in ribose grow cells. In addition, GlpK and GlpD, the glycerol kinase and glycerol-3-phosphate dehydrogenase were detected in higher quantities in most of the strains after growth on ribose. Conversely, six proteins were down-regulated on glucose, of which four were involved in glycolysis. The inosine-5-monophosphate dehydrogenase (GuaB), involved in purine metabolism, and the putative oxidoreductase Lsa0165 were down-regulated, whereas the elongation factor Ts (EF-Ts) was up-regulated on ribose. An overview of the catabolic pathways for glucose (glycolysis) and ribose (phosphoketolase pathway) utilization in *L. sakei* is shown in Figure 2. Proteins whose expression was modified in cells grown on ribose are shown.

It is likely that the induction of RbsK and Xpk and hence the phosphoketolase pathway in the cells restricts the flow of carbon down the glycolytic route. In many microorganisms, the glycolytic flux depends on the activity of 6-phosphofructokinase (Pfk) and pyruvate kinase (Pyk) [47,48]. Similar to several other LAB [48-50] these two enzymes are encoded from a *pfk-pyk* operon [34], and as reflected at the level of genetic structure, a lower expression of both enzymes was seen on ribose in all strains examined. A lower expression of Pfk was also observed by Stentz *et al*. [17] during growth on ribose. The glycolytic enzymes fructose-1,6-bisphosphate aldolase (Fba) and a phosphoglycerate mutase (Gpm3) showed a lower expression in most of the strains, and interestingly, strains LS 25 and MF1058 showed a lower expression of three more glycolytic enzymes compared to the rest of the strains. It is possible that these strains have a more efficient mechanism of down-regulating the glycolytic pathway. LS 25 is an industrially used starter culture for fermented sausages, while MF1058 is suitable as a protective culture in vacuum packed fresh meat [9,10]. From a meat model system based on minced meat fermentation we previously observed that these two strains performed the fastest acidification of the ten strains, and also had the ability to compete with the indigenous microbiota of the meat batter [30]. Although the triosephosphate isomerase (Tpi), GapA, phosphoglycerate kinase (Pgk), and enolase (Eno) are all encoded from the *gap* operon [20], our proteome data showed a significantly lower expression only for GapA, Pgk and Eno. In addition, expression of the L-lactate dehydrogenase (LdhL) responsible for the reduction of pyruvate to lactic acid was observed to be lower in the two strains.

The bacterium alters its pyruvate metabolism growing on ribose compared to glucose, possibly since during ribose utilization, more ATP is generated from pyruvate per ribose unit when acetate is produced than when lactate is produced [51]. The up-regulated pyruvate oxidases convert pyruvate into acetyl-phosphate, and the PDC catalyses the transformation of pyruvate to acetyl-CoA (Figure 2).

The increased GlpD enzyme belongs to the glycerol/glycerolipid catabolic pathway, a pathway linked to membrane properties as glycerol-3-phosphate can be converted to phosphatidic acid, which leads to membrane phospholipid synthesis. Also when exposed to low temperature, this protein shows an increased expression in *L. sakei* [34]. Modified membrane properties could potentially also exist as a response to the higher level of acetate produced when utilizing ribose. Acetate has a higher antimicrobial effect than lactate, with pK_a values of 4.74 and 3.86, respectively, and the proportion of antimicrobial undissociated acetic acid molecules is increased as the pH is lowered. The *glpD* gene is associated in a *glp* operon with glycerol kinase (*glpK*), which also showed an increased expression on ribose, and glycerol uptake facilitator protein (*glpF*) genes [34].

The role of CcpA in CCR in *L. plantarum* has previously been established, and CcpA was shown to mediate regulation of the *pox* genes encoding pyruvate oxidases [52,53]. Rud [54] observed an up-regulation of several genes and operons including the *pox* genes, the *pdh* operon encoding the PDC, and the *glp* operon, during growth on ribose compared with glucose. As putative *cre* sites [55] were identified in promoter regions, their expression was suggested to be regulated by CcpA-mediated CCR. The putative *cre* site found preceding *rbs* in *L. sakei* [25], could indicate that this bacterium possesses global regulation mediated by CcpA. In an *rbsR* mutant overexpressing RbsUDK, the growth on ribose was not accelerated, whereas in a *ptsI* mutant, the transcription of *rbsUDK* was not modified, but transport and phosphorylation of ribose increased. Thus it was concluded that the PTS negatively controls ribose utilization, by a direct or indirect way [17,22]. Nevertheless, a change in expression of the PTS enzymes could not be detected in our ribose 2-DE gels. Further experiments are needed to elucidate the mechanism by which the *rbs* operon is regulated.

The EF-Ts, with an increased expression on ribose, is involved in protein synthesis and translation elongation, and the less expressed GuaB is involved in nucleotide biosynthesis, where ribose is a source for the basic molecule phosphoribosylpyrophosphate (PRPP). Finally, the putative oxidoreductase Lsa0165, also less expressed on ribose, belongs to the short-chain dehydrogenases/reductases family (SDR), possibly a glucose dehydrogenase.

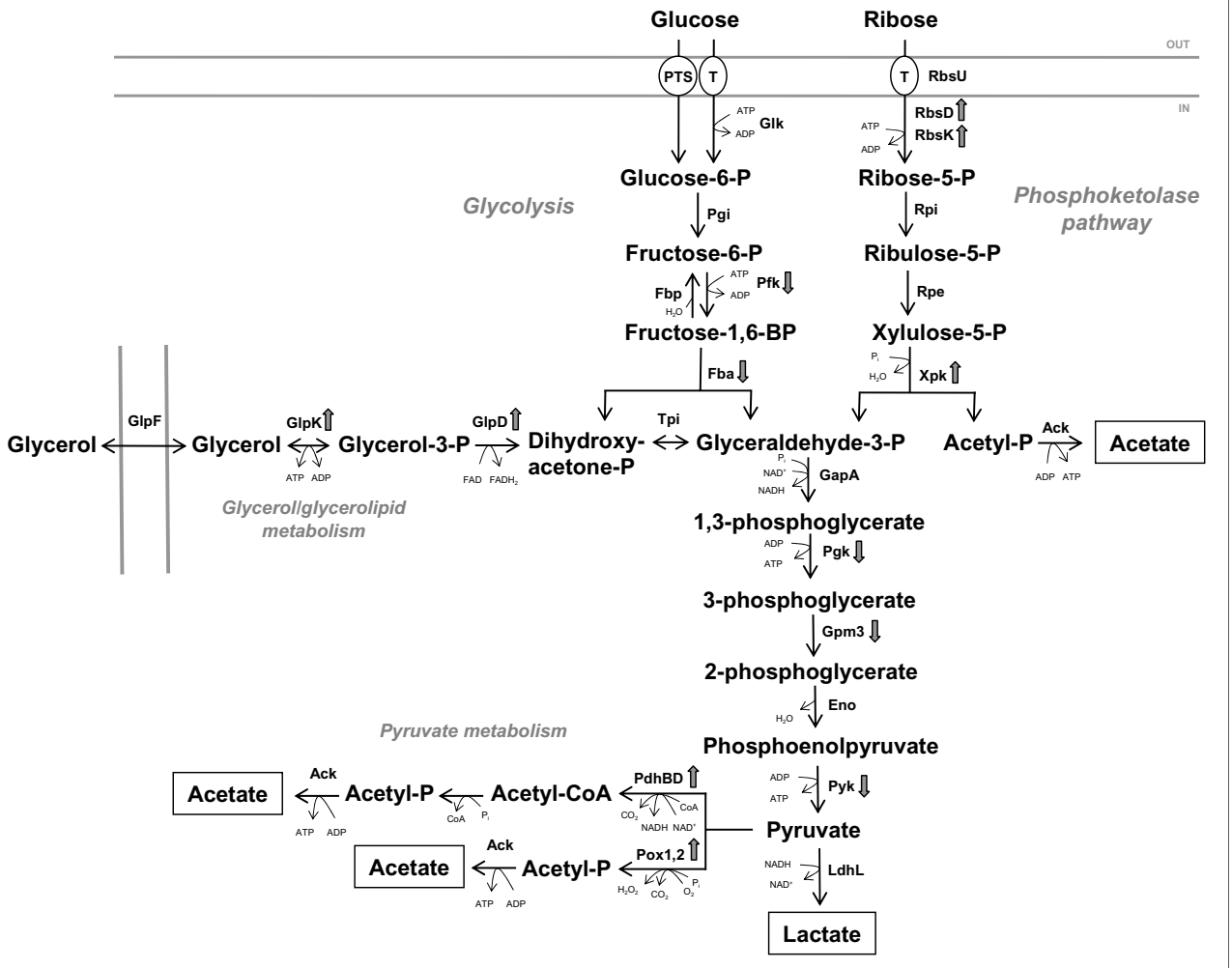


Figure 2 Overview of the metabolic pathways for glucose and ribose fermentation in *L. sakei*. Enzymes which expression is up- or down-regulated on ribose compared with glucose in the majority of the ten *L. sakei* strains (see Additional file 1, Table S2) are indicated with upward and downward pointing arrows, respectively. End-products are boxed. PTS, phosphotransferase system; T, transport protein; P, phosphate; B, bis; Glk, glucokinase; Pgi, phosphoglucoisomerase; Fbp, fructose-1,6-bisphosphatase; Pfk, 6-phosphofructokinase; Fba, fructose-bisphosphate aldolase; RbsU, ribose transporter; RbsD, D-ribose pyranase; RbsK, ribokinase; Rpi, ribose-5-phosphate isomerase; Rpe, ribulose-phosphate 3-epimerase; Xpk, xylulose-5-phosphate phosphoketolase; Tpi, triose-phosphate isomerase; GapA, glyceraldehyde-3-phosphate dehydrogenase; Pgk, phosphoglycerate kinase; Gpm3, phosphoglycerate mutase; Eno, enolase; Pyk, pyruvate kinase; LdhL, L-lactate dehydrogenase; PdhBD, pyruvate dehydrogenase complex subunits B and D; Pox1,2, pyruvate oxidase; Ack, acetate kinase; GlpD, glycerol-3-phosphate dehydrogenase; GlpK, glycerol kinase; GlpF, glycerol uptake facilitator protein.

Proteins over-expressed in L. sakei MF1053

Interestingly, compared to the other strains *L. sakei* MF1053 showed a higher expression of seven proteins related to stress whatever the carbon source used for growth (Figure 1c). A list of the proteins and references where their involvement in different stresses are described [56-65], are listed in Additional file 2, Table S3. The reason for the observed difference in expression of these stress proteins remains to be elucidated.

Conclusions

At present, the complete *L. sakei* genome sequence of strain23K is available [16], and the genome sequence of strain DSM 15831 is currently under assembly http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi. It is obvious from the data obtained in this study that the proteomic approach efficiently identify differentially expressed proteins caused by the change of carbon source. However, the absence of genome sequence remains a limiting factor for the identification of proteins in the non sequenced strains. Sequence analysis has provided valuable information, showing a metabolic repertoire that reflects adaptation to meat, though genomic analyses provide a static view of an organism, whereas proteomic analysis allows a more dynamic observation. Despite the basic similarity in the strains metabolic routes when they ferment glucose

and ribose, there were also differences. We are currently combining proteomic and transcriptomic data of different *L. sakei* strains and hope to reveal more about the primary metabolism. From the application point of view, to understand regulatory mechanisms, actions of catabolic enzymes and proteins, and preference of carbon source is of great importance.

Additional material

Additional file 1 Table S2. Identification of protein spots differentially expressed depending on the carbon source used for growth in ten *L. sakei* strains. Presents identification and characteristics of protein spots with a significant volume change depending on the carbon source used for growth in ten *L. sakei* strains.

Additional file 2 Table S3. Proteins over-expressed in *L. sakei* MF1053. Presents the identification and characteristics of protein spots over-expressed in *L. sakei* MF1053 compared to the other *L. sakei* strains in this study.

Abbreviations

2-DE: two dimensional gel electrophoresis; MALDI-TOF MS: matrix-assisted laser desorption/ionization-time of flight mass spectrometry; MW: molecular weight; pI: isoelectric point; RbsD: D-ribose pyranase; RbsK: ribokinase; Fbp: fructose-1,6-bisphosphatase; Pfk: 6-phosphofructokinase; Fba: fructose-bisphosphate aldolase; Xpk: xylulose-5-phosphate phosphoketolase; Tpi: triosephosphate isomerase; GapA: glyceraldehyde-3-phosphate dehydrogenase; Pgk: phosphoglycerate kinase; Gpm: phosphoglycerate mutase; Eno: enolase; Pyk: pyruvate kinase; LdhL: L-lactate dehydrogenase; PDC: pyruvate dehydrogenase complex; Pox: pyruvate oxidase; Ack: acetate kinase; GlpD: glycerol-3-phosphate dehydrogenase; GlpK: glycerol kinase; GuaB: inosine-5-monophosphate dehydrogenase; EFTs: elongation factor Ts; CCR: carbon catabolite repression; CcpA: catabolite control protein A.

Authors' contributions

AM participated in the design of the study, conducted the experimental work, image and statistical analysis, analyzed and interpreted data, and drafted the manuscript. MZ, MCCV, KN and LA conceived the study, participated in the study design process, and helped write the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by Grant 159058/I10 from the Norwegian Research Council and by a Short Term Fellowship from the European Molecular Biology Organization (EMBO). The authors would like to thank Fabienne Baraige and Paricia Anglade for their contribution during the preliminary 2-DE and MS analyses. We also thank Morten Skaugen for excellent technical assistance during MS analysis. Ellen Mosleth Færgestad and Stefania Gudrun Bjarnadottir are acknowledged for their contribution during statistical analysis.

Author Details

[1]Nofima Mat AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Osloveien 1, NO-1430 Ås, Norway, [2]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway and [3]Unité Flore Lactique et Environnement Carné, UR309, INRA, Domaine de Vilvert, F-78350 Jouy en Josas, France

Received: 10 February 2010 Accepted: 22 April 2010
Published: 22 April 2010

References

1. Katagiri H, Kitahara K, Fukami K: **The characteristics of the lactic acid bacteria isolated from moto, yeast mashes for sake manufacture. Part IV. Classification of the lactic acid bacteria.** *Bulletin of Agricultural and Chemical Society of Japan* 1934, **10:**156-157.
2. Klaenhammer T, Altermann E, Arigoni F, Bolotin A, Breidt F, Broadbent J, Cano R, Chaillou S, Deutscher J, Gasson M, Guchte M van de, Guzzo J, Hartke A, Hawkins T, Hols P, Hutkins R, Kleerebezem M, Kok J, Kuipers O, Lubbers M, Maguin E, McKay L, Mills D, Nauta A, Overbeek R, Pel H, Pridmore D, Saier M, van Sinderen D, Sorokin A, *et al.*: **Discovering lactic acid bacteria by genomics.** *Antonie Van Leeuwenhoek* 2002, **82:**29-58.
3. Vogel RF, Lohmann M, Nguyen M, Weller AN, Hammes WP: **Molecular characterization of *Lactobacillus curvatus* and *Lact. sake* isolated from sauerkraut and their application in sausage fermentations.** *J Appl Bacteriol* 1993, **74:**295-300.
4. Leroi F, Joffraud JJ, Chevalier F, Cardinal M: **Study of the microbial ecology of cold-smoked salmon during storage at 8 degrees C.** *Int J Food Microbiol* 1998, **39:**111-121.
5. Lyhs U, Bjorkroth J, Korkeala H: **Characterisation of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping.** *Int J Food Microbiol* 1999, **52:**77-84.
6. Hammes WP, Bantleon A, Min S: **Lactic acid bacteria in meat fermentation.** *FEMS Microbiol Rev* 1990, **87:**165-174.
7. Hammes WP, Hertel C: **New developments in meat starter cultures.** *Meat Science* 1998, **49:**125-138.
8. Vermeiren L, Devlieghere F, Debevere J: **Evaluation of meat born lactic acid bacteria as protective cultures for biopreservation of cooked meat products.** *Int J Food Microbiol* 2004, **96:**149-164.
9. Bredholt S, Nesbakken T, Holck A: **Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat.** *Int J Food Microbiol* 1999, **53:**43-52.
10. Bredholt S, Nesbakken T, Holck A: **Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats.** *Int J Food Microbiol* 2001, **66:**191-196.
11. Axelsson L, Ahrné S: **Lactic acid bacteria.** In *Applied microbial systematics* Edited by: Priest FG, Goodfellow M. Dordrechet, The Netherlands: Kluwer Academic Press; 2000:365-386.
12. Chiaramonte F, Blugeon S, Chaillou S, Langella P, Zagorec M: **Behavior of the meat-borne bacterium *Lactobacillus sakei* during its transit through the gastrointestinal tracts of axenic and conventional mice.** *Appl Environ Microbiol* 2009, **75:**4498-4505.
13. Dal Bello F, Walter J, Hammes WP, Hertel C: **Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition.** *Microb Ecol* 2003, **45:**455-463.
14. Heilig HG, Zoetendal EG, Vaughan EE, Marteau P, Akkermans AD, de Vos WM: **Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA.** *Appl Environ Microbiol* 2002, **68:**114-123.
15. Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP: **Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis.** *Appl Environ Microbiol* 2001, **67:**2578-2585.
16. Chaillou S, Champomier-Vergès MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V, Beaufils S, Darbon-Rongere E, Bossy R, Loux V, Zagorec M: **The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K.** *Nat Biotechnol* 2005, **23:**1527-1533.
17. Stentz R, Cornet M, Chaillou S, Zagorec M: **Adaption of *Lactobacillus sakei* to meat: a new regulatory mechanism of ribose utilization?** *INRA, EDP Sciences* 2001, **81:**131-138.
18. Lauret R, Morel-Deville F, Berthier F, Champomier Vergès MC, Postma P, Erlich SD, Zagorec M: **Carbohydrate utilization in *Lactobacillus sake*.** *Appl Environ Microbiol* 1996, **62:**1922-1927.
19. Champomier-Vergès MC, Chaillou S, Cornet M, Zagorec M: **Erratum to " *Lactobacillus sakei* : recent developments and future prospects".** *Res Microbiol* 2002, **153:**115-123.
20. Naterstad K, Rud I, Kvam I, Axelsson L: **Characterisation of the *gap* operon from *Lactobacillus plantarum* and *Lactobacillus sakei*.** *Curr Microbiol* 2007, **54:**180-185.
21. Stentz R, Zagorec M: **Ribose utilization in *Lactobacillus sakei* : analysis of the regulation of the *rbs* operon and putative involvement of a new transporter.** *J Mol Microbiol Biotechnol* 1999, **1:**165-173.
22. Stentz R, Lauret R, Ehrlich SD, Morel-Deville F, Zagorec M: **Molecular cloning and analysis of the *ptsHI* operon in *Lactobacillus sake*.** *Appl Environ Microbiol* 1997, **63:**2111-2116.
23. Stulke J, Hillen W: **Carbon catabolite repression in bacteria.** *Curr Opin Microbiol* 1999, **2:**195-201.

24. Titgemeyer F, Hillen W: **Global control of sugar metabolism: a gram-positive solution.** *Antonie Van Leeuwenhoek* 2002, **82:**59-71.
25. Rodionov DA, Mironov AA, Gelfand MS: **Transcriptional regulation of pentose utilisation systems in the *Bacillus/Clostridium* group of bacteria.** *FEMS Microbiol Lett* 2001, **205:**305-314.
26. Klein G, Dicks LMT, Pack A, Hack B, Zimmerman K, Dellaglio F, Reuter G: **Emended description of *Lactobacillus sake* (Katahiri, Katahara and Fukami) and *Lactobacillus curvatus* (Abo-Elnega Kandler): Numerical classification revealed by protein fingerprinting and identification based on biochemical patterns and DNA-DNA hybridizations.** *Int J Syst Bacteriol* 1996, **46:**367-376.
27. Torriani S, Van Reenen GA, Klein G, Reuter G, Dellaglio F, Dicks LM: ***Lactobacillus curvatus* subsp. *curvatus* subsp. nov. and *Lactobacillus curvatus* subsp. *melibiosus* subsp. nov. and *Lactobacillus sake* subsp. *sake* subsp. nov. and *Lactobacillus sake* subsp. *carnosus* subsp. nov., new subspecies of *Lactobacillus curvatus* Abo-Elnaga and Kandler 1965 and *Lactobacillus sake* Katagiri, Kitahara, and Fukami 1934 (Klein et al. emended descriptions), respectively.** *Int J Syst Bacteriol* 1996, **46:**1158-1163.
28. Berthier F, Ehrlich SD: **Genetic diversity within *Lactobacillus sakei* and *Lactobacillus curvatus* and design of PCR primers for its detection using randomly amplified polymorphic DNA.** *Int J Syst Bacteriol* 1999, **49:**997-1007.
29. Chaillou S, Daty M, Baraige F, Dudez AM, Anglade P, Jones R, Alpert CA, Champomier-Vergès MC, Zagorec M: **Intraspecies genomic diversity and natural population structure of the meat-borne lactic acid bacterium *Lactobacillus sakei*.** *Appl Environ Microbiol* 2009, **75:**970-980.
30. McLeod A, Nyquist OL, Snipen L, Naterstad K, Axelsson L: **Diversity of *Lactobacillus sakei* strains investigated by phenotypic and genotypic methods.** *Syst Appl Microbiol* 2008, **31:**393-403.
31. Moretro T, Hagen BF, Axelsson L: **A new, completely defined medium for meat lactobacilli.** *J Appl Microbiol* 1998, **85:**715-722.
32. Marceau A, Mera T, Zagorec M, Champomier-Vergès MC: **Protein expression under uracil privation in *Lactobacillus sakei* .** *FEMS Microbiol Lett* 2001, **200:**49-52.
33. Champomier-Vergès MC, Marceau A, Mera T, Zagorec M: **The *pepR* gene of *Lactobacillus sakei* is positively regulated by anaerobiosis at the transcriptional level.** *Appl Environ Microbiol* 2002, **68:**3873-3877.
34. Marceau A, Zagorec M, Chaillou S, Mera T, Champomier-Vergès MC: **Evidence for involvement of at least six proteins in adaptation of *Lactobacillus sakei* to cold temperatures and addition of NaCl.** *Appl Environ Microbiol* 2004, **70:**7260-7268.
35. Jofre A, Champomier-Vergès M, Anglade P, Baraige F, Martin B, Garriga M, Zagorec M, Aymerich T: **Protein synthesis in lactic acid and pathogenic bacteria during recovery from a high pressure treatment.** *Res Microbiol* 2007, **158:**512-520.
36. De Man JC, Rogosa M, Shape ME: **A medium for the cultivation of lactobacilli.** *J Appl Microbiol* 1960, **23:**130-135.
37. Blum H, Beier H, Gross HJ: **Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels.** *Electrophoresis* 1987, **8:**93-99.
38. Shevchenko A, Wilm M, Vorm O, Mann M: **Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels.** *Anal Chem* 1996, **68:**850-858.
39. Faergestad EM, Rye M, Walczak B, Gidskehaug L, Wold JP, Grove H, Jia X, Hollung K, Indahl UG, Westad F, Berg F van den, Martens H: **Pixel-based analysis of multiple images for the identification of changes: a novel approach applied to unravel proteome patters of 2-D electrophoresis gel images.** *Proteomics* 2007, **7:**3450-3461.
40. Karp NA, Feret R, Rubtsov DV, Lilley KS: **Comparison of DIGE and post-stained gel electrophoresis with both traditional and SameSpots analysis for quantitative proteomics.** *Proteomics* 2008, **8:**948-960.
41. Storey JD, Tibshirani R: **Statistical significance for genomewide studies.** *Proc Natl Acad Sci USA* 2003, **100:**9440-9445.
42. Jensen ON, Larsen MR, Roepstorff P: **Mass spectrometric identification and microcharacterization of proteins from electrophoretic gels: strategies and applications.** *Proteins* 1998, **2:**74-89.
43. Jia X, Ekman M, Grove H, Faergestad EM, Aass L, Hildrum KI, Hollung K: **Proteome changes in bovine *longissimus thoracis* muscle during the early postmortem storage period.** *J Proteome Res* 2007, **6:**2720-2731.
44. Rabilloud T: **Solubilization of proteins for electrophoretic analyses.** *Electrophoresis* 1996, **17:**813-829.
45. Deutscher J, Francke C, Postma PW: **How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria.** *Microbiology and Molecular Biology Reviews* 2006, **70:**939-1031.
46. Manning G, Plowman GD, Hunter T, Sudarsanam S: **Evolution of protein kinase signaling from yeast to man.** *Trends Biochem Sci* 2002, **27:**514-520.
47. Kandler O: **Carbohydrate metabolism in lactic acid bacteria.** *Antonie Van Leeuwenhoek* 1983, **49:**209-224.
48. Branny P, De La Torre F, Garel JR: **Cloning, sequencing, and expression in *Escherichia coli* of the gene coding for phosphofructokinase in *Lactobacillus bulgaricus*.** *J Bacteriol* 1993, **175:**5344-5349.
49. Crispie F, Anba J, Renault P, Ehrlich D, Fitzgerald G, van Sinderen D: **Identification of a phosphofructokinase-encoding gene from *Streptococcus thermophilus* CNRZ1205-a novel link between carbon metabolism and gene regulation?** *Mol Genet Genomics* 2002, **268:**500-509.
50. Viana R, Perez-Martinez G, Deutscher J, Monedero V: **The glycolytic genes *pfk* and *pyk* from *Lactobacillus casei* are induced by sugars transported by the phosphoenolpyruvate:sugar phosphotransferase system and repressed by CcpA.** *Arch Microbiol* 2005, **183:**385-393.
51. Axelsson L: **Lactic acid bacteria: classification and physiology.** In *Lactic acid bacteria: microbiological and functional aspects* 3rd edition. Edited by: Salminen S, von Wright A, Ouwehand A. New York, USA: Marcel Dekker, Inc. CRC Press; 2004:1-66.
52. Muscariello L, Marasco R, De Felice M, Sacco M: **The functional *ccpA* gene is required for carbon catabolite repression in *Lactobacillus plantarum*.** *Appl Environ Microbiol* 2001, **67:**2903-2907.
53. Lorquet F, Goffin P, Muscariello L, Baudry JB, Ladero V, Sacco M, Kleerebezem M, Hols P: **Characterization and functional analysis of the *poxB* gene, which encodes pyruvate oxidase in *Lactobacillus plantarum*.** *J Bacteriol* 2004, **186:**3749-3759.
54. Rud I: **Primary metabolism in *Lactobacillus* - a study of control and regulation of acid production.** In *PhD thesis* Ås, Norway: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences; 2008.
55. Weickert MJ, Chambliss GH: **Site-directed mutagenesis of a catabolite repression operator sequence in *Bacillus subtilis*.** *Proc Natl Acad Sci USA* 1990, **87:**6238-6242.
56. Antelmann H, Bernhardt J, Schmid R, Mach H, Volker U, Hecker M: **First steps from a two-dimensional protein index towards a response-regulation map for *Bacillus subtilis*.** *Electrophoresis* 1997, **18:**1451-1463.
57. Duche O, Tremoulet F, Glaser P, Labadie J: **Salt stress proteins induced in *Listeria monocytogenes*.** *Appl Environ Microbiol* 2002, **68:**1491-1498.
58. Duche O, Tremoulet F, Namane A, Labadie J: **A proteomic analysis of the salt stress response of *Listeria monocytogenes*.** *FEMS Microbiol Lett* 2002, **215:**183-188.
59. Drews O, Weiss W, Reil G, Parlar H, Wait R, Gorg A: **High pressure effects stepwise altered protein expression in *Lactobacillus sanfranciscensis*.** *Proteomics* 2002, **2:**765-774.
60. Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R, Tarchini R, Peters SA, Sandbrink HM, Fiers MW, Stiekema W, Lankhorst RM, Bron PA, Hoffer SM, Groot MN, Kerkhoven R, de Vries M, Ursing B, de Vos WM, Siezen RJ: **Complete genome sequence of *Lactobacillus plantarum* WCFS1.** *Proc Natl Acad Sci USA* 2003, **100:**1990-1995.
61. Almiron M, Link AJ, Furlong D, Kolter R: **A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*.** *Genes Dev* 1992, **6:**2646-2654.
62. Choi SH, Baumler DJ, Kaspar CW: **Contribution of *dps* to acid stress tolerance and oxidative stress tolerance in *Escherichia coli* O157:H7.** *Appl Environ Microbiol* 2000, **66:**3911-3916.
63. Malone AS, Chung YK, Yousef AE: **Genes of *Escherichia coli* O157:H7 that are involved in high-pressure resistance.** *Appl Environ Microbiol* 2006, **72:**2661-2671.
64. Weber A, Kogl SA, Jung K: **Time-dependent proteome alterations under osmotic stress during aerobic and anaerobic growth in *Escherichia coli*.** *J Bacteriol* 2006, **188:**7165-7175.
65. Hengge R, Bukau B: **Proteolysis in prokaryotes: protein quality control and regulatory principles.** *Mol Microbiol* 2003, **49:**1451-1462.

66. Berthier F, Zagorec M, Champomier-Vergès MC, Ehrlich SD, Morel-Deville F: **Efficient transformation of *Lactobacillus sake* by electroporation.** *Microbiol* 1996, **142:**1273-1279.
67. Dudez AM, Chaillou S, Hissler L, Stentz R, Champomier-Vergès MC, Alpert CA, Zagorec M: **Physical and genetic map of the *Lactobacillus sakei* 23K chromosome.** *Microbiology* 2002, **148:**421-431.
68. Hagen BF, Naes H, Holck AL: **Meat starters have individual requirements for Mn2+.** *Meat Science* 2000, **55:**161-168.
69. Axelsson L, Lindstad G, Naterstad K: **Development of an inducible gene expression system for *Lactobacillus sakei*.** *Lett Appl Microbiol* 2003, **37:**115-120.
70. Eijsink VG, Brurberg MB, Middelhoven PH, Nes IF: **Induction of bacteriocin production in *Lactobacillus sake* by a secreted peptide.** *J Bacteriol* 1996, **178:**2232-2237.
71. Tichaczek PS, Vogel RF, Hammes WP: **Cloning and sequencing of *sakP* encoding sakacin P, the bacteriocin produced by *Lactobacillus sake* LTH 673.** *Microbiology* 1994, **140:**361-367.
72. Koort J, Vandamme P, Schillinger U, Holzapfel W, Bjorkroth J: ***Lactobacillus curvatu* s subsp. *melibiosus* is a later synonym of *Lactobacillus sakei* subsp. *carnosus*.** *Int J Syst Evol Microbiol* 2004, **54:**1621-1626.
73. Aasen IM, Moretro T, Katla T, Axelsson L, Storro I: **Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687.** *Appl Microbiol Biotechnol* 2000, **53:**159-166.

doi: 10.1186/1471-2180-10-120
Cite this article as: McLeod *et al.*, Primary metabolism in Lactobacillus sakei food isolates by proteomic analysis *BMC Microbiology* 2010, **10**:120

Table S2. Protein spots with a volume change depending on the carbon source used for growth in ten *L. sakei* strains. The proteins were identified against *L. sakei* 23K (Lsa) proteins. "+" and "-" refer to a statistically significant ($q < 0.05$) higher and lower expression on ribose compared with glucose, respectively.

Functional category	Spot name[a]	Protein identification	NCBI GI identifier	aa	Matched peptides / % sequence coverage[b]	MW[c] / MW[d] (x 10^3)	pI[c] / pI[d]	Average protein level ratio (fold change)[e, f]									
								23K	MF 1053	LS 25	Lb 790x	LTH 673	MF 1328	MF 1058	CCUG 31331	DSM 20017	Lb 16
Carbohydrate metabolism																	
Phosphoketolase pathway	RbsK	Ribokinase Lsa0202	gi|81427812	302	18 / 74	31.66 / 34	5.01 / 4.9, 5.0, 5.05	++	++	++	++	++	++	++	++	++	++
	Xpk	Putative phosphoketolase Lsa0289	gi|81427904	787	19 / 21	88.70 / 86	5.14 / 5.10, 5.15, 5.2	++	++	++	+++	++	+++	++	++	++	++
Pyruvate metabolism	Pox1	Pyruvate oxidase Lsa1188	gi|81428800	611	31 / 66	66.76 / 66	4.97 / 4.9	+	++	++	++	++	++	++	++	++	+
	Pox2	Pyruvate oxidase Lsa1830	gi|81429441	577	17 / 30	62.36 / 62	4.99 / 4.95	+	no	no	+	no	+	+	+	no	no
	PdhB	Pyruvate dehydrogenase complex E1-β subunit Lsa1084	gi|81428694	332	10 / 41	36.13 / 36	5.09 / 4.8	++	++	++	++	++	++	++	++	++	++
	PdhD	Dihydrolipoamide dehydrogenase Lsa1082	gi|81428692	468	10 / 24	49.64 / 52	5.07 / 5.0	+	++	+	++	+[g]	+	+[g]	++	+	+
Glycolysis	Fba	Fructose-bisphosphate aldolase Lsa1527	gi|81429140	287	11 / 46	30.88 / 28	4.93 / 4.95	-	-	-[g]	no	-	-	- -	-	-[g]	no
	Pyk	Pyruvate kinase Lsa1032	gi|81428643	586	31 / 66	62.92 / 68	5.19 / 5.15, 5.2	-	- -	-	-	- -	-[g]	- -	-	-[g]	-
	Gpm3	Phosphoglycerate mutase Lsa0206	gi|81427816	229	9 / 54	25.85 / 25	5.04 / 5.1	-	-	-	-[g]	-	no	- -	-[g]	-	-
	Pfk	6-phosphofructokinase Lsa1033	gi|81428644	319	24 / 70	34.34 / 32	5.36 / 5.4	-	-	-	-[g]	-	-[g]	-	-	-	-
	Pgk	Phosphoglycerate kinase Lsa0605	gi|81428220	404	20 / 62	42.72 / 42	5.03 / 5.0, 5.05	no	no	-	no	no	no	- -	no	no	no
	GapA	Glyceraldehyde-3-phosphate dehydrogenase Lsa0604	gi|81428219	338	16 / 53	35.47 / 35	5.19 / 5.0, 5.1, 5.2, 5.3	no	no	-	no	no	no	-	no	no	no
	LdhL	L-lactate dehydrogenase Lsa1606	gi|81429218	325	28 /82	35.42 / 34	4.99 / 4.9	no	no	-	no	no	no	-	no	no	no
	Eno	Phosphopyruvate hydratase / enolase Lsa0607	gi|81428222	431	28 /66	46.55 / 41	4.70 / 4.6	no	no	-	no	no	no	-	no	no	no
Membrane transport	RbsD	D-ribose pyranase Lsa0201	gi|81427811	131	5 / 52	14.30 / 14	5.32 / 5.3	+++	+++	+++	++	++	+++	++	++	+++	++
Glycerol/glycerolipid metabolism	GlpD	Glycerol-3-phosphate dehydrogenase Lsa0650	gi|81428265	608	44 / 74	66.77 / 66	5.13 / 5.05	++	+++	++	++	++	+++	+++	+++	++	++
	GlpK	Glycerol kinase Lsa0276	gi|81428263	505	22 / 39	55.89 / 51	4,89 / 4.75	++	++	+	no	++	no	+	+	no	++
Nucleotide metabolism	GuaB	Inositol-5-monophosphate dehydrogenase Lsa0276	gi|81427891	493	28 / 68	52.30 / 52	5.39 / 5.35	no	-	-	no	-	-	no	no	-	-
Oxidoreductase activity	Lsa0165	Putative oxidoreductase Lsa0165	gi|81427775	296	15 / 60	31.33 / 31	5.09 / 5.1	no	-	-	no	- -	-	-	no	no	-
Protein synthesis	EF-Ts	Elongation factor Ts Lsa1264	gi|81428875	291	15 / 51	31.31 / 38	4.82 / 4.65, 4.7	+	no	no	+	++	no	++	+	no	+

[a] Spot name referring to the spots labelled in Figure 1.
[b] Matched peptides and percentage of amino acid coverage for the 23K strain.
[c] Theoretical MW (kDa) and pI values.
[d] Estimated MW (kDa) and pI values by 2-DE from strain 23K. Several pI values for one protein refer to different isoforms.
[e] Average fold change increase (+) or decrease (-) on ribose gels compared to glucose gels. +++, > 5; ++, 2-5; +, 1.5-2; -, 1.5-2; - -, 2-5.
[f] no, no change in expression.
[g] $0.05 > q > 0.1$.

Table S3. Protein spots over-expressed in *L. sakei* MF1053 compared to the other *L. sakei* strains in this study. "+" refers to a statistically significant ($q < 0.05$) higher expression on 2-DE gels from growth in both DMLG and DMLRg compared to the reference *L. sakei* 23K.

Spot name[a]	Protein identification[b]	NCBI GI identifier	aa	Matched peptides / % sequence coverage	MW[c] / MW[d] (x 10^3)	pI[c]/pI[d]	Average protein level ratio (fold change)[e, f]		Protein function, stress, reference
							23K	MF1053	
GuaB	Inositol-5-monophosphate dehydrogenase Lsa0276	gi|81427891	493	28 / 68[g]	52.30 / 52[g]	5.39 / 5.35[g]	p	++	Nucleotide biosynthesis. Superoxide stress in *Bacillus subtilis* [56] and salt stress in *Listeria monocytogenes* [57,58].
EF-Tu	Elongation factor Tu Lsa1063	gi|81428673	396	15 / 40[g]	43.27 / 43[g]	4.72 / 4.8[g]	p	+++	Translation elongation, protein folding and protection from stress. High-pressure in *Lactobacillus sanfranciscensis* [59] and salt stress in *Listeria monocytogenes* [65,66].
Lsa0169	Putative general stress protein Lsa0169	gi|81427779	142	7 / 38[g]	15.62 / 15[g]	4.71 / 4.55[g]	p	+	General stress. High-pressure in *L. sakei* [35]. Related to alkaline shock proteins (Asp) in *L. plantarum* [60].
Lsa0170	Putative general stress protein Lsa0170	gi|81427780	153	13 / 69[g]	17.06 / 17[g]	4.68 / 4.55, 4.65[g]	p	++	General stress. High-pressure [35] and at cold temperature in *L. sakei* [34]. Related to alkaline shock proteins (Asp) in *L. plantarum* [60].
Usp4	Universal stress protein Lsa1173	gi|81428783	144	8 / 65[g]	15.64 / 16[g]	5.93 / 5.9[g]	p	+++	Universal stress UspA protein family. Six Usp paralogs are present in the *L. sakei* 23K genome [16]. High-pressure in *L. sakei* [35].
Lp3128	Stress induced DNA binding protein from *L. plantarum* WCFS1	gi|28379536	155	9 / 47[h]	18.01 / 16[h]	4.70 / 4.35, 4.7[h]	np	++	DNA protection during starvation (Dps) protein family. Oxidative, acid, osmotic, high-pressure stresses in *E. coli* [61-64].
	Putative ferritin-like DNA binding protein from *L. sakei*	gi|189085575	155	9 / 47[h]	18.04 / 16[h]	4.70 / 4.35, 4.7[h]			
PepC56	C56 family peptidase from *L. sakei* DSM 15831 (genome sequencing status 'assembly')	gi|227361883	167	8 / 55[h]	18.16 / 17[h]	4.47 / 4.45, 4.5[h]	np	+++	Degradation of aberrant and non-functional proteins [65].
	C56 family peptidase from *L. buchneri* ATCC 11577	gi|227513849	167	8 / 55[h]	18.36 / 17[h]	4.65 / 4.45, 4.5[h]			

[a] Spot name referring to the spots labelled in Figure 1.
[b] Lsa refers to *L. sakei* 23K.
[c] Theoretical MW (kDa) and pI values.
[d] Estimated MW (kDa) and pI values by 2-DE. Several pI values for one protein refer to different isoforms.
[e] p, spot present; np, spot not present.
[f] Average fold change increase (+) on glucose gels using strain 23K as a reference. +++, > 5; ++, 2-5; +, 1-2.
[g] Estimated MW (kDa) and pI values, and matched peptides and percentage of amino acid coverage are shown from strain 23K.
[h] Estimated MW (kDa) and pI values, and matched peptides and percentage of amino acid coverage are shown from strain MF1053.

Paper III

Comparative genomics of *Lactobacillus sakei* with emphasis on strains from meat

O. Ludvig Nyquist[1,*], Anette McLeod[1,3,*], Dag A. Brede[1], Lars Snipen[2], Ågot Aakra[1], Ingolf F. Nes[1,§]

[1]Laboratory of Microbial Gene Technology and Food Microbiology, Department of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

[2]Biostatistics, Department of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

[3]Nofima Mat AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Osloveien 1, NO-1430 Ås, Norway

*These authors contributed equally to this work

[§]Corresponding author

E-mail addresses:

OLN: otto.nyquist@umb.no

AM: anette.mcleod@nofima.no

DAB: dag.anders.brede@umb.no

LS: lars.snipen@umb.no

ÅAa: agot.aakra@umb.no

IFN: ingolf.nes@umb.no

Abbreviations: LAB, lactic acid bacteria; CGH, comparative genome hybridization; HGT, horizontal gene transfer.

Abstract

Lactobacillus sakei is a lactic acid bacterium (LAB) important in food microbiology mainly due to its ability to ferment and preserve meat. The genome sequence of *L. sakei* strain 23K has revealed specialized metabolic capacities that reflect the bacterium's adaption to meat products, and that differentiate it from other LAB. We have investigated 18 *L. sakei* isolates, mainly from processed meat, and the sequenced strain by comparative genome hybridization (CGH) experiments. For this we used a microarray based on the 23K genome with an additional set of sequenced *L. sakei* genes including bacteriocin genes. The strains genome sizes varied from 1.880 to 2.175 Mb, and the 23K genome was among the smallest. Consequently, a large part of the genome of this strain belongs to a common gene pool invariant in this species. Furthermore, the genomic divergence corresponded mainly to five regions in the 23K genome, which showed features consistent with horizontal gene transfer. Carbohydrate-fermentation profiles of the strains were evaluated in light of the CGH data, and in general the results correlated. The majority of genes important in adaption to meat products, the ability to flexibly use meat components, and robustness during meat processing and storage were conserved, such as genes involved in nucleoside scavenging, catabolism of arginine, and the ability to cope with changing redox and oxygen levels, indicative of the role these genes play in niche specialization within the *L. sakei* species. Moreover, all the strains carried remnants of or complete bacteriocin operons. The results demonstrate a highly conserved organization of the *L. sakei* genomes, and the 23K strain is a suitable model organism to investigate core features of *L. sakei*.

Introduction

Lactobacillus is the largest and most diverse genus among the lactic acid bacteria (LAB), which currently includes more than 150 species (http://www.bacterio.cict.fr) [1-3]. Their natural habitat ranges from dairy, meat and plant material fermentations to being members of the normal microbiota of the gastrointestinal (GI) tract and oral cavity of humans and animals, some with probiotic effects [4,5]. With the influx of complete genome sequences and analysis thereof, microbial comparative genomics is a rapidly emerging field, providing information that aid to redefine genotypic, and the resulting phenotypic differences between species. Several comparative analyses of LAB genomes included *Lactobacillus* species and have revealed wide phylogenetic and phenotypic diversities of the different species [1,3], as well as genetic niche-specific adaption features [2,6]. The number of predicted protein-coding genes differs from ~1,725 to ~3,000 between *Lactobacillus* genomes, and comparative genomics has suggested that evolution of LAB has been driven by gain of functions by horizontal gene transfer (HGT) from other LAB genomes and loss of dispensable ancestral functions [1,7]. HGT is important in bacterial evolution, whereby integrating phages, transposons and other mobile elements are inserted within the host bacterial genome creating genotypic and phenotypic diversity [8,9]. Microarrays are increasingly used within the microbial comparative genomics field, and whole-genome DNA microarrays are used to assess genomic diversity and compare gene content between genomes of related organisms, as well as different bacterial strains within a species [10-15].

Lactobacillus sakei is known as a food-associated and commercially important lactobacillus, which is frequently isolated from fresh or fermented meat. It is widely used as a starter culture in the meat industry, as it exhibits technological properties optimal for sausage production [16,17]. The bacterium has the ability to change the meat environment in ways that discourage growth of spoilage and pathogenic microorganisms, and thus allow improved preservation and microbial safety of meat products [16,18-20]. This is mainly due to the production of lactic acid as well as other antagonistic molecules such as bacteriocins [21-23]. Many strains of *L. sakei* produce sakacin A or P, which possess strong activity against the food-borne pathogen *Listeria monocytogenes* [24,25]. Besides meat, the bacterium is also found in fish [26,27] or fermented vegetal products [28,29]. In fact, it was initially described in saké, an alcoholic beverage made by fermenting rice [30], thereby its name. The species has been observed as a transient member of the human GI tract [31,32], and mutant strains

were recently reported to colonize the GI tract of axenic mice [31,33], a finding which could lead to increased interest for this species.

The genome sequence analysis of strain 23K [17] revealed specific features that could account for its adaptation to meat and its ability to sustain life under challenging environmental conditions. Its capability to make use of nutritional compounds in meat includes arginine utilization, salvage of exogenous nucleosides, and heme acquisition, in addition to its important traits such as salt tolerance, detoxification, psychrotrophic character, and its ability to cope with changing oxygen conditions have made *L. sakei* a very adaptable and useful LAB in meat processing [17]. *L. sakei* is divided into two subspecies (*sakei* and *carnosus*) [34,35], and strains are known to display a range of phenotypic differences [19,36,37]. An intraspecies genomic diversity study of the species has demonstrated a 25% variation in genome size and a wide genetic variation between different strains [38]. A PCR-based method was used that detected the possession of 60 chromosomal genes belonging to the flexible gene pool of the species, and the results showed that different genotypes may be isolated from similar types of meat products [38]. However, studies to obtain a more detailed view of the gene content between various strains compared to the sequenced genome have not been reported.

In the present paper, we explored the gene content, as well as the genomic and metabolic variation among *L. sakei* strains using microarray-based comparative genome hybridization (CGH), pulsed field gel electrophoresis (PFGE) and carbohydrate fermentation profiling (API 50 CH assay). We have used data from a previous study by McLeod et al. [37] where the diversity of *L. sakei* strains was investigated by the same methods, and examined additional strains; in total 18 *L. sakei* strains are compared with the sequenced strain 23K. The microarray used represents the *L. sakei* 23K genome [17] and an additional set of sequenced *L. sakei* genes including bacteriocin genes. Most of the investigated strains were obtained from meat, while two strains had been isolated from different environments. Moreover, the type strains of both subspecies were included. It was assumed that by identifying the common gene pool invariant among the *L. sakei* species, we would be closer to understanding what genomic components assemble this group of phenotypically diverse organisms. We also expected to get some insight of the regions of the 23K chromosome where variation is prominent and contribute to genetic diversity in this species. A particular focus was put on the meat adaption genes identified in 23K, and an aim of the study was also to examine the abundance of bacteriocin genes in the species.

Methods

Bacterial strains and cultivation

The bacterial strains used in this study are listed in Table 1. All strains were grown in MRS medium (Oxoid) [39] at 30°C, unless stated otherwise.

Table 1. Strains used in this study.

Strain	Description	Origin	Reference
23K	Plasmid cured. Sequenced	French fermented sausage	[17,34,45]
CCUG 31331 (R 14 b/a, DSM 15831)	Type strain for *L. sakei* subsp. *carnosus*	Fermented sausage	[35,36,44]
CTC372	Bacteriocin	Spanish fermented sausage	[52]
CTC460		Spanish fermented sausage	[52]
CTC494	Sakacin K	Spanish fermented sausage	[52,83,84]
DSM 20017 (ATCC 15521)	Type strain for *L. sakei* subsp. *sakei*	Saké, alcoholic beverage made by fermenting rice	[30,35]
L45	Lactocin S producer Two plasmids	Norwegian fermented dry sausage	[21]
Lb16 (Lb1048, CCUG 42687)	Sakacin P producer	Minced meat	[74,85]
Lb77		Spanish fermented dry sausage	Luis Cintas, Madrid, Spain
Lb148		Fermented Spanish dry sausage	[78]
Lb156		Spanish fermented dry sausage	Luis Cintas, Madrid, Spain
Lb706	Sakacin A producer	Meat	[75,86]
Lb790	Two plasmids	Meat	[87]
LMGT2380		Fermented sausage	This study
LS 25	Commercial starter culture for salami sausage	Meat	[37,88]
LTH673	Sakacin P and sakacin Q producer	German fermented dry sausage	[24,89,90]
MF1053	Sakacin P producer	Fermented fish (Norwegian 'Rakfisk')	[37]
MF1058 (TH1)	Protective culture	Vacuum-packed, cooked meat	[18,91]
MF1328		Fermented sausage	[37]

Pulsed Field Gel Electrophoresis

Pulsed field gel electrophoresis (PFGE) and I-*Ceu*-I digestion analysis of *L. sakei* strains CTC372, CTC460, CTC494, L45, Lb77, Lb148, Lb156, Lb706, Lb790, and LMGT2380 were performed as described by McLeod et al. [37] to estimate the chromosomal sizes. *L. sakei* strain 23K was included as a reference, and the molecular mass markers used were yeast chromosome PFG marker (NO345S; New England BioLabs) and Lambda ladder PFG marker (NO340S; New England BioLabs). Two gels were prepared for each strain.

Carbohydrate fermentation

The *L. sakei* strains CTC372, CTC460, CTC494, L45, Lb77, Lb148, Lb156, Lb706, Lb790, and LMGT2380 were subjected to the API 50 CH assay (BioMérieux), according to the manufacturer's instruction. Results for each carbohydrate were read after 24 h and 48 h, and each strain was tested in duplicate.

DNA extraction and labelling of genomic DNA for CGH analysis

Cells from 3 ml of overnight cultures of the *L. sakei* strains (Table 1) were pelleted and used for genomic DNA extraction using Advamax beads (EdgeBioSystems), as described previously [11]. In each CGH experiment, 5 µg DNA from a test strain (non-sequenced strain) and from the reference strain 23K was labelled with either Cyanine 5-dUTP (Cy5; PerkinElmer Life Sciences) or Cyanine 3-dUTP (Cy3; PerkinElmer Life Sciences). The DNA was labelled using the BioPrime DNA labelling kit (Invitrogen) as described previously [11].

Microarray design

The microarray used in this work is based on the *L. sakei* 23K genome [17] and an additional set of sequenced *L. sakei* genes ('non-23K genes'). A detailed description of the array is available at http://migale.jouy.inra.fr/sakei/Supplement.html/. The gene probe sequences were 70-mer oligonucleotides supplied as an Array Ready Oligo Set (Operon Biotechnologies), which were spotted in triplicates onto epoxy coated glass slides (Corning). The probes from 23K consisted of protein coding genes, RNA genes, dual sense-strand controls, and non-coding regions ~500 bp. The additional 'non-23K genes' from other *L. sakei* strains included bacteriocin genes, lactose PTS transposon, plasmid pRV500, glucan sucrase, and plasmidic tetracycline resistance. Alien probe sequences (SpotReport Alien Oligo Array Validation System, Stratagene) without homology to any known nucleic acid sequences in public databases, were spotted as negative controls on the arrays. In order to reduce biases due to positional effects, the replicate probes were spotted at different positions on the array. The *L. sakei* 23K genome has been re-annotated since the oligonucleotide probes of this array were designed, and by BLASTing the probe sequences against the genome sequence (GeneBank acc. no. CR936503.1), we found that the probes have one or more matches good enough to yield hybridization (70% identity) in 1879 coding genes and 27 RNA genes according to the

most recent annotation. Our downstream analysis of the 23K genes is focused on these 1906 genes unless stated otherwise.

Hybridization protocol

Labelled DNA from test strains (Table 1) and reference strain 23K was dried through vacuum centrifugation (Savant SPD2010 Speedvac concentrator, Thermo). DNA of each test strain was cohybridized to a microarray along with the reference DNA from strain 23K. The dried labelled DNA was combined and dissolved in 140 µl of hybridization buffer, which contained 50% formamide, 0.1% SDS, 5x SSC-buffer, 40 µg/ml heat denatured salmon sperm DNA, and 1% BSA. The DNA was denatured by boiling twice for 2 min, vortexed at low speed for 10 sec between boiling, and loaded into the hybridization chambers of the HS 400 Pro (Tecan) hybridization station according to the instructions provided by the manufacturer. Hybridization, washing and drying of the arrays was performed as follows: 1) Wash with 2x SSC, 0.2% SDS (42°C) for 30 sec; 2) Sample injection (42°C) with agitation; 3) Hybridization (42°C) with medium agitation frequency for 16 hrs; 4) Wash twice with 2x SSC, 0.2% SDS (42°C) for 1 min, soaking for 3 min; 5) Wash twice with 2x SSC (23°C) for 1 min; 6) Wash with 0.2x SSC (23°C) for 30 sec; 7) Wash with filtered MilliQ water (23°C) for 10 sec; 8) Drying of slides with N_2 (*g*) (23°C) flushing for 3 min. Dye-swap replicates were performed for all microarray experiments in order to remove potential biases associated with labelling and subsequent scanning.

Scanning and image analysis

The hybridized arrays were scanned at wavelengths 532 nm (Cy3) and 635 nm (Cy5) using an LS reloaded scanner (Tecan). Image analysis was performed using GenePix Pro v6.0 (Molecular Devices). After localization of the spots, manual filtering was performed to ensure that the spots were properly located, and spots were excluded on the basis of slide or morphology abnormalities.

Data analysis

Microarray data were preprocessed by using the LIMMA package [40] in the R computing environment (http://www.r-project.org). Predictions of presence and divergence of genes based on the CGH data were made by the bias-corrected S-signal prediction method described

by Snipen et al. [41]. This method computes a predicted sequence identity between each probe and the test strain genome. An identity score of 0.7 was used for discriminating present from divergent, i.e. probes with more than 70% identity with the genome are assumed to give hybridization signals, and are defined as 'present'. In the cases where a gene was represented by several probes, the average identity score over all probes was used. Genes with identity score between 0.6 and 0.8 are defined as 'uncertain', reflecting that these genes have a score close to the cutoff.

For visualizing the CGH results for the 18 test strains, a genome atlas presentation was created where the CGH data is compared to the *L. sakei* 23K genome. Interactive Genewiz atlases [42] of CGH data are available at http://ws.cbs.dtu.dk/cgi-bin/gwBrowser/edit.cgi?hexkey=1fc31f07efd0f6fca723c2c859820b00, http://ws.cbs.dtu.dk/cgi-bin/gwBrowser/edit.cgi?hexkey=25856482a5c235c587a216b3498cd7d8, http://ws.cbs. dtu.dk/cgi-bin/gwBrowser/edit.cgi?hexkey=4c2c4c7b908b659fa88cf87d7936b815, and http://ws.cbs.dtu.dk/cgi-bin/gwBrowser/edit.cgi?hexkey=ac2415c7c209612d1e23743ae42ecd45. In order to look for divergent regions along the 23K chromosome, we defined a cumulative divergence score. Based on the total number of present and divergent genes in all 18 strains, we found that if at least four out of 18 strains lack a gene, this gene is significantly 'enriched with divergence'. Based on this observation, we defined the divergence score for every gene as the number of strains lacking the gene (maximum 18), but if the gene is divergent in less than 4 strains the score is set to -10. The cumulative sum of divergence score along the chromosome was computed, but any sum less than zero were re-set to zero. This means the cumulative score will have certain peaks along the chromosome, and the higher peak the more divergent region, indicating many strains lacking genes, and/or many consecutive genes are divergent.

A dendrogram was created by hierarchical clustering using average linkage on the Manhattan distance between strains, i.e. the number of genes where they differ in presence/divergence status, leaving out all genes with uncertain status. The 23K strain was included, having all genes present.

From the J. Craig Venter Institute (JCVI; www.jcvi.org) we downloaded functional categories for all annotated *L. sakei* 23K genes. Unfortunately, the JCVI annotation differs from the GenBank annotation, and functional category information lack for a subset of the 23K genes that we examined. The number of core genes in each functional group was compared to that of the 23K genome, and the Fisher exact test was used to test for over- or under-representation of core genes.

Microarray data accession numbers

The CGH data has been deposited in the Array Express database (http://www.ebi.ac.uk/arrayexpress/) under the accession numbers A-MEXP-1166 (array design), E-MEXP-1639 (hybridization data for strains CCUG 31331, DSM 20017, Lb16, LTH673, LS 25, MF1053, MF1058, and MF1328 presented by McLeod et al. [37]), and E-MEXP-1969 (hybridization data for strains CTC372, CTC460, CTC494, L45, Lb77, Lb790, Lb148, Lb156, and LMGT2380).

Results and Discussion

Strains of the species *L. sakei* are encountered largely from fresh and fermented meat, although also found in fish or fermented vegetal products. Only one complete genome sequence is currently available of the species [17], and this strain originates from meat [34]. In the present work, we have assessed the variation in gene content between 18 *L. sakei* isolates and the sequenced *L. sakei* strain 23K from data obtained by microarray-based CGH analyses. The gene content of 16 strains from meat was examined for the purpose of identifying variation in the strains adaption to this environment, and in addition we examined the gene content of one strain isolated from fermented fish, and one from saké, to determine if niche specific variation exists. The isolates genome size and carbohydrate fermentation profile were also evaluated in light of the CGH data.

Methodical considerations with respect to CGH

Using a 70-mer oligonucleotide-array constructed from the genome sequence of *L. sakei* strain 23K [17] and an additional set of sequenced *L. sakei* genes ('non-23K genes'), a detailed view of the gene content between the various isolates was obtained. We used a recent developed method for the data analysis which computes a predicted sequence identity between each probe and the test strain genome based on the array signals and sequence identity between probes and the reference genome. Genes were considered present or divergent using an identity score of 0.7, thus probes with more than 70% identity with the genome were assumed to give hybridization signals, and were defined as present [41]. This cutoff has previously been a reasonable choice for other 70-mer oligonucleotide arrays [43]. The competitive hybridizations in the CGH experiments included DNA from the reference

strain 23K which then functioned both as an internal standard as well as positive and negative control. Alien probes did not produce signals significantly above background levels from neither the reference nor the test strains in any of the hybridizations. Furthermore, the set of 'non-23K genes' investigated produced the expected hybridization patterns, as divergent in 23K and present in the strains from which they were originally identified and sequenced. In addition, the correlations between the dye-swap experiments ($R^2 > 0.92$ for all the strains; average 0.974) showed that the reproducibility and accuracy of the method were good. In conclusion, we consider the data to be of good quality and reliable. However, a limitation of this type of analysis is that it cannot identify genes present in the test strain but absent from the reference strain represented on the array. Thus, strain-specific genes that are not represented on the array are not identified. Moreover, genes that are present in the test strain but have undergone diversification such that hybridization with the array does not occur, cannot be discriminated from absent genes. The CGH analysis provides information on the gene composition but not the chromosomal locations.

Genomic diversity among *L. sakei* strains compared to strain 23K

To summarize the CGH results, the number of 23K genes present varied between 1674 (87.8%) in strain LMGT2380 and 1852 (97.1%) in L45, with an average of 1764 (92.5%), as shown in Table 2. Thus a large part of the 23K genes are present in all the test strains. The number of uncertain genes were at most only 59 (3.1%; in MF1058), and divergent genes varied from 46 (2.4%) in L45 to 172 (9.0%) in Lb706 (Table 2). In total, 430 genes were divergent or uncertain in at least one of the test strains, as shown in Table 4. A projection of the CGH results for the 18 test strains onto the 23K genome map is shown in Figure 1.

Based on number of genes with different presence/divergence status, leaving out genes with uncertain status, the dendrogram created shows similarity between strains, which represents genomic diversity (Figure 2). Clustering based on the gene specific CGH data was previously reported [37] to be comparable to global clustering profiles based on genetic fingerprinting techniques used to differentiate between the subspecies [34-36,44]. The dendrogram in Figure 2 shows a clear separation of strains LMGT2380, LS 25, and DSM 20017 from the rest of the strains. Suggesting that the method reflects the subspecies division of *L. sakei*, 16 of our strains including type strain CCUG 31331 and the fish strain MF1053 belong to *L. sakei* subsp. *carnosus*, while LMGT2380, LS 25, and the type strain DSM 20017 from saké belong to *L. sakei* subsp. *sakei*. Some variation in the grouping of the strains within

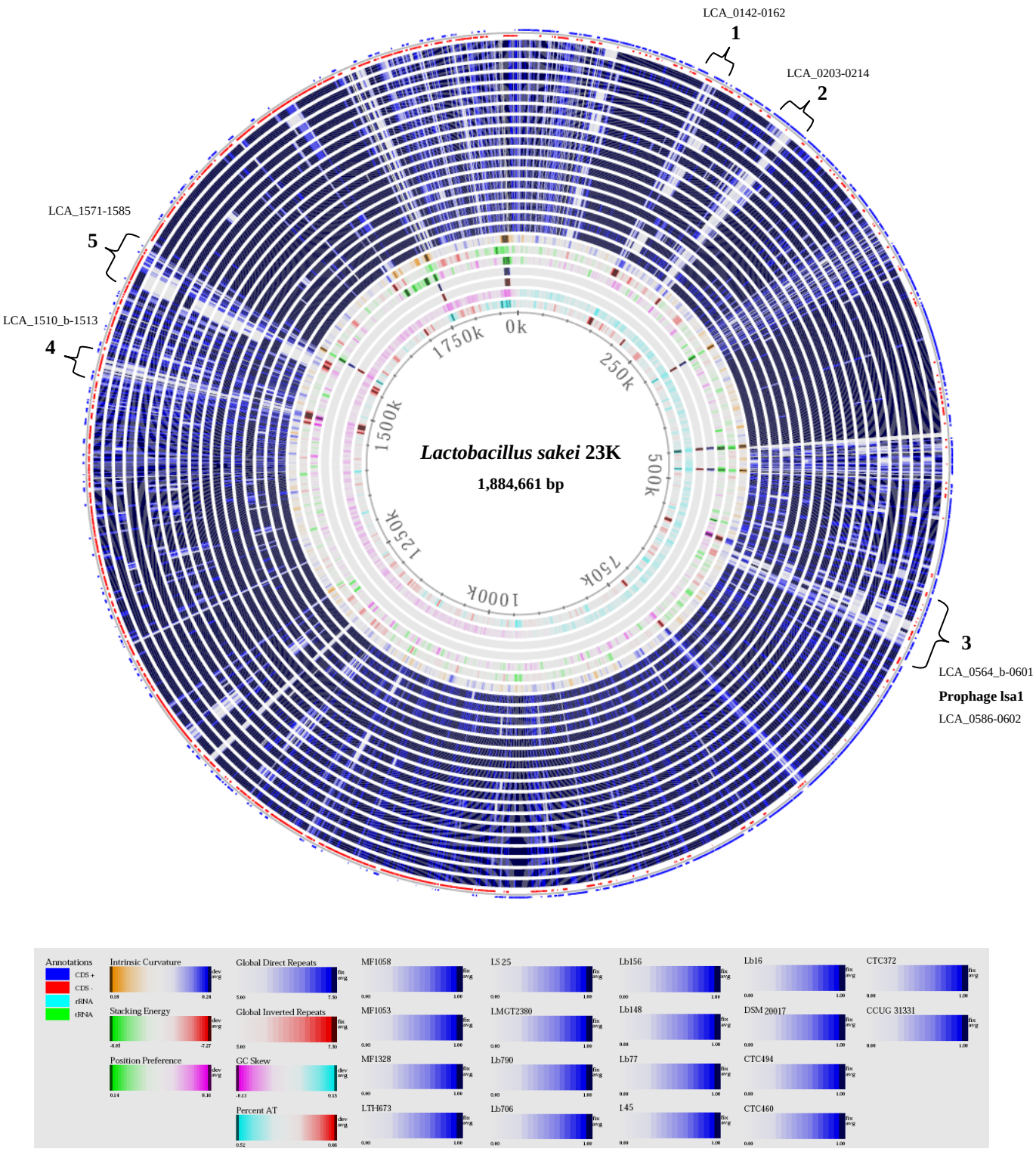


Figure 1. Genome atlas [42] presentation of CGH analysis of 18 *L. sakei* strains compared to the *L. sakei* 23K genome. Regions giving a cumulative divergence score (see text) of at least 75 are indicated by brackets 1-5, the prophage lsa1 is indicated, and locations in the 23K genome (LCA_ numbers) are shown. From outer to inner lanes: 1) 23K annotated CDS, 2) MF1058, 3) MF1053, 4) MF1328, 5) LTH673, 6) LS 25, 7) LMGT2380, 8) Lb790, 9) Lb706, 10) Lb156 11) Lb148, 12) Lb77, 13) L45, 15) Lb16, 16) DSM 20017, 17) CTC494, 18) CTC460, 19) CTC372, 20) CCUG 31331, 21) Intrinsic curvature, 22) Stacking energy, 23) Position preference, 24) Global direct repeats, 25) Global inverted repeats, 26) GC skew, 27) AT percent.

the subspecies *carnosus* was seen when comparing to previous reported clustering based on genomic diversity [37,38].

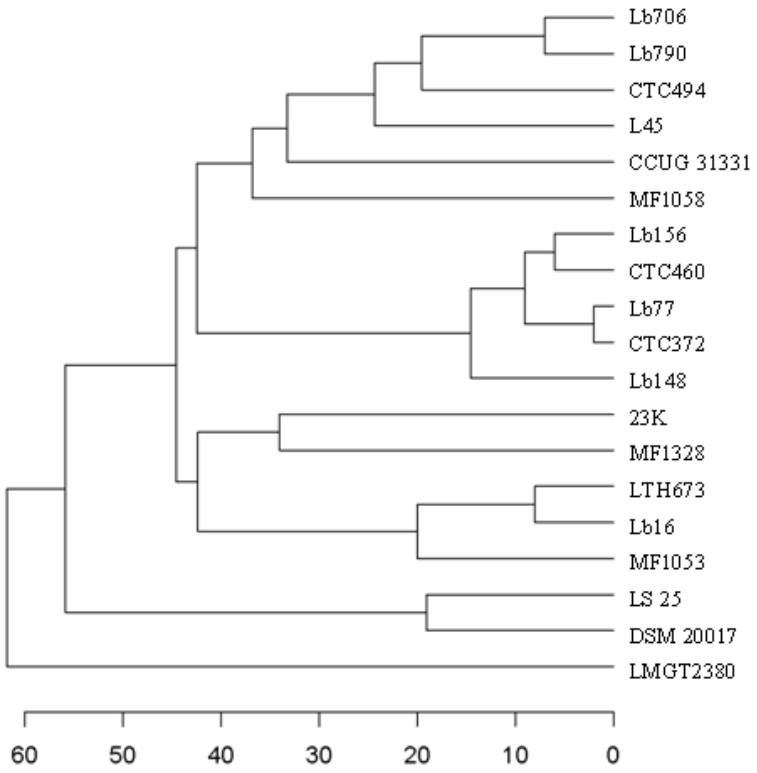


Figure 2. Hierarchical clustering using average linkage based on CGH results. Scale under the dendrogram shows the Manhattan distance which reflects the number of *L. sakei* 23K genes with different presence/divergence status between the strains, leaving out genes with uncertain status. Strain 23K is included, having all genes present.

The extent of genome size variation investigated by PFGE analysis of I-*Ceu*I-digested fragments showed a variation from 1.880 Mb in CTC460 to 2.175 Mb in CCUG 31331 (Table 2), a variation of approximately 300 kb. For some of the strains, the estimated size varied slightly compared to previous findings [38], though well within the expected error of the technique [45]. However, we noticed a larger discrepancy for two of the strains (approximately 100 kb for CCUG 31331 and CTC494). As the I-*Ceu*I recognition site is located in the 23S rRNA gene, the number of DNA bands after I-*Ceu*I digestion and PFGE indicates the number of *rrn* operons. According to our results (Table S1) and previous findings [37], seven DNA fragments were obtained for all the *L. sakei* strains examined, though the presence of a 5-6 kb band for strains CTC494 and DSM 20017 is in disagreement with the findings by Chaillou et al. [38] who described loss of one *rrn* copy for these strains. The reasons for the discrepancies are unclear, but possibly a result of variations in the experimental protocols leading to differences in gel resolution. Genome size could not be

used to distinguish between the two subspecies, as observed previously [38]. The 23K genome was among the smallest in this work, as also reported previously in genome size comparisons within the species [38]. Although microarray-based CGH is a powerful means of exploring the gene content, the analysis is limited by the microarray which represented mainly the reference strain, and the PFGE analysis clearly shows that there are potentially many extra genes in the test strains that are not represented by the relatively small genome of 23K.

Table 2. Numbers and percentages (parenthesis) of divergent (D), uncertain (U) and present (P) genes in the *L. sakei* strains examined by CGH. The threshold between present and divergent in the data analysis was defined at identity score 0.7 (see Method section) and genes with score between 0.6 and 0.8 were counted as uncertain. Genome sizes are listed in the last column.

Strain	D (%)	U (%)	P (%)	Genome size in kb
CCUG 31331	111 (5.8)	27 (1.4)	1769 (92.8)	2175
CTC372	113 (5.9)	2 (0.1)	1792 (94.0)	1928
CTC460	140 (7.3)	32 (1.7)	1735 (91.0)	1880
CTC494	146 (7.7)	40 (2.1)	1721 (90.2)	2011
DSM 20017	68 (3.6)	53 (2.8)	1786 (93.6)	1925
L45	46 (2.4)	9 (0.5)	1852 (97.1)	2029
Lb16	80 (4.2)	36 (1.9)	1791 (93.9)	1918
Lb77	73 (3.8)	35 (1.8)	1799 (94.3)	1919
Lb148	107 (5.6)	56 (2.9)	1744 (91.5)	1939
Lb156	122 (6.4)	30 (1.6)	1755 (92.0)	1970
Lb706	172 (9.0)	53 (2.8)	1682 (88.2)	2056
Lb790	102 (5.3)	40 (2.1)	1765 (92.6)	2025
LMGT2380	177 (9.3)	56 (2.9)	1674 (87.8)	2027
LS 25	73 (3.8)	21 (1.1)	1813 (95.1)	2133
LTH673	97 (5.1)	14 (0.7)	1796 (94.2)	2085
MF1053	106 (5.6)	13 (0.7)	1788 (93.7)	2050
MF1058	128 (6.7)	59 (3.1)	1720 (90.2)	1966
MF1328	117 (6.1)	28 (1.5)	1762 (92.4)	2117
23K	*0 (0)*	*0 (0)*	*1907 (100)*	*1884*
Average	**110 (5.8)**	**34 (1.8)**	**1764 (92.5)**	**2009**

Core Genome and distribution of core genes into functional categories

We found that 1449 of the 23K genes were invariant among the strains investigated and thus most likely constitute the core of functional genes that define *L. sakei* as a species. This finding is in accordance with Cortez et al. [46] who previously investigated bacterial genomes for 'clusters of atypical genes' (CAGs), which likely represent recently integrated foreign DNA elements, and the species specific core genes were reported to be 80% of the 23K genes.

Using the JCVI database (www.jcvi.org), genes were divided into 19 (including unclassified and unknown functions) functional categories and the distribution of the genes in the 23K genome and the core over these functional categories is shown in Table 3. As expected, genes related to signal transduction, transcription, and protein synthesis were over-represented in the core genome, as well as genes related to DNA metabolism. Genes in the categories of mobile and extrachromosomal element functions were under-represented in the core genome.

Table 3. Distribution of genes into functional categories. The first column (23K) lists the number of JCVI-annotated 23K genes in each functional category. Only genes annotated both at JCVI and GenBank are used here, hence the smaller total number of genes. The second column (Core) gives the same result for the core genes, i.e. the subset of genes found present in all 18 test strains. The third column lists the oddsratio for over- or under-representation of core genes in a given functional category. Only oddsratios significantly different from 1.0 in a Fisher test are displayed.

Functional category	23K	Core	Oddsratio
Amino acid biosynthesis	20	17	
Biosynthesis of cofactors, prosthetic groups, and carriers	51	39	
Cell envelope	166	126	
Cellular processes	71	61	
Central intermediary metabolism	66	55	
DNA metabolism	101	87	1.65
Energy metabolism	150	112	
Fatty acid and phospholipid metabolism	35	31	
Hypothetical proteins	178	141	
Mobile and extrachromosomal element functions	14	5	0.14
Protein fate	79	66	
Protein synthesis	126	117	3.57
Purines, pyrimidines, nucleosides, and nucleotides	71	62	
Regulatory functions	115	86	
Signal transduction	15	15	Inf
Transcription	26	26	Inf
Transport and binding proteins	207	161	
Unclassified	83	63	
Unknown function	101	82	

Metabolic diversity

Within *L. sakei* a wide variation with respect to carbohydrate fermentation exists [37,44,47]. Strains 23K and MF1328 was able to ferment 11 of the 49 substrates tested for by API 50 CH, while LS 25 and MF1053 fermented 18 (Table S2). In accordance with the presence of genes encoding phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTSs) for sugar uptake, all the strains fermented glucose, mannose, fructose, N-acetylglucosamine, and sucrose. Also genes encoding putative cellobiose- and trehalose-specific PTS's were present in all the test strains, though fermentation of these two sugars was not confirmed for all the

strains. Among the few sugars available in meat and fish, *L. sakei* utilizes mainly glucose and ribose for growth. Ribose was metabolized by all the strains (Table S2), however the CGH results showed considerable deviation between strains in the *rbsUDKR* operon (LCA_0200-0203) shown to be responsible for ribose catabolism [48-50]. While *rbsUD* encoding a ribose transporter and a D-ribose pyranase, respectively, were conserved, both *rbsK* and *rbsR* were divergent in several strains. Absence of *rbsR* encoding the *rbs* operon repressor has been shown to result in derepression of *rbsUDK* which does not affect ribose utilization, while inactivation of *rbsK* encoding a ribokinase has been shown to impair growth on ribose [48]. The gene encoding a putative carbohydrate kinase (LCA_0254) with possible ribokinase activity was present in all the test strains, and could indicate a second functional ribokinase which in some of the strains function as the main ribokinase.

By the API 50 CH assay, all the strains were able to ferment galactose, and strains CTC372, Lb148, Lb156, CTC460, LS 25 and Lb77 were found to ferment lactose (Table S2). The ability to ferment lactose may be useful for *L. sakei* growing in certain environments, but is considered an unfavourable phenotype for meat strains as lactose is regularly added as a water binding agent in fermented sausage [51]. The *lacLM* operon (LCA_1711-1710) encoding a β-galactosidase which catalyzes the hydrolysis of β-galactosides into monosaccharides, was present in all the test strains, except in LMGT2380 where *lacM* was determined as uncertain, likely due to sequence difference to that of the 23K strain. CTC372 has previously been shown to ferment lactose by a plasmid encoded *lac* operon [52]. The CGH results indicated that the CTC372, Lb148, and Lb156 strains harbour genes similar to the *lac* genes from strain T332, which are among the 'non-23K genes' on the array (Table 5). A lactose-specific PTS system is encoded by the *lac* operon. Notably, all of the lactose metabolizing strains except LS 25 originate from Spanish fermented sausage (Table 1), and they clustered together in the CGH-based dendrogram (Figure 2).

Also catabolic pathways for melibiose, gluconate, arabinose, malate and glycerol are present in the 23K genome, and fermentation was observed for the three first mentioned carbohydrates for several strains (Table S2). However, as genetic information on the uptake/metabolism of carbohydrates in *L. sakei*, except for ribose and lactose, is largely restricted to bioinformatically inferred gene functions, the CGH data could not provide metabolic explanation for all the carbohydrates. Strains MF1053 and MF1058 were the only strains to ferment methyl-β-glucopyranoside, LS 25 fermented xylose and rhamnose, and Lb77 fermented inulin (Table S2). Lactobacilli in general display large intra species variation

in their carbohydrate utilizations capabilities, which is reflected in the genomes of sequenced strains [1].

Improved resistance to the toxic effects of lactic acid and low pH has previously been linked to citrate metabolism in LAB [53]. This occurs through a reduction in acid stress by removal of lactate from the cytoplasm and thereby prolonging their survival in fermented products [53]. Citrate is not naturally found in meat but can be found in natural raw materials such as milk, vegetables, and fruits. Catabolic genes are present in the 23K genome (LCA_1220-1232), and for six of the test strains, the majority of these genes were divergent. Whereas the ability to ferment citrate may be potentially advantageous for survival in environments other than meat, several of the genes were divergent in the DSM 20017 strain originating from saké (Table 4).

Adaptation to meat and survival during meat processing

As the 23K genome shows features that explain why this bacterium is competitive on meat surface and survives meat processing [17], we were especially interested in the presence or divergence of the respective genes presumed to confer these features. Sugars are rapidly exhausted in meat and, accordingly, *L. sakei* is described as well adapted to derive energy from other compounds [17]. It has a well-developed potential for amino acid catabolism, and the effective survival on meat products presumably involves catabolism of arginine, which is abundant in meat, by the arginine deiminase (ADI) pathway [54,55]. The *arc* operon (LCA_0370-0375) responsible for arginine catabolism, was conserved in all the test strains. However, the second putative ADI pathway (LCA_0067-0073) present in 23K suggested to further enhance its ability to survive in meat [17], was only present in ten of the test strains (Table 4). Among these strain was MF1053 isolated from fermented fish, and as fish contains relatively little carbohydrates but many free amino acids [56], catabolic pathways for amino acids could be especially advantageous when living in this environment. The *tdcB* gene (LCA_0572) encoding the threonine deaminase, was divergent only in MF1053 and LMGT2380 while all other genes necessary for catabolism of threonine were present in all the strains. The genes necessary for catabolism of aspartate were present in all strains.

The presence of methylglyoxal bypass of glycolysis has been described as a unique feature that may occur in 23K in response to glucose starvation or to modulate carbon flux during co-metabolism of alternative carbon sources [17]. The bypass is thought to involve methylglyoxal synthase encoded from *mgs*A (LCA_1157), a putative oxidoreductase

(LCA_1158), and an iron containing aldehyde dehydrogenase (LCA_0379) [17]. The *mgsA* gene was divergent in several strains, while the two other genes were present in all the strains. Thus it seems that this bypass pathway could potentially be advantageous for only some *L. sakei* strains. The redundancy of 25 catabolic genes involved in exogenous nucleoside scavenging is described in the 23K genome, including the ability to catabolize inosine and adenosine for energy production [17]. Only *iunH1* (LCA_0252) encoding one of the three inosine-uridine preferring nucleoside hydrolases was divergent in CTC494 and Lb706, and *deoD* (LCA_0797) coding for a purine-nucleoside phosphorylase was divergent in LMGT2380 (Table 4), supporting the importance of using nucleosides as carbon source within *L. sakei* species.

L. sakei is described to resist a variety of adverse conditions [57]. Refrigeration (4°C) and curing (3-9% NaCl) are two common preservative conditions employed in meat processing [58], which obviously impose severe challenges to the organisms living in such environments. All the test strains harboured the uptake systems and a sodium-dependent symporter thought to drive the accumulation of osmo- and cryoprotective solutes such as betaine and carnitine, as well as genes encoding the putative cold stress proteins Csp1-4 and general stress proteins [17]. Genes encoding several Na^+/H^+ antiporters are found in the 23K genome, and only one putative Na^+/H^+ antiporter (LCA_0233) was divergent in three strains. Thus the ability to tolerate low temperature and high salt content is well conserved. Moreover, changing oxygen levels and redox potentials are other challenges for microorganisms during meat processing [59]. Compared to other sequenced lactobacilli, *L. sakei* appears to be the most competent species in coping with changing redox and oxygen levels, which typically fluctuate during meat processing, and deals effectively with toxic byproducts [17,60]. The production of H_2O_2 from O_2^- as well as reduction of peroxide is thought to take place through the actions of the enzymes coded by *nox* (LCA_0802), *sodA* (LCA_0896), *tpx* (LCA_0104), *npr* (LCA_0575), *katA* (LCA_0171), and *ohrA* (LCA_0552). These genes were all present in the test strains, except *npr* (LCA_0575) encoding an NADH peroxidase divergent in LMGT2380. However, reducing mechanisms for restoring protein activity after oxidation, such as a cluster of genes encoding a putative thiosulfate sulfurtransferase (LCA_0217), a putative cyanate transporter (LCA_0219_b), and a thioredoxin (LCA_0218), were divergent in several strains. A gene encoding a methionine sulfoxide reductase (LCA_0866), which counter methionine oxidation was divergent in Lb706. Oxidoreductases are beneficial for growth in meat by maintaining the redox balance, and more than 30 putative oxidoreductases are encoded in the 23K genome [17], most of these present in the test strains. The genome of

strain 23K also showed the presence of putative cytochromes and a mutated cytochrome P450 gene (LCA_1182, LCA_1183_c), the latter gene being intact in some other *L. sakei* strains [38]. All the test strains harboured parts of the mutated cytochrome P450 gene, indicating that some strains may even be capable of some form of respiration, similar to what has been observed for *Lactococcus lactis* [61].

L. sakei is devoid of heme biosynthesis machinery which makes it necessary to take up/utilize heme from the environment. LAB are generally considered not to have iron requirements [62,63], and it was shown for *L. sakei* that iron is dispensable [64]. Nevertheless, Duhutrel et al. [64] showed that iron present in the meat environment highly benefits *L. sakei* by sustaining a long term survival. Most of the putative iron transport systems found in the 23K genome were intact in all the test strains, and of the three iron-dependent transcriptional regulators (LCA_0109, LCA_0466 and LCA_1029), only one (LCA_0466) was divergent in a few strains [17]. As already mentioned, the *katA* gene coding for a heme-dependent catalase was present in all the strains. These findings indicate that heme acquisition is important for the oxidative stress response in this organism, and contributes to the survival on meat [64-66].

Divergent regions and horizontal gene transfer

Wide phylogenetic and functional diversity mediated by extensive gene loss and horizontal HGT of genes from other LAB genomes has been described within the *Lactobacillales* [1]. The projection of CGH results onto the 23K genome map reveals that a considerable part of the genomic divergence corresponds to five distinct regions in the 23K genome (Figure 1 and Table 4). By calculating a cumulative divergence score for each gene on the array, we identified these five divergent regions along the 23K chromosome. The G+C-content of the regions in 23K is 37.7%, which is lower than the average G+C-content of the rest of the genome (41.3%) [17]. DNA base composition skew is an indication of horizontally transferred genomic regions [8,9]. Integrative elements (IEs) are found in these low %G+C genomic regions. Divergent regions 1 and 3 contain genes coding for transposases and a prophage, which are typically elements associated with HGT [67]. The 23K genome contains 17 genes of the prophage lsa1 (LCA_0586-0602), and five more phage-related genes (LCA_0923-0924, LCA_1292, LCA_1294, LCA_1788). None of the examined strains harboured the complete 23K prophage lsa1 (Table 4), although parts of the prophage were present in LTH673, Lb790 and Lb16, suggesting that these strains harbour one or more

prophages with modules similar to lsa1. Only LCA_0602 gene of lsa1 and three of the five 23K phage-related genes were present in all the test strains. Since all the strains harboured some phage related genes, it might suggest the presence of more prophage genes in these strains and even complete set of genes for functional temperate phages. Within the majority of low G+C Gram-positive organisms, evidence of phage-mediated evolution has been reported [68]. In conclusion, the genome of all the *L. sakei* test strains contained prophages, or remnants thereof, suggesting that phage DNA sequences contribute in the evolution of *L. sakei* as well.

Variable regions in other *Lactobacillus* species have been shown to contain an over-representation of genes involved in carbohydrate utilization and transcriptional regulation, proposed as lifestyle adaption regions [6,12]. It is likely that such regions also exist within the *L. sakei* species. Part of the *rbs* operon (LCA_0200-0203) involved in ribose catabolism is located in divergent region 2 in the 23K genome (Figure 1 and Table 4), and the operon is described to be of chimeric genetic origin [48,49]. In the same divergent region, *gpm3* (LCA_0206) encoding a phosphoglycerate mutase involved in glycolysis is located, as well as a bile salt hydrolase encoding gene (LCA_0210).

Whereas proteins that may be involved in adhesion to intestinal mucus are not encoded in the 23K genome, several genes are present encoding proteins suggested to be involved in biofilm formation and cellular auto- and co-aggregation, allowing the bacterium to proliferate and colonize meat surfaces [17]. However, many of the genes belonging to the category of cell wall associated proteins are located in the divergent regions and show typical features of HGT. The two distinct surface polysaccharide gene clusters (LCA_1510_[a-g]-1513 and LCA_1571-1585) described to potentially be involved in attachment to meat surfaces or intestinal mucosa [17] are located in divergent regions 4 and 5 and were defined as divergent in the majority of test strains (Table 4). Eight gene clusters coding for a novel type cell-surface protein complexes (Csc) have been identified in 23K and suggested to be involved in microbe-microbe and/or surface-microbe interactions [17]. Similar *csc* gene clusters to those of 23K have been identified in several gram-positive bacteria associated with plants and plant fermentations, while they are not found in typical gut bacteria [69]. One of the clusters (LCA_0211-0215) is located in divergent region 2 and was divergent in the majority of test strains (Table 4). The erratic presence of these genes could indicate cell wall composition differences and variation in the strains abilities to attach to meat surfaces. However, *csc* gene clusters LCA_1283-1287 and LCA_1821-1819 were conserved in all test strains, and cluster LCA_0172-0177 was conserved in all strains except CTC494. For the

remaining four *csc* gene clusters (LCA_0613-0610, LCA_1731-1735, LCA_1811-1810, LCA_1815-1816) the hybridization patterns for some of the strains indicate the presence of alleles with sequences deviating considerably from the 23K sequence. Moreover, a gene (LCA_0534) encoding a hypothetical cell surface protein with a LPXTG cell wall anchoring signal proposed as a candidate for biofilm formation [17], was present in all strains except DSM 20017, LS 25, and MF1328 (Table 4), and genes coding for two sets of paralogous proteins (LCA_1159/LCA_1165 and LCA_0194/LCA_0313) predicted to mediate auto-aggregation and adherence to collagen [17] were present in all test strains.

The isolation and characterization of a dextransucrase gene *dsrB* encoding a cell-surface dextransucrase precursor (sucrose 6-glycosyltransferase) (acc. no. AY697434) from *L. sakei* strain Kg15 has been reported [70]. This gene was among the 'non-23K genes' on the array, and was divergent in all the strains. Twelve genes of the plasmid pRV500 (acc. no AF438419) from *L. sakei* strain T332 [71], and a *tetM* gene encoding a plasmidic tetracycline resistance protein [72,73] were also represented on the array, and also these genes were divergent in the test strains.

Bacteriocin genes

Genes associated with production of bacteriocins obtained from various *L. sakei* strains were among the 'non-23K genes' included on the array. Table 5 shows the status of these bacteriocin associated genes in the test strains. Previous studies have shown that strains Lb16, LTH673, MF1053 and LMGT2380 produce sakacin P [24,74] (and our unpublished results), Lb706 produces sakacin A [25,75], and L45 produces lactocin S [76,77]. The presence of these bacteriocin genes was confirmed by our CGH results (Table 5). Lactocin S genes were also present in Lb77, Lb148, and Lb156, strains which have previously been observed to produce bacteriocin-like substances [78] (and unpublished data). Sakacin A genes were present in CTC494 (Table 5), confirming previous observations that the CTC494 bacteriocin, 'sakacin K', is identical with sakacin A [79]. The CGH results also indicated the presence of lactocin S genes in strain CTC460 and sakacin Q genes in Lb16, LMGT2380, and MF1053 (Table 5). An incomplete and rearranged chromosome-encoded sakacin P gene (*spp*) cluster (LCA_0560_a-0569_b) is found in the genome sequence of 23K [17], and *spp* homologs have been found in several non-sakacin P producing *L. sakei* strains [80]. This correlates well with our findings of genes of the Lb674 and 23K *spp* clusters present in the test strains (Table 5). The sakacin TX cluster from *L. sakei* 5 described by Vaughan et al. [81] was divergent in all

the strains. Only gene *skgD* encoding sakacin G ABC transporter of the sakacin G cluster from *L. sakei* 2512 describe by Simon et al. [82], was found present in the same strains that harboured the complete sakacin P gene cluster. Our results confirm previous findings in suggesting that bacteriocin-associated genes are highly abundant among *L. sakei* strains [80]. This strongly indicates that these genes are, or have been, important in the evolution of the species, and as previously suggested may give the strains a competitive advantage in its environment [17,80].

Concluding remarks

To conclude, the comparison of gene content between various *L. sakei* strains to the sequenced strain 23K demonstrates that a large part of the genome of this strain belongs to a common gene pool invariant in this species. This is currently the only complete genome available of the species, and because being among the genetically smallest ones, the 23K strain might be a suitable model organism to investigate core features of the *L. sakei* species. The large number of conserved genes is indicative of the role that these play in niche specialization within the *L. sakei* species. Overall, the metabolic repertoire reflects that the bacterium's ability to flexibly use meat components, to compete on the meat surface, as well as to survive meat processing and storage, were highly conserved. In addition, and all test strains carried remnants of or, complete bacteriocin operons. The genomic divergence corresponded mainly to five distinct regions in the 23K genome, which showed features consistent with HGT. Of the strains examined, two originates from other sources than meat, though our results indicated that these strains do not differ significantly from the meat strains. Also no obvious difference between the two *L. sakei* subspecies could be seen. The application of whole-genome microarrays to study *L. sakei* is in its infancy, and more sequences of larger genomes are needed to include a wider representation of the existing features and potential within the species.

Table 4. *L. sakei* 23K genes with status as divergent/uncertain in at least one of the 18 *L. sakei* test strains after CGH analysis. Genes were considered present or divergent using an identity score of 0.7, thus probes with more than 70% identity with the genome were assumed to give hybridization signals, and were defined as present. Genes with identity score between 0.6 and 0.8 were defined as uncertain, reflecting that these genes have a score close to the cutoff. Strains: 1. CCUG 31331; 2. CTC372; 3. CTC494; 4. CTC460; 5. DSM 20017; 6. L45; 7. Lb148; 8. Lb156; 9. Lb16; 10 Lb790; 11. Lb706; 12. Lb77; 13. LS 25; 14. LMGT2380; 15. LTH673; 16. MF1058; 17. MF1328; 18. MF1053. NA refers to 'not analyzed'. Divergent regions are indicated with a grey background. The values of the divergent genes are shown in bold.

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_0023	*lsa0023*	Putative ribonucleotide reductase (NrdI-like)	**0.42**	1	**0**	1	0.7	1	1	1	**0.47**	**0**	**0**	1	0.69	**0**	0.69	**0.02**	1	**0.48**
LCA_0031	*lsa0031*	Hypothetical protein	1	1	0.99	1	1	1	1	1	1	1	0.74	1	1	1	1	1	1	1
LCA_0052	*lsa0052*	Putative Na(+) ABC exporter, membrane-spanning/permease subunit	0.93	1	1	1	1	1	1	1	1	1	1	1	1	1	0.96	1	**0.53**	1
LCA_0062	*lsa0062*	Hypothetical integral membrane protein	1	1	1	0.67	1	1	0.88	0.6	1	1	1	1	1	1	0.62	1	0.89	1
LCA_0067	*lsa0067*	Putative ornithine carbamoyltransferase (OTCase)	**0**	1	**0**	1	**0.07**	1	1	1	1	**0.23**	**0.21**	1	**0**	1	1	**0.09**	1	1
LCA_0068	*lsa0068*	Putative amino acid/polyamine antiporter (N-terminal fragment), authentic frameshift	**0**	1	**0**	1	**0**	**0.16**	1	1	1	**0.06**	**0**	1	**0**	1	1	**0.09**	1	1
LCA_0069	*lsa0069*	Putative amino acid/polyamine antiporter (C-terminal fragment), authentic frameshift	**0**	1	**0**	1	**0**	**0.47**	1	1	1	**0.05**	**0.04**	1	**0**	1	1	**0.04**	1	1
LCA_0070	*lsa0070*	Putative peptidylarginine deiminase (Amidinotransferase)	**0.13**	1	**0.55**	1	**0**	**0.11**	1	1	1	0.88	**0.35**	1	**0**	1	1	**0.11**	1	1
LCA_0071	*lsa0071*	Putative carbamate kinase	**0.04**	1	**0.28**	1	**0.53**	0.89	1	1	1	0.42	**0.17**	1	**0.12**	0.96	1	**0.12**	1	1
LCA_0072	*lsa0072*	Putative peptidylarginine deiminase (Amidinotransferase)	**0**	1	**0**	1	**0**	**0**	1	1	1	0	**0**	1	**0**	1	1	**0**	1	1
LCA_0073	*lsa0073*	Putative transcriptional regulator, RpiR family	**0**	1	**0.02**	1	**0.5**	**0.01**	1	1	1	0	**0**	1	**0.03**	1	1	**0**	1	1
LCA_0074	*lsa0074*	Putative DNA-binding protein, XRE family	**0**	1	**0**	1	1	0.89	1	1	1	0.29	**0.04**	1	1	1	1	**0.01**	1	1
LCA_0076	*lsa0076*	Putative DNA invertase (Plasmidic resolvase)	0.8	1	**0.37**	1	1	1	1	1	1	0.85	**0.37**	1	1	1	1	**0.31**	1	1
LCA_0077	*lsa0077*	Two-component system, response regulator	0.84	1	0.84	1	1	1	1	1	1	1	0.71	1	1	1	1	1	1	1
LCA_0079	*lsa0079*	Hypothetical protein	**0.25**	**0.5**	**0.39**	**0.42**	0.79	1	0.61	**0.14**	0.65	0.71	**0.37**	1	1	**0.39**	1	1	**0.43**	1
LCA_0080	*lsa0080*	Hypothetical protein	**0.42**	**0**	**0.32**	**0.3**	0.83	1	0.66	**0.28**	0.76	0.64	**0.32**	1	0.71	**0.32**	**0.51**	1	**0.37**	1
LCA_0128	*lsa0128*	Putative antimicrobial peptide ABC exporter, membrane-spanning/permease subunit	1	1	1	1	1	1	1	1	1	0.98	0.67	1	1	0.95	1	1	1	1
LCA_0135	*lsa0135*	Hypothetical integral membrane protein, similar to CcrB	1	1	1	1	1	1	1	1	1	**0.2**	**0**	1	1	1	1	1	1	1
LCA_0142	*lsa0142*	Hypothetical protein	**0.05**	**0**	**0**	**0**	1	0.77	**0.58**	**0.06**	**0.14**	**0**	**0**	**0.36**	**0.28**	**0**	**0.12**	**0**	**0.14**	**0**
LCA_0143	*lsa0143*	Putative adenine-specific DNA methyltransferase	**0.32**	**0**	**0**	**0**	1	1	**0.53**	**0.42**	0.6	**0.15**	**0.14**	**0.02**	**0.19**	**0.22**	**0.2**	**0.37**	**0.11**	**0.12**
LCA_0146	*lsa0146*	Putative adenine-specifique DNA methyltransferase	**0.38**	**0.04**	**0**	**0**	1	1	**0.54**	**0.04**	0.85	**0.31**	**0.06**	0.65	0.62	**0**	0.65	**0.08**	**0.24**	0.83
LCA_0147	*lsa0147*	Putative DNA-repair helicase	**0.26**	**0**	**0.58**	**0.5**	1	1	0.72	0.79	0.75	0.88	**0.46**	**0**	**0.07**	0.74	**0**	**0.33**	**0.58**	**0**
LCA_0149	*lsa0149*	Hypothetical protein	**0**	**0**	**0.04**	**0**	**1**	**0.24**	**0.5**	**0.01**	**0.48**	**0.21**	**0**	**0**	**0.02**	**0.06**	**0.1**	**0.16**	**0.24**	**0.02**
LCA_0150	*lsa0150*	Putative deacetylase (acetyl esterase)	1	**0**	0.97	**0.24**	1	1	0.76	0.77	1	1	0.95	**0**	0.8	**0.42**	1	1	1	1
LCA_0151	*lsa0151*	Hypothetical protein	1	**0**	1	**0**	1	1	**0**	**0**	1	1	1	**0**	**0.33**	**0**	1	1	1	1
LCA_0152	*lsa0152*	Hypothetical integral membrane protein	1	**0**	1	**0**	1	1	**0**	**0**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0153	*lsa0153*	Hypothetical protein	1	**0**	1	**0**	1	1	**0.29**	**0**	1	1	1	**0**	1	**0.06**	1	1	1	1
LCA_0154	*lsa0154*	Putative DNA-binding protein, XRE family	1	**0**	1	**0**	1	1	**0.5**	**0.01**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0155	*lsa0155*	Putative hydrolase, haloacid dehalogenase-like family	1	**0**	1	**0.44**	1	1	0.74	0.65	1	1	1	**0**	1	0.72	1	1	1	1
LCA_0156	*lsa0156*	Hypothetical protein	1	**0**	1	**0**	1	1	**0.16**	**0.02**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0157	*lsa0157*	Putative hydroxyl/aromatic amino acid symporter	1	**0**	1	**0**	1	1	**0**	**0**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0158	*lsa0158*	Hypothetical small peptide	1	**0**	1	**0**	1	1	**0.5**	**0**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0159	*lsa0159*	Hypothetical protein	1	**0**	1	**0**	1	1	**0.51**	**0.54**	1	1	1	**0**	1	**0.39**	1	1	1	1
LCA_0160	*lsa0160*	Hypothetical protein	1	**0**	1	**0**	1	1	**0**	**0.01**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0161	*lsa0161*	Putative transcriptional regulator, ArsR family	1	**0**	1	**0**	1	1	**0.5**	**0.35**	1	1	1	**0**	1	**0**	1	1	1	1
LCA_0162	*lsa0162*	Putative Bifunctional glycosyl transferase, family 8	1	**0**	1	**0.14**	0.79	1	0.73	**0.27**	1	1	1	0.64	1	**0**	1	1	1	1
LCA_0163	*lsa0163*	Hypothetical protein	1	1	1	1	0.77	1	1	1	1	1	1	1	1	0.74	1	1	1	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_0164	*lsa0164*	Putative serine/tyrosine protein phosphatase	1	1	1	1	0.96	1	1	1	1	1	1	1	1	**0.53**	1	1	1	1
LCA_0165	*lsa0165*	Putative oxidoreductase, short-chain dehydrogenase/reductase family	1	1	1	1	**0.59**	1	1	1	1	1	1	1	1	**0**	1	1	1	1
LCA_0172	*lsa0172*	Hypothetical cell surface protein precursor	1	1	**0.45**	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	0.74
LCA_0173	*lsa0173*	Hypothetical cell surface protein precursor	1	1	**0.59**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_0174	*lsa0174*	Hypothetical protein	1	1	**0.56**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_0175	*lsa0175*	Hypothetical cell surface protein precursor	1	1	**0**	1	1	1	1	1	1	1	1	1	0.76	1	1	1	1	1
LCA_0176	*lsa0176*	Hypothetical protein	1	1	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_0180	*mtsC*	Manganese ABC transporter, ATP-binding subunit	0.62	1	**0**	1	0.88	1	1	1	1	**0.04**	**0.05**	1	0.91	1	1	1	1	1
LCA_0183	*lsa0183*	Putative hydrolase, isochorismatase/nicotamidase family	0.78	1	**0.27**	1	0.9	1	1	1	1	0.86	**0.37**	1	1	1	1	1	1	1
LCA_0193	*lsa0193*	Hypothetical protein	0.83	1	0.71	1	0.86	1	1	1	1	0.78	0.61	1	1	0.69	1	1	1	1
LCA_0195	*lsa0195*	Hypothetical lipoprotein precursor	**0.25**	1	**0.07**	1	0.79	1	1	1	**0.45**	**0.09**	**0**	1	1	**0**	**0.53**	1	1	1
LCA_0198	*ack1*	Acetate kinase (Acetokinase)	1	1	0.63	0.7	1	1	0.67	0.81	0.95	0.91	**0.44**	1	1	0.66	1	0.68	0.82	1
LCA_0199	*lsa0199*	Hypothetical protein	**0.42**	1	**0.16**	**0.13**	1	1	**0.59**	**0.35**	0.65	**0.07**	**0**	1	1	**0.02**	1	**0.45**	**0.34**	0.93
LCA_0202	*rbsK*	Ribokinase	0.9	**0**	0.71	0.89	1	0.76	0.79	0.99	0.97	0.92	0.82	**0.36**	1	0.79	1	0.6	0.85	1
LCA_0203	*rbsR*	Ribose operon transcriptional regulator, LacI family	0.99	1	**0.34**	**0.31**	1	1	0.76	0.88	1	0.78	**0.51**	1	1	0.92	1	0.81	0.6	1
LCA_0204	*lsa0204*	Putative transcriptional regulator, PadR family	0.83	1	0.68	0.92	1	1	**0.51**	**0.07**	0.89	1	**0.46**	1	1	**0.03**	1	**0.28**	**0.28**	1
LCA_0205	*lsa0205*	Putative drug:H(+) antiporter	0.96	1	**0.38**	**0.43**	1	1	0.63	**0.19**	0.86	0.59	**0.2**	1	1	**0.08**	1	**0.43**	**0.51**	1
LCA_0207	*clpL*	ATPase/chaperone ClpL, putative specificity factor for ClpP protease	0.97	**0**	**0.06**	**0.3**	1	**0**	**0.37**	**0**	0.86	**0.33**	**0.08**	**0**	1	**0.27**	1	**0.45**	0.69	1
LCA_0208	*lsa0208*	Hypothetical integral membrane protein	1	**0**	**0.14**	**0.07**	1	**0.25**	**0.5**	**0.05**	1	**0.15**	**0.18**	**0**	0.98	1	1	1	**0.52**	1
LCA_0209	*lsa0209*	Hypothetical integral membrane protein	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**
LCA_0210	*lsa0210*	Choloylglycine hydrolase (Bile salt hydrolase)	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	**0.03**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**
LCA_0211	*lsa0211*	Hypothetical cell surface protein precursor	**0**	**0**	**0.31**	**0**	1	**0.25**	0.7	0.61	**0.4**	**0.44**	**0.17**	**0**	1	**0**	**0**	**0.15**	1	**0**
LCA_0212	*lsa0212*	Hypothetical cell surface protein precursor	**0.14**	**0**	**0.3**	**0.42**	1	**0.34**	**0.27**	**0.16**	**0.15**	**0.5**	**0.25**	**0.22**	1	**0.05**	**0.07**	**0.08**	1	**0.01**
LCA_0213	*lsa0213*	Hypothetical cell surface protein precursor	**0**	**0**	**0**	**0**	1	**0.58**	**0**	**0**	**0.33**	**0**	**0**	**0**	1	**0**	**0**	**0**	1	**0**
LCA_0214	*lsa214*	Hypothetical protein	**0**	**0**	**0**	**0**	1	**0.35**	**0.01**	**0**	**0.01**	**0**	**0**	**0**	1	**0**	**0**	**0**	0.83	**0.08**
LCA_0215	*lsa0215*	Hypothetical cell surface protein precursor	0.9	1	0.69	1	1	1	1	1	1	0.91	0.81	1	1	**0.54**	0.9	0.72	1	1
LCA_0216_b	*lsa0216_b*	Putative transcriptional regulator, MarR family, truncated	1	1	0.87	1	1	1	1	1	1	0.73	0.76	1	1	1	1	1	1	1
LCA_0217	*lsa0217*	Putative thiosulfate sulfurtransferase with a ArsR-HTH domain, Rhodanese family	**0**	1	**0**	1	1	**0.25**	1	1	1	**0**	**0**	1	1	1	1	1	1	1
LCA_0218	*trxA1*	Thioredoxin	**0**	1	**0**	1	1	0.94	1	1	1	**0.49**	**0.03**	1	1	1	1	1	1	1
LCA_0219_a	*lsa0219_a*	Putative DNA-binding protein, XRE family	**0.28**	1	0.73	1	1	1	1	1	1	0.94	0.72	1	1	1	1	1	1	1
LCA_0219_b	*lsa0219_b*	Putative cyanate transport protein	**0**	1	**0**	1	1	**0**	1	1	1	**0**	**0**	1	1	1	1	1	1	1
LCA_0220_c	*dapE*	Succinyl-diaminopimelate desuccinylase	1	1	1	1	0.96	1	1	1	1	1	1	1	1	**0.25**	1	0.78	1	1
LCA_0229	*lsa0229*	Putative transcriptional regulator, MerR family (N-terminal fragment), authentic frameshift	1	1	1	1	1	1	1	1	0.95	1	1	1	1	0.91	1	0.79	1	1
LCA_0231	*lsa0231*	Putative transcriptional regulator, MarR family	1	1	1	1	1	1	1	1	1	1	1	1	1	0.84	1	0.72	1	1
LCA_0232	*lmrA*	Multidrug ABC exporter, ATP-binding and membrane-spanning/permease subunits	1	1	1	1	1	1	1	1	1	1	1	1	1	0.66	1	0.9	1	1
LCA_0233	*lsa0233*	Putative Na(+):H(+) antiporter (N-terminal fragment), authentic frameshift	1	1	1	1	1	1	1	1	**0.41**	1	1	1	1	**0**	**0.9**	**0**	1	1
LCA_0236	*lsa0236*	Hypothetical extracellular peptide precursor	1	1	1	1	1	1	1	1	0.99	1	1	1	1	0.4	1	0.84	1	1
LCA_0239	*lsa0239*	Putative transcriptional regulator, MerR family	1	1	1	1	1	1	1	1	1	1	1	1	1	0.77	1	1	1	1
LCA_0248	*lsa0248*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	0.83	1	0.74	1	1
LCA_0252	*iunH1*	Inosine-uridine preferring nucleoside hydrolase	1	1	**0.57**	0.77	1	1	0.87	0.97	1	0.75	**0.19**	1	1	1	1	1	0.81	1
LCA_0261	*tnpB-ISLsa2*	Transposase (orfB) of ISLsa2 (IS150 family)	0.94	1	0.66	0.87	0.82	1	0.93	0.95	1	0.82	0.69	1	1	0.65	1	0.78	1	1
LCA_0262	*tnpA-ISLsa2*	Transposase (orfA) of ISLsa2 (IS150 family)	0.99	1	0.85	0.94	**0.53**	1	0.88	0.96	0.96	1	0.8	1	0.96	0.89	1	0.69	0.97	1
LCA_0269	*lsa0269*	Putative transcriptional regulator, TetR family	1	1	0.78	0.84	1	1	0.9	0.87	1	1	**0.57**	1	1	1	1	1	0.91	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_0274	*lsa0274*	Putative DNA helicase (N-terminal fragment), authentic frameshift	0.85	1	0.69	0.65	1	1	0.81	**0.29**	1	1	0.81	1	1	1	1	0.74	0.63	1
LCA_0276	*guaB*	Inosine-5-monophosphate dehydrogenase	1	1	0.7	0.68	1	1	0.87	0.97	0.9	0.95	**0.54**	1	1	**0.49**	0.85	0.66	0.83	0.68
LCA_0283	*lsa0283*	Putative zinc/iron ABC transporter, ATP-bindingsubunit	0.72	1	**0.41**	**0.35**	1	1	**0.59**	**0.43**	0.99	**0.58**	**0.2**	1	1	**0.38**	1	**0.58**	**0.51**	0.93
LCA_0294	*lsa0294*	Hypothetical lipoprotein precursor	1	0.84	**0**	**0.04**	1	1	0.64	0.74	1	**0.41**	**0.14**	1	1	1	1	**0.26**	**0.46**	1
LCA_0299	*gntP*	Gluconate:H(+) symporter	1	1	0.84	0.62	1	1	0.87	0.98	0.98	0.84	**0.56**	1	1	1	1	0.75	0.95	1
LCA_0303	*lsa0303*	Hypothetical protein	1	1	1	1	1	1	1	1	0.89	1	1	1	1	**0.33**	1	**0.48**	1	1
LCA_0309	*lsa0309*	Hypothetical membrane protein	1	1	1	1	1	1	1	1	0.93	1	1	1	1	**0.59**	1	0.75	1	1
LCA_0315	*lsa0315*	Hypothetical protein	0.81	1	**0.59**	0.71	1	1	1	0.76	1	0.74	0.6	1	1	0.8	1	1	0.86	1
LCA_0316	*sdhB*	L-serine dehydratase, beta subunit (L-serine deaminase)	0.44	1	1	1	1	1	1	1	0.83	1	1	1	1	**0.25**	0.9	0.63	1	0.99
LCA_0317	*sdhA*	L-serine dehydratase, alpha subunit (L-serine deaminase)	1	1	1	1	1	1	1	1	0.94	1	1	1	1	**0.28**	1	0.7	1	1
LCA_0326	*lsa0326*	Putative DNA helicase	1	1	1	1	1	1	1	1	1	1	1	1	0.78	1	1	1	1	1
LCA_0328	*lsa0328*	Putative drug:Na(+) antiporter (drug efflux pump)	1	1	1	1	**0**	1	1	1	1	1	1	1	**0**	1	1	1	1	**0**
LCA_0329	*lsa0329*	Putative DNA-entry nuclease precursor (N-terminal fragment), authentic frameshift	0.65	1	1	1	0.77	1	1	1	0.77	1	1	1	1	**0.12**	1	**0.26**	1	1
LCA_0330	*lsa0330*	Putative DNA-entry nuclease precursor (C-terminal fragment), authentic framesshift	0.76	1	1	1	0.85	1	1	1	0.85	1	1	1	1	**0.04**	0.97	**0.12**	1	0.97
LCA_0331	*lsa0331*	Hypothetical protein	1	1	1	1	1	1	1	1	0.97	1	1	1	0.88	**0.59**	0.92	0.84	1	0.86
LCA_0346	*lsa0346*	Hypothetical protein	1	1	**0.46**	**0.49**	1	1	0.96	0.92	1	0.83	0.63	0.83	1	1	1	0.65	0.83	0.97
LCA_0349	*lsa0349*	Putative O-sialoglycoprotein metallo-endopeptidase (M22 family)	1	1	1	1	1	1	0.96	1	1	1	1	1	1	0.78	1	0.93	0.95	1
LCA_0377	*tgt*	Queuine tRNA-ribosyltransferase	1	1	**0.47**	0.6	1	1	0.77	0.77	0.94	0.85	**0.52**	1	1	**0.39**	1	0.63	0.75	1
LCA_0410	*lsa0410*	Putative mechanosensitive ion chanel	1	1	0.71	1	1	1	1	1	1	0.79	**0.54**	1	1	0.72	1	0.8	1	0.97
LCA_0439	*lsa0439*	Hypothetical extracellular lipase/esterase precursor	1	0.84	**0.24**	**0.22**	0.93	1	0.62	0.6	1	**0.35**	**0.24**	0.78	1	1	1	1	1	1
LCA_0440	*mleR*	Transcriptional regulator of the malolactic fermentation, LysR family	1	**0**	**0.55**	**0.49**	0.97	0.68	**0.51**	**0.18**	1	**0.52**	**0.11**	**0.59**	**0.4**	1	1	1	**0.42**	1
LCA_0441	*mleS*	Malolactic enzyme	1	1	**0.59**	0.75	0.98	1	0.86	0.88	1	0.86	**0.42**	1	1	1	1	1	0.95	1
LCA_0444	*lsa0444*	Putative malate dehydrogenase	**0.58**	1	**0**	1	1	1	1	1	**0.59**	**0**	**0**	1	1	**0**	0.73	**0.27**	1	**0.53**
LCA_0445	*mleP2*	L-malate uniport protein	1	1	**0.44**	1	0.93	1	1	1	**0.59**	0.87	**0.28**	1	1	**0.45**	0.85	**0.48**	1	0.74
LCA_0446	*pyrDB2*	Putative dihydroorotate oxidase, catalytic subunit	0.96	1	**0.06**	1	1	1	1	1	0.89	**0.58**	**0.14**	1	1	**0.03**	1	**0.44**	**0.45**	1
LCA_0447	*lsa0447*	Putative hydrolase, haloacid dehalogenase family	0.85	1	0.87	1	1	1	1	1	0.91	1	0.74	1	1	1	1	0.77	1	1
LCA_0448	*gltP*	Putative glutamate/aspartate:cation symporter	0.67	1	0.14	1	1	1	1	1	0.93	0.67	**0.25**	1	1	**0.31**	1	**0.52**	1	1
LCA_0454	*lsa0454*	Putative oxidoreductase, short-chain dehydrogenase/reductase family	**0.46**	1	1	1	1	1	1	1	0.46	1	1	1	1	**0**	**0.29**	1	1	**0.29**
LCA_0455	*lsa0455*	Hypothetical protein	**0.5**	1	1	1	1	1	1	1	0.74	1	1	1	1	0.62	**0.59**	1	1	**0.58**
LCA_0456	*lsa0456*	Hypothetical protein	**0.06**	1	1	1	1	1	1	1	**0.22**	1	1	1	1	**0**	**0.13**	1	1	**0.24**
LCA_0457	*lsa0457*	Putative transport protein	**0.2**	1	1	1	1	1	1	1	**0.07**	1	1	1	1	**0**	**0.41**	1	1	**0.51**
LCA_0458	*lsa0458*	Hypothetical integral membrane protein	0.63	1	1	1	1	1	1	1	0.71	1	1	1	1	**0**	0.81	1	1	0.96
LCA_0460	*lsa0460*	Hypothetical small peptide	1	1	1	1	1	1	1	1	0.93	1	1	1	1	**0.34**	1	0.77	1	1
LCA_0461	*lsa0461*	Putative glycosyltransferase, group 1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.75	1	1	1	1
LCA_0462	*lsa0462*	hypothetical integral membrane protein	0.86	1	1	1	1	1	1	1	0.77	1	1	1	1	**0.51**	1	1	1	1
LCA_0466	*lsa0466*	Putative transcriptional regulator, Fur family	1	1	**0**	**0.19**	0.89	1	0.69	**0.43**	1	**0.33**	**0.11**	1	1	1	1	**0.39**	0.7	1
LCA_0467	*lsa0467*	Hypothetical integral membrane protein	1	**0**	0.75	0.87	0.95	1	0.93	0.87	1	0.74	**0.18**	0.65	**0.53**	1	1	0.86	0.87	0.69
LCA_0472	*lsa472*	Putative transport protein	0.92	1	1	1	1	1	1	1	0.76	1	1	1	1	0.4	1	1	1	1
LCA_0473	*tnpA-ISLsa2*	Transposase (orfA) of ISLsa2 (IS150 family)	0.99	1	0.85	0.94	**0.53**	1	0.88	0.96	0.96	1	0.8	1	0.96	0.89	1	0.69	0.97	1
LCA_0474	*tnpB-ISLsa2*	Transposase (orfB) of ISLsa2 (IS150 family)	0.94	1	0.66	0.87	0.82	1	0.93	0.95	1	0.82	0.69	1	1	0.65	1	0.78	1	1
LCA_0476	*guaC*	Guanosine 5'-monophosphate reductase (GMP reductase)	0.98	1	1	1	1	1	1	1	0.93	1	1	1	1	**0.11**	1	1	1	1
LCA_0478	*lsa0478*	Putative rRNA large subunit methyltransferase A	1	1	0.83	0.76	1	1	0.87	1	1	0.89	0.7	1	1	0.67	1	0.81	1	1
LCA_0483	*lsa0483*	Hypothetical protein	**0.57**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_0485	*lsa0485*	Hypothetical protein	0.96	1	1	1	1	1	1	1	0.95	1	1	1	1	**0.57**	1	0.72	1	1
LCA_0489	*lsa0489*	Putative metal-dependent phosphohydrolase precursor	0.96	1	0.92	1	1	1	1	1	1	1	0.88	1	1	0.61	1	0.72	0.86	1
LCA_0491	*lsa0491*	Hypothetical protein	0.95	1	**0.04**	**0.19**	1	1	0.61	0.61	0.76	0.72	**0.21**	1	1	**0.13**	1	**0.47**	**0.41**	1
LCA_0492	*lsa0492*	Putative bacterial type II secretion/competence system, ATP-dependent DNA helicase ComFA-like	**0.54**	0.87	**0.1**	**0**	1	1	**0.51**	**0.01**	0.82	0.66	**0.03**	1	1	**0**	1	**0.3**	**0.26**	1
LCA_0493	*lsa0493*	Putative bacterial type II secretion/competence system, protein ComFC-like	0.75	0.92	**0**	**0.1**	1	1	**0.51**	**0.11**	0.61	**0.3**	**0**	1	1	**0.04**	1	**0.16**	**0.22**	1
LCA_0504	*ptsA*	Phosphate ABC transporter, membrane-spanning subunit	1	**0.53**	0.66	0.84	1	1	0.99	1	1	0.83	**0.33**	0.65	1	1	1	1	1	1
LCA_0509	*kbl*	2-amino-3-ketobutyrate coenzyme A ligase (Glycine acetyltransferase)	0.88	1	**0.5**	0.75	1	1	0.88	0.92	0.81	0.85	**0.54**	1	1	**0.58**	1	0.62	0.68	1
LCA_0511	*lsa0511*	L-threonine dehydrogenase (C-terminal fragment), authentic frameshift	1	1	0.75	0.87	1	1	0.87	0.89	1	1	0.98	1	1	0.92	1	0.85	1	1
LCA_0516	*hprK*	Hpr kinase	1	1	1	1	1	1	1	1	1	1	1	0.78	1	1	1	1	1	1
LCA_0517	*lgt*	Prolipoprotein diacylglyceryl transferase	1	1	0.86	0.95	1	1	1	1	1	0.9	**0.54**	1	1	1	1	0.9	1	1
LCA_0518	*gps*	[NAD(P)+]-dependent glycerol-3-phosphate dehydrogenase	1	1	1	1	1	1	1	1	1	0.97	0.77	1	1	1	1	1	1	1
LCA_0521	*pgm*	Phosphoglucomutase	1	1	0.73	1	1	1	1	1	1	0.87	0.68	1	1	1	1	1	0.98	1
LCA_0523	*uvrB*	Excinuclease ABC, subunit B	1	0.99	0.62	0.61	1	1	0.77	0.89	1	0.83	**0.55**	1	1	0.78	0.9	0.84	0.75	0.89
LCA_0525	*lsa0525*	Hypothetical integral membrane protein	0.96	1	**0.09**	**0.22**	1	1	**0.56**	**0.09**	0.91	0.63	0.65	1	1	0.73	1	**0.39**	**0.35**	1
LCA_0526	*lsa0526*	Hypothetical protein	**0.59**	**0.14**	**0**	**0**	1	1	**0.51**	**0.27**	0.65	**0.27**	**0.06**	0.65	1	**0**	**0.55**	**0.13**	**0.32**	1
LCA_0527	*lsa0527*	Putative extracellular lipase/esterase precursor	0.86	0.65	**0.22**	**0.36**	1	1	**0.58**	**0.21**	0.9	**0.58**	**0.06**	1	1	**0.14**	0.86	**0.44**	**0.41**	0.92
LCA_0534	*lsa0534*	Hypothetical cell surface protein precursor (with LPQTG sorting signal)	0.94	1	1	1	**0.5**	1	1	1	1	1	1	1	**0.54**	1	1	1	**0.34**	1
LCA_0539	*lsa0539*	Putative NADPH-quinone oxidoreductase (N-terminal fragment), authentic frameshift	**0**	1	1	1	1	1	1	1	**0**	1	1	1	**0.21**	**0**	**0.08**	**0**	1	1
LCA_0540	*lsa0540*	Putative NADPH-quinone oxidoreductase (C-terminal fragment), authentic frameshift	**0.03**	1	1	1	0.99	1	1	1	**0.08**	1	1	1	**0.05**	**0**	**0**	**0**	1	1
LCA_0541	*lsa0541*	Putative DNA-binding protein, XRE family	**0.22**	1	1	1	1	1	1	1	**0.55**	1	1	1	**0.18**	**0**	**0.31**	**0.04**	1	1
LCA_0543	*lsa0543*	Putative 3-methy-adenine DNA glycosylase I	1	1	1	1	0.86	1	1	1	0.89	1	1	1	1	**0.29**	1	0.6	1	1
LCA_0553	*lsa0553*	Hypothetical protein	1	1	1	1	0.66	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_0564_b	*lsa0564_b*	Hypothetical small peptide	1	**0.54**	1	1	0.66	1	**0.5**	**0**	**0.48**	1	1	1	1	**0.22**	**0.26**	1	**0**	**0.3**
LCA_0564_c	*lsa0564_c*	Putative bacteriocin immunity protein	1	**0**	1	1	**0.55**	1	**0.51**	**0.07**	**0**	0.61	**0.4**	1	1	**0.19**	**0**	1	**0.03**	**0**
LCA_0565	*lsa0565*	Hypothetical small peptide	1	**0**	1	1	1	1	**0**	**0**	**0**	1	1	1	1	**0**	**0**	1	**0**	**0**
LCA_0566	*lsa0566*	Hypothetical small peptide	1	1	**0.27**	0.61	1	1	0.65	**0.3**	**0.09**	**0.46**	**0**	1	1	**0**	**0**	1	**0.03**	**0**
LCA_0567	*lsa0567*	Hypothetical protein	1	1	1	1	1	1	1	1	**0.09**	1	1	1	1	**0**	**0**	1	**0.17**	**0**
LCA_0568	*lsa0568*	Putative sakacin P immunity protein, SpiA	1	1	1	1	1	1	1	1	**0.03**	1	1	1	1	**0**	**0**	1	**0.23**	**0**
LCA_0569_b	*lsa0569_b*	Hypothetical small protein	1	1	1	1	1	1	1	1	**0.22**	1	1	1	1	**0**	**0**	1	1	**0**
LCA_0570	*lsa0570*	Hypothetical small protein	1	1	1	1	1	1	1	1	**0**	1	1	1	1	**0**	**0**	1	1	**0**
LCA_0571	*lsa0571*	Hypothetical small protein	1	1	1	1	1	1	1	1	0.85	1	1	1	1	1	0.81	1	1	0.68
LCA_0572	*tdcB*	Threonine deaminase (Threonineammonia-lyase, Threonine dehydratase, IlvA homolog)	1	1	1	1	1	1	1	1	0.84	1	1	1	1	**0.26**	0.7	1	1	**0.45**
LCA_0573	*lsa0573*	Hypothetical protein, CAAX protease family	1	1	1	1	1	1	1	1	0.63	1	1	1	1	0.73	**0.29**	1	1	**0.48**
LCA_0574	*lsa0574*	Hypothetical integral membrane protein	1	1	1	1	1	1	1	1	**0**	1	1	1	1	**0**	**0**	0.98	1	**0**
LCA_0575	*npr*	NADH peroxidase	1	1	1	1	1	1	1	1	0.85	1	1	1	1	**0.39**	0.93	1	1	0.76
LCA_0576	*lsa0576*	Hypothetical protein	0.66	1	0.69	0.82	0.97	1	0.91	0.88	**0**	**0.56**	**0.18**	1	1	**0**	**0**	**0.48**	0.71	**0**
LCA_0577	*lsa0577*	Hypothetical protein	0.77	0.94	**0.29**	**0.25**	1	1	**0.59**	**0.19**	**0.12**	0.68	**0.29**	1	1	**0.07**	**0**	**0.36**	**0.42**	**0**
LCA_0578	*lsa0578*	Hypothetical integral membrane protein	**0.24**	**0**	**0**	**0**	1	1	**0.35**	**0.02**	**0.01**	**0.19**	**0**	0.65	1	**0**	**0**	**0**	**0**	**0**
LCA_0579	*lsa0579*	Putative transcriptional regulator, LysR family	**0**	**0**	**0**	**0**	1	**0.28**	**0**	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**
LCA_0580	*ubiD*	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	**0.12**	**0**	**0.17**	**0.1**	1	0.75	0.76	0.63	**0.25**	0.74	**0.21**	**0**	1	**0.25**	**0**	**0.2**	**0.42**	**0**
LCA_0581	*ubiX*	Putative 3-octaprenyl 4-hydroxybenzoatecarboxylyase	**0.2**	**0**	**0.56**	0.83	1	0.92	0.78	0.67	**0.41**	0.91	**0.5**	**0.19**	1	**0.38**	**0.01**	**0.3**	**0.32**	**0**
LCA_0582	*lsa0582*	Hypothetical protein	**0**	**0**	**0**	**0**	**1**	**0.47**	**0**	**0**	**0.21**	**0.44**	**0**	**0**	1	**0**	**0**	**0**	**0**	**0**

Continued on following page

Table 4. *Continued*

Locus tag*	Gene	Description	Strain																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
LCA_0583	*glpQ*	Glycerophosphodiester phosphodiesterase	0.73	0.94	**0**	**0**	**1**	**1**	**0.51**	**0.15**	**0.08**	**0.22**	**0**	1	1	**0**	**0**	**0.3**	**0.32**	**0**
LCA_0584	*pepD4*	Dipeptidase D-type (U34 family)	1	1	0.98	1	1	1	1	1	**0**	1	1	1	1	**0**	**0**	1	1	**0**
LCA_0585	*lsa0585*	Hypothetical protein	1	1	1	1	**0.58**	1	1	1	**0.05**	1	1	1	0.92	**0**	**0**	1	1	**0**
LCA_0586	*lsa0586*	Site-specific recombinase, prophage lsa1 integrase	**0.09**	**0**	**0**	**0**	0.67	**0.56**	0.78	**0.4**	0.82	**0.48**	**0.08**	**0.28**	**0.33**	**0**	0.9	**0.22**	**0.4**	**0**
LCA_0587	*lsa0587*	Hypothetical prophage lsa1 protein	**0**	**0**	**0.3**	**0.59**	**0.5**	**0.47**	0.61	0.64	0.83	0.77	**0.3**	**0**	**0.1**	**0.34**	0.71	**0.09**	0.72	**0**
LCA_0588	*lsa0588*	Hypothetical prophage lsa1 protein	**0.2**	**0**	**0.08**	**0.02**	0.71	**0.5**	0.84	0.58	0.76	**0.59**	**0.19**	**0.21**	**0.31**	**0.11**	**0.55**	**0.3**	**0.49**	**0**
LCA_0589	*lsa0589*	Putative prophage lsa1 DNA-binding protein, XREfamily	**0**	**0**	**0.41**	**0.05**	**0.05**	**0.53**	**0.53**	**0.56**	0.6	**0.41**	**0.13**	**0**	**0.27**	**0.37**	**0.14**	**0.56**	**0.27**	**0.07**
LCA_0590	*lsa0590*	Hypothetical prophage lsa1 protein	**0.14**	**0**	**0.56**	0.73	0.76	0.65	1	1	0.94	1	0.8	0.65	0.68	0.64	0.99	0.71	0.91	**0.37**
LCA_0591	*lsa0591*	Hypothetical prophage lsa1 protein	**0.11**	**0**	1	1	**0.44**	**0.43**	0.79	0.73	0.81	1	0.79	**0**	**0.17**	0.73	1	**0.33**	**0.35**	**0**
LCA_0592	*lsa0592*	Hypothetical prophage lsa1 peptide	**0.21**	**0**	**0.18**	**0.42**	**0.02**	1	0.73	**0.38**	0.89	0.81	0.65	0.65	**0.31**	**0.32**	1	**0.48**	**0.56**	**0.15**
LCA_0593	*lsa0593*	Hypothetical prophage lsa1 protein	**0**	**0**	**0.11**	**0.04**	0.72	**0.25**	**0.55**	**0.4**	0.63	**0.42**	**0.27**	**0**	**0**	0.62	1	**0.45**	**0.19**	**0.17**
LCA_0594	*lsa0594*	Hypothetical prophage lsa1protein	**0**	**0**	**0.39**	**0.47**	0.7	**0.04**	0.71	**0.16**	0.7	0.91	**0.5**	**0**	**0.03**	**0.12**	0.71	**0.04**	**0.41**	**0**
LCA_0595	*lsa0595*	Putative prophage lsa1 DNA primase	0.64	**0.5**	1	1	0.69	0.92	0.94	1	0.9	1	0.9	0.65	0.74	0.84	**0.57**	0.69	0.87	**0.33**
LCA_0596	*lsa0596*	Hypothetical prophage lsa1 protein	**0**	**0**	**0**	**0**	**0**	**0**	0.82	**0.27**	**0.09**	**0.24**	**0.06**	**0**	**0**	**0.04**	**0**	**0.27**	**0**	**0**
LCA_0597	*lsa0597*	Hypothetical prophage lsa1 protein	**0.28**	**0**	**0.4**	0.64	**0.03**	0.76	0.73	0.75	**0.33**	0.77	**0.54**	**0.36**	**0.37**	**0.44**	**0.53**	**0.44**	**0.36**	**0.16**
LCA_0598	*lsa0598*	Hypothetical prophage lsa1 protein	**0**	**0**	**0.07**	**0.05**	**0**	**0.19**	0.79	**0.29**	**0.29**	**0.31**	**0.13**	**0**	**0.04**	**0.18**	0.9	**0.42**	**0.05**	**0**
LCA_0599	*lsa0599*	Hypothetical prophage lsa1 protein	**0.11**	**0**	**0.08**	**0.26**	**0.5**	0.85	**0.55**	**0.03**	0.92	0.63	**0.22**	**0.51**	**0.14**	**0.04**	1	**0.2**	**0.33**	**0**
LCA_0600	*lsa0600*	Hypothetical prophage lsa1 protein	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.09**	**0**	**0**	**0**	**0**	**0**	1	**0**	**0**	**0**
LCA_0601	*lsa0601*	Hypothetical prophage lsa1 protein	0.69	**0.5**	0.84	**0.5**	0.73	1	0.91	0.98	1	0.81	0.6	0.65	0.92	1	1	0.61	0.8	0.61
LCA_0612	*lsa0612*	Hypothetical cell surface protein precursor (with LPQTG sorting signal)	1	1	**0.58**	0.88	1	1	0.83	0.6	1	1	**0.49**	1	1	1	1	1	1	1
LCA_0623	*lsa0623*	Putative metallo-phosphoesterase	0.74	**0.13**	**0.05**	**0.01**	0.87	1	**0.51**	**0.1**	0.83	**0.31**	**0**	0.65	1	**0.04**	0.9	**0.33**	**0.34**	0.94
LCA_0665	*loxL1I*	L-lactate oxidase (central fragment), degenerate	1	1	1	0.94	1	1	1	1	1	1	0.85	1	1	0.72	1	0.68	1	1
LCA_0710	*lsa0710*	Hypothetical protein	1	1	1	1	**0.53**	1	1	1	1	1	1	1	0.62	1	1	1	1	1
LCA_0724	*lsa0724*	Hypothetical protein	**0.41**	**0**	**0.29**	**0.19**	0.79	1	0.74	0.77	0.81	0.65	**0.26**	**0.49**	**0.42**	**0.04**	**0.08**	**0.31**	**0.25**	**0.04**
LCA_0727	*lsa0727*	Hypothetical cell surface protein precursor	**0**	1	0.7	0.82	**0.36**	1	0.75	0.71	**0.16**	1	**0.45**	1	**0.03**	**0**	**0**	**0**	**0**	**0**
LCA_0740	*lsa0740*	Hypothetical integral membrane protein	1	1	1	1	1	1	1	1	1	1	0.75	1	1	1	1	1	1	1
LCA_0770	*lsa0770*	Putative drug:H(+) antiporter	0.96	**0**	1	**0.57**	1	1	0.98	1	1	1	1	1	1	1	1	1	0.97	1
LCA_0771	*lsa0771*	Hypothetical integral membrane protein	**0.52**	**0**	**0**	**0**	1	1	**0.53**	**0.16**	1	**0.39**	**0**	0.65	1	1	1	0.65	**0.31**	**0.58**
LCA_0781	*lsa0781*	Putative cobalt ABC transporter, membrane-spanning/permease subunit	1	1	1	1	1	1	1	1	**0.28**	1	1	1	1	**0**	1	1	1	1
LCA_0797	*deoD*	Purine-nucleoside phosphorylase	1	1	1	1	1	1	1	1	0.97	1	1	1	1	**0.48**	1	1	1	1
LCA_0824	*birA1*	Biotin-[acetyl-CoA carboxylase] ligase	1	1	1	1	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_0827	*lsa0827*	Hypothetical lipoprotein precursor	1	1	1	1	0.76	1	1	1	1	1	1	1	0.8	1	1	1	1	1
LCA_0866	*msrA*	Protein methionine sulfoxide reductase	1	1	0.9	1	1	1	1	1	1	0.92	0.57	1	1	0.7	1	0.8	1	1
LCA_0871	*lsa0871*	Putative nitroreductase (oxidoreductase)	1	1	0.86	1	1	1	1	1	1	0.96	0.62	1	1	0.92	1	0.89	1	1
LCA_0872	*lsa0872*	Hypothetical protein	1	1	**0.38**	0.72	1	1	0.9	1	1	0.77	**0.49**	1	1	**0.39**	1	0.86	1	1
LCA_0902	*lsa0902*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0.37**
LCA_0903_b	*tnpA3C-ISLsa2*	Transposase (orfB) of ISLsa2 (IS150 family)	0.94	1	0.66	0.87	0.82	1	0.93	0.95	1	0.82	0.69	1	1	0.65	1	0.78	1	1
LCA_0904	*tnpA3N-ISLsa2*	Transposase (orfA) of ISLsa2 (N-terminal fragment), authentic frameshift	0.99	1	0.85	0.94	**0.53**	1	0.88	0.96	0.96	1	0.8	1	0.96	0.89	1	0.69	0.97	1
LCA_0912	*lsa0912*	Putative ATP-dependent helicase, DinG family	1	1	0.88	1	1	1	0.95	0.97	0.94	1	0.79	1	1	0.78	1	0.85	0.99	1
LCA_0923	*lsa0923*	Putative phage envelope protein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.79
LCA_0928	*lsa0928*	Hypothetical protein	1	**0**	**0.07**	**0**	1	1	**0.51**	**0.31**	1	**0**	**0**	0.65	1	1	1	1	**0.22**	1
LCA_0930	*lsa0930*	Putative ABC transporter, ATP-binding subunit	1	1	1	1	**0.5**	1	1	1	1	1	1	1	**0.31**	1	1	1	1	1
LCA_0931	*lsa0931*	Hypothetical integral membrane protein	1	1	**0.26**	**0.22**	0.64	1	0.65	**0.57**	1	**0.58**	**0.12**	1	**0.13**	**0.12**	1	1	**0.56**	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_0932	*lsa0932*	Hypothetical small peptide	1	1	1	1	1	1	1	1	0.95	1	1	1	1	0.71	1	1	1	1
LCA_0937	*lsa0937*	Putative drug ABC exporter, membrane-spanning/permease subunit	1	1	1	1	1	0.94	1	1	1	1	1	0.65	1	1	1	1	1	1
LCA_0938	*lsa0938*	Putative drug ABC exporter, ATP-binding subunit	1	1	1	1	0.88	1	1	1	1	1	1	1	**0.59**	1	1	1	1	1
LCA_0956	*pyrDA*	Dihydroorotate oxidase, electron transfer subunit	1	1	**0.59**	0.83	1	1	0.76	0.85	1	0.97	**0.43**	1	1	0.62	1	0.74	0.97	1
LCA_0972	*lsa0972*	Putative transcriptional regulator, LysR family	1	1	**0.02**	**0**	0.98	1	0.83	**0.59**	1	**0.49**	**0.02**	1	1	1	1	1	**0.51**	1
LCA_0973	*pflA*	Formate C-acetyltransferase activating enzyme (Pyruvate formate-lyase activating enzyme)	1	1	**0.38**	**0.41**	1	1	0.68	0.83	1	**0.69**	**0.12**	1	1	1	1	1	0.69	1
LCA_0999	*lsa0999*	Hypothetical protein, DegV family	1	1	**0.58**	**0.56**	1	1	0.85	0.9	0.98	0.87	**0.59**	1	1	**0.5**	1	0.79	0.78	1
LCA_1002	*lsa1002*	Putative drug resistance ABC transporter, two ATP-binding subunits	1	1	0.85	1	1	1	1	1	0.97	0.97	0.75	1	1	0.79	1	0.84	1	1
LCA_1007	*lsa1007*	Putative transcriptional regulator, DUF24 family (related to MarR/PadR families)	**0**	1	1	1	**0.5**	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1008	*lsa1008*	Putative extracellular chitin-binding protein precursor	**0.52**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1009	*lsa1009*	Putative drug:H(+) antiporter (C-terminal fragment), authentic frameshift	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1010	*lsa1010*	Putative drug:H(+) antiporter (N-terminal fragment), authentic frameshift	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1011	*lsa1011*	Hypothetical protein	0.6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1030	*lsa1030*	Hypothetical protein	1	1	1	1	0.74	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1064	*lsa1064*	Hypothetical protein	0.62	1	1	1	1	1	1	1	1	1	1	1	1	**0.44**	1	0.73	1	1
LCA_1076	*lsa1076*	Hypothetical extracellular protein precursor	1	1	1	1	0.76	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1094	*lsa1094*	Hypothetical integral membrane protein	1	1	1	1	**0.05**	1	1	1	1	1	1	1	0.67	1	1	1	1	1
LCA_1097	*lsa1097*	Putative ADP-ribose phosphorylase, NUDIX family	1	**0**	0.98	**0**	1	1	**0.36**	**0**	1	1	0.69	**0**	1	1	1	1	1	1
LCA_1098	*folP*	Dihydropteroate synthase	1	**0**	0.82	**0**	1	1	**0.01**	**0.07**	1	0.85	0.45	**0**	1	1	1	1	0.99	1
LCA_1099	*folC2*	Folylpolyglutamate synthase	0.92	**0**	1	**0**	0.89	1	**0**	**0**	0.93	1	1	**0**	1	**0.34**	1	0.72	1	0.89
LCA_1100	*folE*	GTP cyclohydrolase I	1	**0**	1	**0**	1	1	**0**	**0**	1	1	1	**0**	1	1	1	1	1	1
LCA_1101	*folK*	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	0.96	**0**	**0.59**	**0**	1	1	**0**	**0**	0.84	0.8	**0.14**	**0**	1	**0.36**	0.62	**0.49**	0.78	0.68
LCA_1107	*lsa1107*	Hypothetical protein	0.65	1	1	1	1	1	1	1	0.8	1	1	1	1	**0.7**	0.77	**0.52**	0.76	**0.59**
LCA_1108	*lsa1108*	Putative chromosome segregation ATPase	0.66	1	1	1	1	1	1	1	0.79	1	1	1	**0.7**	**0.57**	**0.45**	**0.45**	1	**0.52**
LCA_1116	*lsa1116*	Putative methionine ABC transporter, ATP-bindingsubunit	1	1	0.89	0.93	1	1	0.87	0.96	1	1	0.61	1	1	0.84	1	0.89	0.99	1
LCA_1117	*lsa1117*	Putative aminomethyltransferase, family ofglycine cleavage H protein	1	1	**0.59**	**0.52**	1	1	0.75	0.82	1	0.86	0.61	1	1	**0.44**	1	0.77	**0.46**	1
LCA_1120	*lsa1120*	Hypothetical protein	0.69	0.64	**0**	**0**	0.63	1	**0.5**	**0.02**	0.67	**0**	**0**	1	1	**0**	0.93	**0.15**	**0.04**	0.96
LCA_1121	*lsa1121*	Hypothetical extracellular peptide precursor	1	1	0.91	0.96	1	1	1	1	1	0.85	**0.34**	1	1	0.67	1	0.97	1	0.96
LCA_1150	*lsa1150*	Putative transcriptional regulator, TetR family	1	1	0.83	0.67	1	1	1	1	1	0.99	0.88	1	1	1	1	1	1	1
LCA_1151	*lsa1151*	Potassium/ion channel protein	1	**0**	**0**	**0**	1	1	**0**	**0**	1	**0**	**0**	**0.42**	1	1	1	1	**0**	1
LCA_1153	*lsa1153*	Hypothetical protein, CAAX protease family	1	1	0.91	1	1	1	1	1	1	1	**0.5**	1	1	1	1	1	0.9	1
LCA_1155	*lsa1155*	Hypothetical integral membrane protein	1	1	1	1	1	1	1	1	1	1	1	0.65	1	1	1	1	1	1
LCA_1156	*lsa1156*	Hypothetical integral membrane protein	1	**0**	**0**	**0**	1	1	**0**	**0**	1	**0**	**0**	0.65	1	1	1	1	**0**	1
LCA_1157	*mgsA*	Methylglyoxal synthase	1	1	**0.06**	**0.12**	1	1	**0.56**	**0.04**	1	0.65	**0.08**	1	1	1	1	1	**0.42**	1
LCA_1160	*lsa1160*	Putative uncharacterized protein	1	1	0.83	0.93	1	1	0.91	0.97	1	0.93	0.78	1	1	0.76	1	0.88	0.94	1
LCA_1162	*lsa1162*	DNA-repair protein (SOS response UmuC-like protein)	1	1	**0**	**0**	1	1	**0.5**	**0**	1	**0.04**	**0.19**	1	1	**0.16**	1	0.97	0.06	1
LCA_1164	*lsa1164*	Hypothetical protein	1	1	**0.52**	**0.48**	1	1	0.71	0.76	1	0.84	**0.58**	1	1	**0.31**	1	1	**0.45**	1
LCA_1166	*lsa1166*	Putative potassium transport protein	1	1	**0.23**	**0.41**	1	1	0.71	0.65	1	**0.35**	**0.17**	1	1	1	1	1	**0.4**	1
LCA_1167	*lsa1167*	Hypothetical protein	1	**0**	**0**	**0**	1	1	**0.1**	**0.02**	1	**0**	**0**	0.65	1	1	1	1	**0**	1
LCA_1168	*lsa1168*	Putative nucleoside deoxyribosyltransferase	1	1	**0.52**	**0.57**	1	1	0.94	0.98	1	0.9	**0.41**	1	1	1	1	1	0.85	1
LCA_1169	*lsa1169*	Putative 6-phospho-beta-glucosidase, glycosidehydrolase family 1	1	1	0.68	1	1	1	1	1	1	0.95	0.9	1	1	1	1	1	1	1
LCA_1172	*lsa1172*	Hypothetical lipoprotein precursor	1	1	1	1	0.71	1	1	1	1	1	1	1	0.76	1	1	1	1	1
LCA_1176	*lsa1176*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	0.71	1	1	1	1	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_1178	*lsa1178*	Hypothetical protein	1	1	1	1	0.79	1	1	1	1	1	1	1	0.84	1	1	1	1	**0.34**
LCA_1181	*tnpB-ISLsa3*	Transposase (orfB) of ISLsa3 (IS4 family)	1	1	1	1	0.67	1	1	1	1	1	1	1	**0.39**	1	1	1	1	1
LCA_1182	*lsa1182*	Cytochrome P450 (C-terminal fragment), truncated	1	1	1	1	**0.5**	1	1	1	1	1	1	1	**0.1**	1	1	1	1	1
LCA_1201	*lsa1201*	Putative transcriptional regulator, GntR family	**0.16**	**0**	**0**	**0**	**0.47**	1	**0.5**	**0.18**	1	**0**	**0**	**0**	**0.31**	1	1	**0.03**	**0**	**0.28**
LCA_1202	*lsa1202*	Hypothetical protein	**0.03**	**0**	**0**	**0**	**0.6**	**0**	**0**	**0**	1	**0**	**0**	**0**	**0**	1	1	**0**	**0**	**0**
LCA_1203	*lsa1203*	Hypothetical membrane protein	**0.02**	**0**	**0**	**0**	**0**	0.98	**0**	**0**	1	**0.23**	**0**	**0.24**	**0.06**	1	1	**0**	**0**	**0.14**
LCA_1220	*citG*	Triphosphoribosyl-dephospho-CoA synthase	1	1	**0.28**	**0.3**	**0.4**	1	0.77	0.88	0.83	0.73	**0.42**	1	**0.45**	0.63	**0.24**	**0.43**	0.73	1
LCA_1221	*citR*	Citrate catabolism transcriptional regulator, GntR family	1	1	1	1	0.66	1	1	1	**0.55**	1	1	1	**0.06**	**0.17**	**0**	**0.13**	1	1
LCA_1222	*oadA*	Oxaloacetate decarboxylase, alpha subunit	1	1	1	1	**0.57**	1	1	1	0.62	1	1	1	**0.45**	**0.69**	**0.2**	0.76	1	1
LCA_1223	*citX*	Apo-citrate lyase phosphoribosyl-dephospho-CoA transferase	1	1	1	1	**0.03**	1	1	1	**0**	1	1	1	**0**	**0**	**0**	**0**	1	1
LCA_1224	*citF*	Citrate lyase, alpha subunit	1	1	1	1	**0**	1	1	1	**0.29**	1	1	1	**0.07**	**0.25**	**0**	**0.24**	1	1
LCA_1225	*citE*	Citrate lyase, beta subunit	1	1	1	1	0.72	1	1	1	**0.27**	1	1	1	**0.54**	**0.22**	**0**	**0.39**	1	1
LCA_1227	*citC*	[citrate (pro-3S)-lyase] ligase (citrate lyasesynthetase)	1	1	1	1	0.74	1	1	1	**0.31**	1	1	1	**0.09**	0.67	**0.01**	**0.47**	1	1
LCA_1228	*lsa1228*	Hypothetical small peptide	1	1	1	1	**0**	1	1	1	**0**	1	1	1	**0**	**0**	**0**	**0**	1	1
LCA_1229	*oadB*	Oxaloacetate decarboxylase, beta subunit	1	1	0.96	1	**0**	1	1	1	**0**	0.95	**0.58**	1	**0**	**0**	**0**	**0**	1	0.78
LCA_1230	*oadG*	Oxaloacetate decarboxylase, gamma subunit	1	1	1	1	1	1	1	1	0.83	1	1	1	1	NA	0.76	0.93	1	1
LCA_1231	*lsa1231*	Hypothetical protein	1	1	0.62	0.75	**0.5**	1	0.88	0.95	**0**	0.87	0.61	1	**0**	**0.01**	**0**	**0.03**	0.87	1
LCA_1232	*citM*	Citrate:Mg(2+)(H+) symporter	1	1	1	1	1	1	1	1	0.94	1	1	1	0.92	1	**0.43**	0.98	1	1
LCA_1233	*birA2*	Biotin-[acetyl-CoA-carboxylase] ligase	1	1	**0.29**	**0.05**	**0**	1	**0.54**	**0.19**	**0.58**	0.73	**0.23**	1	**0.18**	**0.13**	**0.2**	**0.12**	**0.32**	1
LCA_1234	*lsa1234*	Hypothetical protein	1	1	1	1	0.83	1	1	1	1	1	1	1	1	0.63	0.86	**0.56**	1	1
LCA_1240	*lsa1240*	Putative coproporphyrinogen oxidase, HemN	1	1	1	1	1	1	1	1	0.81	1	1	1	1	**0.17**	1	1	1	1
LCA_1245	*lsa1245*	Hypothetical protein	0.97	1	**0.32**	**0.39**	0.89	1	0.67	**0.56**	0.66	0.78	**0.13**	1	1	**0**	1	**0.45**	**0.53**	1
LCA_1254	*lsa1254*	Hypothetical protein	**0**	**0**	**0**	**0**	0.71	**0.26**	**0**	**0**	**0.5**	**0**	**0**	0.65	1	**0**	**0.11**	**0**	**0**	**0.18**
LCA_1255	*lsa1255*	Hypothetical protein	**0.52**	**0.31**	1	0.93	1	1	1	0.92	1	1	1	0.65	1	0.83	0.79	0.73	1	0.82
LCA_1293	*lsa1293*	Hypothetical protein	1	1	0.81	1	1	1	1	1	1	1	0.76	1	1	0.76	1	1	1	1
LCA_1306	*lsa1306*	Putative bacterial type II secretion/competencesystem, protein ComGA-like	1	1	1	1	**0.59**	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1310	*lsa1310*	Hypothetical membrane protein	**0**	**0**	**0**	**0**	**0**	**0.08**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
LCA_1314	*lsa1314*	Putative drug:Na(+) antiporter (drug effluxpump)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.72	1	1
LCA_1318	*lsa1318*	Hypothetical integral membrane protein	0.9	1	**0**	**0**	1	1	**0.5**	**0.05**	0.85	**0.15**	**0**	1	1	**0**	1	**0.36**	**0.32**	0.99
LCA_1321	*glnA*	Glutamate-ammonia ligase (Glutamine synthetase)	1	1	0.94	0.96	1	1	1	1	1	0.9	**0.46**	1	1	0.74	1	1	1	1
LCA_1327	*lsa1327*	Hypothetical extracellular peptide precursor	1	1	1	1	**0.5**	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1328	*lsa1328*	Putative rhodanese-sulfurtransferase	1	1	1	1	1	1	1	1	1	1	0.77	1	1	1	1	1	1	1
LCA_1346	*lsa1346*	Hypothetical protein	**0.48**	1	**0.01**	**0.03**	1	1	**0.56**	**0.44**	0.77	**0.16**	**0**	1	1	**0.1**	1	**0.45**	**0.32**	1
LCA_1348	*lsa1348*	Hypothetical extracellular protein precursor	1	1	1	1	1	1	1	1	1	0.94	0.76	1	1	1	1	1	1	1
LCA_1352	*lsa1352*	Putative phosphomethylpyrimidine kinase	1	1	**0.31**	**0.35**	0.89	1	0.62	**0.36**	0.96	**0.4**	**0.09**	1	1	**0.39**	1	0.72	**0.52**	1
LCA_1362	*potB*	Spermidine/putrescine ABC transporter, membrane-spanning subunit	1	1	1	1	1	1	1	1	0.97	1	1	1	1	0.78	0.94	1	1	1
LCA_1364	*lsa1364*	Putative zinc-containing alcohol dehydrogenase (oxidoreductase)	1	**0.25**	**0.09**	**0.12**	1	1	**0.53**	**0.06**	1	**0.12**	**0.01**	**0.65**	1	1	1	1	**0.05**	1
LCA_1366	*lsa1366*	Putative ABC exporter, ATP-binding subunit	1	1	0.97	0.95	1	1	1	1	1	0.64	**0.33**	1	1	1	1	1	0.95	1
LCA_1367	*lsa1367*	Putative ABC exporter, membrane-spanning/permease subunit	1	1	0.95	0.68	1	1	0.79	0.87	1	0.85	**0.57**	1	1	1	1	1	0.64	1
LCA_1370	*lsa1370*	Two-component system, sensor histidine kinase	1	1	**0**	**0.01**	1	1	0.63	0.65	1	**0.27**	**0.13**	1	1	1	1	1	**0.28**	1
LCA_1371	*lsa1371*	Hypothetical membrane protein	1	1	0.99	0.75	1	1	0.96	1	1	0.86	0.69	1	1	0.75	1	0.89	1	1
LCA_1375	*pheT*	Phenylalanyl-tRNA synthetase, beta subunit	1	1	1	1	0.98	1	1	1	0.85	1	1	1	1	**0.47**	1	1	1	0.92
LCA_1380	*lsa1380*	Putative tRNA/rRNA methyltransferase	1	1	1	1	1	1	1	1	1	1	1	1	0.92	1	1	**0.5**	1	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_1381	*lsa1381*	Putative acylphosphatase	1	1	1	1	0.89	1	1	1	1	1	1	1	1	1	1	**0.23**	1	1
LCA_1399	*loxL2*	L-lactate oxidase	0.99	0.86	**0.19**	**0.17**	1	1	0.66	0.74	0.96	0.69	**0.36**	1	0.98	0.68	0.88	0.81	**0.56**	0.77
LCA_1403	*lsa1403*	Hypothetical domain	0.74	1	0.61	0.63	1	1	0.65	0.77	0.93	0.66	**0.35**	1	1	**0.18**	1	**0.57**	**0.4**	1
LCA_1407	*lsa1407*	Putative transcriptional regulator, MarR family	1	1	0.64	1	1	1	1	1	1	0.83	0.61	1	1	1	1	1	0.73	1
LCA_1408	*lsa1408*	Hypothetical protein	0.87	1	0.96	1	1	1	1	1	0.86	0.85	**0.35**	1	1	1	1	0.98	0.95	1
LCA_1409	*lsa1409*	Hypothetical cell surface protein precursor	0.88	1	**0.48**	1	1	1	1	1	0.83	**0.56**	**0.22**	1	1	**0.56**	1	**0.38**	**0.2**	0.84
LCA_1410	*smpB*	t/mRNA binding protein (SsrA RNA binding protein)	0.88	1	0.74	**0.24**	1	1	**0.9**	1	0.93	0.81	**0.52**	1	1	**0.52**	1	0.89	0.94	1
LCA_1412	*lsa1412*	Putative lipase/esterase	1	1	0.74	0.93	1	1	0.98	1	0.96	0.96	0.7	1	1	**0.57**	1	0.95	1	1
LCA_1413	*secG*	Preprotein translocase, SecG subunit	0.99	1	1	1	1	1	1	1	0.96	1	1	1	1	**0.5**	**0.2**	**0.58**	1	**0.56**
LCA_1416	*lsa1416*	Putative ABC transporter, membrane-spanning/permease subunit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.75	1	1
LCA_1422	*lsa1422*	Putative drug ABC exporter, ATP-binding and membrane-spanning/permease subunits	1	1	0.67	1	1	1	0.91	1	0.94	0.77	**0.25**	1	1	0.77	1	1	0.7	1
LCA_1426	*lsa1426*	Putative aminopeptidase	1	**0.59**	**0**	**0**	0.69	1	**0.5**	**0.01**	0.63	**0.11**	**0**	0.94	1	**0**	0.97	**0.19**	**0.06**	0.87
LCA_1427	*lsa1427*	Putative hydrolase, haloacid dehalogenase family	1	1	0.8	0.75	1	1	1	1	0.89	1	0.78	1	1	0.84	1	0.76	1	1
LCA_1430	*lsa1430*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.77	1	1
LCA_1431	*rsuA*	Pseudouridylate synthase, 16S-rRNA-specific	1	1	1	1	0.61	1	1	1	1	1	1	1	1	1	1	1	0.01	1
LCA_1443	*lsa1443*	Putative inorganic polyphosphate/ATP-NAD kinase	1	1	0.96	1	1	1	1	1	1	0.89	0.6	1	1	0.81	1	1	1	1
LCA_1445	*lsa1445*	Hypothetical protein	**0.57**	1	**0.28**	**0.4**	0.98	1	**0.59**	**0.53**	0.9	**0.57**	**0.11**	1	1	**0**	0.96	0.45	1	1
LCA_1452	*lsa1452*	Putative ABC exporter, membrane-spanning/permease subunit	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0.09**	1
LCA_1473	*lsa1473*	Hypothetical integral membrane protein	1	1	1	0.82	1	1	1	1	1	1	1	1	1	**0.58**	1	1	1	1
LCA_1474	*lsa1474*	Putative bifunctional glycosyl transferase, family 8	1	0.97	1	**0**	1	1	**0.51**	**0.09**	1	1	1	1	1	1	0.94	1	1	1
LCA_1480	*lsa1480*	Hypothetical membrane protein	1	1	1	1	1	1	1	1	1	1	1	1	1	**0**	1	1	1	**0.58**
LCA_1481	*lsa1481*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0**	1
LCA_1485	*mvaA*	Hydroxymethylglutaryl-CoA reductase	0.98	1	1	1	1	1	1	1	0.94	0.63	**0.23**	1	1	**0.26**	1	1	0.63	1
LCA_1489	*lsa1489*	Hypothetical integral membrane protein	1	1	**0.38**	1	0.85	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1490	*lsa1490*	Hypothetical protein	1	1	**0**	1	0.68	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1491	*lsa1491*	Hypothetical protein	1	1	**0.03**	1	0.93	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1495	*rfbB*	Putative dTDP-glucose 4,6-dehydratase	1	1	0.83	1	1	1	1	1	1	0.86	**0.57**	1	1	1	1	1	0.95	0.95
LCA_1503	*lsa1503*	Hypothetical protein	1	1	**0.44**	1	1	1	1	1	0.84	1	0.74	1	1	**0.54**	1	0.83	1	1
LCA_1507	*galU*	UTP-glucose-1-phosphate uridylyltransferase	1	1	0.65	**0.55**	1	1	0.78	0.83	0.94	0.97	0.6	1	1	**0.5**	1	0.74	0.75	1
LCA_1508	*lsa1508*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	0.84	1	**0.47**	1	1
LCA_1510_b	*lsa1510_b*	Hypothetical protein	**0**	**0**	1	**0**	0.79	**0.07**	**0.47**	**0**	0.67	**0.27**	**0.4**	**0**	**0**	**0.45**	**0**	**0.16**	**0.01**	**0**
LCA_1510_d	*lsa1510_d*	Putative glycosyl transferase, family 2	**0**	**0**	0.63	**0**	**0.59**	**0**	**0**	**0**	**0.4**	**0**	**0.04**	**0**	**0**	**0.01**	**0**	**0**	**0**	**0**
LCA_1510_e	*lsa1510_e*	Putative glycosyl transferase, group 1	**0**	**0**	1	**0.48**	**0.04**	**0.14**	0.66	0.64	**0.18**	**0.37**	**0.28**	**0**	**0**	**0.11**	**0**	**0.23**	**0.41**	**0**
LCA_1510_f	*lsa1510_f*	Putative glycosyl transferase, family 8	**0**	**0**	1	**0**	**0.5**	0.95	**0.5**	**0.02**	**0**	**0.36**	**0.24**	**0**	**0.48**	**0**	**0**	**0.01**	**0**	**0.08**
LCA_1511	*lsa1511*	Putative polysaccharide biosynthesis protein, chain length determination	1	1	1	**0.33**	0.61	1	**0.51**	**0.15**	0.79	1	1	1	1	1	**0.34**	1	**0.16**	0.95
LCA_1512	*lsa1512*	Putative polysaccharide biosynthesis protein, chain length determination	**0.58**	**0**	1	**0**	**0**	1	**0.51**	**0.01**	**0.56**	**0.28**	**0**	0.65	1	**0**	**0**	**0**	**0.13**	**0.42**
LCA_1513	*lsa1513*	Putative transcriptional regulator (involved in exocellular polysaccharide biosynthesis)	**0.5**	**0**	**0.02**	**0**	**0**	1	**0.5**	**0.51**	0.62	**0.05**	**0.08**	0.65	0.62	**0.17**	**0**	**0.14**	**0.02**	**0.58**
LCA_1514	*lsa1514*	Putative ribonuclease	1	1	1	1	0.6	1	0.99	1	0.98	1	0.98	1	1	1	**0**	1	**0.35**	1
LCA_1522	*pepS*	Aminopeptidase S	1	**0**	0.61	**0.04**	1	1	0.6	**0.14**	1	1	1	0.65	1	1	1	1	1	1
LCA_1523	*lsa1523*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0.54**	0.81	1	1
LCA_1525	*lsa1525*	Small multidrug resistance efflux protein, SMR family	1	1	1	1	1	1	1	1	1	1	1	1	1	0.61	1	1	1	1
LCA_1529	*lsa1529*	Putative aromatic acid transport protein (N-terminal fragment), authentic frameshift	0.72	1	**0.13**	**0.15**	1	1	**0.59**	**0.53**	0.91	**0.49**	**0.13**	1	1	0.05	1	1	**0.45**	0.94
LCA_1530	*lsa1530*	Putative small molecules-binding protein, 3Hdomain	0.63	0.99	0.06	0.07	0.87	1	**0.51**	**0.06**	0.86	**0.38**	**0.05**	1	1	**0.23**	1	**0.39**	**0.41**	0.97

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_1532	*lsa1532*	Hypothetical protein	1	1	0.63	0.76	1	1	0.89	0.95	1	1	0.91	1	1	0.61	1	0.8	0.77	1
LCA_1538	*lsa1538*	Putative extracellular glycosyl hydrolase precursor, family 25 (Lysosyme M1 family)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.76	1	1
LCA_1552	*pcrA*	ATP-dependent DNA helicase	0.81	1	**0.54**	0.7	0.99	1	1	0.94	0.97	0.85	**0.56**	1	1	0.67	1	0.66	1	1
LCA_1560	*lsa1560*	Putative N-acetyltransferase, GNAT family	1	1	1	1	1	1	1	1	1	0.79	0.47	1	1	1	1	0.82	1	1
LCA_1562	*lsa1562*	Hypothetical lipoprotein precursor	1	1	1	1	1	1	1	1	1	**0.24**	**0**	1	1	1	1	**0.18**	1	1
LCA_1568	*lsa1568*	Putative calcium-transporting P-type ATPase	1	1	**0.59**	0.63	1	1	**0.36**	**0.59**	0.95	0.8	0.64	1	1	**0.53**	1	0.84	0.68	1
LCA_1569	*nadE*	NH(3)-dependent NAD(+) synthetase	0.96	1	1	1	1	1	1	1	1	1	1	1	1	0.69	1	1	0.97	0.99
LCA_1570	*nadC*	Nicotinate phosphoribosyltransferase	**0.1**	1	1	1	1	1	1	1	1	1	1	1	1	**0**	1	1	**0.19**	0.88
LCA_1571	*tagA*	N-acetylglucosamyldiphospho-undecaprenol-N-acetyl-beta-D-mannosaminyltransferase (Teichoic acidbiosynthesis protein A)	0.8	**0**	1	**0**	1	1	**0.5**	**0.39**	**0.43**	1	1	0.65	1	**0.27**	**0.06**	1	**0.48**	**0.1**
LCA_1572	*lsa1572*	Putative teichoic acid/polysaccharide glycosyltransferase, family 2	**0.26**	**0**	1	0.62	1	1	0.6	**0.28**	**0.16**	0.83	0.67	**0.28**	1	1	**0.07**	1	**0.25**	**0**
LCA_1573	*lsa1573*	Putative teichoic acid/polysaccharide glycosyltransferase, group 1	**0**	**0**	1	**0.13**	1	**0.15**	**0**	**0**	**0.07**	**0.16**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1574	*tagD*	Glycerol-3-phosphate cytidyltransferase (CDP-glycerol pyrophosphorylase) (Teichoic acid biosynthesis protein D)	**0**	**0**	1	**0**	1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1575	*lsa1575*	Putative teichoic acid/polysaccharide exportprotein	**0**	**0**	1	**0**	1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1576	*tagB*	CDP-glycerol:glycerophosphate glycerophosphotransferase (Teichoic acid biosynthesisprotein B)	**0**	**0**	1	**0.07**	1	**0.21**	**0.5**	**0.01**	**0**	**0.41**	**0.16**	**0**	1	1	**0**	1	**0**	**0**
LCA_1577	*lsa1577*	Hypothetical protein	**0**	**0**	1	**0**	1	0.92	**0.14**	**0**	**0**	**0.07**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1578	*lsa1578*	Putative teichoic acid/polysaccharide biosynthesis protein	**0**	**0**	1	**0**	1	0	**0**	**0**	**0**	**0**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1579	*lsa1579*	Putative teichoic acid/polysaccharide exportprotein	**0**	**0**	1	**0**	0.61	0	**0**	**0**	**0**	**0**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1580	*lsa1580*	Hypothetical integral membrane protein	**0**	**0**	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**	**0**	1	1	**0**	1	**0**	**0**
LCA_1581	*lsa1581*	Putative teichoic acid-binding N-acetylmuramoyl L-alalanine amidase (cell wall hydrolase)	**0.18**	**0**	0.84	**0.26**	0.79	0.73	0.7	0.83	0.72	0.76	**0.44**	0.65	1	0.85	**0.27**	0.83	0.6	**0.46**
LCA_1582	*lsa1582*	Hypothetical small cell surface protein precursor	**0.24**	**0**	**0.48**	**0.46**	0.71	**0.49**	0.77	0.77	0.25	0.96	0.86	0.61	1	0.79	**0.09**	0.7	0.73	**0.12**
LCA_1584	*lsa1584*	Putative teichoic acid/polysaccharide glycosyltransferase	**0**	**0**	**0.07**	**0**	**0**	**0.01**	**0.5**	**0**	**0.46**	0.64	**0.08**	**0**	1	**0**	**0.01**	**0.01**	**0.01**	**0**
LCA_1585	*lsa1585*	Putative teichoic acid/polysaccharide phosphoglycerol transferase	0.7	**0.07**	1	0.99	1	1	1	1	0.9	1	1	0.65	1	1	**0.1**	0.87	0.95	**0.24**
LCA_1587	*lsa1587*	Putative transcriptional regulator, GntR family	1	1	1	1	1	1	1	1	1	0.97	0.8	1	1	**0.25**	1	**0.48**	1	1
LCA_1589	*lsa1589*	Putative oxidoreductase	1	1	1	1	1	1	1	0.99	1	1	1	1	1	1	1	0.73	1	1
LCA_1593	*lsa1593*	Putative nucleotide diphosphate hydrolase, NUDIXfamily	1	1	**0.16**	**0.17**	1	1	0.62	**0.15**	0.92	**0.29**	**0.06**	1	1	**0.03**	1	**0.17**	1	0.76
LCA_1594	*lysS*	Lysyl-tRNA synthetase	1	1	0.75	0.78	1	1	0.74	0.6	0.91	0.96	0.7	1	1	0.67	1	**0.52**	0.88	1
LCA_1596	*hslO*	Molecular chaperone, Hsp33 family	1	1	0.82	1	1	1	1	1	0.92	1	0.87	1	1	0.75	1	1	1	1
LCA_1610	*lsa1610*	Hypothetical integral membrane protein	1	1	0.97	1	1	1	1	1	0.98	0.98	0.73	1	1	0.74	1	0.94	1	1
LCA_1623	*lsa1623*	Hypothetical integral membrane protein	1	1	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1624	*lsa1624*	Hypothetical integral membrane protein	1	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1634	*lsa1634*	Putative metal-dependent phosphohydrolase	1	1	1	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1640	*nanA*	N-acetylneuraminate lyase	1	1	1	1	**0.32**	1	1	1	1	1	1	1	**0.33**	1	1	1	1	1
LCA_1641	*nanE*	N-acylglucosamine-6-phosphate 2-epimerase (N-acetylmannosamine-6-phosphate 2-epimerase)	1	1	1	1	**0.07**	1	1	1	1	1	1	1	0.67	1	1	1	1	1
LCA_1642	*lsa1642*	Putative solute:Na(+) symporter	1	1	1	1	**0.06**	1	1	1	1	1	1	1	**0**	1	1	1	1	1
LCA_1643	*lsa1643*	Putative sugar kinase, ROK family	1	1	1	1	**0.5**	1	1	1	1	1	1	1	**0.07**	1	1	1	1	1
LCA_1644	*lsa1644*	Hypothetical protein	1	1	1	1	**0.5**	1	1	1	1	1	1	1	**0**	1	1	1	1	1
LCA_1645	*lsa1645*	Putative Na(+)/(+) antiporter	1	1	1	1	**0**	1	1	1	1	1	1	1	**0**	1	1	1	1	1
LCA_1647	*lsa1647*	Hypothetical membrane protein	1	1	1	1	1	1	1	1	1	1	1	1	**0**	1	1	1	1	1
LCA_1657	*lsa1657*	Putative deoxyribonuclease, TatD family	0.96	1	1	1	0.79	1	1	1	1	1	1	1	1	0.93	1	0.94	1	1

Continued on following page

Table 4. *Continued*

			Strain																	
Locus tag*	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
LCA_1673	*lsa1673*	Putative lipase/esterase precursor	**0.35**	**0.52**	**0.31**	**0.34**	0.91	1	0.79	**0.33**	0.97	0.63	**0.28**	0.86	1	**0.35**	1	**0.56**	**0.46**	0.93
LCA_1681	*cysS*	Cysteinyl-tRNA synthetase	1	1	0.75	0.93	1	1	0.9	0.97	0.98	1	**0.56**	1	1	0.62	1	0.8	0.85	1
LCA_1690	*lsa1690*	Putative cellobiose-specific PTS, enzyme IIC	1	**0**	**0**	**0**	**0.52**	1	**0.44**	**0**	**0.21**	1	1	0.65	0.81	**0**	0.75	**0**	**0**	1
LCA_1691	*lsa1691*	Hypothetical protein	1	1	0.85	**0.33**	0.9	1	0.78	0.84	0.84	1	1	1	1	0.84	1	0.85	0.66	1
LCA_1693	*asnB2*	L-asparaginase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.73	1	1
LCA_1710	*lacM*	Beta-galactosidase, small subunit (Lactase, small subunit)	1	1	1	1	1	1	1	1	1	1	1	1	1	0.69	1	1	1	1
LCA_1719	*lsa1719*	Hypothetical protein	1	1	**0.48**	1	0.65	1	1	1	1	1	1	1	0.99	1	1	1	1	1
LCA_1720_a	*lsa1720_a*	Hypothetical protein (C-terminal fragment), authentic frameshift	**0.13**	1	1	1	0.62	1	1	1	0.76	1	1	1	**0.45**	**0.51**	**0.37**	0.68	1	1
LCA_1725	*lsa1725*	Hypothetical cell surface protein precursor	**0.13**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0.1**
LCA_1726	*lsa1726*	Hypothetical protein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1727	*lsa1727*	Hypothetical protein	**0.01**	**0**	**0.2**	0.78	1	1	0.75	0.67	1	0.66	**0.55**	0.65	1	1	1	1	**0.5**	**0.01**
LCA_1728	*lsa1728*	Hypothetical cell surface protein precursor	**0**	**0**	**0.09**	**0**	0.66	1	**0.5**	**0**	1	**0.39**	**0.21**	0.65	1	1	1	1	**0**	**0**
LCA_1729	*lsa1729*	Hypothetical cell surface protein precursor	**0.18**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0.04**
LCA_1730	*lsa1730*	Hypothetical cell surface protein	**0.25**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**0.01**
LCA_1731	*lsa1731*	Hypothetical cell surface protein precursor	**0.5**	1	1	1	0.79	1	1	1	0.9	1	1	1	0.74	0.84	0.76	0.82	0.7	**0.15**
LCA_1732	*lsa1732*	Hypothetical protein	1	1	1	1	**0.5**	1	1	1	0.62	1	1	1	0.75	1	**0.46**	**0.55**	0.72	**0.34**
LCA_1734	*lsa1734*	Hypothetical extracellular protein precursor	1	1	1	1	**0**	1	1	1	**0.41**	1	1	1	**0**	**0.03**	**0**	**0**	**0**	**0**
LCA_1735	*lsa1735*	Putative cobalt ABC transporter, membrane-spanning subunit	1	1	1	1	0.77	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1776	*lsa1776*	Putative 4-carboxymuconolactone decarboxylase	1	1	1	1	1	1	1	1	1	1	1	1	**0.37**	1	1	1	1	1
LCA_1777	*lsa1777*	Putative phosphate ABC transporter, substrate binding lipoprotein precursor	0.6	1	0.62	0.69	0.9	1	0.87	0.84	1	0.91	0.6	1	1	**0.55**	1	0.63	0.68	1
LCA_1779	*clpC*	ATPase/chaperone ClpC, putative specificityfactor for ClpP protease	1	1	0.82	1	1	1	1	1	1	0.99	0.88	1	1	0.78	1	0.81	1	1
LCA_1785	*lsa1785*	Hypothetical protein	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.03**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1	**0**
LCA_1786	*lsa1786*	Putative melibiose:Na(+) transport protein	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1	**0**
LCA_1787	*lsa1787*	Hypothetical cell surface protein precursor	1	1	**0.53**	0.72	1	1	0.85	0.79	0.79	0.75	**0.21**	1	1	**0.3**	1	**0.5**	1	0.71
LCA_1788	*lsa1788*	Putative phage-related 1,4-beta-N-acetylmuramidase (cell wall hydrolase)	**0**	**0**	**0**	**0**	0.84	1	**0**	**0**	**0.09**	**0**	**0**	**0.33**	**0.37**	**0**	**0.04**	**0**	1	**0.17**
LCA_1789	*lsa1789*	Putative transcriptional regulator with a sugar-binding domain, LacI family	**0.55**	**0**	0.61	0.86	0.76	1	0.77	**0.51**	0.73	0.94	0.65	0.65	0.63	**0.47**	**0.39**	**0.32**	**0.51**	**0.22**
LCA_1798	*lsa1798*	Putative oxidoreductase, short chain dehydrogenase/reductase family	1	1	1	1	0.88	1	1	1	1	1	1	1	0.93	1	1	1	**0.53**	0.84
LCA_1799	*lsa1799*	Putative transcriptional regulator, GntR family	1	1	1	1	0.8	1	1	1	1	1	1	1	1	1	1	1	**0.48**	1
LCA_1800	*lsa1800*	Putative autotransport protein	**0.5**	1	1	1	0.76	1	1	1	**0.56**	1	1	1	0.8	**0.5**	**0.5**	**0.5**	1	**0.58**
LCA_1801	*lsa1801*	Putative drug ABC exporter, membrane-spanning subunit	1	1	1	1	1	1	1	1	1	1	1	1	1	0.95	1	**0**	1	0.1
LCA_1802	*lsa1802*	Putative drug ABC exporter, ATP-binding subunit	**0.41**	1	1	1	0.89	1	1	1	0.82	1	1	1	1	**0.24**	1	**0**	1	**0**
LCA_1803	*serS*	Seryl-tRNA synthetase	0.94	1	1	1	1	1	1	1	0.96	1	1	1	1	1	1	1	1	1
LCA_1804	*lsa1804*	Hypothetical protein	1	1	0.69	0.73	1	1	0.8	0.75	0.86	1	0.94	1	1	0.88	1	0.72	1	1
LCA_1808	*lsa1808*	Hypothetical protein	1	1	1	1	**0.5**	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1810	*lsa1810*	Hypothetical cell surface protein precursor	1	1	1	1	**0**	1	1	1	1	1	0.93	1	**0.48**	1	1	0.99	1	1
LCA_1811	*lsa1811*	Hypothetical cell surface protein precursor	1	1	1	1	**0**	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1812	*lsa1812*	Putative surface polysaccharide deacetylase	1	1	1	1	**0.51**	1	1	1	1	1	1	1	1	1	1	1	1	1
LCA_1813	*lsa1813*	Hypothetical protein	1	1	1	1	**0**	1	1	1	0.63	1	1	1	1	**0.08**	1	**0.49**	1	1
LCA_1815	*lsa1815*	Hypothetical cell surface protein precursor	0.99	1	0.89	0.92	0.73	1	1	0.99	1	0.88	0.71	1	1	1	1	1	1	1
LCA_1850	*lsa1850*	Hypothetical protein	1	1	**0**	**0**	0.87	1	**0.51**	**0.02**	1	**0**	**0**	1	1	1	1	1	1	1
LCA_1852	*lsa1852*	Putative hydrolase, isochorismatase/nicotamidase family	1	**0**	**0**	**0**	1	0.85	**0**	**0**	1	**0**	**0**	0.65	1	1	1	1	1	1
LCA_1853	*lsa1853*	Transcriptional regulator, AsnC/Lrp family	1	**0**	**0**	**0**	1	**0.37**	**0**	**0**	1	**0**	**0**	**0.36**	1	1	1	1	1	1
LCA_1856	*araA*	L-arabinose isomerase	1	1	0.97	1	1	1	1	1	1	0.89	0.75	1	1	1	1	1	1	1
LCA_1860	*araT*	D-arabinose:H(+) symporter	1	1	1	1	0.63	1	1		1	1	1	1	0.82	1	1	1	1	1

* The locus tag indication LCA_ and LSA are both used for *L. sakei*.

Table 5. Bacteriocin-associated genes and plasmid encoded *lac* genes from various *L. sakei* strains investigated by CGH in 18 *L. sakei* strains. Genes were considered present (bold) or divergent using an identity score of 0.7, thus probes with more than 70% identity with the genome were assumed to give hybridization signals, and were defined as present. Genes with identity score between 0.6 and 0.8 were defined as uncertain, reflecting that these genes have a score close to the cutoff. Strains: 1. CCUG 31331; 2. CTC372; 3. CTC494; 4. CTC460; 5. DSM 20017; 6. L45; 7. Lb148; 8. Lb156; 9. Lb16; 10 Lb790; 11. Lb706; 12. Lb77; 13. LS 25; 14. LMGT2380; 15. LTH673; 16. MF1058; 17. MF1328; 18. MF1053.

			Strain																	
Locus tag	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
***L. sakei* Lb674: Sakacin P cluster (acc. no. Z48542)**																				
SKP0001	*sppIP*	Bacteriocin sakacin P inducing peptide	**1**	0	0	0	0	0	0	0	**1**	0	0	0	0	**1**	**1**	0	0	**1**
SKP0004	*sppA*	Bacteriocin sakacin P precursor	**1**	0	0	0	0	0	0	0.01	**1**	0	0	0	0	**1**	**1**	0	0	**1**
SKP0005	*spiA*	Bacteriocin sakacin P immunity protein, SpiA	**1**	0	0	0	0	0	0	0	**1**	0	0	0	0	**1**	**1**	0	0	**0.92**
SKP0006	*sppT*	Sakacin P ABC transporter	0	0	0	0	0	0	0	0	**1**	0	0	0	0	**1**	**1**	0	0	**1**
SKP0007	*sppE*	Sakacin P accesory transport protein	0	0	0	0	0	0	0.5	0	**1**	0	0	0	0	**1**	**1**	0	0.01	**1**
***L. sakei* Lb706: Sakacin A cluster (acc. no. Z46867)**																				
SKA0002	*saiA*	Bacteriocin sakacin A immunity protein	0	0	**1**	0	0	0	0.31	0	0	0	**1**	0	0	0	0	0	0	0
SKA0003	*sapA*	Bacteriocin sakacin A precursor	0	0	**1**	0	0	0	0.01	0.01	0	0	**1**	0	0	0	0	0	0	0
SKA0005	*sapK*	Two-component system, sensor histidine kinase SapK	0	0	**1**	0	0	0	0.26	0	0	0	**1**	0	0	0	0	0	0	0
SKA0006	*sapR*	Two-component system, response regulator SapR	0	0	**1**	0	0	0	0.32	0.02	0	0	**1**	0	0	0	0	0	0	0
SKA0007	*sapT*	Sakacin A ABC transporter	0	0	**1**	0	0	0	0.01	0.12	0	0	**1**	0	0	0	0	0	0	0
SKA0008	*sapE*	Sakacin A accesory transporter protein	0	0	**1**	0	0	0	0.24	0	0	0	**1**	0	0	0	0	0.03	0	0
***L. sakei* 2512: Sakacin G cluster (acc. no. AF395533)**																				
SKG0001	*skgI*	Bacteriocin sakacin G immunity protein	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0
SKG0002	*skgA2*	Bacteriocin sakacin G precursor, beta subunit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKG0003	*skgA1*	Bacteriocin sakacin G precursor, alpha subunit	0	0	0	0	0	0	0.5	0.01	0	0	0	0	0	0	0	0	0	0
SKG0004	*skgD*	Sakacin G ABC transporter	0	0	0	0	0	0	0.04	0.1	**1**	0	0	0	0	**1**	**1**	0	0	**1**
***L. sakei* L45 Lactocin S cluster (acc. no. Z54312)**																				
LCS0009	*lcs0009*	ABC transporter, ATP-binding protein	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
LCS0010	*lasX*	Putative Rgg-type transcriptional regulator	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
LCS0011	*lasA*	Pre-lactocin S	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0.01	0	0
LCS0012	*lasM*	Lantibiotic mersacidin modifying enzyme	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
LCS0013	*lasN*	Putative iron-sulfur flavoprotein	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
LCS0014	*lasT*	Lactocin S ABC transporter	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
LCS0017	*lasP*	Putative serine protease	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
LCS0019	*lasW*	Lactocin S ABC transporter permease component	0	0	0	**1**	0	**1**	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
***L. sakei* LTH673: Sakacin Q cluster (acc. no. AJ844595)**																				
SKQ0001	*sppQ*	Bacteriocin sakacin Q preprotein	0	0	0	0	0	0	0	0	**1**	0	0	0	0	**1**	**1**	0	0	**1**
SKQ0002	*spiQ*	Bacteriocin sakacin Q immunity protein	0	0	0	0	0	0	0.37	0	**1**	0	0	0	0	**1**	**1**	0	0	**1**
SKQ0003	*skq0003*	Hypothetical protein (ORF-4) paralogous of *lsa567* in 23K	0	0	0	0	0	0	0	0	**1**	0	0	0	0.08	**1**	**1**	0	0	**1**
SKQ0004	*skq0004*	Hypothetical protein (ORF-5) paralogous of *lsa568* in 23K	0	0	0	0	0	0	0.29	0	**1**	0	0	0	0	**1**	**1**	0	0	**1**
SKQ0005	*skq0005*	Hypothetical protein (ORF-x) paralogous of *lsa570* in 23K	0	0	0	0	0	0	0	0	**1**	0	0	0	0	**1**	**1**	0	0	**1**

Continued on following page

Table 5. *Continued*

			Strain																	
Locus tag	**Gene**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
***L. sakei* 5: SakacinTX cluster (acc. no. AY206863)**																				
SKX0003	*bcrD*	Putative transport accessory protein	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKX0004	*stxP*	Sakacin X inducing peptide	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0
SKX0005	*stxR*	Two-component system, response regulator (sakacin X production)	0	0	0	0	0	0	0.16	0	0	0	0	0	0	0	0	0	0	0
SKX0006	*stxK*	Two-component system, histidine sensor kinase (sakacin X production)	0	0	0	0	0	0	0.32	0	0	0	0	0	0	0	0	0	0	0
SKX0007	*stxT*	Sakacin X ABC exporter, ATP-binding subunit	0	0	0	0	0	0	0.01	0.01	0	0	0	0	0	0	0	0	0	0
SKX0008	*sakT1*	Bacteriocin sakacin T precursor, alpha subunit	0	0	0	0	0	0	0.43	0.02	0	0	0	0	0	0	0	0.06	0	0
SKX0009	*sakT2*	Bacteriocin sakacin T precursor, beta subunit	0	0	0	0	0	0	0.22	0.34	0	0	0	0	0	0	0	0	0	0
SKX0010	*sakTIM*	Bacteriocin sakacin T immunity protein	0	0	0	0	0	0	0.5	0.03	0	0	0	0	0	0	0	0.02	0	0
SKX0011	*sakX*	Bacteriocin sakacin X precursor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKX0012	*sakXIM*	Bacteriocin sakacin X immunity protein	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKX0013	*skx0008*	BcrD-like transport accesory protein	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKX0019	*skx0019*	Putative DNA-binding protein, XRE family	0	0	0	0	0	0	0.1	0.01	0	0	0	0	0	0	0	0	0	0
***L. sakei* 23K: Degenerate sakacin P cluster**																				
LCA_0560_b	*lsa560_a*	Putative bacteriocin inducing peptide	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
LCA_0561	*sppK*	Two-component system, sensor histidine kinase, (SppK fragment) , degenerate	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
LCA_0562	*sppK*	Two-component system, sensor histidine kinase, (SppK fragment) , degenerate	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
LCA_0563	*sppR*	Two-component system, response regulator SppR (sakacin P production)	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
LCA_0564_c	*lsa564_c*	Putative bacteriocin immunity protein	**1**	0	**1**	**1**	0.55	**1**	0.51	0.07	0	0.61	0.4	**1**	**1**	0.19	0	**1**	0.03	0
LCA_0568	*lsa568*	Putative SakacinP immunity protein, SpiA	1	1	1	1	1	1	1	1	0.03	1	1	1	1	0	0	**1**	0.23	0
***L. sakei* T332: Lactose PTS plasmid (unpublished)**																				
TNL0009	*lacR*	Lactose PTS repressor, DeoR family	0	**1**	0	0	0	0	**1**	1	0	0	0	0	0	0	0	0	0	0
TNL0010	*lacA*	Galactose-6-phosphate isomerase, lacA subunit	0	0.24	0	0	0	0	**1**	0.39	0	0	0	0	0.04	0	0	0	0.01	0
TNL0011	*lacB*	Galactose-6-phosphate isomerase, lacB subunit	0	**1**	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0.01	0.01	0
TNL0012	*lacC*	Tagatose-6-phosphate kinase	0	**1**	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0
TNL0014	*lacF*	Lactose-specific PTS, enzyme IIA	0	**1**	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0
TNL0015	*lacE*	Lactose-specific PTS, enzymes IIBC	0	**1**	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0
TNL0016	*lacG*	6-phospho-beta-galactosidase	0	**1**	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0.03	0	0
TNL0019	*lacX*	Putative aldose 1-epimerase, LacX protein	0	**1**	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0

Author's contributions

OLN and AM primary authors participated in the design of the study, conducted the experiments, analyzed and interpreted data, and drafted the manuscript. LS conducted the programming and statistical analysis. DAB analyzed and interpreted data, and helped write the manuscript. ÅAa and IFN conceived the study, participated in the study design process and reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was financially supported by grants from the Norwegian University of Life Sciences and from the Norwegian Research Council. OLN was supported by a fellowship from the Norwegian University of Life Sciences. The authors would like to thank Professor Monique Zagorec, INRA, France, for the invitation to collaborate on production of the *L. sakei* microarray. We also thank Bjørn E. Kristiansen, the Norwegian Microarray Consortium (NMC), Oslo, for printing of the microarrays.

References

1. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, Pavlov A, Pavlova N, Karamychev V, Polouchine N, et al: **Comparative genomics of the lactic acid bacteria.** *Proc Natl Acad Sci U S A* 2006, **103:**15611-15616.
2. O'Sullivan O, O'Callaghan J, Sangrador-Vegas A, McAuliffe O, Slattery L, Kaleta P, Callanan M, Fitzgerald GF, Ross RP, Beresford T: **Comparative genomics of lactic acid bacteria reveals a niche-specific gene set.** *BMC Microbiol* 2009, **9:**50.
3. Canchaya C, Claesson MJ, Fitzgerald GF, van Sinderen D, O'Toole PW: **Diversity of the genus *Lactobacillus* revealed by comparative genomics of five species.** *Microbiology* 2006, **152:**3185-3196.
4. Hammes WP, Vogel RF: **The genus *Lactobacillus*.** In *The genera of lactic acid bacteria. Volume* 2. Edited by Wood BJB, Hilzapfel WH. Glasgow, UK: Blackie Academic, Professional; 1995: 19-54
5. Ouwehand AC, Salminen S, Isolauri E: **Probiotics: an overview of beneficial effects.** *Antonie Van Leeuwenhoek* 2002, **82:**279-289.
6. Cai H, Thompson R, Budinich MF, Broadbent JR, Steele JL: **Genome Sequence and Comparative Genome Analysis of *Lactobacillus casei*: Insights into Their Niche-Associated Evolution.** *Genome Biol Evol* 2009, **2009:**239-257.
7. Pfeiler EA, Klaenhammer TR: **The genomics of lactic acid bacteria.** *Trends Microbiol* 2007, **15:**546-553.
8. Lawrence JG, Ochman H: **Amelioration of bacterial genomes: rates of change and exchange.** *J Mol Evol* 1997, **44:**383-397.
9. Daubin V, Lerat E, Perriere G: **The source of laterally transferred genes in bacterial genomes.** *Genome Biol* 2003, **4:**R57.
10. Siezen RJ, Tzeneva VA, Castioni A, Wels M, Phan HT, Rademaker JL, Starrenburg MJ, Kleerebezem M, Molenaar D, van Hylckama Vlieg JE: **Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches.** *Environ Microbiol* 2009.
11. Aakra A, Nyquist OL, Snipen L, Reiersen TS, Nes IF: **Survey of genomic diversity among *Enterococcus faecalis* strains by microarray-based comparative genomic hybridization.** *Appl Environ Microbiol* 2007, **73:**2207-2217.

12. Molenaar D, Bringel F, Schuren FH, de Vos WM, Siezen RJ, Kleerebezem M: **Exploring *Lactobacillus plantarum* genome diversity by using microarrays.** *J Bacteriol* 2005, **187:**6119-6127.
13. Chen YT, Liao TL, Wu KM, Lauderdale TL, Yan JJ, Huang IW, Lu MC, Lai YC, Liu YM, Shu HY, et al: **Genomic diversity of citrate fermentation in *Klebsiella pneumoniae*.** *BMC Microbiol* 2009, **9:**168.
14. Murray AE, Lies D, Li G, Nealson K, Zhou J, Tiedje JM: **DNA/DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes.** *Proc Natl Acad Sci U S A* 2001, **98:**9853-9858.
15. Dong QJ, Wang Q, Xin YN, Li N, Xuan SY: **Comparative genomics of *Helicobacter pylori*.** *World J Gastroenterol* 2009, **15:**3984-3991.
16. Hammes WP, Hertel C: **New developments in meat starter cultures.** *Meat Science* 1998, **49:**125-138.
17. Chaillou S, Champomier-Verges MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V, Beaufils S, Darbon-Rongere E, Bossy R, Loux V, Zagorec M: **The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K.** *Nat Biotechnol* 2005, **23:**1527-1533.
18. Bredholt S, Nesbakken T, Holck A: **Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats.** *Int J Food Microbiol* 2001, **66:**191-196.
19. Champomier-Verges MC, Chaillou S, Cornet M, Zagorec M: **Erratum to "*Lactobacillus sakei*: recent developments and future prospects" [Research in Microbiology 152 (2001) 839].** *Res Microbiol* 2002, **153:**115-123.
20. Vermeiren L, Devlieghere F, Debevere J: **Evaluation of meat born lactic acid bacteria as protective cultures for biopreservation of cooked meat products.** *Int J Food Microbiol* 2004, **96:**149-164.
21. Mortvedt CI, Nissen-Meyer J, Sletten K, Nes IF: **Purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus sake* L45.** *Appl Environ Microbiol* 1991, **57:**1829-1834.
22. Axelsson L, Holck A: **The genes involved in production of and immunity to sakacin A, a bacteriocin from *Lactobacillus sake* Lb706.** *J Bacteriol* 1995, **177:**2125-2137.

23. Riley MA, Wertz JE: **Bacteriocin diversity: ecological and evolutionary perspectives.** *Biochimie* 2002, **84:**357-364.
24. Tichaczek PS, Vogel RF, Hammes WP: **Cloning and sequencing of *sakP* encoding sakacin P, the bacteriocin produced by *Lactobacillus sake* LTH 673.** *Microbiology* 1994, **140:**361-367.
25. Schillinger U, Lucke FK: **Antibacterial activity of *Lactobacillus sake* isolated from meat.** *Appl Environ Microbiol* 1989, **55:**1901-1906.
26. Lyhs U, Bjorkroth J, Korkeala H: **Characterisation of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping.** *Int J Food Microbiol* 1999, **52:**77-84.
27. Leroi F, Joffraud JJ, Chevalier F, Cardinal M: **Study of the microbial ecology of cold-smoked salmon during storage at 8 degrees C.** *Int J Food Microbiol* 1998, **39:**111-121.
28. Vogel RF, Lohmann M, Nguyen M, Weller AN, Hammes WP: **Molecular characterization of *Lactobacillus curvatus* and *Lact. sake* isolated from sauerkraut and their application in sausage fermentations.** *J Appl Bacteriol* 1993, **74:**295-300.
29. Klaenhammer T, Altermann E, Arigoni F, Bolotin A, Breidt F, Broadbent J, Cano R, Chaillou S, Deutscher J, Gasson M, et al: **Discovering lactic acid bacteria by genomics.** *Antonie Van Leeuwenhoek* 2002, **82:**29-58.
30. Katagiri H, Kitahara K, Fukami K: **The characteristics of the lactic acid bacteria isolated from moto, yeast mashes for sake manufacture. Part IV. Classification of the lactic acid bacteria.** *Bulletin of Agricultural and Chemical Society of Japan* 1934, **10:**156–157.
31. Chiaramonte F, Blugeon S, Chaillou S, Langella P, Zagorec M: **Behavior of the meat-borne bacterium *Lactobacillus sakei* during its transit through the gastrointestinal tracts of axenic and conventional mice.** *Appl Environ Microbiol* 2009, **75:**4498-4505.
32. Dal Bello F, Walter J, Hammes WP, Hertel C: **Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition.** *Microb Ecol* 2003, **45:**455-463.
33. Chiaramonte F, Anglade P, Baraige F, Gratadoux JJ, Langella P, Champomier-Verges MC, Zagorec M: **Analysis of *Lactobacillus sakei* mutants selected after adaptation**

to the gastrointestinal tract of axenic mice. *Appl Environ Microbiol* 2010, **76:**2932-2939.

34. Berthier F, Ehrlich SD: **Genetic diversity within *Lactobacillus sakei* and *Lactobacillus curvatus* and design of PCR primers for its detection using randomly amplified polymorphic DNA.** *Int J Syst Bacteriol* 1999, **49:**997-1007.

35. Torriani S, Van Reenen GA, Klein G, Reuter G, Dellaglio F, Dicks LM: ***Lactobacillus curvatus* subsp. *curvatus* subsp. nov. and *Lactobacillus curvatus* subsp. *melibiosus* subsp. nov. and *Lactobacillus sake* subsp. *sake* subsp. nov. and *Lactobacillus sake* subsp. *carnosus* subsp. nov., new subspecies of *Lactobacillus curvatus* Abo-Elnaga and Kandler 1965 and *Lactobacillus sake* Katagiri, Kitahara, and Fukami 1934 (Klein et al. 1996, emended descriptions), respectively.** *Int J Syst Bacteriol* 1996, **46:**1158-1163.

36. Klein G, Dicks LMT, Pack A, Hack B, Zimmerman K, Dellaglio F, Reuter G: **Emended description of *Lactobacillus sake* (Katahiri, Katahara and Fukami) and *Lactobacillus curvatus* (Abo-Elnega Kandler): Numerical classification revealed by protein fingerprinting and identification based on biochemical patterns and DNA–DNA hybridizations.** *Int J Syst Bacteriol* 1996, **46**.

37. McLeod A, Nyquist OL, Snipen L, Naterstad K, Axelsson L: **Diversity of *Lactobacillus sakei* strains investigated by phenotypic and genotypic methods.** *Syst Appl Microbiol* 2008, **31:**393-403.

38. Chaillou S, Daty M, Baraige F, Dudez AM, Anglade P, Jones R, Alpert CA, Champomier-Verges MC, Zagorec M: **Intraspecies genomic diversity and natural population structure of the meat-borne lactic acid bacterium *Lactobacillus sakei*.** *Appl Environ Microbiol* 2009, **75:**970-980.

39. De Man JC, Rogosa M, Shape ME: **A medium for the cultivation of lactobacilli.** *J Appl Microbiol* 1960, **23:**130-135.

40. Smyth GK, Speed T: **Normalization of cDNA microarray data.** *Methods* 2003, **31:**265-273.

41. Snipen L, Nyquist OL, Solheim M, Aakra A, Nes IF: **Improved analysis of bacterial CGH data beyond the log-ratio paradigm.** *BMC Bioinformatics* 2009, **10:**91.

42. Hallin PF, Binnewies TT, Ussery DW: **The genome BLASTatlas-a GeneWiz extension for visualization of whole-genome homology.** *Mol Biosyst* 2008, **4:**363-371.

43. Solheim M, Aakra A, Snipen LG, Brede DA, Nes IF: **Comparative genomics of *Enterococcus faecalis* from healthy Norwegian infants.** *BMC Genomics* 2009, **10:**194.

44. Koort J, Vandamme P, Schillinger U, Holzapfel W, Bjorkroth J: ***Lactobacillus curvatus* subsp. *melibiosus* is a later synonym of *Lactobacillus sakei* subsp. *carnosus*.** *Int J Syst Evol Microbiol* 2004, **54:**1621-1626.

45. Dudez AM, Chaillou S, Hissler L, Stentz R, Champomier-Verges MC, Alpert CA, Zagorec M: **Physical and genetic map of the *Lactobacillus sakei* 23K chromosome.** *Microbiology* 2002, **148:**421-431.

46. Cortez D, Forterre P, Gribaldo S: **A hidden reservoir of integrative elements is the major source of recently acquired foreign genes and ORFans in archaeal and bacterial genomes.** *Genome Biol* 2009, **10:**R65.

47. Nigatu A: **Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate *Lactobacillus plantarum, Lact. fermentum, Lact. rhamnosus, Lact. sake, Lact. parabuchneri, Lact. gallinarum, Lact. casei, Weissella* minor and related taxa isolated from kocho and tef.** *J Appl Microbiol* 2000, **89:**969-978.

48. Stentz R, Zagorec M: **Ribose utilization in *Lactobacillus sakei*: analysis of the regulation of the *rbs* operon and putative involvement of a new transporter.** *J Mol Microbiol Biotechnol* 1999, **1:**165-173.

49. Stentz R, Cornet M, Chaillou S, Zagorec M: **Adaption of *Lactobacillus sakei* to meat: a new regulatory mechanism of ribose utilization?** *INRA, EDP Sciences* 2001, **81:**131-138.

50. McLeod A, Zagorec M, Champomier Verges MC, Naterstad K, Axelsson L: **Primary metabolism in *Lactobacillus sakei* by proteomic analysis.** *BMC Microbiol* 2010, **10**.

51. Obst M, Meding ER, Vogel RF, Hammes WP: **Two genes encoding the beta-galactosidase of *Lactobacillus sake*.** *Microbiology* 1995, **141:**3059-3066.

52. Garriga M, Hugas M, Aymerich T, Monfort JM: **Bacteriocinogenic activity of lactobacilli from fermented sausages.** *J Appl Bacteriol* 1993, **75:**142-148.

53. Magni C, de Mendoza D, Konings WN, Lolkema JS: **Mechanism of citrate metabolism in *Lactococcus lactis*: resistance against lactate toxicity at low pH.** *J Bacteriol* 1999, **181:**1451-1457.

54. Zuniga M, Champomier-Verges M, Zagorec M, Perez-Martinez G: **Structural and functional analysis of the gene cluster encoding the enzymes of the arginine deiminase pathway of *Lactobacillus sake*.** *J Bacteriol* 1998, **180:**4154-4159.

55. Champomier Verges MC, Zuniga M, Morel-Deville F, Perez-Martinez G, Zagorec M, Ehrlich SD: **Relationships between arginine degradation, pH and survival in *Lactobacillus sakei*.** *FEMS Microbiol Lett* 1999, **180:**297-304.

56. Jacquot R: **Organic constituents of fish and other aquatic animal foods.** In *Fish as food. Volume* 1. Edited by Borgstrom G. San Diego, USA: Academic press Inc; 1961: 145-209

57. Axelsson L, Ahrné S: **Lactic acid bacteria.** In *Applied Microbial Systematics.* Edited by Priest FG, Goodfellow M. Dordrechet, The Netherlands: Kluwer Academic Press; 2000: 365-386

58. Leistner L: **Basic aspects of food preservation by hurdle technology.** *Int J Food Microbiol* 2000, **55:**181-186.

59. Condon S: **Responses of lactic acid bacteria to oxygen.** *FEMS Microbiol Rev* 1987, **46:**269-280.

60. Claesson MJ, van Sinderen D, O'Toole PW: **The genus *Lactobacillus* - a genomic basis for understanding its diversity.** *FEMS Microbiol Lett* 2007, **269:**22-28.

61. Duwat P, Sourice S, Cesselin B, Lamberet G, Vido K, Gaudu P, Le Loir Y, Violet F, Loubiere P, Gruss A: **Respiration capacity of the fermenting bacterium *Lactococcus lactis* and its positive effects on growth and survival.** *J Bacteriol* 2001, **183:**4509-4516.

62. Bruyneel B, Vanderwoestyne M, Verstraete W: **Lactic acid bacteria - Microorganisms able to grow in the absence of iron and copper.** *Biotechnol Lett* 1989, **11:**401-406.

63. Weinberg ED: **The *Lactobacillus* anomaly: total iron abstinence.** *Perspect Biol Med* 1997, **40:**578-583.

64. Duhutrel P, Bordat C, Wu TD, Zagorec M, Guerquin-Kern JL, Champomier-Verges MC: **Iron sources used by the nonpathogenic lactic acid bacterium *Lactobacillus sakei* as revealed by electron energy loss spectroscopy and secondary-ion mass spectrometry.** *Appl Environ Microbiol* 2010, **76:**560-565.

65. Knauf HJ, Vogel RF, Hammes WP: **Cloning, sequence, and phenotypic expression of *katA*, which encodes the catalase of *Lactobacillus sake* LTH677.** *Appl Environ Microbiol* 1992, **58:**832-839.

66. Hertel C, Schmidt G, Fischer M, Oellers K, Hammes WP: **Oxygen-dependent regulation of the expression of the catalase gene *katA* of *Lactobacillus sakei* LTH677.** *Appl Environ Microbiol* 1998, **64:**1359-1365.

67. Cheetham BF, Katz ME: **A role for bacteriophages in the evolution and transfer of bacterial virulence determinants.** *Mol Microbiol* 1995, **18:**201-208.

68. Brussow H, Canchaya C, Hardt WD: **Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion.** *Microbiol Mol Biol Rev* 2004, **68:**560-602.

69. Siezen R, Boekhorst J, Muscariello L, Molenaar D, Renckens B, Kleerebezem M: ***Lactobacillus plantarum*** **gene clusters encoding putative cell-surface protein complexes for carbohydrate utilization are conserved in specific gram-positive bacteria.** *BMC Genomics* 2006, **7:**126.

70. Kralj S, van Geel-Schutten GH, Dondorff MM, Kirsanovs S, van der Maarel MJ, Dijkhuizen L: **Glucan synthesis in the genus *Lactobacillus*: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains.** *Microbiology* 2004, **150:**3681-3690.

71. Alpert CA, Crutz-Le Coq AM, Malleret C, Zagorec M: **Characterization of a theta-type plasmid from *Lactobacillus sakei*: a potential basis for low-copy-number vectors in lactobacilli.** *Appl Environ Microbiol* 2003, **69:**5574-5584.

72. Danielsen M: **Characterization of the tetracycline resistance plasmid pMD5057 from *Lactobacillus plantarum* 5057 reveals a composite structure.** *Plasmid* 2002, **48:**98-103.

73. Gevers D, Danielsen M, Huys G, Swings J: **Molecular characterization of *tet(M)* genes in *Lactobacillus* isolates from different types of fermented dry sausage.** *Appl Environ Microbiol* 2003, **69:**1270-1275.

74. Aasen IM, Moretro T, Katla T, Axelsson L, Storro I: **Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687.** *Appl Microbiol Biotechnol* 2000, **53:**159-166.

75. Axelsson L, Holck A, Birkeland SE, Aukrust T, Blom H: **Cloning and nucleotide sequence of a gene from *Lactobacillus sake* Lb706 necessary for sakacin A production and immunity.** *Appl Environ Microbiol* 1993, **59:**2868-2875.

76. Skaugen M, Abildgaard CI, Nes IF: **Organization and expression of a gene cluster involved in the biosynthesis of the lantibiotic lactocin S.** *Mol Gen Genet* 1997, **253:**674-686.

77. Skaugen M, Andersen EL, Christie VH, Nes IF: **Identification, characterization, and expression of a second, bicistronic, operon involved in the production of lactocin S in *Lactobacillus sakei* L45.** *Appl Environ Microbiol* 2002, **68:**720-727.

78. Rodriguez JM, Cintas LM, Casaus P, Suarez A, Hernandez PE: **PCR detection of the lactocin S structural gene in bacteriocin-producing lactobacilli from meat.** *Appl Environ Microbiol* 1995, **61:**2802-2805.

79. Leroy F, De Vuyst L: **Sakacins.** In *Natural food antimicrobial systems.* Edited by Naidu AS. Boca Raton, USA: CRC Press; 2000: 589-610

80. Moretro T, Naterstad K, Wang E, Aasen IM, Chaillou S, Zagorec M, Axelsson L: **Sakacin P non-producing *Lactobacillus sakei* strains contain homologues of the sakacin P gene cluster.** *Res Microbiol* 2005, **156:**949-960.

81. Vaughan A, Eijsink VG, Van Sinderen D: **Functional characterization of a composite bacteriocin locus from malt isolate *Lactobacillus sakei* 5.** *Appl Environ Microbiol* 2003, **69:**7194-7203.

82. Simon L, Fremaux C, Cenatiempo Y, Berjeaud JM: **Sakacin g, a new type of antilisterial bacteriocin.** *Appl Environ Microbiol* 2002, **68:**6416-6420.

83. Leroy F, de Vuyst L: **The presence of salt and a curing agent reduces bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation.** *Appl Environ Microbiol* 1999, **65:**5350-5356.

84. Hugas M, Garriga M, Aymerich T, Monfort JM: **Inhibition of Listeria in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC 494.** *J Appl Bacteriol* 1995, **79:**322-330.

85. Holck AL, Axelsson L, Huhne K, Krockel L: **Purification and cloning of sakacin 674, a bacteriocin from *Lactobacillus sake* Lb674.** *FEMS Microbiol Lett* 1994, **115:**143-149.

86. Schillinger U, Lücke F-K: **Identification of lactobacilli from meat and meat products.** *Food Microbiology* 1987, **4:**199-208.

87. Axelsson L, Katla T, Bjornslett M, Eijsink VG, Holck A: **A system for heterologous expression of bacteriocins in *Lactobacillus sake*.** *FEMS Microbiol Lett* 1998, **168:**137-143.

88. Hagen BF, Naes H, Holck AL: **Meat starters have individual requirements for Mn^{2+}.** *Meat Science* 2000, **55:**161-168.

89. Eijsink VG, Brurberg MB, Middelhoven PH, Nes IF: **Induction of bacteriocin production in *Lactobacillus sake* by a secreted peptide.** *J Bacteriol* 1996, **178:**2232-2237.

90. Mathiesen G, Huehne K, Kroeckel L, Axelsson L, Eijsink VG: **Characterization of a new bacteriocin operon in sakacin P-producing *Lactobacillus sakei*, showing**

strong translational coupling between the bacteriocin and immunity genes. *Appl Environ Microbiol* 2005, **71:**3565-3574.

91. Bredholt S, Nesbakken T, Holck A: **Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat.** *Int J Food Microbiol* 1999, **53:**43-52.

Table S1. Estimated fragment sizes of restriction enzyme digests with I-*Ceu*I of the chromosomes of *L. sakei* strains.

Strain	Fragment size (kb) obtained with I-*Ceu*I							
	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6	Fragment 7	Estimated genome size (kb)
CTC460	1125	315	172	135	95	33	5	1880
23K	1104	331	175	135	98	36	5	1884
Lb77	1150	319	176	137	98	34	5	1919
CTC372	1175	314	174	132	95	33	5	1928
Lb148	1170	322	176	135	98	33	5	1939
Lb156	1235	307	168	129	93	33	5	1970
Lb790	1230	340	181	137	98	34	5	2025
LMGT2380	1260	331	170	130	98	33	5	2027
L45	1230	343	178	137	103	33	5	2029
CTC494	1190	343	192	140	105	36	5	2011
Lb706	1280	335	172	133	98	33	5	2056

Table S2. Fermentation patterns of *L. sakei* strains used in this work. The values indicate positive (+), negative (-), and weak (w) fermentation by the respective strains. The substrates that were not fermented by any of the strains are not shown.

Strain / Substrate	L-Arabinose	D-Ribose	D-Xylose	D-Galactose	D-Glucose	D-Fructose	D-Mannose	L-Rhamnose	Methyl-βD-glucopyranoside	N-acetylglucosamine	Amygdalin	Arbutin	Esculin ferric citrate	Salicin	D-Cellobiose	D-Maltose	D-Lactose	D-Melibiose	D-Saccharose (sucrose)	D-Trehalose	Inulin	Gentiobiose	Potassium gluconate
23K*	+	+	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	w
CCUG 31331*	+	+	-	+	+	+	+	-	-	+	-	-	+	w	-	+	-	+	+	+	-	+	w
CTC372	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	+	+	+	+	-	-	w
CTC460	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	w	-	+	+	-	-	-
CTC494	-	+	-	+	+	+	+	-	-	+	-	-	+	-	+	-	-	+	+	+	-	w	w
DSM 20017*	+	+	-	+	+	+	+	-	-	+	-	-	+	+	-	+	-	+	+	+	-	w	w
L45	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	-	+	+	+	-	w	w
Lb16*	+	+	-	+	+	+	+	-	-	+	-	+	+	+	w	-	-	+	+	-	-	-	w
Lb77	-	+	-	+	+	+	+	-	-	+	-	-	+	w	-	+	+	-	+	+	+	-	w
Lb148	-	+	-	+	+	+	+	-	-	+	-	-	+	w	-	-	+	+	+	+	-	-	w
Lb156	-	+	-	+	+	+	+	-	-	+	-	-	+	w	+	-	+	+	+	+	-	-	w
Lb706	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	-	+	+	+	-	+	w
Lb790	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	-	+	+	+	-	+	w
LMGT2380	+	+	-	+	+	+	+	-	-	+	w	+	+	+	w	-	-	+	+	-	-	-	w
LS 25*	+	+	+	+	+	+	+	w	-	+	-	-	+	w	-	+	+	+	+	+	-	w	w
LTH673*	+	+	-	+	+	+	+	-	-	+	-	+	+	+	w	-	-	+	+	-	-	-	w
MF1053*	+	+	-	+	+	+	+	-	+	+	-	w	+	w	w	+	w	+	+	+	-	-	w
MF1058*	-	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	-	+	+	+	-	-	w
MF1328*	+	+	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	w

* Presented by McLeod et al. [37].

Paper IV

Global transcriptome response in *Lactobacillus sakei* during growth on ribose

Anette McLeod[1,2,*], Lars Snipen[2], Kristine Naterstad[1] and Lars Axelsson[1]

[1]Nofima Mat AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Osloveien 1, NO-1430 Ås, Norway

[2]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

E-mail adresses:

AM: anette.mcleod@nofima.no

LS: lars.snipen@umb.no

KN: kristine.naterstad@nofima.no

LA: lars.axelsson@nofima.no

*Corresponding author

Abstract

Lactobacillus sakei is valuable in the fermentation of meat products and exhibits properties that allow for better preservation of meat and fish. On these substrates, glucose and ribose are the main carbon sources available for growth. We used a whole-genome microarray based on the genome sequence of *L. sakei* strain 23K to investigate the global transcriptome response of three *L. sakei* strains when grown on ribose compared with glucose. An up-regulation with genes directly involved in ribose catabolism, the phosphoketolase pathway, and in alternative fates of pyruvate was observed. The gene encoding methylglyoxal synthase, an enzyme unique for *L. sakei* among lactobacilli, and genes involved in transport and metabolism of various carbohydrates, as well as glycerol/glycerolipid metabolism were also up-regulated. Among the down-regulated genes were those encoding enzymes involved in glucose metabolism including glycolytic enzymes, and the L-lactate dehydrogenase. Ribose catabolism seems to be closely linked with catabolism of nucleosides in *L. sakei*. The *deoR* gene encoding the deoxyribonucleoside synthesis operon transcriptional regulator, DeoR, with sigma (σ) factor activity and two gene clusters involved in nucleoside catabolism including a ribokinase gene, were induced. Moreover, *hprK* encoding the HPr kinase/phosphatase, which plays a major role in the regulation of carbon metabolism and sugar transport, was up-regulated, as were genes encoding the general PTS enzyme I, and the mannose-specific enzyme II complex (EII^{man}) encoding glucose uptake. Putative catabolite-responsive element (*cre*) sites were found in proximity to the promoter of several genes and operons affected by the change of carbon source. It seems likely that a global catabolite control protein A (CcpA)-mediated carbon catabolite repression (CCR) mechanism permit a fine tuning of the expression of enzymes that control the efficient exploitation of available carbon sources in *L. sakei*. Moreover, the EII^{man} is possibly indirectly involved in this regulation during growth on ribose.

Introduction

The *Lactobacillus sakei* species belongs to the lactic acid bacteria (LAB), a group of Gram-positive organisms with a low G+C content which produce lactic acid as the main end product of carbohydrate fermentation. This trait has, throughout history, made LAB suitable for production of food, as acidification suppresses the growth and survival of undesirable spoilage bacteria and human pathogens. *L. sakei* is naturally associated with the meat and fish environment, and is important in the meat industry where it is used as starter culture for sausage fermentation [1,2]. The bacterium shows great potential as a protective culture and biopreservative to extend storage life and ensure microbial safety of meat and fish products [3-6]. The genome sequence of *L. sakei* strain 23K has revealed a metabolic repertoire which reflects the bacterium's adaption to meat products and the ability to flexibly use meat components [7]. Only a few carbohydrates are available in meat and fish, and *L. sakei* can utilize mainly glucose and ribose for growth, a utilization biased in favour of glucose [7-9]. This hexose is primarily transported and phosphorylated by the phosphoenolpyruvate (PEP)-dependent carbohydrate phosphotransferase system (PTS), where a phosphorylation cascade is driven from PEP through the general components enzyme I (EI) and the histidine protein (HPr), then via the mannose-specific enzyme II complex (EII^{man}) to the incoming sugar. Moreover, glucose is fermented through glycolysis leading to lactate [7,8,10]. Ribose transport and subsequent phosphorylation are induced by the ribose itself and mediated by a ribose transporter (RbsU), a D-ribose pyranase (RbsD), and a ribokinase (RbsK) encoded by *rbsUDK*, respectively, forming an operon with *rbsR* which encodes the local repressor RbsR [11,12]. The phosphoketolase pathway (PKP) is used for pentose fermentation ending with lactate and other end products [8]. *L. sakei* also has the ability to catabolize arginine, which is abundant in meat, and it has the ability to catabolize the nucleosides inosine and adenine, a property which is uncommon among lactobacilli [7,13].

By proteomics, we recently identified proteins involved in ribose catabolism and the phosphoketolase pathway as over-expressed during growth on ribose compared with glucose, while several glycolytic enzymes were observed to be less expressed [14]. Moreover, also enzymes involved in pyruvate- and glycerol/glycerolipid metabolism were over-expressed on ribose [14]. Bacteria often use carbon catabolite repression (CCR) in order to control hierarchical utilization of different carbon sources. In low G+C content Gram-positive bacteria, the dominant CCR pathway is mediated by the three main components: catabolite control protein A (CcpA) transcriptional regulator, the histidine protein (HPr), and catabolite-

responsive element (*cre*) DNA sites located in proximity to catabolic genes and operons, which are bound by CcpA [15-17]. The HPr protein has diverse regulatory functions in carbon metabolism depending on its phosphorylation state. In response to high throughput through glycolysis, the enzyme is phosphorylated at Ser46 by HPr kinase/phosphorylase (HPrK/P) to give P-Ser-HPr which then binds to CcpA and converts it into its DNA-binding-competent conformation, whereas it dephosphorylates P-Ser-HPr when the concentration of glycolytic intermediates drop [16-18]. Under low glucose concentrations, HPr is phosphorylated by E1 of the PTS at His15 to give P-His-HPr, which has a catalytic function in the PTS and regulatory functions by phosphorylation of catabolic enzymes and transcriptional regulators with a PTS regulation domain (PRD). Several P-EIIBs also phosphorylate different types of non-PTS proteins and regulate their activities [15-17]. Evidence for regulatory processes resembling glucose repression was shown both during lactose utilization [19] and catabolism of arginine [20,21] in *L. sakei*, and as a *cre* site has been found upstream of the *rbs* operon [22], CcpA could likely be acting on the *rbs* operon as well as other catabolic genes and operons in this bacterium.

In the present study we use a microarray representing the *L. sakei* 23K genome and an additional set of sequenced *L. sakei* genes, to investigate the global transcriptome response of three *L. sakei* strains when grown on ribose compared with glucose. Moreover, we predict the frequency of *cre* sites presumed to be involved in CCR in the *L. sakei* 23K genome sequence. Our objective was to identify differentially expressed genes between growth on the two sugars, and to increase the understanding of how the primary metabolism is regulated.

Methods

Bacterial strains, media and growth conditions

L. sakei 23K is a plasmid-cured sausage isolate [23], and its complete genome sequence has been published [7]. *L. sakei* LS 25 is a commercial starter culture strain for salami sausage [24]. *L. sakei* MF1053 originates from fermented fish (Norwegian "rakfisk") [9]. The strains were maintained at -80°C in MRS broth (Oxoid) supplemented with 20% glycerol. Growth experiments were performed in a defined medium for lactobacilli [25] supplemented with 0.5% glucose (DMLG) or 0.5% ribose + 0.02% glucose (DMLRg) as described previously [14]. Samples were extracted at three different days from independent DMLG and DMLRg

cultures from each strain grown at 30°C to mid-exponential phase (OD_{600} = 0.5-0.6) for a total of three sample sets (parallels).

Microarrays

The microarrays used have been described by Nyquist et al. [26], and a description is available at http://migale.jouy.inra.fr/sakei/Supplement.html/. 70-mer oligonucleotide probes representing the *L. sakei* strain 23K genome and an additional set of sequenced *L. sakei* genes were printed in three copies onto epoxy glass slides (Corning).

RNA extraction

Total RNA extraction was performed using the RNeasy Protect Mini Prep Kit (Qiagen) as described by Rud [27]. The concentration and purity of the total RNA was analysed using NanoDrop ND-1000 (NanoDrop Technologies), and the quality using Agilent 2100 Bioanalyzer (Agilent Technologies). Sample criteria for further use in the transcriptome analysis were A_{260}/A_{280} ratio superior to 1.9 and 23S/16S RNA ratio superior to 1.6.

cDNA synthesis, labeling, and hybridization

cDNA was synthesized and labeled with the Fairplay III Microarray Labeling Kit (Stratagene, Agilent Technologies) as described previously [28]. After labeling, unincorporated dyes were removed from the samples using the QIAQuick PCR purification kit (Qiagen). The following prehybridization, hybridization, washing, and drying of the arrays were performed in a Tecan HS 400 Pro hybridization station (Tecan) as described by Nyquist et al. [26]. For studying the carbon effects, samples from DMLG and DMLRg were co-hybridized for each of the three strains. Separate hybridizations were performed for each strain on all three biological parallels. In order to remove potential biases associated with labelling and subsequent scanning, a replicate hybridization was performed for each strain for one of the three parallels, where the Cy3 and Cy5 dyes (GE Healthcare) used during cDNA synthesis were swapped. The hybridized arrays were scanned at wavelengths 532 nm (Cy3) and 635 nm (Cy5) with a Tecan scanner LS (Tecan). GenePix Pro 6.0 (Molecular Devices) was used for image analysis, and spots were excluded based on slide or morphology abnormalities.

Microarray data analysis

Downstream analysis was done by the Limma package (www.bioconductor.org) in the R computing environment (www.r-project.org). Pre-processing and normalization followed a standard procedure using methods described by Smyth & Speed [29], and testing for differential expressed genes were done by using a linear mixed model as described by Smyth [30]. A mixed-model approach was chosen to adequately describe between-array variation and still utilize probe-replicates (three replicates of each probe in each array). An empirical Bayes smoothing of gene-wise variances was conducted according to Smyth et al. [31], and for each gene the p-value was adjusted to control the false discovery rate (FDR), hence all p-values displayed are FDR-adjusted (often referred to as q-values in the literature).

Microarray accession numbers

The microarray data have been deposited in the Array Express database (http://www.ebi.ac.uk/arrayexpress/) under the accession numbers A-MEXP-1166 (array design) and E-MEXP-XXXX (experiment; will be submitted).

Sequence analysis

A prediction of *cre* sites in the *L. sakei* 23K genome sequence (GeneBank acc. no. CR936503.1), both strands, was performed based on the consensus sequence TGWNAN**CG**NTNWCA (W=A/T, N=A/T/G/C), confirmed in Gram-positive bacteria [32]. We made a search with the consensus sequence described by the regular expression T-G-[AT]-X-A-X-C-G-X-T-X-[AT]-C-A, allowing up to two mismatches in the conserved positions except for the two center position, highlighted in boldface. All computations were done in R (www.r-project.org).

Results and Discussion

Selection of *L. sakei* strains and growth conditions

We have previously investigated *L. sakei* strain variation [9], and used proteomics to study the bacterium's primary metabolism [14], providing us with a basis for choosing strains with interesting differences for further studies. The starter culture strain LS 25 showed the fastest growth rates in a variety of media, and together with strain MF1053 from fish, it fermented the highest number of carbohydrates [9]. The LS 25 strain belongs to the *L. sakei* subsp. *sakei*, whereas the 23K and MF1053 strains belong to *L. sakei* subsp. *carnosus* [9,14]. By

identification of differentially expressed proteins caused by the change of carbon source from glucose to ribose, LS 25 seemed to down-regulate the glycolytic pathway more efficiently than other strains during growth on ribose [14]. For these reasons, LS 25 and MF1053 were chosen in addition to 23K for which the microarray is based on. We recently investigated the genomes of various *L. sakei* strains compared to the sequenced strain 23K by comparative genome hybridization (CGH) using the same microarray as in the present study [26]. A detailed view of the gene content between the strains was obtained, with a large part of the 23K genes defined as present in all the strains investigated. Thus the status for each gene on the array is known for all the three strains.

As glucose is the preferred sugar, *L. sakei* grows faster when glucose is utilized as the sole carbon source compared with ribose [8,9,11]. However, glucose stimulates ribose uptake and a possible co-metabolism of these two sugars present in meat and fish has been suggested, a possibility that give the organism an advantage in competition with other microbiota [11,12,33]. To obtain comparable 2-DE gels between samples issued from bacteria grown on the two carbohydrates in our recent proteomic analysis, growth on ribose was enhanced by adding small amounts of glucose [14]. For the present transcriptome analysis we therefore chose the same growth conditions.

Global gene expression patterns

An oligonucleotide-based microarray representing the *L. sakei* 23K genome and an additional set of sequenced *L. sakei* genes was used for studying the effect of carbon source on the transcriptome of *L. sakei* strains 23K, MF1053 and LS 25. Genes displaying a significant differential expression with a $\log_2$ ratio > 0.5 or < -0.5 were classified into functional categories according to the *L. sakei* 23K genome database (http://migale.jouy.inra.fr/sakei/genome-server) and are listed in Table 1. The 23K strain showed differential expression for 364 genes within these limits, MF1053 and LS 25 for 223 and 316 genes, respectively. Among these, 90, 49 and 83, respectively, were genes belonging to the category of genes of 'unknown' function.

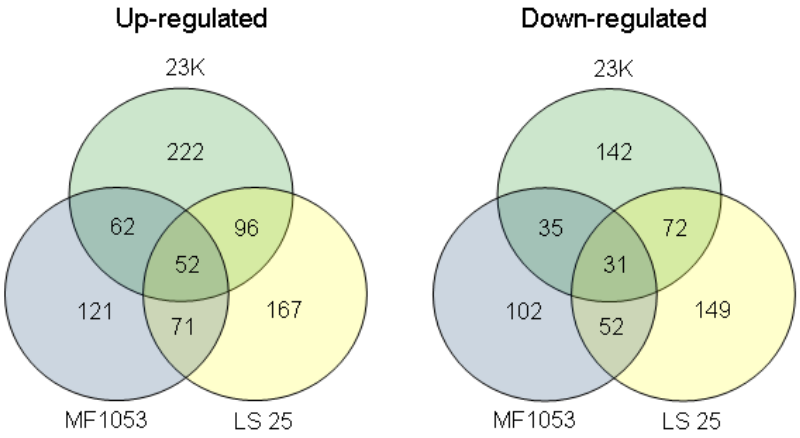


Figure 1. Venn diagram showing the number of unique and common up- and down-regulated genes in *L. sakei* strains 23K, MF1053 and LS 25 when grown on ribose compared with glucose.

Eighty three genes, the expression of which varied depending on the carbon source, were common to the three strains, among which 52 were up-regulated and 31 down-regulated during growth on ribose (Figure 1). The function of these common regulated genes was mostly related to carbohydrate transport and metabolism (34 genes, Table 1).

Several of the up-regulated genes are located in operons, an organisation believed to provide the advantage of coordinated regulation. In addition, in order to discriminate genes induced by growth on ribose from those repressed by glucose (submitted to CCR mediated by CcpA), a search of the complete genome sequence of *L. sakei* 23K [7] was undertaken, with the aim to identify putative *cre* sites. The search revealed 1962 hits, most of which did not have any biological significance considering their unsuitable location in relation to promoters. Relief of CcpA-mediated CCR likely occur for many of the up-regulated genes in the category of carbohydrate transport and metabolism, of which putative *cre* sites were identified in their promoter region, as well as for some genes involved in nucleoside and amino acid transport and metabolism (Table 2). In the other gene categories, the presences of putative *cre* sites were rare. With regard to gene product, the *L. sakei* genome shares high level of conservation with *Lactobacillus plantarum* [7], and high similarity of catabolic operon organization. The role of CcpA in CCR in *L. plantarum* has been established, and it was shown to mediate regulation of the *pox* genes encoding pyruvate oxidases [34,35]. During growth on ribose, *L. plantarum* was reported to induce a similar set of genes as observed in the present study, and putative *cre* sites were also identified in the upstream region of several genes involved [27].

Ribose catabolism and PKP

Confirming its major role in ribose transport and utilization in *L. sakei*, and in agreement with previous findings [12], our microarray data revealed a strong up-regulation (Table 1; 2.8-4.3) of *rbsUDK*. The genes encoding an additional putative carbohydrate kinase belonging to the ribokinase family and a putative phosphoribosyl isomerase, *lsa0254* and *lsa0255*, respectively, previously suggested to be involved in catabolism of ribose in *L. sakei* [7], were induced in all the strains (Table 1). Recent CGH studies revealed that some *L. sakei* strains which were able to grow on ribose did not harbour the *rbsK* gene, whereas *lsa0254* was present in all strains investigated [26]. This second ribokinase could therefore function as the main ribokinase in some *L. sakei* strains. The *rbsK* sequence could also differ considerably from that of 23K in these strains. The PKP showed an obvious induction with an up-regulation (2.2-3.2) of the *xpk* gene encoding the key enzyme xylulose-5-phosphate phosphoketolase (Xpk). This enzyme connects the upper part of the PKP to the lower part of glycolysis by converting xylulose-5-phosphate into glyceraldehyde-3-phosphate and acetyl-phosphate. Acetyl-phosphate is then converted to acetate and ATP by acetate kinase (Ack). Supporting our results, previous proteomic analysis showed an over-expression of RbsK, RbsD and Xpk during growth on ribose [11,12,14]. The induction of ribose transport and phosphorylation, and increased phosphoketolase and acetate kinase activities were previously observed during growth on ribose [11]. Three genes encoding Ack are present in the 23K genome [7], as well as in MF1053 and LS 25 [26], and a preferential expression of different *ack* genes for the acetate kinase activity seem to exist. The *ack2* gene was up-regulated in all the strains, while *ack1* was up-regulated and *ack3* down-regulated in 23K and LS 25 (Table 1). An illustration of the metabolic pathways with genes affected by the change of carbon source from glucose to ribose in *L. sakei* is shown in Figure 2.

As a consequence of the pentose-induced PKP, genes involved in PKP-metabolism of glucose, such as *gntZ*, *gntK* and *zwf*, were down-regulated (Table 1, Figure 2). The glycolytic pathway was clearly repressed, with several genes encoding glycolytic enzymes being among the down-regulated genes, supporting previous results [11,14]. Among these genes were *pfk* (0.5-1.1) encoding 6-phosphofructokinase (Pfk), and *fba* (0.7-1.1) coding for fructose-bisphosphate aldolase, both acting at the initial steps of glycolysis. In addition, *gpm3* encoding one of the five phosphoglycerate mutases present in the 23K genome, acting in the lower part of glycolysis, was also down-regulated (0.7-0.9). MF1053 down-regulated *pyk* (0.7) encoding pyruvate kinase (Pyk) that competes for PEP with the PTS (Figure 2). Its

activity results in the production of pyruvate and ATP, and it is of major importance in glycolysis and energy production in the cell. MF1053 also showed a stronger down-regulation of *pfk* than the other strains (Table 1). Similar to several other lactobacilli, *pfk* is transcribed together with *pyk* [36,37], and in many microorganisms the glycolytic flux depends on the activity of the two enzymes encoded from this operon [36,38]. At the protein level, we previously observed both Pfk and Pyk expressed at a lower level for all the three strains [14], however this was not confirmed at the level of gene expression for 23K and LS 25. We could also not confirm the lower protein expression of glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and enolase previously seen in LS 25 [14]. The latter three enzymes are encoded from the central glycolytic operon (*cggR-gap-pgk-tpi-eno*) together with triose-phosphate isomerase and the putative central glycolytic genes regulator (CggR) [39]. Besides the *cggR* gene being down-regulated in MF1053 and LS 25, no change in gene expression was seen of these central glycolytic genes. Thus at the transcription level it is not obvious that the LS 25 strain down-regulate the glycolytic pathway more efficiently than the other strains, as previously suggested [14].

Interestingly, all the strains showed an induction (1.4-2.3) of *mgsA* encoding methylglyoxal synthase, which catalyzes the conversion of dihydroxyacetone-phosphate to methylglyoxal (Figure 2). The presence of this gene is uncommon among LAB and so far a unique feature among the sequenced lactobacilli. The methylglyoxal pathway represents an energetically unfavourable bypass to the glycolysis, which in *E. coli* occurs as a response to phosphate starvation or uncontrolled carbohydrate metabolism, and enhanced ribose uptake was shown to lead to the accumulation of methylglyoxal [40,41]. As suggested by Chaillou et al. [7], such flexibility in the glycolytic process in *L. sakei* may reflect the requirement to deal with glucose starvation or to modulate carbon flux during co-metabolism of alternative carbon sources. Breakdown of methylglyoxal is important as it is toxic to the cells [42]. An induction of the *lsa1158* gene contiguous with *mgsA* was seen for 23K and MF1053. This gene encodes a hypothetical protein, also suggested as a putative oxidoreductase, which may reduce methylglyoxal to lactaldehyde [7]. However, no induction of the *adhE* (*lsa0379*) gene encoding an iron-containing aldehyde dehydrogenase suggested to further reduce lactaldehyde to L-lactate [7] was seen. By CGH [26] *lsa1158* and *adhE* were present in all the *L. sakei* strains investigated, whereas *mgsA* was lacking in some strains, indicating that the MgsA function is not vital.

Pyruvate metabolism

Pyruvate is important in both glycolysis and PKP. It can be converted into lactate by the NAD-dependent L-lactate dehydrogenase, which regenerates NAD^+ and maintains the redox balance. This enzyme is encoded by the *ldhL* gene which was down-regulated (0.7-1.4) in all three strains, in accordance with previous findings [43], and the down-regulation was strongest for the LS 25 strain. At the protein level, only LS 25 showed a lower expression of this enzyme during growth on ribose [14]. Genes responsible for alternative fates of pyruvate (Figure 2) were highly induced in all the strains, however with some interesting strain variation (Table 1). The shift in pyruvate metabolism can benefit the bacteria by generating ATP, or by gaining NAD^+ for maintaining the redox balance and may lead to various end products in addition to lactate [44].

In all the strains, a strongly up-regulated (2.1-3.0) *pox1* gene was observed, and in 23K an up-regulated *pox2* (0.7), encoding pyruvate oxidases which under aerobic conditions convert pyruvate to acetyl-phosphate with hydrogen peroxide (H_2O_2) and CO_2 as side products. Accumulation of peroxide ultimately leads to aerobic growth arrest [45]. H_2O_2 belongs to a group of compounds known as reactive oxygen species and reacts readily with metal ions to yield hydroxyl radicals that damage DNA, proteins and membranes [46]. Remarkable differences in redox activities exist among *Lactobacillus* species and *L. sakei* is among those extensively well equipped to cope with changing oxygen conditions, as well as dealing effectively with toxic oxygen byproducts [7]. 23K up-regulated *npr* (1.0) encoding NADH peroxidase which decomposes low concentrations of H_2O_2 to H_2O and O_2, and all the strains up-regulated the *sodA* gene (1.7-3.4) encoding a superoxide dismutase which produces hydrogen peroxide from superoxide (O_2^-). Various oxidoreductases showed an up-regulation in all the strain (Table 1), indicating the need for the bacterium to maintain its redox balance.

The *pdhABCD* gene cluster encoding components of the pyruvate dehydrogenase enzyme complex (PDC) which transforms pyruvate into acetyl-CoA and CO_2 were among the strongly up-regulated (2.1-3.7) genes. The *eutD* gene encoding a phosphate acetyltransferase which further forms acetyl-phosphate from acetyl-CoA was also induced (1.0-2.0). Pyruvate can be transformed to acetolactate by acetolactate synthase and further to acetoin by acetolactate decarboxylase, before 2,3-butanediol may be formed by an acetoin recuctase (Figure 2). While the *budC* gene encoding the acetoin reductase showed a strong up-regulation in all three strains, the *als-aldB* operon was only strongly up-regulated in LS 25 (1.9). Pyruvate formate lyase produces acetyl-CoA and formate from pyruvate. Only in 23K,

the *pflAB* genes encoding formate C-acetyltransferase and its activating enzyme involved in formate formation were strongly up-regulated (4.0 and 1.7, respectively). This strain was the only one to strongly induce L-lactate oxidase encoding genes which are responsible for conversion of lactate to acetate when oxygen is present (Table 1). In 23K and LS 25, the *ppdK* gene coding for the pyruvate phosphate dikinase involved in regenerating PEP, was induced, as was also *lsa0444* encoding a putative malate dehydrogenase that catalyzes the conversion of malate into oxaloacetate using NAD^+ and vice versa (Table 1).

During growth on ribose, *L. sakei* was shown to require thiamine (vitamine B1) [11]. The E1 component subunit α of the PDC, as well as Pox and Xpk, require thiamine pyrophosphate, the active form of thiamine, as a coenzyme [47]. This could explain the induction of the *thiMDE* operon and *lsa0055* in LS 25, as well as *lsa0980* in 23K, encoding enzymes involved in thiamine uptake and biosynthesis (Table 1). The up-regulation of *lsa1664* (1.1-1.6) encoding a putative dihydrofolate reductase involved in biosynthesis of riboflavin (vitamin B2) in all the strains could indicate a requirement for flavin nucleotides as enzyme cofactors. Riboflavin is the precursor for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) redox cofactors in flavoproteins, and the E3 component of PDC as well as glycerol-3-phosphate dehydrogenase encoded from the up-regulated *glpD*, are among enzymes requiring FAD. Another cofactor which seems to be important during growth on ribose is lipoate, essential of the E2 component of the PDC. An up-regulation of *lplA* (1.0-1.6) encoding lipoate-protein ligase, which facilitates attachment of the lipoyl moiety to metabolic enzyme complexes, was seen in all the strains, allowing the bacterium to scavenge extracellular lipoate [48, 49].

Nucleoside catabolism

The *L. sakei* genome contains a multiplicity of catabolic genes involved in exogenous nucleoside salvage pathways, and the bacterium has been shown to catabolize inosine and adenosine for energy [7]. Three *iunH* genes are present in the 23K genome, which encode inosine-uridine preferring nucleoside hydrolases responsible for conversion of inosine to ribose and purine base. The *iunH1* gene was up-regulated in all the strains when grown on ribose (1.8-2.6), as was also the *iunH2* gene in 23K (1.2). The *deoC* gene encodes a deoxyribose-phosphate aldolase, and is located in an operon structure preceding the genes *deoB*, *deoD*, *lsa0798*, *lsa0799*, *deoR* and *pdp* which encode phosphopentomutase, purine nucleoside phosphorylase, pyrimidine-specific nucleoside symporter, a putative purine transport protein, the deoxyribonucleoside synthesis operon transcriptional regulator (DeoR),

and a pyrimidine-nucleoside phosphorylase, respectively. The complete operon was induced in all the strains, except for *pdp* only induced in 23K (Table 1). The phosphorylases catalyze cleavage of ribonucleosides and deoxyribonucleosides to the free base pluss ribose-1-phosphate or deoxyribose-1-phosphate. The bases are further utilized in nucleotide synthesis or as nitrogen sources. The pentomutase converts ribose-1-phosphate or deoxyribose-1-phosphate to ribose-5-phosphate or deoxyribose-5-phosphate, respectively, which can be cleaved by the aldolase to glyceraldehyde-3-phosphate and acetaldehyde. Glyceraldehyde-3-phosphate enters the glycolysis, while a putative iron containing alcohol dehydrogenase, encoded by *lsa0258* up-regulated in all the strains (0.5-1.6), could further reduce acetaldehyde to ethanol (Figure 2). The obvious induced nucleoside catabolism at the level of gene expression was not seen by proteomic analysis [14].

Genes involved in glycerol/glycerolipid/fatty acid metabolism

During growth on ribose, a strong induction of the *glpKDF* operon encoding glycerol kinase (GlpK), glycerol-3-phosphate dehydrogenase (GlpD), and glycerol uptake facilitator protein was observed (Table 1), which is in correlation with the over-expression of GlpD and GlpK seen by proteomic analysis [14]. GlpD is $FADH_2$ linked and converts glycerol-3-phosphate to dihydroxyacetone-phosphate. An over-expression of GlpD was also reported when *L. sakei* was exposed to low temperature [50]. A *glpD* mutant showed enhanced survival at low temperature, and it was suggested that this was a result of the glycerol metabolism being redirected into phosphatidic acid synthesis which leads to membrane phospholipid biosynthesis [50]. Nevertheless, a down-regulation was observed of the *lsa1493* gene (0.6-0.9) encoding a putative diacylglycerol kinase involved in the synthesis of phosphatidic acid, and of *cfa* (1.3-1.4) encoding cyclopropane-fatty-acyl-phospholipid synthase directly linked to modifications in the bacterial membrane fatty acid composition that reduce membrane fluidity and helps cells adapt to their environment [51]. Interestingly, LS 25 up-regulated several genes (LSA0812-0823), including *accD* and *accA* encoding the α- and ß-subunits of the multi-subunit acetyl-CoA carboxylase (Table 1). This is a biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA, an essential intermediate in fatty acid biosynthesis which relieves repression of the *fab* genes in *B. subtilis* [52]. Moreover, also *acpP*, *fabZ1*, *fabH*, *fabD* and *fabI* (Table 1) encoding enzymes involved in fatty acid biosynthesis were induced in this strain. The altered flux to malonyl-CoA may be a result of the decreased glycolytic rate. MF1053, on the other hand, showed a down-regulation of several genes in the same gene cluster. A higher level of acetate is produced

when the bacterium utilizes ribose, and acetate lowers the pH and has a higher antimicrobial effect than lactate. Changes in the phospholipid composition could be a response to changes in intracellular pH. Protons need to be expelled at a higher rate when the pH drops. The LS 25 strain which showed faster growth rates than the other strains [9], was the only strain to up-regulate the F_0F_1 ATP synthase (Table 1), which at the expense of ATP expels protons during low pH.

Regulation mechanisms

Little is known about the regulation of catabolic pathways in *L. sakei*. Starting from ribose uptake, the *rbs* operon may be both relieved from repression and ribose induced. Presumably, a dual regulation of this operon by two opposite mechanisms, substrate induction by ribose and CCR by glucose may occur in *L. sakei*. The *ccpA* gene was not regulated, consistent with this gene commonly showing constitutive expression in lactobacilli [35,53]. The local repressor RbsR is homologous with CcpA, both belonging to the same LacI/GalR family of transcriptional regulators. RbsR was proposed to bind a *cre*-like consensus sequence located close to a putative CcpA *cre* site, both preceding *rbsU* [22]. RbsR in the Gram-positive soil bacterium *Corynebacterium glutamicum* was shown to bind a *cre*-like sequence, and using microarrays, the transcription of no other genes but the *rbs* operon was affected positively in an *rbsR* deletion mutant. It was concluded that RbsR influences the expression of only the *rbs* operon [54]. Similarily, in the *L. sakei* sequence, no other candidate members of RbsR regulation could be found [22]. However, experiments are needed to confirm RbsR binding in *L. sakei*. In *Bacillus subtilis*, RbsR represent a novel interaction partner of P-Ser-HPr in a similar fashion to CcpA [55]. The P-Ser-HPr interaction is possible also in *L. sakei* as the bacterium exhibits HPr-kinase/phosphatase activity.

A putative *cre* site is present in the promoter of *lsa0254* encoding the second ribokinase (Table 2), and this gene is preceeded by the opposite oriented gene *lsa0253* encoding a transcriptional regulator with a sugar binding domain which belongs to the GntR family. This family of transcriptional regulators, as well as the LacI family which RbsR and CcpA belong to, are among the families to which regulators involved in carbohydrate uptake or metabolism usually belong [56]. The GntR-type regulator could possibly be involved in regulating the expression of the second ribokinase, or of the inosine-uridine preferring nucleoside hydrolase encoding *iunH1* gene which is located further upstream of *lsa0254*. *C. glutamicum* possesses an operon encoding a ribokinase, a uridine transporter, and a uridine-preferring nucleoside hydrolase which is co-controlled by a local repressor together with the

RbsR repressor of the *rbs* operon [53,54,57]. It is possible that such co-control could exist also in *L. sakei*. In the meat environment, ribose as well as nucleosides are products of the degradation of organic materials such as DNA, RNA and ATP. The simultaneous expression of the *rbs* and *deo* operons as well as the other genes involved in ribose and nucleoside catabolism (Figure 2) allows the bacterium to access the different substrates simultaneously and use both ribose as well as nucleosides as carbon and energy source. DeoR shows 51% identity to the *B. subtilis* DeoR repressor protein [58,59]. Genes encoding deoxyribose-phosphate aldolase, nucleoside uptake protein and pyrimidine nucleoside phosphorylase in *B. subtilis* are organized in a *dra-nupC-pdp* operon followed by *deoR*, and ribose was shown to release DeoR from DNA binding and thus repression of the operon genes are alleviated [58-60]. The *B. subtilis* pentomutase and purine-nucleoside phosphorylase are encoded from a *drm-pupG* operon which is not negatively regulated by DeoR, though both operons are subject to CcpA mediated CCR [58,59,61]. As a *cre* site is found preceding the *L. sakei deoC* (Table 2), the operon could be regulated by CcpA as well. It is interesting that DeoR is the only strongly induced transcriptional regulator in all three strains, and this regulator has sigma (σ) factor activity. We can only speculate whether it could function as activator of transcription on some of the regulated genes in this study.

Expression of the Xpk encoding gene of *Lactobacillus pentosus* was reported to be induced by sugars fermented through the PKP and repressed by glucose mediated by CcpA [62]. Indeed, the *cre* site overlapping ATG start codon of *L. sakei xpk* (Table 2) indicates relief of CcpA-mediated CCR during growth on ribose. Also for several genes involved in alternative fates of pyruvate, putative *cre* sites were present (Table 2).

Several genes and operons involved in transport and metabolism of various carbohydrates such as mannose, galactose, fructose, lactose, cellobiose, N-acetylglucosamine, including putative sugar kinases and PTSs, were induced during growth on ribose (Table 1), and as shown in Table 2, putative *cre* sites are located in the promoter region of many of these up-regulated genes and operons. 23K showed an up-regulation of genes involved in the arginine deiminase pathway, and 23K and LS 25 showed an up-regulated threonine deaminase (Table 1), and both *arcA* and *tdcB* have putative *cre* sites in their promoter regions (Table 2). Thus ribose seems to induce a global regulation of carbon metabolism in *L. sakei*.

A putative *cre* site precedes the *glp* operon (Table 2), suggesting regulation mediated by CcpA. However, regulation of the *L. sakei* GlpK may also occur by an inducer exclusion-based CcpA-independent CCR mechanism as described in enterococci and *B. subtilis* [63,64], and as previously suggested by Stentz et al. [11]. By this mechanism, glycerol metabolism is

regulated by PEP-dependent, EI- and HPr-catalyzed phosphorylation of GlpK in response to the presence or absence of a PTS substrate. In the absence of a PTS sugar, GlpK is phosphorylated by P-His-HPr at a conserved histidyl residue, forming the active P-GlpK form, whereas during growth on a PTS sugar, phosphoryl transfer flux through the PTS is high, concentration of P-His-HPr is low, and GlpK is present in a less active dephospho form [16,63,64]. This conserved histidyl residue (His232) is present in *L. sakei* GlpK [16], and Stentz et al. [11] reported that whereas *L. sakei* can grow poorly on glycerol, this growth was abolished in *ptsI* mutants.

Mannose-PTS

As mentioned in the introduction, the PTS plays a central role, in both the uptake of a number of carbohydrates and regulatory mechanisms [15-17]. Encoding the general components, *ptsH* showed an up-regulation in MF1053 and LS 25 (1.2 and 0.9, respectively), while all the strains up-regulated *ptsI* (0.8-1.7). The *manLMN* operon encoding the EII^{man} complex was surprisingly strongly up-regulated during growth on ribose in all the strains (Table 1). By proteomic analysis, no regulation of the PTS enzymes was seen [14]. The expression of HPr and EI in *L. sakei* during growth on glucose or ribose was previously suggested to be constitutive [10], and in other lactobacilli, the EII^{man} complex was reported to be consistently highly expressed, regardless of carbohydrate source [65-67]. Notably, PEP-dependent phosphorylation of PTS sugars has been detected in ribose-grown cells, indicating that the EII^{man} complex is active, and since no transport and phosphorylation via EII^{man} occurs, the complex is phosphorylated, while it is unphosphorylated in the presence of the substrates of the EII^{man} complex [8,66]. The stimulating effect exerted by small amounts of glucose on ribose uptake in *L. sakei*, which has also been reported in other lactobacilli [65,68], was suggested to be caused by dephosphorylation of the PTS proteins in the presence of glucose, as a *ptsI* mutant lacking EI, as well as P-His-HPr, was shown to enhance ribose uptake [11,12,69]. Stentz et al. [11] observed that a *L. sakei* mutant (strain RV52) resistant to 2 deoxy-D-glucose, a glucose toxic analog transported by EII^{man}, and thus assumed to be affected in the EII^{man}, did not show the same enhanced uptake [11]. It was concluded that EII^{man} is not involved in the PTS-mediated regulation of ribose metabolism in *L. sakei*. The mutation was though not reported verified by sequencing [11], and other mutations could be responsible for the observed phenotype. The *L. sakei* $EIIAB^{man}$, $EIIC^{man}$ and $EIID^{man}$ show 72, 81, and 82% identity, respectively, with the same enzymes in *L. casei*, in which mutations rendering the EII^{man} complex inactive were shown to derepress *rbs* genes, resulting in a loss

of the preferential use of glucose over ribose [68]. Furthermore, in *L. pentosus*, EII^{man} was shown to provide a strong signal to the CcpA-dependent repression pathway [66]. The *hprK* gene encoding HPrK/P which controls the phosphorylation state of HPr was strongly up-regulated (1.2-2.0) in all three strains as well. HPrK/P dephosphorylates P-Ser-HPr when the concentration of glycolytic intermediates such as fructose-1,6-bisphosphate, glucose-6-phosphate or fructose-1-phosphate drop, which is likely the situation during growth on ribose [16-18].

Numerous genes encoding hypothetical proteins with unknown function were also found to be differentially expressed (Table 1), as well as several other genes belonging to various functional categories. For most of these, their direct connection with ribose metabolism is unknown, and is likely an indirect effect.

Concluding remarks

The ability to ferment meat and fish is related to the capacity of the bacterium to rapidly take up the available carbohydrates and other components for growth. The importance of this process, especially to the meat industry, stimulates research aimed at understanding its mechanisms for transport and metabolism of these compounds, with the ultimate goal to be able to select improved strains. Genome-wide transcriptome analyses with DNA microarrays efficiently allowed the identification of genes differentially expressed between growth on the two carbohydrates which *L. sakei* can utilize from these substrates. Moreover, microarrays were a powerful tool to increase the understanding of the bacterium's primary metabolism and revealed a global regulatory mechanism. In summary, the ribose uptake and catabolic machinery is highly regulated at the transcription level, and it is closely linked with catabolism of nucleosides. A global regulation mechanism seems to permit a fine tuning of the expression of enzymes that control efficient exploitation of available carbon sources.

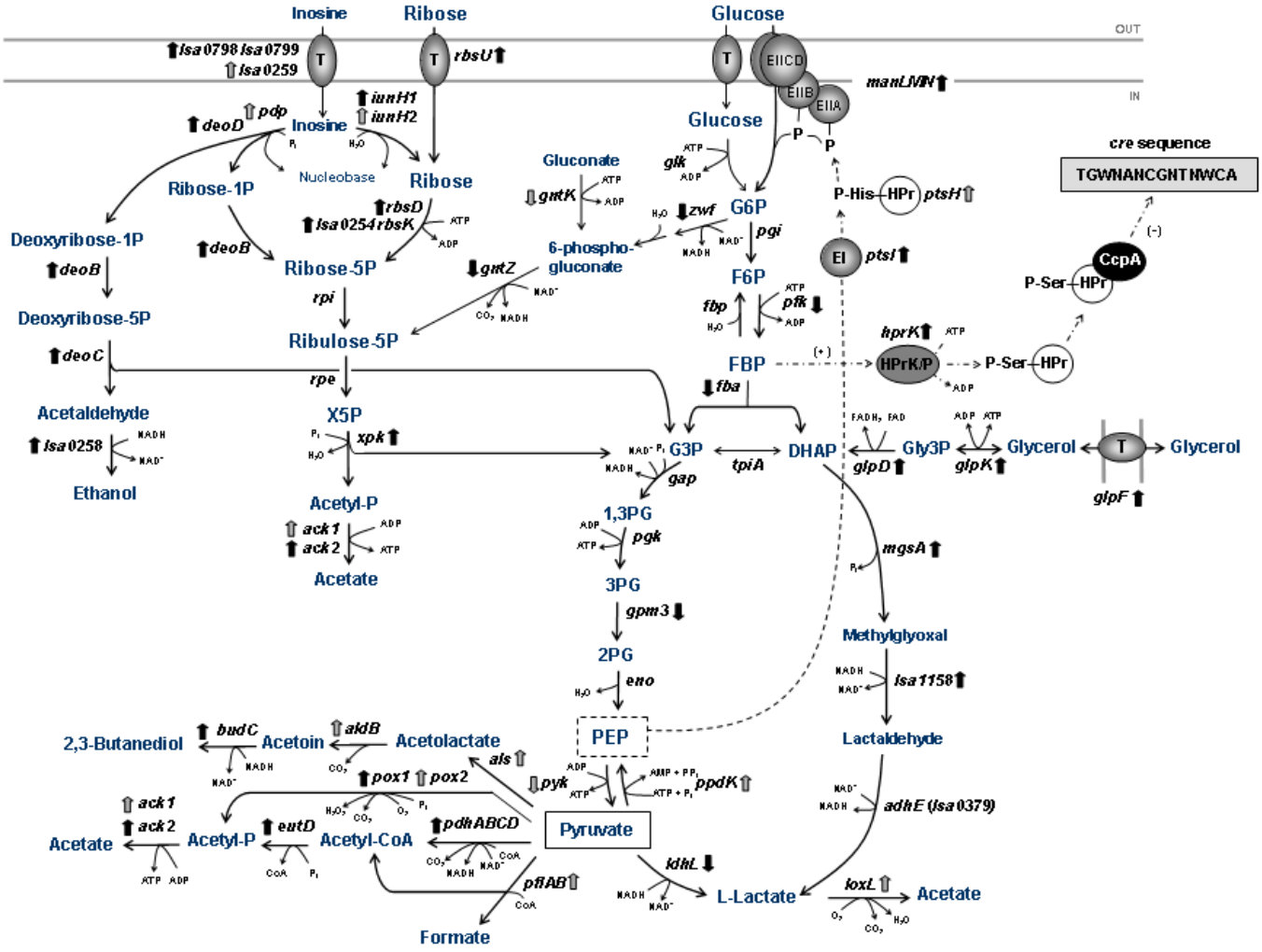


Figure 2. Overview of the glycolysis, phosphoketolase pathway and nucleoside catabolic pathway affected by the change of carbon source from glucose to ribose in three *L. sakei* strains in this study. Genes which expression is up- or down-regulated are indicated with upward and downward pointing arrows, respectively, and are listed in Table 1. Black arrows indicate regulation in all three strains, and grey arrows indicate regulation in one or two strains. Schematic representation of CcpA-mediated CCR pathway is shown in the upper right corner. EII, enzyme II of the phosphotransferase system (PTS); EI, enzyme I, HPr, Histidine-containing protein; T, transport protein; P, phosphate; HPrK/P, HPr kinase/phosphatase; G6P, glucose-6-phosphate; F6P; fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; G3P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; Gly3P, glycerol-3-phosphate; X5P, xylulose-5-phosphate; 1,3PG, 1,3-phosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolepyruvate; *glk*, glucokinase; *pgi*, phosphoglucoisomerase; *fbp*, fructose-1,6-bisphosphatase; *tpi*, triose-phosphate isomerase; *gap*, glyceraldehyde-3-phosphate dehydrogenase; *pgk*, phosphoglycerate kinase; *eno*, enolase; *rpi*, ribose-5-phosphate isomerase; *rpe*, ribulose-phosphate 3-epimerase.

Table 1. Genes with significant differential expression in three *L. sakei* strains grown on ribose compared with glucose, FDR adjusted p-value less than 0.01 and $\log_2$ of > 0.5 or < -0.5 ($\log_2$ values > 1.0 or < -1.0 are shown in bold). The microarray used has been described previously [26]. Asterix (*) relates the gene to Table 2. D and U refer to genes classified as 'divergent' and 'uncertain', respectively, by CGH analysis [26].

Gene locus	Gene	Description	23K	MF1053	LS 25
Carbohydrate transport and metabolism					
Transport/binding of carbohydrates					
LSA0185*	*galP*	Galactose:cation symporter	**1.2**		**1.7**
LSA0200*	*rbsU*	Ribose transport protein	**2.8**	**3.5**	**4.3**
LSA0353*	*lsa0353*	Putative cellobiose-specific PTS, enzyme IIB	**3.6**	**1.3**	**2.5**
LSA0449*	*manL*	Mannose-specific PTS, enzyme IIAB	**2.1**	**2.5**	**1.5**
LSA0450*	*manN*	Mannose-specific PTS, enzyme IIC	**1.9**	**2.0**	**1.4**
LSA0451*	*manM*	Mannose-specific PTS, enzyme IID	**2.4**	**1.0**	**2.1**
LSA0651*	*glpF*	Glycerol uptake facilitator protein, MIP family	**3.4**	**4.7**	**3.4**
LSA1050*	*fruA*	Fructose-specific PTS, enzyme IIABC			0.9
LSA1204*	*lsa1204*	Putative sugar transporter		**1.1**	
LSA1457*	*lsa1457*	Putative cellobiose-specific PTS, enzyme IIC		**2.3**	
LSA1462*	*ptsI*	PTS, enzyme I	0.8	**1.7**	0.9
LSA1463*	*ptsH*	Phosphocarrier protein HPr (Histidine protein)		**1.2**	0.9
LSA1533	*lsa1533*	Putative cellobiose-specific PTS, enzyme IIA		**2.5**	**2.1**
LSA1690	*lsa1690*	Putative cellobiose-specific PTS, enzyme IIC	0.9		
LSA1792*	*scrA*	Sucrose-specific PTS, enzyme IIBCA	0.8		**1.1**
Metabolism of carbohydrates and related molecules					
LSA0123*	*lsa0123*	Putative sugar kinase, ROK family	**1.2**		
LSA0198	*ack1*	Acetate kinase (Acetokinase)	**1.7**		**1.3**
LSA0254*	*lsa0254*	Putative carbohydrate kinase	**2.4**	0.8	**1.8**
LSA0292*	*budC*	Acetoin reductase (acetoin dehydrogenase) (Meso-2,3-butanediol dehydrogenase)	**3.4**	**2.3**	**3.4**
LSA0444	*lsa0444*	Putative malate dehydrogenase	**3.4**	D	**2.1**
LSA0516	*hprK*	Hpr kinase/phosphorylase	**2.0**	**1.6**	**1.2**
LSA0664*	*loxL1N*	L-lactate oxidase (N-terminal fragment), degenerate	**1.2**		0.7
LSA0665*	*loxLI*	L-lactate oxidase (central fragment), degenerate	**1.0**		
LSA0666*	*loxL1C*	L-lactate oxidase (C-terminal fragment), degenerate	**1.0**		
LSA0974*	*pflB*	Formate C-acetyltransferase (Pyruvate formate-lyase) (Formate acetyltransferase)	**4.0**		
LSA0981	*aldB*	Acetolactate decarboxylase (Alpha-acetolactate decarboxylase)		0.6	**1.9**
LSA0982	*als*	Acetolactate synthase (Alpha-acetolactate synthase)			**1.9**
LSA0983	*lsa0983*	Putative aldose-1 epimerase	0.6		
LSA1032	*pyk*	Pyruvate kinase		-0.7	
LSA1080	*lsa1080*	Myo-inositol monophosphatase	0.6		0.8
LSA1082	*pdhD*	Pyruvate dehydrogenase complex, E3 component, dihydrolipoamide dehydrogenase	**2.8**	**2.5**	**2.1**
LSA1083	*pdhC*	Puruvate dehydrogenase complex, E2 component, dihydrolipoamide acetyltransferase	**3.4**	**3.7**	**2.7**
LSA1084	*pdhB*	Pyruvate dehydrogenase complex, E1 component, beta subunit	**3.2**	**3.3**	**2.2**
LSA1085	*pdhA*	Pyruvate dehydrogenase complex, E1 component, alpha subunit	**2.9**	**3.5**	**2.4**
LSA1141*	*ppdK*	Pyruvate phosphate dikinase	**1.0**		0.9
LSA1188*	*pox1*	Pyruvate oxidase	**2.3**	**3.1**	**2.1**
LSA1298	*ack2*	Acetate kinase (Acetokinase)	**1.1**	0.9	0.9
LSA1343*	*eutD*	Phosphate acetyltransferase (Phosphotransacetylase)	**2.0**	**1.0**	**1.6**
LSA1381	*lsa1381*	Putative acylphosphatase	-0.6	-0.5	
LSA1399*	*loxL2*	L-lactate oxidase	**3.4**	U	
LSA1630	*lsa1630*	Putative sugar kinase, ROK family	-0.6		-0.6
LSA1640*	*nanA*	N-acetylneuraminate lyase	**2.0**		D
LSA1641*	*nanE*	N-acylglucosamine/mannosamine-6-phosphate 2-epimerase	0.9		D
LSA1643*	*lsa1643*	Putative sugar kinase, ROK family	**1.8**		
LSA1668	*ack3*	Acetate kinase (Acetokinase)	-0.7		**-1.1**
LSA1830*	*pox2*	Pyruvate oxidase	0.7		
Intermediary metabolism					
LSA0255*	*lsa0255*	Putative phosphoribosyl isomerase	**2.0**	**1.0**	**1.6**
Specific carbohydrate metabolic pathway					
LSA0201*	*rbsD*	D-ribose pyranase	**2.5**	**2.5**	**3.4**
LSA0202*	*rbsK*	Ribokinase	**3.0**	**3.9**	**4.3**
LSA0289*	*xpk*	Xylulose-5-phosphate phosphoketolase	**3.2**	**2.3**	**2.6**

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0297	*gntZ*	6-phosphogluconate dehydrogenase	**-1.2**	-0.9	**-1.7**
LSA0298	*gntK*	Gluconokinase	-0.8		
LSA0381	*zwf*	Glucose-6-phosphate 1-dehydrogenase	-0.6	-0.6	-0.6
LSA0649*	*glpK*	Glycerol kinase	**3.4**	**4.8**	**2.1**
LSA0650*	*glpD*	Glycerol-3-phosphate dehydrogenase	**2.3**	**2.2**	**2.0**
LSA0764*	*galK*	Galactokinase	**1.1**	0.7	**1.8**
LSA0765*	*galE1*	UDP-glucose 4-epimerase			**1.2**
LSA0766*	*galT*	Galactose-1-phosphate uridylyltransferase	**1.2**	0.8	**2.0**
LSA0767*	*galM*	Aldose 1-epimerase (mutarotase)	**1.3**		**2.0**
LSA1146*	*manA*	Mannose-6-phosphate isomerase	**1.4**	**1.3**	**1.5**
LSA1531	*lsa1531*	Putative beta-glucosidase		0.7	0.9
LSA1588	*nagA*	N-acetylglucosamine-6-phosphate deacetylase	0.6		
LSA1685	*rpiA*	Ribose 5-phosphate epimerase (Ribose 5-phosphate isomerase)		**1.1**	0.8
LSA1710*	*lacM*	Beta-galactosidase, small subunit (Lactase, small subunit)	**3.3**		**1.2**
LSA1711*	*lacL*	Beta-galactosidase, large subunit (Lactase, large subunit)	**3.0**	**1.5**	**1.7**
LSA1790*	*scrK*	Fructokinase		**1.0**	**1.1**
LSA1791*	*dexB*	Glucan 1,6-alpha-glucosidase (dextran glucosidase)			**1.1**
LSA1795	*melA*	Alpha-galactosidase (Melibiase)			-0.6
Glycolytic pathway					
LSA0131	*gpm2*	Phosphoglycerate mutase		0.7	
LSA0206	*gpm3*	Phosphoglycerate mutase	-0.7	-0.8	-0.9
LSA0609*	*gloAC*	Lactoylglutathione lyase (C-terminal fragment), authentic frameshift	**1.1**		0.7
LSA0803	*gpm4*	Phosphoglycerate mutase	0.5		0.5
LSA1033	*pfk*	6-phosphofructokinase	-0.6	**-1.1**	-0.5
LSA1157	*mgsA*	Methylglyoxal synthase	**2.3**	**1.4**	**1.7**
LSA1179	*pgi*	Glucose-6-phosphate isomerase	0.5		
LSA1527	*fba*	Fructose-bisphosphate aldolase	**-1.0**	-0.7	**-1.1**
LSA1606	*ldhL*	L-lactate dehydrogenase	**-1.0**	-0.9	**-1.5**
Nucleotide transport and metabolism					
Transport/binding of nucleosides, nucleotides, purines and pyrimidines					
LSA0013	*lsa0013*	Putative nucleobase:cation symporter	-0.9		**-1.5**
LSA0055	*lsa0055*	Putative thiamine/thiamine precursor:cation symporter			**1.6**
LSA0064	*lsa0064*	Putative nucleobase:cation symporter		-0.8	
LSA0259	*lsa0259*	Pyrimidine-specific nucleoside symporter	**1.5**		**1.3**
LSA0798*	*lsa0798*	Pyrimidine-specific nucleoside symporter	**3.5**	**2.2**	**1.7**
LSA0799*	*lsa0799*	Putative purine transport protein	**4.4**	**2.7**	**2.9**
LSA1210	*lsa1210*	Putative cytosine:cation symporter (C-terminal fragment), authentic frameshift	-0.8		-0.6
LSA1211	*lsa1211*	Putative cytosine:cation symporter (N-terminal fragment), authentic frameshit	**-1.1**		-0.9
Metabolism of nucleotides and nucleic acids					
LSA0010	*lsa0010*	Putative nucleotide-binding phosphoesterase			-0.6
LSA0023	*lsa0023*	Putative ribonucleotide reductase (NrdI-like)	-0.5	D	D
LSA0063	*purA*	Adenylosuccinate synthetase (IMP-aspartate ligase)		-0.8	
LSA0139	*guaA*	Guanosine monophosphate synthase (Glutamine amidotransferase)		-0.5	-0.8
LSA0252	*iunH1*	Inosine-uridine preferring nucleoside hydrolase	**2.6**	**2.6**	**1.8**
LSA0446	*pyrDB*	Putative dihydroorotate oxidase, catalytic subunit			0.9
LSA0489	*lsa0489*	Putative metal-dependent phosphohydrolase precursor	0.5		
LSA0533*	*iunH2*	Inosine-uridine preferring nucleoside hydrolase	**1.2**		
LSA0785	*lsa0785*	Putative NCAIR mutase, PurE-related protein	**-2.3**		**-1.3**
LSA0795*	*deoC*	2 Deoxyribose-5 phosphate aldolase	**4.0**	**2.1**	**2.2**
LSA0796*	*deoB*	Phosphopentomutase (Phosphodeoxyribomutase)	**5.5**	**4.1**	**3.2**
LSA0797*	*deoD*	Purine-nucleoside phosphorylase	**4.5**	**2.6**	**1.9**
LSA0801*	*pdp*	Pyrimidine-nucleoside phosphorylase	**1.8**		
LSA0940	*nrdF*	Ribonucleoside-diphosphate reductase, beta chain		**1.0**	0.6
LSA0941	*nrdE*	Ribonucleoside-diphosphate reductase, alpha chain		**1.0**	0.6
LSA0942	*nrdH*	Ribonucleotide reductase, NrdH-redoxin		**1.1**	
LSA0950	*pyrR*	Bifunctional protein: uracil phosphoribosyltransferase and pyrimidine operon transcriptional regulator	-0.6		

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0993	*rnhB*	Ribonuclease HII (RNase HII)			0.6
LSA1018	*cmk*	Cytidylate kinase			0.6
LSA1097	*lsa1097*	Putative ADP-ribose phosphorylase, NUDIX family	0.5		
LSA1352	*lsa1352*	Putative phosphomethylpyrimidine kinase	-0.8		
LSA1651	*lsa1651*	Putative purine phosphoribosyltransferase, PRT family		0.8	
LSA1661	*lsa1661*	Putative nucleotide hydrolase, NUDIX family		-0.5	
LSA1805	*dgk*	Deoxyguanosine kinase	**-1.0**		-0.8
Transcription					
Transcription regulation					
LSA0130	*lsa0130*	Putative transcriptional regulator, LacI family	-0.6		
LSA0132	*lsa0132*	Putative transcriptional regulator, MarR family	-0.6		
LSA0161	*lsa0161*	Putative transcriptional regulator, ArsR family	-0.6		
LSA0186	*lsa0186*	Putative transcriptional regulator, LytR family		0.8	0.6
LSA0203	*rbsR*	Ribose operon transcriptional regulator, LacI family	**1.7**		
LSA0217	*lsa0217*	Putative thiosulfate sulfurtransferase with a ArsR-HTH domain, Rhodanese family		**-1.0**	-0.7
LSA0229	*lsa0229*	Putative transcriptional regulator, MerR family (N-terminal fragment), authentic frameshift	-0.5		
LSA0269	*lsa0269*	Putative transcriptional regulator, TetR family			-0.6
LSA0293	*lsa0293*	Putative DNA-binding protein, XRE family			-0.6
LSA0603	*cggR*	Glycolytic genes regulator		-0.6	-0.6
LSA0669	*lsa0669*	Putative transcription regulator, TetR family		-0.6	
LSA0783	*lsa0783*	Putative transcriptional regulator, Fnr/Crp Family	-0.6		
LSA0800	*deoR*	Deoxyribonucleoside synthesis operon transcriptional regulator, GntR family	**3.8**	**2.1**	**1.9**
LSA0835	*lsa0835*	Putative DNA-binding protein, XRE family	-0.6		
LSA0972	*lsa0972*	Putative transcriptional regulator, LysR family	0.9		
LSA1201	*lsa1201*	Putative transcriptional regulator, GntR family	**1.4**	D	D
LSA1322	*glnR*	Glutamine synthetase transcriptional regulator, MerR family	**-1.4**	**-1.3**	
LSA1351	*lsa1351*	Putative transcritional regulator with aminotransferase domain, GntR family		-0.5	-0.6
LSA1434	*lsa1434*	Putative transcriptional regulator, DUF24 family (related to MarR/PadR families)	-0.8		
LSA1449	*spxA*	Transcriptional regulator Spx	**1.0**		0.6
LSA1521	*lsa1521*	Putative transcriptional regulator, TetR family	0.6		
LSA1554	*lsa1554*	Putative transcriptional regulator, LacI family	-0.7	-0.9	-0.5
LSA1587	*lsa1587*	Putative transcriptional regulator, GntR family	0.6		
LSA1611	*lsa1611*	Putative DNA-binding protein, PemK family		-0.5	-0.7
LSA1653	*lsa1653*	Putative transcriptional regulator, MarR family			-0.6
LSA1692	*lsa1692*	Putative transcriptional regulator, GntR family	0.7		0.7
CoEnzyme transport and metabolism					
Metabolism of coenzymes and prostethic groups					
LSA0041	*panE*	2-dehydropantoate 2-reductase		0.8	
LSA0057	*thiE*	Thiamine-phosphate pyrophosphorylase (Thiamine-phosphate synthase)			**1.9**
LSA0058	*thiD*	Phosphomethylpyrimidine kinase (HMP-phosphate kinase)			**1.4**
LSA0059	*thiM*	Hydroxyethylthiazole kinase (4-methyl-5-beta- hydroxyethylthiazole kinase)	**1.0**		**1.8**
LSA0183	*lsa0183*	Putative hydrolase, isochorismatase/nicotamidase family	-0.7		
LSA0840	*lsa0840*	Putative glutamate-cysteine ligase	0.6		
LSA0947	*fhs*	Formate-tetrahydrofolate ligase (Formyltetrahydrofolate synthetase)	0.6		
LSA0980	*lsa0980*	Putative hydroxymethylpyrimidine / phosphomethylpyrimidine kinase, PfkB family	0.6		
LSA1101	*folK*	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	0.6	U	
LSA1614	*acpS*	Holo-[acyl-carrier protein] synthase (Holo-ACP synthase) (4'-phosphopantetheine transferase AcpS)	**-1.0**	-0.9	-0.9
LSA1664	*lsa1664*	Putative dihydrofolate reductase	**1.6**	**1.1**	**1.5**
Energy production and conversion					
Membrane bioenergetics (ATP synthase)					
LSA1125	*atpC*	H(+)-transporting two-sector ATPase (ATP synthase), epsilon subunit	0.6		
LSA1126	*atpD*	H(+)-transporting two-sector ATPase (ATP synthase), beta subunit			0.6
LSA1127	*atpG*	H(+)-transporting two-sector ATPase (ATP synthase), gamma subunit			0.8

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA1128	*atpA*	H(+)-transporting two-sector ATPase (ATP synthase), alpha subunit			0.6
LSA1129	*atpH*	H(+)-transporting two-sector ATPase (ATP synthase), delta subunit			0.6
LSA1130	*atpF*	H(+)-transporting two-sector ATPase (ATP synthase), B subunit			0.5
LSA1131	*atpE*	H(+)-transporting two-sector ATPase (ATP synthase), C subunit			0.7
Inorganic ion transport and metabolism					
Transport/binding of inorganic ions					
LSA0029	*lsa0029*	Putative ion Mg(2+)/Co(2+) transport protein, hemolysinC-family			-0.7
LSA0134	*lsa0134*	Putative Na(+)/H(+) antiporter			-0.6
LSA0180	*mtsC*	Manganese ABC transporter, ATP-binding subunit	-0.8		
LSA0181	*mtsB*	Manganese ABC transporter, membrane-spanning subunit	-0.8		**-1.0**
LSA0182	*mtsA*	Manganese ABC transporter, substrate-binding lipoprotein precursor	-0.7		-0.6
LSA0246	*mntH1*	Mn(2+)/Fe(2+) transport protein	-0.9		**-1.3**
LSA0283	*lsa0283*	Putative zinc/iron ABC transporter, ATP-binding subunit			-0.5
LSA0284	*lsa0284*	Putative zinc/iron ABC transporter, membrane-spanning subunit			-0.6
LSA0399	*lsa0399*	Iron(III)-compound ABC transporter, substrate-binding lipoprotein precursor	**1.1**	0.9	
LSA0400	*lsa0400*	Iron(III)-compound ABC transporter, ATP-binding subunit		0.7	
LSA0401	*lsa0401*	Iron(III)-compound ABC transporter, membrane-spanning subunit			0.5
LSA0402	*lsa0402*	Iron(III)-compound ABC transporter, membrane-spanning subunit	0.5		0.6
LSA0503	*pstC*	Phosphate ABC transporter, membrane-spanning subunit	0.5		
LSA0504	*pstA*	Phosphate ABC transporter, membrane-spanning subunit	0.6		
LSA0781	*lsa0781*	Putative cobalt ABC transporter, membrane-spanning/permease subunit	-0.9		
LSA0782	*lsa0782*	Putative cobalt ABC transporter, membrane-spanning/permease subunit	**-2.1**		
LSA1166	*lsa1166*	Putative potassium transport protein	0.7		
LSA1440	*cutC*	Copper homeostasis protein, CutC family	-0.6		
LSA1460	*atkB*	Copper-transporting P-type ATPase	0.6		
LSA1638	*lsa1638*	Putative large conductance mechanosensitive channel		**-1.0**	-0.8
LSA1645	*lsa1645*	Putative Na(+)/(+) antiporter	**1.4**		D
LSA1699	*mntH2*	Mn(2+)/Fe(2+) transport protein			-0.6
LSA1703	*lsa1703*	Putative Na(+)/H(+) antiporter	**-1.2**		
LSA1704	*lsa1704*	Putative calcium-transporting P-type ATPase			-0.8
LSA1735	*lsa1735*	Putative cobalt ABC transporter, membrane-spanning subunit			-0.6
LSA1736	*lsa1736*	Putative cobalt ABC transporter, ATP-binding subunit	-0.6		
LSA1737	*lsa1737*	Putative cobalt ABC transporter, ATP-binding subunit	-0.7		
LSA1838	*lsa1838*	Putative metal ion ABC transporter, membrane-spanning subunit			-0.5
LSA1839	*lsa1839*	Putative metal ion ABC transporter, substrate-binding lipoprotein precursor			-0.6
Amino acid transport and metabolism					
Transport/binding of amino acids					
LSA0125	*lsa0125*	Putative amino acid/polyamine transport protein	0.6		
LSA0189	*lsa0189*	Putative amino acid/polyamine transport protein			-0.7
LSA0311	*lsa0311*	Putative glutamate/aspartate:cation symporter	**-1.1**		**-1.0**
LSA1037	*lsa1037*	Putative amino acid/polyamine transport protein	**1.0**	0.8	0.5
LSA1219	*lsa1219*	Putative cationic amino acid transport protein	0.7		
LSA1415	*lsa1415*	Putative amino acid/polyamine transport protein	**1.1**		0.7
LSA1424	*lsa1424*	Putative L-aspartate transport protein	**-1.4**	-0.9	**-1.2**
LSA1435	*lsa1435*	Putative amino acid:H(+) symporter	**1.0**		0.8
LSA1496	*lsa1496*	Putative glutamine/glutamate ABC transporter, ATP-binding subunit		**1.2**	
LSA1497	*lsa1497*	Putative glutamine/glutamate ABC transporter, membrane-spanning/substrate-binding subunit precursor		0.7	
Transport/binding of proteins/peptides					
LSA0702	*oppA*	Oligopeptide ABC transporter, substrate-binding lipoprotein precursor		**1.3**	**1.0**
LSA0703	*oppB*	Oligopeptide ABC transporter, membrane-spanning subunit		0.8	0.8
LSA0704	*oppC*	Oligopeptide ABC transporter, membrane-spanning subunit		**1.8**	**1.0**
LSA0705	*oppD*	Oligopeptide ABC transporter, ATP-binding subunit		**1.2**	**1.1**
LSA0706	*oppF*	Oligopeptide ABC transporter, ATP-binding subunit		**1.2**	**1.2**
Protein fate					
LSA0053	*pepO*	Endopeptidase O	0.6		

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0133	*pepR*	Prolyl aminopeptidase	**1.5**		
LSA0226	*pepN*	Aminopeptidase N (Lysyl-aminopeptidase-Alanyl aminopeptidase)			-0.7
LSA0285	*pepF1*	Oligoendopeptidase F1			-0.7
LSA0320	*pepD3*	Dipeptidase D-type (U34 family)		-0.8	-0.5
LSA0424	*pepV*	Xaa-His dipeptidase V (Carnosinase)	**1.6**		
LSA0643	*pepX*	X-Prolyl dipeptidyl-aminopeptidase	0.6		
LSA0888	*pepT*	Tripeptide aminopeptidase T	0.6		
LSA1522	*pepS*	Aminopeptidase S	0.5		
LSA1686	*pepC1N*	Cysteine aminopeptidase C1 (Bleomycin hydrolase) (N-terminal fragment), authentic frameshift		**1.6**	
LSA1688	*pepC2*	Cysteine aminopeptidase C2 (Bleomycin hydrolase)		0.7	
LSA1689	*lsa1689*	Putative peptidase M20 family	**1.0**		**1.1**
Metabolism of amino acids and related molecules					
LSA0220_c	*dapE*	Succinyl-diaminopimelate desuccinylase	**-1.4**		**-1.5**
LSA0316	*sdhB*	L-serine dehydratase, beta subunit (L-serine deaminase)	-0.7		
LSA0370*	*arcA*	Arginine deiminase (Arginine dihydrolase)	**1.9**		
LSA0372*	*arcC*	Carbamate kinase	0.5		
LSA0463	*lsa0463*	Putative 2-hydroxyacid dehydrogenase	-0.7		
LSA0509	*kbl*	2-amino-3-ketobutyrate coenzyme A ligase (Glycine acetyltransferase)	**1.5**		
LSA0510	*lsa0510*	L-threonine dehydrogenase (N-terminal fragment), authentic frameshift	**2.0**	0.5	
LSA0572*	*tdcB*	Threonine deaminase (Threonine ammonia-lyase, Threonine dehydratase, IlvA homolog)	**2.2**		**1.7**
LSA0922	*serA*	D-3-phosphoglycerate dehydrogenase	0.9		
LSA1134	*glyA*	Glycine/Serine hydroxymethyltransferase		0.7	
LSA1321	*glnA*	Glutamate-ammonia ligase (Glutamine synthetase)	**-1.3**	**-1.0**	
LSA1484	*mvaS*	Hydroxymethylglutaryl-CoA synthase	-0.7	-0.6	-0.7
LSA1693	*asnA2*	L-asparaginase	0.8		
Lipid transport and metabolism					
Metabolism of lipids					
LSA0045	*cfa*	Cyclopropane-fatty-acyl-phospholipid synthase	**-1.3**	**-1.4**	**-1.4**
LSA0644	*lsa0644*	Putative acyl-CoA thioester hydrolase	0.6		
LSA0812	*fabZ1*	(3R)-hydroxymyristoyl-[acyl-carrier protein] dehydratase		-0.7	0.5
LSA0813	*fabH*	3-oxoacyl-[acyl carrier protein] synthetase III			0.6
LSA0814	*acpP*	Acyl carrier protein			0.6
LSA0815	*fabD*	Malonyl-CoA:ACP transacylase		-0.7	0.7
LSA0816	*fabG*	3-oxoacyl-acyl carrier protein reductase		-0.7	
LSA0817	*fabF*	3-oxoacyl-[acyl carrier protein] synthetase II		-0.7	
LSA0819	*fabZ*	(3R)-hydroxymyristoyl-[acyl carrier proetin] dehydratase			0.7
LSA0820	*accC*	Acetyl-CoA carboxylase (biotin carbooxylase subunit)		-0.7	
LSA0821	*accD*	Acetyl-CoA carboxylase (carboxyl transferase beta subunit)			0.8
LSA0822	*accA*	Acetyl-CoA carboxylase (carboxyl transferase alpha subunit)			0.6
LSA0823	*fabI*	Enoyl [acyl carrier protein] reductase			0.9
LSA0891	*lsa0891*	Putative lipase/esterase	1.2		
LSA1485	*mvaA*	Hydroxymethylglutaryl-CoA reductase	-0.5		
LSA1493	*lsa1493*	Putative diacylglycerol kinase	-0.6	-0.9	-0.7
LSA1652	*ipk*	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	-0.6		-0.7
Secondary metabolites transport and metabolism					
Transport/binding proteins and lipoproteins					
LSA0046	*lsa0046*	Putative transport protein	**-1.0**	-0.6	**-1.3**
LSA0089	*lsa0089*	Putative drug transport protein	**-2.1**	-0.9	-0.8
LSA0094	*lsa0094*	Putative transport protein, Major Facilitator Superfamily	-0.7		-0.7
LSA0095	*lsa0095*	Putative transport protein	**1.3**	0.5	
LSA0128	*lsa0128*	Putative antimicrobial peptide ABC exporter, membrane-spanning/permease subunit			-0.5
LSA0187	*lsa0187*	Putative drug-resistance ABC transporter, two ATP-binding subunits		0.7	
LSA0219_b	*lsa0219_b*	Putative cyanate transport protein	-0.6		
LSA0232	*lmrA*	Multidrug ABC exporter, ATP-binding and membrane-spanning/permease subunits	-0.7		-0.7
LSA0270	*lsa0270*	Putative multidrug ABC exporter, membrane-spanning/permease subunit	-0.7		

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0271	*lsa0271*	Putative multidrug ABC exporter, ATP-binding subunit	-0.7		-0.6
LSA0272	*lsa0272*	Putative multidrug ABC exporter, ATP-binding and membrane-spanning/permease subunits	-0.6		-0.6
LSA0308	*lsa0308*	Putative drug:H(+) antiporter			-0.7
LSA0376	*lsa0376*	Putative transport protein	0.7		
LSA0420	*lsa0420*	Putative drug:H(+) antiporter (N-terminal fragment), authentic frameshift	-0.8		**-1.1**
LSA0469	*lsa0469*	Putative drug:H(+) antiporter	-0.6		-0.5
LSA0788	*lsa0788*	Putative facilitator protein, MIP family	**-2.6**		
LSA0936	*lsa0936*	Putative drug ABC exporter, membrane-spanning/permease subunit	**1.1**		
LSA0937	*lsa0937*	Putative drug ABC exporter, membrane-spanning/permease subunit	**1.3**		
LSA0938	*lsa0938*	Putative drug ABC exporter, ATP-binding subunit	**1.2**		
LSA0963	*lsa0963*	Integral membrane protein, hemolysin III related			
LSA1088	*lsa1088*	Putative multidrug ABC exporter, ATP-binding and membrane-spanning/permease subunits	0.5		
LSA1261	*lsa1261*	Putative autotransport protein	0.5		
LSA1340	*lsa1340*	Putative transport protein		-0.7	
LSA1366	*lsa1366*	Putative ABC exporter, ATP-binding subunit	-0.8		**-1.0**
LSA1367	*lsa1367*	Putative ABC exporter, membrane-spanning/permease subunit	-0.8	-0.5	-0.8
LSA1420	*lsa1417*	Putative lipase/esterase		**-1.1**	
LSA1621	*lsa1621*	Putative drug:H(+) antiporter		**-1.1**	
LSA1642	*lsa1642*	Putative Solute:Na(+) symporter	**3.4**	**1.8**	D
LSA1872	*lsa1872*	Putative drug:H(+) antiporter		0.7	
LSA1878	*lsa1878*	Putative drug resistance ABC transporter, two ATP-binding subunits	-0.6		
Detoxification					
LSA0772	*lsa0772*	Hypothetical protein (TelA, Telluric resistance family)	**1.0**		0.7
LSA1317	*lsa1317*	Putative chromate reductase	0.6	-0.7	
LSA1450	*lsa1450*	Putative metal-dependent hydrolase (Beta-lactamase family III)			0.6
LSA1776	*lsa1776*	Putative 4-carboxymuconolactone decarboxylase	0.6		D
Translation, ribosomal structure and biogenesis					
Translation initiation					
LSA1135	*lsa1135*	Putative translation factor, Sua5 family		0.7	0.6
Translation elongation					
LSA0251	*efp1*	Elongation factor P (EF-P)	0.5		
LSA1063	*tuf*	Elongation factor Tu (EF-Tu)	0.6		
Ribosomal proteins					
LSA0011	*rplI*	50S Ribosomal protein L9			-0.8
LSA0266	*rpsN*	30S ribosomal protein S14		0.7	-0.5
LSA0494	*lsa0494*	30S ribosomal interface protein S30EA	**1.7**		
LSA0696	*rpmB*	50S ribosomal protein L28			0.8
LSA1017	*rpsA*	30S Ribosomal protein S1	0.9		0.6
LSA1333	*rpmG*	50S ribosomal protein L33			0.6
LSA1666	*rplL*	50S ribosomal protein L7/L12	-0.6		
LSA1676	*rpmG2*	50S ribosomal protein L33			-0.6
LSA1750	*rplF*	50S ribosomal protein L6		0.6	
LSA1755	*rpsQ*	30S ribosomal protein S17		0.5	
LSA1761	*rplB*	50S ribosomal protein L2		0.6	
LSA1765	*rpsJ*	30S ribosomal protein S10	-0.7		
Protein synthesis					
LSA0377	*tgt*	Queuine tRNA-ribosyltransferase	-0.6		
LSA1546	*gatB*	Glutamyl-tRNA amidotransferase, subunit B		-0.5	
LSA1547	*gatA*	Glutamyl-tRNA amidotransferase, subunit A	-0.5		-0.5
RNA restriction and modification					
LSA0437	*lsa0437*	Hypothetical protein with an RNA-binding domain	-0.7		
LSA0443	*lsa0443*	Putative single-stranded mRNA endoribonuclease	**2.7**		**1.9**
LSA0738	*dtd*	D-tyrosyl-tRNA(tyr) deacylase	0.5		
LSA0794	*trmU*	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase		-0.9	
LSA1534	*lsa1534*	Putative ATP-dependent RNA helicase		0.9	

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA1615	*lsa1615*	Putative ATP-dependent RNA helicase	-0.7	-0.8	**-1.0**
LSA1723	*truA*	tRNA pseudouridylate synthase A (Pseudouridylate synthase I)	-0.7		-0.6
LSA1880	*trmE*	tRNA modification GTPase trmE	-0.7		
Aminoacyl-tRNA synthetases					
LSA0880	*glyQ*	Glycyl-tRNA synthetase, alpha subunit		0.7	
LSA0881	*glyS*	Glycyl-tRNA synthetase, beta subunit		0.7	
LSA1400	*thrS*	Threonyl-tRNA synthetase	0.6		
LSA1681	*cysS*	Cysteinyl-tRNA synthetase	-0.6		
DNA replication, recombination and repair					
DNA replication					
LSA0221	*lsa0221*	Putative transcriptional regulator, LysR family (C-terminal fragment), degenerate	-0.8	-0.9	**-1.1**
LSA0976	*parE*	Topoisomerase IV, subunit B		0.5	
Transposon and IS					
LSA1152_a	*tnpA3-ISLsa1*	Transposase of ISLsa1 (IS30 family)	-0.6		
Phage-related function					
LSA1292	*lsa1292*	Putative prophage protein	0.6		
LSA1788	*lsa1788*	Putative phage-related 1,4-beta-N-acetyl muramidase (cell wall hydrolase)	**-1.0**	D	D
DNA recombination and repair					
LSA0076	*lsa0076*	Putative DNA invertase (Plasmidic Resolvase)	**-1.1**	**-1.5**	**-1.4**
LSA0366	*ruvA*	Holliday junction DNA helicase RuvA			-0.5
LSA0382	*dinP*	DNA-damage-inducible protein P	-0.5		
LSA0487	*recA*	DNA recombinase A	-0.8		**-1.1**
LSA0523	*uvrB*	Excinuclease ABC, subunit B	-0.7		-0.5
LSA0524	*uvrA1*	Excinuclease ABC, subunit A	**-1.2**		-0.7
LSA0910	*rexAN*	ATP-dependent exonuclease, subunit A (N terminal fragment), authentic frameshift	0.6		
LSA0911	*rexAC*	ATP-dependent exonuclease, subunit A (C terminal fragment), authentic frameshift	0.7		
LSA0912	*lsa0912*	Putative ATP-dependent helicase, DinG family	0.6		0.8
LSA1162	*lsa1162*	DNA-repair protein (SOS response UmuC-like protein)		0.8	-0.6
LSA1405	*fpg*	Formamidopyrimidine-DNA glycosylase	-0.5	-0.6	-0.6
LSA1477	*recX*	Putative regulatory protein, RecX family	-0.6		
LSA1843	*ogt*	Methylated-DNA-protein-cysteine S-methyltransferase	-0.6		
DNA restriction and modification					
LSA0143	*lsa0143*	Putative adenine-specific DNA methyltransferase	-0.7	D	D
LSA0921	*lsa0921*	Putative adenine-specific DNA methyltransferase	0.8		
LSA1299	*lsa1299*	Putative adenine-specific DNA methyltransferase	0.9	0.7	**1.2**
Information pathways					
LSA0326	*lsa0326*	Putative DNA helicase		-0.6	U
DNA packaging and segregation					
LSA0135	*lsa0135*	Hypothetical integral membrane protein, similar to CcrB			-0.6
LSA1015	*hbsU*	Histone-like DNA-binding protein HU	**1.0**		0.9
Cell division and chromosome partitioning					
Cell division					
LSA0755	*divIVA*	Cell-division initiation protein (septum placement)			0.5
LSA0845	*lsa0845*	Putative negative regulator of septum ring formation	0.7		0.6
LSA1118	*lsa1118*	Rod-shape determining protein		0.6	0.5
LSA1597	*ftsH*	ATP-dependent zinc metalloendopeptidase FtsH (cell division protein FtsH)			-0.6
LSA1879	*gidA*	Cell division protein GidA	-0.6		
Cell envelope biogenesis, outer membrane					
Cell wall					
LSA0280	*murE*	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	-0.6	-0.6	-0.7
LSA0621	*pbp2A*	Bifunctional glycolsyltransferase/transpeptidase penicillin binding protein 2A			0.7
LSA0648	*lsa0648*	Putative penicillin-binding protein precursor (Beta-lactamase class C)			**1.0**
LSA0862	*lsa0862*	N-acetylmuramoyl-L-alanine amidase precursor (cell wall hydrolase) (autolysin)	0.6		0.8
LSA0917	*pbp1A*	Bifunctional glycosyltransferase/transpeptidase penicillin-binding protein 1A			0.5

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA1123	*murA1*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase I		-0.5	
LSA1334	*pbp2B2*	Bifuntional dimerisation/transpeptidase penicillin-binding protein 2B		0.7	0.7
LSA1437	*lsa1437*	N-acetylmuramoyl-L-alanine amidase precursor (cell wall hydrolase) (Autolysin)		-0.7	
LSA1441	*bacA*	Putative undecaprenol kinase (Bacitracine resistance protein A)		0.6	
LSA1613	*alr*	Alanine racemase	-0.8	-0.9	-0.7
LSA1616	*murF*	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase			-0.5
Cell envelope and cellular processes					
LSA0162	*lsa0162*	Putative Bifunctional glycosyl transferase, family 8		**-1.2**	**-1.5**
LSA1246	*lsa1246*	Putative glycosyl transferase, family 2		-0.9	
LSA1558	*lsa1558*	Putative extracellular N-acetylmuramoyl-L-alanine amidase precursor (cell wall hydrolase/Lysosyme subfamily 2)			-0.6
Cell motility and secretion					
Protein secretion					
LSA0948	*lspA*	Signal peptidase II (Lipoprotein signal peptidase) (Prolipoprotein signal peptidase)			0.5
LSA1884	*oxaA2*	Membrane protein chaperone oxaA			-0.6
Signal transduction					
Signal transduction					
LSA0561	*sppKN*	Two-component system, sensor histidine kinase, (SppK fragment), degenerate		0.5	
LSA0692	*lsa0692*	Putative serine/threonine protein kinase		0.5	0.6
LSA1384	*lsa1384*	Two-component system, response regulator		0.5	
Post translational modifications, protein turnover, chaperones					
Protein folding					
LSA0050	*lsa0050*	Putative molecular chaperone, small heat shock protein, Hsp20 family			-0.7
LSA0082	*htrA*	Serine protease HtrA precursor, Trypsin family		-0.6	
LSA0207	*clpL*	ATPase/chaperone ClpL, putative specificity factor for ClpP protease	0.6		
LSA0358	*groS*	Co-chaperonin GroES (10 kD chaperonin) (Protein Cpn10)			-0.5
LSA0359	*groEL*	Chaperonin GroEL (60 kDa chaperonin) (Protein Cpn60)			-0.5
LSA0436	*lsa0436*	Putative peptidylprolyl isomerase (Peptidylprolyl cis-trans isomerase) (PPIase)			-0.6
LSA0984	*hslU*	ATP-dependent Hsl protease, ATP-binding subunit HslU	0.7		0.7
LSA1465	*clpE*	ATPase/chaperone ClpE, putative specificity factor for ClpP protease	-0.7	-0.6	-0.6
LSA1618	*htpX*	Membrane metalloprotease, HtpX homolog		0.8	
Adaption to atypical conditions					
LSA0170	*lsa0170*	Putative general stress protein	0.5		**-1.5**
LSA0247	*usp2*	Similar to universal stress protein, UspA family			-0.5
LSA0264	*lsa0264*	Putative glycine/betaine/carnitine/choline transport protein	-0.6		-0.6
LSA0513	*lsa0513*	Putative stress-responsive transcriptional regulator		-0.8	
LSA0552	*lsa0552*	Organic hydroperoxide resistance protein		0.6	
LSA0616	*lsa0616*	Putative glycine/betaine/carnitine/choline ABC transporter, ATP-binding subunit	0.9		
LSA0617	*lsa0617*	Putative glycine/betaine/carnitine/choline ABC transporter, membrane-spanning subunit	**1.3**		
LSA0618	*lsa0618*	Putative glycine/betaine/carnitine/choline ABC transporter, substrate-binding lipoprotein	0.6		
LSA0619	*lsa0619*	Putative glycine/betaine/carnitine/choline ABC transporter, membrane-spanning subunit	**1.5**	0.5	
LSA0642	*usp3*	Similar to universal stress protein, UspA	0.9		
LSA0768	*csp1*	Similar to cold shock protein, CspA family	**2.1**	0.6	**1.8**
LSA0836	*usp6*	Similar to universal stress protein, UspA family	0.6		
LSA0946	*csp4*	Similar to cold shock protein, CspA family	0.6		
LSA1110	*lsa1110*	Putative NifU-homolog involved in Fe-S cluster assembly		0.6	
LSA1111	*lsa1111*	Putative cysteine desulfurase (Class-V aminotransferase, putative SufS protein homologue)		0.7	
LSA1173	*usp4*	Similar to universal stress protein, UspA family	**1.5**	**-2.1**	
LSA1694	*lsa1694*	Putative glycine/betaine/carnitine ABC transporter, substrate binding lipoprotein precursor	**-1.7**		**-1.1**
LSA1695	*lsa1695*	Putative glycine/betaine/carnitine ABC transporter, membrane-spanning subunit	**-2.1**	**-2.0**	**-1.9**
LSA1696	*lsa1696*	Putative glycine/betaine/carnitine ABC transporter, ATP-binding subunit	**-1.6**		-0.9
LSA1870	*lsa1870*	Putative glycine betaine/carnitine/choline ABC transporter, ATP-binding subunit	-0.6		-0.6
Protein modification					
LSA0865	*lsa0865*	Putative protein methionine sulfoxide reductase		-0.6	

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0866	*msrA*	Protein methionine sulfoxide reductase		-0.7	
LSA0934	*lplA*	Lipoate-protein ligase	**1.6**	**1.4**	**1.0**
LSA0973	*pflA*	Pyruvate formate-lyase activating enzyme	**1.7**		
General function prediction only					
Miscellaneous					
LSA0030	*lsa0030*	Putative aldo/keto reductase (oxidoreductase)		-0.7	-0.8
LSA0120	*lsa0120*	Putative GTP-binding protein	-0.5		
LSA0164	*lsa0164*	Putative serine/tyrosine protein phosphatase	0.2	**-1.1**	**-1.2**
LSA0165	*lsa0165*	Putative oxidoreductase, short chain dehydrogenase/reductase family		-0.9	**-1.2**
LSA0218	*trxA1*	Thioredoxin		-0.9	
LSA0258	*lsa0258*	Putative iron-containing alcohol dehydrogenase	**1.6**	0.5	**1.6**
LSA0260	*lsa0260*	Putative aldo/keto reductase (oxidoreductase)	**1.9**	1.2	**1.7**
LSA0312	*lsa0312*	Putative NADH oxidase	-0.9		**-1.0**
LSA0324	*lsa0324*	Putative hydrolase, haloacid dehalogenase family (N-terminal fragment), authentic frameshift	**1.9**		
LSA0325	*lsa0325*	Putative hydrolase, haloacid dehalogenase family (C-terminal fragment), authentic frameshift	**1.8**		
LSA0350	*lsa0350*	Putative N-acetyltransferase, GNAT family	-0.5		
LSA0369	*lsa0369*	Putative N-acetyltransferase, GNAT family	-0.5		-0.5
LSA0384	*lsa0384*	Putative phosphoesterase, DHH family	-0.5		
LSA0403	*lsa0403*	Putative thioredoxin reductase		0.9	
LSA0447	*lsa0447*	Putative hydrolase, haloacid dehalogenase family			0.6
LSA0475	*lsa0475*	Putative N-acetyltransferase, GNAT family		-0.6	
LSA0520	*trxB2*	Thioredoxin reductase	-0.8		
LSA0575	*npr*	NADH peroxidase	**1.0**	U	
LSA0802	*nox*	NADH oxidase	**1.5**		
LSA0806	*lsa0806*	Putative N-acetyltransferase, GNAT family	0.6		
LSA0831	*lsa0831*	Putative nitroreductase (oxidoreductase)		**1.6**	
LSA0896	*sodA*	Iron/Manganese superoxide dismutase	**3.4**	**1.7**	**1.7**
LSA0925	*adh*	Putative zinc-containg alcohol dehydrogenase (oxidoreductase)	0.5		
LSA0971	*ppa*	Inorganic pyrophosphatase (Pyrophosphate phosphohydrolase)	0.7		
LSA0994	*lsa0994*	Putative GTP-binding protein			0.6
LSA1016	*engA*	Putative GTP-binding protein	0.6		0.7
LSA1045	*obgE*	Putative GTP-binding protein	0.6		
LSA1153	*lsa1153*	Hypothetical protein, CAAX protease family	0.5		
LSA1311	*lsa1311*	Hypothetical protein containing a possible heme/steroid binding domain	0.7	-0.6	
LSA1320	*lsa1320*	Putative NADPH-quinone oxidoreductase		-0.8	
LSA1345	*lsa1345*	Putative hydrolase, haloacid dehalogenase family	0.5		
LSA1349	*lsa1349*	Putative N-acetyltransferase, GNAT family		-0.5	
LSA1365	*lsa1365*	Hypothetical protein		-0.5	-0.7
LSA1368	*lsa1368*	Hypothetical protein	0.9		0.6
LSA1371	*lsa1371*	Hypothetical membrane protein	0.6		
LSA1395	*lsa1395*	Putative zinc-containing alcohol dehydrogenase (oxidoreductase)	0.9		
LSA1427	*lsa1427*	Putative hydrolase, haloacid dehalogenase	**1.3**		0.6
LSA1472	*lsa1472*	Putative N-acetyl transferase, GNAT family	0.6		
LSA1535	*lsa1535*	Putative oxidoreductase	0.5	**1.1**	0.7
LSA1553	*lsa1553*	Putative hydrolase, haloacid dehalogenase family	-0.6		
LSA1559	*lsa1559*	Putative oxidoreductase	0.6	**1.1**	0.7
LSA1702	*lsa1702*	Putative zinc-containing alcohol dehydrogenase (oxidoreductase)	**1.1**		
LSA1712	*lsa1712*	Putative nitroreductase (oxidoreductase)		-0.7	-0.8
LSA1832	*lsa1832*	Putative zinc-containing alcohol dehydrogenase (oxidoreductase)		**1.0**	
LSA1835	*lsa1835*	Putative zinc-containing alcohol dehydrogenase (oxidoreductase)	-0.7		**-1.0**
LSA1867	*lsa1867*	Putative acetyltransferase, isoleucine patch superfamily	-0.5	-0.6	-0.7
LSA1871	*gshR*	Glutathione reductase	-0.6		

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
Unknown					
Proteins of unknown function that are similar to other proteins					
LSA0018	*lsa0018*	Hypothetical protein		0.5	
LSA0027	*lsa0027*	Hypothetical protein			**-1.1**
LSA0028	*lsa0028*	Hypothetical protein, DegV family	-0.5		
LSA0044	*lsa0044*	Hypothetical protein			-0.7
LSA0061	*lsa0061*	Hypothetical extracellular protein precursor	-0.5		
LSA0106	*lsa0106*	Hypothetical cell surface protein precursor	0.5		
LSA0160	*lsa0160*	Hypothetical protein	-0.7		
LSA0166	*lsa0166*	Hypothetical Integral membrane protein			**-1.2**
LSA0190	*lsa0190*	Hypothetical integral membrane protein	-0.7		-0.6
LSA0191	*lsa0191*	Hypothetical integral membrane protein	-0.6		-0.6
LSA0199	*lsa0199*	Hypothetical protein	**1.1**	**1.0**	**1.1**
LSA0208	*lsa0208*	Hypothetical integral membrane protein	0.7		
LSA0235	*lsa0235*	Hypothetical extracellular protein precursor	**2.1**	**1.6**	**1.7**
LSA0236	*lsa0236*	Hypothetical extracellular peptide precursor	**2.0**	**1.3**	**1.5**
LSA0244	*lsa0244*	Hypothetical integral membrane protein			-0.5
LSA0245	*lsa0245*	Hypothetical lipoprotein precursor	-0.9	**-1.0**	**-1.1**
LSA0249	*lsa0249*	Hypothetical protein	**1.1**	**1.0**	
LSA0263	*lsa0263*	Hypothetical integral membrane protein	-0.6		-0.9
LSA0300	*lsa0300*	Hypothetical protein			0.7
LSA0315	*lsa0315*	Hypothetical protein	-0.7		
LSA0319	*lsa0319*	Hypothetical protein		-0.8	-0.8
LSA0323	*lsa0323*	Hypothetical protein			-0.5
LSA0337	*lsa0337*	Hypothetical protein	-0.7		
LSA0348	*lsa0348*	Hypothetical integral membrane protein	-0.9		-0.7
LSA0352	*lsa0352*	Hypothetical integral membrane protein	-0.6		
LSA0354	*lsa0354*	Hypothetical integral membrane protein			**-1.1**
LSA0356	*lsa0356*	Hypothetical protein	-0.8	-0.5	-0.9
LSA0388	*lsa0388*	Hypothetical protein		-0.6	
LSA0389	*lsa0389*	Hypothetical protein		-0.7	-0.7
LSA0390	*lsa0390*	Hypothetical protein		-0.5	
LSA0409	*lsa0409*	Hypothetical integral membrane protein			-0.8
LSA0418	*lsa0418*	Hypothetical protein			-0.8
LSA0464	*lsa0464*	Hypothetical protein		-0.6	
LSA0470	*lsa0470*	Hypothetical protein	0.9		0.7
LSA0512	*lsa0512*	Hypothetical protein		-0.6	
LSA0515	*lsa0515*	Hypothetical integral membrane protein		-0.5	
LSA0536	*lsa0536*	Hypothetical protein		0.7	
LSA0716	*lsa0716*	Hypothetical protein			0.6
LSA0752	*lsa0752*	Hypothetical protein	0.5		0.6
LSA0757	*lsa0757*	Hypothetical protein		0.8	
LSA0773	*lsa0773*	Hypothetical protein	0.9		0.6
LSA0784	*lsa0784*	Hypothetical protein	**-2.6**		
LSA0786	*lsa0786*	Hypothetical protein	**-2.0**		
LSA0787	*lsa0787*	Hypothetical protein	**-1.7**		
LSA0790	*lsa0790*	Hypothetical protein, ATP utilizing enzyme PP-loop family	**-2.5**		
LSA0827	*lsa0827*	Hypothetical lipoprotein precursor	0.8		U
LSA0828	*lsa0828*	Hypothetical protein	0.7		
LSA0829	*lsa0829*	Hypothetical integral membrane protein			0.5
LSA0848	*lsa0848*	Hypothetical protein	**1.6**	0.7	
LSA0874	*lsa0874*	Hypothetical protein	0.5		
LSA0901	*lsa0901*	Hypothetical protein			0.5
LSA0913	*lsa0913*	Hypothetical extracellular protein precursor	0.5		0.7
LSA0919	*lsa0919*	Hypothetical protein			0.7
LSA0933	*lsa0933*	Hypothetical protein	0.6		0.6
LSA0961	*lsa0961*	Hypothetical protein, DegV family		-0.5	

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0968	*lsa0968*	Hypothetical integral membrane protein	0.7		
LSA0977	*lsa0977*	Hypothetical integral membrane protein	0.7		0.8
LSA0987	*lsa0987*	Hypotehtical protein, GidA family (C-terminal fragment)	0.5		
LSA0996	*lsa0996*	Hypothetical protein			0.5
LSA1003	*lsa1003*	Hypothetical protein	**2.0**		**1.2**
LSA1005	*lsa1005*	Hypothetical membrane protein	0.9	0.6	0.7
LSA1008	*lsa1008*	Putative extracellular chitin-binding protein precursor		0.9	**1.2**
LSA1027	*lsa1027*	Hypothetical protein			0.6
LSA1047	*lsa1047*	Hypothetical protein	**3.5**	**1.2**	**1.3**
LSA1064	*lsa1064*	Hypothetical protein	0.5		0.7
LSA1075	*lsa1075*	Hypothetical protein			0.5
LSA1078	*lsa1078*	Hypothetical protein			0.6
LSA1081	*lsa1081*	Hypothetical protein	**1.0**		**1.0**
LSA1091	*lsa1091*	Hypothetical protein			0.6
LSA1096	*lsa1096*	Hypothetical protein	0.6		
LSA1124	*lsa1124*	Hypothetical protein		-0.7	
LSA1154	*lsa1154*	Hypothetical protein	0.6		0.6
LSA1158	*lsa1158*	Hypothetical protein	**1.7**	**1.4**	
LSA1189	*lsa1189*	Hypothetical integral membrane protein	**-1.6**		**-1.1**
LSA1282	*lsa1282*	Hypothetical protein		-0.5	
LSA1296	*lsa1296*	Hypothetical integral membrane protein		**-1.2**	-0.8
LSA1342	*lsa1342*	Hypothetical protein		-0.7	
LSA1346	*lsa1346*	Hypothetical protein	0.8		
LSA1350	*lsa1350*	Hypothetical protein		-0.6	**-1.0**
LSA1353	*lsa1353*	Hypothetical integral membrane protein	-0.9	-0.5	
LSA1446	*lsa1446*	Hypothetical protein	-0.6	-0.6	-0.7
LSA1466	*lsa1466*	Hypothetical protein	0.6		
LSA1467	*lsa1467*	Hypothetical protein		-0.6	**-1.1**
LSA1524	*lsa1524*	Hypothetical protein	0.7		
LSA1540	*lsa1540*	Hypothetical extracellular protein precursor	0.7		
LSA1563	*lsa1563*	Hypothetical integral membrane protein		-0.6	-0.6
LSA1610	*lsa1610*	Hypothetical integral membrane protein	-0.7		-0.9
LSA1617	*lsa1617*	Hypothetical protein			-0.7
LSA1620	*lsa1620*	Hypothetical protein			-0.6
LSA1623	*lsa1623*	Hypothetical integral membrane protein	-0.5		-0.6
LSA1637	*lsa1637*	Hypothetical integral membrane protein, TerC family	**-1.7**	**-1.0**	**-1.6**
LSA1644	*lsa1644*	Hypothetical protein	**1.7**		D
LSA1649	*lsa1649*	Hypothetical extracellular protein precursor			-0.5
LSA1659	*lsa1659*	Hypothetical protein	-0.5		
LSA1662	*lsa1662*	Hypothetical protein	**-1.0**	-0.6	-0.7
LSA1663	*lsa1663*	Hypothetical protein	-0.8		
LSA1678	*lsa1678*	Hypothetical protein	-0.6		
LSA1680	*lsa1680*	Hypothetical protein	-0.6		
LSA1716	*lsa1716*	Hypothetical protein		-0.5	
LSA1822	*lsa1822*	Hypothetical protein			-0.5
LSA1828	*lsa1828*	Hypothetical integral membrane protein	0.6	0.7	
LSA1850	*lsa1850*	Hypothetical protein		-0.6	
LSA1876	*lsa1876*	Hypothetical integral membrane protein			-0.6
LSA1877	*lsa1877*	Hypothetical protein			-0.6
Proteins of unknown function only similar to other proteins from the same organism					
LSA1159	*lsa1159*	Hypothetical cell surface protein precursor	**2.0**		0.5
LSA1165	*lsa1165*	Hypothetical cell surface protein precursor	**1.8**		
LSA1700	*lsa1700*	Hypothetical protein	**2.1**	0.8	
LSA1814	*lsa1814*	Hypothetical protein			-0.5
Proteins of unknown function. without similarity to other proteins					
LSA0065	*lsa0065*	Hypothetical integral membrane protein	-0.5		
LSA0093	*lsa0093*	Hypothetical integral membrane protein	-0.9		**-1.2**

Continued on following page

Table 1. *Continued*

Gene locus	Gene	Description	23K	MF1053	LS 25
LSA0121	*lsa0121*	Hypothetical small peptide	-0.7	-0.6	-0.5
LSA0163	*lsa0163*	Hypothetical protein		**-1.1**	**-1.3**
LSA0167	*lsa0167*	Hypothetical protein			**-1.4**
LSA0168	*lsa0168*	Hypothetical protein			**-1.4**
LSA0188	*lsa0188*	Hypothetical small peptide			-0.8
LSA0256_a	*lsa0256_a*	Hypothetical protein	**2.3**	**1.0**	**2.2**
LSA0257	*lsa0257*	Hypothetical protein	**1.4**		
LSA0281	*lsa0281*	Hypothetical lipoprotein precursor		-0.5	-0.6
LSA0301	*lsa0301*	Hypothetical protein			0.6
LSA0334	*lsa0334*	Hypothetical extracellular protein precursor	**1.1**		
LSA0339	*lsa0339*	Hypothetical protein	-0.5		
LSA0378	*lsa0378*	Hypothetical protein	-0.7		
LSA0514	*lsa0514*	Hypothetical small extracellular protein precursor		-0.8	
LSA0534	*lsa0534*	Hypothetical cell surface protein precursor (with LPQTG sorting signal)	**1.0**		D
LSA0576	*lsa0576*	Hypothetical protein	0.5	D	
LSA0641	*lsa0641*	Hypothetical extracellular peptide precursor		-0.5	
LSA0647	*lsa0647*	Hypothetical extracellular protein precursor	0.6		
LSA0667	*lsa0667*	Hypothetical protein	**1.0**		0.9
LSA0753	*lsa0753*	Hypothetical integral membrane protein			0.5
LSA0789	*lsa0789*	Hypothetical protein	**-1.9**		
LSA0837	*lsa0837*	Hypothetical protein	**1.2**	**1.3**	**1.4**
LSA0885	*lsa0885*	Hypothetical protein	**1.8**		
LSA0902	*lsa0902*	Hypothetical protein	0.7	D	
LSA0945	*lsa0945*	Hypothetical protein			0.9
LSA1019	*lsa1019*	Hypothetical cell surface protein precursor			0.8
LSA1035	*lsa1035*	Hypothetical small integral membrane protein			0.6
LSA1086	*lsa1086*	hypothetical protein	0.8		0.5
LSA1104	*lsa1104*	Hypothetical protein	-0.5		
LSA1155	*lsa1155*	Hypothetical integral membrane protein	0.5		
LSA1174	*lsa1174*	Hypothetical protein	**1.0**		
LSA1176	*lsa1176*	Hypothetical protein		**-1.0**	U
LSA1319	*lsa1319*	Hypothetical small protein		-0.8	
LSA1408	*lsa1408*	Hypothetical protein			0.6
LSA1464	*lsa1464*	Hypothetical protein	-0.6		
LSA1478	*lsa1478*	Hypothetical protein	-0.7	-0.6	-0.6
LSA1480	*lsa1480*	Hypothetical membrane protein	0.5	D	
LSA1524	*lsa1524*	Hypothetical protein	0.8		
LSA1539	*lsa1539*	Hypothetical protein	0.9		
LSA1713	*lsa1713*	Hypothtical small peptide			-0.6
LSA1787	*lsa1787*	Hypothetical cell surface protein precursor	-0.5	U	
LSA1820	*lsa1820*	Hypothetical cell surface protein precursor			-0.6
LSA1821	*lsa1821*	Hypothetical cell surface protein precursor		-0.6	
LSA1845	*lsa1845*	Hypothetical small protein		0.8	
LSA1848	*lsa1848*	Hypothetical protein			-0.5
LSA1851	*lsa1851*	Hypothetical extracellular small protein	-0.6		-0.7
LSA1883	*lsa1883*	Hypothetical small protein	**1.2**		**1.5**
Bacteriocin associated genes					
SKP0001	*sppIP*	Bacteriocin sakacin P inducing peptide	D	0.5	D
SKP0006	*sppT*	Sakacin P ABC transporter	D	0.6	D
SKP0007	*sppE*	Sakacin P accesory transport protein	D	0.6	D

Table 2. Putative *cre* sites present in the promoter region of some *L. sakei* genes up-regulated in the present study. The identification is based on the genome sequence of *L. sakei* strain 23K, and the consensus sequence TGWNAN**CG**NTNWCA (W=A/T, N=A/T/G/C), confirmed in Gram-positive bacteria [32] was used in the search, allowing up to two mismatches (underlined) in the conserved positions except for the two center positions, highlighted in boldface.

Gene locus	Gene	*cre* site sequence[a]	Position[b]	Co-transcribed genes/operon[c]	Gene locus
LSA0123	*lsa0123*	TGAAAG**CG**TTACAA	-93		
LSA0185	*galP*	GAACAT**CG**TTATCA	-46		
LSA0200	*rbsU*	GTAAAC**CG**TTTTCA	-113	*rbsUDK*	LSA0200-0202
LSA0254	*lsa0254*	TGTAAG**CG**TTTTAT	-56	*lsa0254-lsa0255-lsa0256_a*	LSA0254-0256_a
LSA0289	*xpk*	CTATTA**CG**ATGACA	-8		
LSA0292	*budC*	TGTAAC**CG**TTTTAA	-51		
LSA0353	*lsa0353*	AGAAAG**CG**CTTATA	-102		
LSA0370	*arcA*	TGAAAG**CG**ATTACC	-58	*arcA-arcB*[e]*-arcC-arcT*[e]*-arcD*[e]	LSA0370-0374
LSA0449	*manL*	TGTTAG**CG**TTTTTA	-56	*manL-manM-manN*	LSA0449-0451
LSA0533	*iunH2*	AAAAAG**CG**TTCACA	-35		
LSA0572	*tdcB*	TGAAAA**CG**TTCTAA	-134		
LSA0608	*gloAN*	TGTAAC**CG**TTTTAA	-100	*gloAN-gloAC*	LSA0608-0609
LSA0649	*glpK*	AGGAAA**CG**TTTTCC	-42	*glpK-glpD-glpF*	LSA0649-0651
LSA0664	*loxL1*	AGAAAG**CG**AGTACA	-82	*loxL1N-loxLI-loxL1C*	LSA0664-0666
LSA0764	*galK*	TGAAAG**CG**ATTAAT	-30	*galK-galE1-galT-galM*	LSA0764-0767
LSA0795	*deoC*	TGAAAG**CG**TTAACA	-33	*deoC-deoB-deoD-lsa0798-lsa0799-deoR-pdp*	LSA0795-0801
LSA0974	*pflB*	TACGAA**CG**CTTACA	-147	*pflB-pflA*	LSA0974-0973
LSA1048	*fruR*[e]	TGTAAA**CG**ATGACA	-39	*fruR*[e]*-fruK*[e]*-fruA*	LSA1048-1050
LSA1141	*ppdK*	GGTTAT**CG**ATAAAA	-29		
LSA1146	*manA*	CGAAAT**CG**CTTTAA	-98		
LSA1188	*pox1*	TGTAAT**CG**ATTTCA	-88		
LSA1204	*lsa1204*	TGTAAT**CG**TTTTTT	-127		
LSA1343	*eutD*	GTAAAA**CG**CTCTCA	-94		
LSA1399	*loxL2*	TGTAAA**CG**ATTTCA	-42		
LSA1457	*lsa1457*	TGATAA**CG**CTTACA	-85		
LSA1463[d]	*ptsH*	TGAAAG**CG**GTATAG	-161	*ptsHI*	LSA1463-1462
LSA1641	*nanE*	TGTAAG**CG**GTTAAT	-85	*nanE-nanA*	LSA1641-1640
LSA1643	*lsa1643*	TGATAA**CG**CTTACA	-31		
LSA1651	*lsa1651*	GGTAAG**CG**GTTAAA	-148		
LSA1711	*lacL*	TGAAAC**CG**TTTTAA	-36	*lacL-lacM*	LSA1711-1710
LSA1792	*scrA*	TGTAAA**CG**GTTGTA	-78	*scrA-dexB-scrK*	LSA1792-1790
LSA1830	*pox2*	TTGTAA**CG**CTTACA	-70		

[a] mismatch to consensus sequence is underlined

[b] position of *cre* in relation to the start codon

[c] suggested co-transcribed genes or genes organized in an operon

[d] *cre* in preceding gene encoding hypothetical protein

[e] gene not regulated in this study

Abbreviations

PKP, phosphoketolase pathway; PEP, phosphoenolpyruvate; PTS, PEP-dependent carbohydrate phosphotransferase system; CCR, carbon catabolite repression; cre, catabolite responsive element; RbsK, ribokinase; RbsD, D-Ribose pyranase; Xpk, xylulose-5-phosphate phosphoketolase; Ack, Acetate kinase, Pfk, 6-phosphofructokinase; Pyk, pyruvate kinase; PDC, pyruvate dehydrogenase complex; GlpD, glycerol-3-phosphate dehydrogenase; GlpK, glycerol kinase; EII, enzyme II; EI, enzyme I, HPr, histidine protein; HPrK/P, HPr kinase/phosphatase; DeoR, deoxyribonucleoside synthesis operon transcriptional regulator.

Author's contributions

AM participated in the study design, conducted the experimental work, analyzed and interpreted data, and wrote the manuscript. LS conducted the statistical analysis. KN and LA conceived the study, participated in the study design process and reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was financially supported by Grant 159058/I10 from the Norwegian Research Council. The authors would like to thank Monique Zagorec for helpful suggestions and critically reading the manuscript. We also thank Margrete Solheim and Mari Christine Brekke for their assistance during the microarray experiments, and Hallgeir Bergum, the Norwegian Microarray Consortium (NMC), for printing the microarray slides.

References

1. Hammes WP, Bantleon A, Min S: **Lactic acid bacteria in meat fermentation.** *FEMS Microbiol Rev* 1990, **87:**165-174.
2. Hammes WP, Hertel C: **New developments in meat starter cultures.** *Meat Science* 1998, **49:**125-138.
3. Bredholt S, Nesbakken T, Holck A: **Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat.** *Int J Food Microbiol* 1999, **53:**43-52.
4. Bredholt S, Nesbakken T, Holck A: **Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats.** *Int J Food Microbiol* 2001, **66:**191-196.
5. Katikou P, Georgantelis D, Paleologos EK, Ambrosiadis I, Kontominas MG: **Relation of biogenic amines' formation with microbiological and sensory attributes in *Lactobacillus*-inoculated vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets.** *J Agric Food Chem* 2006, **54:**4277-4283.
6. Vermeiren L, Devlieghere F, Debevere J: **Evaluation of meat born lactic acid bacteria as protective cultures for biopreservation of cooked meat products.** *Int J Food Microbiol* 2004, **96:**149-164.
7. Chaillou S, Champomier-Verges MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V, Beaufils S, Darbon-Rongere E, Bossy R, Loux V, Zagorec M: **The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K.** *Nat Biotechnol* 2005, **23:**1527-1533.
8. Lauret R, Morel-Deville F, Berthier F, Champomier-Verges M, Postma P, Ehrlich SD, Zagorec M: **Carbohydrate utilization in *Lactobacillus sake*.** *Appl Environ Microbiol* 1996, **62:**1922-1927.
9. McLeod A, Nyquist OL, Snipen L, Naterstad K, Axelsson L: **Diversity of *Lactobacillus sakei* strains investigated by phenotypic and genotypic methods.** *Syst Appl Microbiol* 2008, **31:**393-403.
10. Stentz R, Lauret R, Ehrlich SD, Morel-Deville F, Zagorec M: **Molecular cloning and analysis of the *ptsHI* operon in *Lactobacillus sake*.** *Appl Environ Microbiol* 1997, **63:**2111-2116.

11. Stentz R, Cornet M, Chaillou S, Zagorec M: **Adaption of *Lactobacillus sakei* to meat: a new regulatory mechanism of ribose utilization?** *INRA, EDP Sciences* 2001, **81:**131-138.
12. Stentz R, Zagorec M: **Ribose utilization in *Lactobacillus sakei*: analysis of the regulation of the *rbs* operon and putative involvement of a new transporter.** *J Mol Microbiol Biotechnol* 1999, **1:**165-173.
13. Claesson MJ, van Sinderen D, O'Toole PW: **The genus *Lactobacillus* - a genomic basis for understanding its diversity.** *FEMS Microbiol Lett* 2007, **269:**22-28.
14. McLeod A, Zagorec M, Champomier Verges MC, Naterstad K, Axelsson L: **Primary metabolism in *Lactobacillus sakei* by proteomic analysis.** *BMC Microbiol* 2010.
15. Stulke J, Hillen W: **Carbon catabolite repression in bacteria.** *Curr Opin Microbiol* 1999, **2:**195-201.
16. Deutscher J, Francke C, Postma PW: **How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria.** *Microbiol Mol Biol Rev* 2006, **70:**939-1031.
17. Titgemeyer F, Hillen W: **Global control of sugar metabolism: a gram-positive solution.** *Antonie Van Leeuwenhoek* 2002, **82:**59-71.
18. Schumacher MA, Allen GS, Diel M, Seidel G, Hillen W, Brennan RG: **Structural basis for allosteric control of the transcription regulator CcpA by the phosphoprotein HPr-Ser46-P.** *Cell* 2004, **118:**731-741.
19. Obst M, Hehn R, Vogel RF, Hammes WP: **Lactose metabolism in *Lactobacillus curvatus* and *Lactobacillus sake*.** *FEMS Microbiol Lett* 1992, **97:**209-214.
20. Zuniga M, Champomier-Verges M, Zagorec M, Perez-Martinez G: **Structural and functional analysis of the gene cluster encoding the enzymes of the arginine deiminase pathway of *Lactobacillus sake*.** *J Bacteriol* 1998, **180:**4154-4159.
21. Montel MC, Champomier MC: **Arginine catabolism in *Lactobacillus sake* isolated from meat.** *Appl Environ Microbiol* 1987, **53:**2683-2685.
22. Rodionov DA, Mironov AA, Gelfand MS: **Transcriptional regulation of pentose utilisation systems in the *Bacillus/Clostridium* group of bacteria.** *FEMS Microbiol Lett* 2001, **205:**305-314.
23. Berthier F, Zagorec M, Champomier-Verges MC, Ehrlich SD, Morel-Deville F: **Efficient transformation of *Lactobacillus sake* by electroporation.** *Microbiol* 1996, **142:**1273-1279.

24. Hagen BF, Naes H, Holck AL: **Meat starters have individual requirements for Mn^{2+}.** *Meat Science* 2000, **55:**161-168.
25. Moretro T, Hagen BF, Axelsson L: **A new, completely defined medium for meat lactobacilli.** *J Appl Microbiol* 1998, **85:**715-722.
26. Nyquist OL, McLeod A, Brede DA, Snipen L, Nes IF: **Comparative genomics of *Lactobacillus sakei* with emphasis on strains from meat.** *This thesis* 2010.
27. Rud I: **Transcriptome analysis of CggR (Central glycolytic gene Regulator) in *Lactobacillus plantarum*.** In *PhD thesis: Primary metabolism in Lactobacillus - a study of control and regulation of acid production.* Ås, Norway: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences; 2008
28. Vebø HC: **Comparative genomic analysis of pathogenic and probiotic *Enterococcus faecalis* isolates, and their transcriptional responses to growth in human urine.** In *PhD thesis: An explorative study of Enterococcus faecalis transcriptional responses to infection-relavant growth environments.* Ås, Norway: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences; 2010
29. Smyth GK, Speed T: **Normalization of cDNA microarray data.** *Methods* 2003, **31:**265-273.
30. Smyth GK: **Linear models and empirical bayes methods for assessing differential expression in microarray experiments.** *Stat Appl Genet Mol Biol* 2004, **3:**Article3.
31. Smyth GK, Michaud J, Scott HS: **Use of within-array replicate spots for assessing differential expression in microarray experiments.** *Bioinformatics* 2005, **21:**2067-2075.
32. Weickert MJ, Chambliss GH: **Site-directed mutagenesis of a catabolite repression operator sequence in *Bacillus subtilis*.** *Proc Natl Acad Sci U S A* 1990, **87:**6238-6242.
33. Champomier-Verges MC, Chaillou S, Cornet M, Zagorec M: **Erratum to "*Lactobacillus sakei*: recent developments and future prospects".** *Res Microbiol* 2002, **153:**115-123.
34. Lorquet F, Goffin P, Muscariello L, Baudry JB, Ladero V, Sacco M, Kleerebezem M, Hols P: **Characterization and functional analysis of the *poxB* gene, which encodes pyruvate oxidase in *Lactobacillus plantarum*.** *J Bacteriol* 2004, **186:**3749-3759.

35. Muscariello L, Marasco R, De Felice M, Sacco M: **The functional *ccpA* gene is required for carbon catabolite repression in *Lactobacillus plantarum*.** *Appl Environ Microbiol* 2001, **67:**2903-2907.

36. Branny P, De La Torre F, Garel JR: **Cloning, sequencing, and expression in *Escherichia coli* of the gene coding for phosphofructokinase in *Lactobacillus bulgaricus*.** *J Bacteriol* 1993, **175:**5344-5349.

37. Viana R, Perez-Martinez G, Deutscher J, Monedero V: **The glycolytic genes *pfk* and *pyk* from *Lactobacillus casei* are induced by sugars transported by the phosphoenolpyruvate:sugar phosphotransferase system and repressed by CcpA.** *Arch Microbiol* 2005, **183:**385-393.

38. Kandler O: **Carbohydrate metabolism in lactic acid bacteria.** *Antonie Van Leeuwenhoek* 1983, **49:**209-224.

39. Naterstad K, Rud I, Kvam I, Axelsson L: **Characterisation of the *gap* operon from *Lactobacillus plantarum* and *Lactobacillus sakei*.** *Curr Microbiol* 2007, **54:**180-185.

40. Kim I, Kim E, Yoo S, Shin D, Min B, Song J, Park C: **Ribose utilization with an excess of mutarotase causes cell death due to accumulation of methylglyoxal.** *J Bacteriol* 2004, **186:**7229-7235.

41. Weber J, Kayser A, Rinas U: **Metabolic flux analysis of *Escherichia coli* in glucose-limited continuous culture. II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behaviour.** *Microbiology* 2005, **151:**707-716.

42. Totemeyer S, Booth NA, Nichols WW, Dunbar B, Booth IR: **From famine to feast: the role of methylglyoxal production in *Escherichia coli*.** *Mol Microbiol* 1998, **27:**553-562.

43. Malleret C, Lauret R, Ehrlich SD, Morel-Deville F, Zagorec M: **Disruption of the sole *ldhL* gene in *Lactobacillus sakei* prevents the production of both L- and D-lactate.** *Microbiology* 1998, **144:**3327-3333.

44. Axelsson L: **Lactic acid bacteria: classification and physiology.** In *Lactic acid bacteria: microbiological and functional aspects.* Third revised and expanded edition. Edited by Salminen S, von Wright A, Ouwehand A. New York, USA: Marcel Dekker, Inc./CRC Press; 2004: 1-66

45. Condon S: **Responses of lactic acid bacteria to oxygen.** *FEMS Microbiol Rev* 1987, **46:**269-280.

46. Fridovich I: **The biology of oxygen radicals.** *Science* 1978, **201:**875-880.

47. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS: **Comparative genomics of thiamin biosynthesis in procaryotes. New genes and regulatory mechanisms.** *J Biol Chem* 2002, **277:**48949-48959.

48. Jordan A, Reichard P: **Ribonucleotide reductases.** *Annu Rev Biochem* 1998, **67:**71-98.

49. Keeney KM, Stuckey JA, O'Riordan MX: **LplA1-dependent utilization of host lipoyl peptides enables Listeria cytosolic growth and virulence.** *Mol Microbiol* 2007, **66:**758-770.

50. Marceau A, Zagorec M, Chaillou S, Mera T, Champomier-Verges MC: **Evidence for involvement of at least six proteins in adaptation of *Lactobacillus sakei* to cold temperatures and addition of NaCl.** *Appl Environ Microbiol* 2004, **70:**7260-7268.

51. Grogan DW, Cronan JE, Jr.: **Cyclopropane ring formation in membrane lipids of bacteria.** *Microbiol Mol Biol Rev* 1997, **61:**429-441.

52. Schujman GE, Guerin M, Buschiazzo A, Schaeffer F, Llarrull LI, Reh G, Vila AJ, Alzari PM, de Mendoza D: **Structural basis of lipid biosynthesis regulation in Gram-positive bacteria.** *Embo J* 2006, **25:**4074-4083.

53. Mahr K, Hillen W, Titgemeyer F: **Carbon catabolite repression in *Lactobacillus pentosus*: analysis of the *ccpA* region.** *Appl Environ Microbiol* 2000, **66:**277-283.

54. Nentwich SS, Brinkrolf K, Gaigalat L, Huser AT, Rey DA, Mohrbach T, Marin K, Puhler A, Tauch A, Kalinowski J: **Characterization of the LacI-type transcriptional repressor RbsR controlling ribose transport in *Corynebacterium glutamicum* ATCC 13032.** *Microbiology* 2009, **155:**150-164.

55. Muller W, Horstmann N, Hillen W, Sticht H: **The transcription regulator RbsR represents a novel interaction partner of the phosphoprotein HPr-Ser46-P in *Bacillus subtilis*.** *Febs J* 2006, **273:**1251-1261.

56. Perez-Rueda E, Collado-Vides J: **The repertoire of DNA-binding transcriptional regulators in *Escherichia coli* K-12.** *Nucleic Acids Res* 2000, **28:**1838-1847.

57. Brinkrolf K, Ploger S, Solle S, Brune I, Nentwich SS, Huser AT, Kalinowski J, Puhler A, Tauch A: **The LacI/GalR family transcriptional regulator UriR negatively controls uridine utilization of *Corynebacterium glutamicum* by binding to catabolite-responsive element (*cre*)-like sequences.** *Microbiology* 2008, **154:**1068-1081.

58. Saxild HH, Andersen LN, Hammer K: ***dra-nupC-pdp* operon of *Bacillus subtilis*: nucleotide sequence, induction by deoxyribonucleosides, and transcriptional**

regulation by the *deoR*-encoded DeoR repressor protein. *J Bacteriol* 1996, **178:**424-434.

59. Zeng X, Saxild HH: **Identification and characterization of a DeoR-specific operator sequence essential for induction of *dra-nupC-pdp* operon expression in *Bacillus subtilis*.** *J Bacteriol* 1999, **181:**1719-1727.

60. Zeng X, Saxild HH, Switzer RL: **Purification and characterization of the DeoR repressor of *Bacillus subtilis*.** *J Bacteriol* 2000, **182:**1916-1922.

61. Schuch R, Garibian A, Saxild HH, Piggot PJ, Nygaard P: **Nucleosides as a carbon source in *Bacillus subtilis*: characterization of the *drm-pupG* operon.** *Microbiology* 1999, **145:**2957-2966.

62. Posthuma CC, Bader R, Engelmann R, Postma PW, Hengstenberg W, Pouwels PH: **Expression of the xylulose 5-phosphate phosphoketolase gene, *xpkA*, from Lactobacillus pentosus MD363 is induced by sugars that are fermented via the phosphoketolase pathway and is repressed by glucose mediated by CcpA and the mannose phosphoenolpyruvate phosphotransferase system.** *Appl Environ Microbiol* 2002, **68:**831-837.

63. Charrier V, Buckley E, Parsonage D, Galinier A, Darbon E, Jaquinod M, Forest E, Deutscher J, Claiborne A: **Cloning and sequencing of two enterococcal *glpK* genes and regulation of the encoded glycerol kinases by phosphoenolpyruvate-dependent, phosphotransferase system-catalyzed phosphorylation of a single histidyl residue.** *J Biol Chem* 1997, **272:**14166-14174.

64. Darbon E, Servant P, Poncet S, Deutscher J: **Antitermination by GlpP, catabolite repression via CcpA and inducer exclusion triggered by P-GlpK dephosphorylation control *Bacillus subtilis glpFK* expression.** *Mol Microbiol* 2002, **43:**1039-1052.

65. Veyrat A, Gosalbes MJ, Perez-Martinez G: ***Lactobacillus curvatus* has a glucose transport system homologous to the mannose family of phosphoenolpyruvate-dependent phosphotransferase systems.** *Microbiology* 1996, **142 (Pt 12):**3469-3477.

66. Chaillou S, Postma PW, Pouwels PH: **Contribution of the phosphoenolpyruvate:mannose phosphotransferase system to carbon catabolite repression in *Lactobacillus pentosus*.** *Microbiology* 2001, **147:**671-679.

67. Barrangou R, Azcarate-Peril MA, Duong T, Conners SB, Kelly RM, Klaenhammer TR: **Global analysis of carbohydrate utilization by *Lactobacillus acidophilus* using cDNA microarrays.** *Proc Natl Acad Sci U S A* 2006, **103:**3816-3821.

68. Veyrat A, Monedero V, Perez-Martinez G: **Glucose transport by the phosphoenolpyruvate:mannose phosphotransferase system in *Lactobacillus casei* ATCC 393 and its role in carbon catabolite repression.** *Microbiology* 1994, **140:**1141-1149.

69. Viana R, Monedero V, Dossonnet V, Vadeboncoeur C, Perez-Martinez G, Deutscher J: **Enzyme I and HPr from *Lactobacillus casei*: their role in sugar transport, carbon catabolite repression and inducer exclusion.** *Mol Microbiol* 2000, **36:**570-584.