Chapter 1

Updates on Metabolism in Lactic Acid Bacteria in Light of "Omic" Technologies

Magdalena Kowalczyk[1], Baltasar Mayo[2], María Fernández[2], and Tamara Aleksandrzak-Piekarczyk[1,*]

[1] *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland*
[2] *Departamento de Microbiología y Bioquímica, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Spain*

1.1. Sugar Metabolism

Sugars are the primary carbon and energy source for LAB that are grown for fermented food and feed production as well as in laboratory media. Many different transport systems are involved in LAB carbohydrate uptake, including phosphotransferase systems (PTS), ATP-binding cassettes (ABC), and glycoside–pentoside–hexuronide transporters. In *Lactococcus lactis*, glucose is imported by either a mannose or cellobiose PTS or one or more non-PTS permease(s) (Castro et al. 2009). LAB prefer glucose but can also metabolize several common hexoses; however, the ability to ferment other sugars is strain dependent. Dairy LAB can use milk's most abundant sugar, lactose, as a carbon source, whereas plant-associated bacteria utilize a large variety of other carbohydrates, including β-glucosides (Aleksandrzak-Piekarczyk 2013). Furthermore, phenotypic and genotypic analyses of twenty *L. lactis* subsp. *lactis* and *cremoris* genotypes showed strain-to-strain variations (Fernandez et al. 2011). These two groups had distinctive carbohydrate fermentation and enzyme activity profiles with *cremoris* genotypes exhibiting broader profiles.

Monosaccharides incorporated by the cell or liberated in the cytoplasm by disaccharide hydrolysis enter glycolysis at the glucose-6P (G6P) level or are processed by the Leloir pathway (Figure 1.1). In *L. lactis*, lactose that is transported by the PTS system is hydrolyzed to galactose-6P, transformed by the tagatose pathway (Tag6P), and then enters glycolysis at the triose phosphate level. In some LAB, only the glucose moiety of lactose is fermented, while the galactose moiety is excreted; this leads to galactose accumulation in the medium, which yields poor-quality dairy products (Neves et al. 2010; Aleksandrzak-Piekarczyk, 2013). Galactose can be imported by the non-PTS permease GalP and metabolized via the Leloir pathway (*galMKTE*). Alternatively, galactose can be imported by PTS^{Lac} (*lacFE*) and further metabolized to triose phosphates by the Tag6P pathway (*lacABCD*). Recently, an alternative uptake route was discovered that consists of galactose translocation via the

* Corresponding author: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland. tamara@ibb.waw.pl

Biotechnology of Lactic Acid Bacteria: Novel Applications, Second Edition. Edited by Fernanda Mozzi, Raúl R. Raya, and Graciela M. Vignolo.

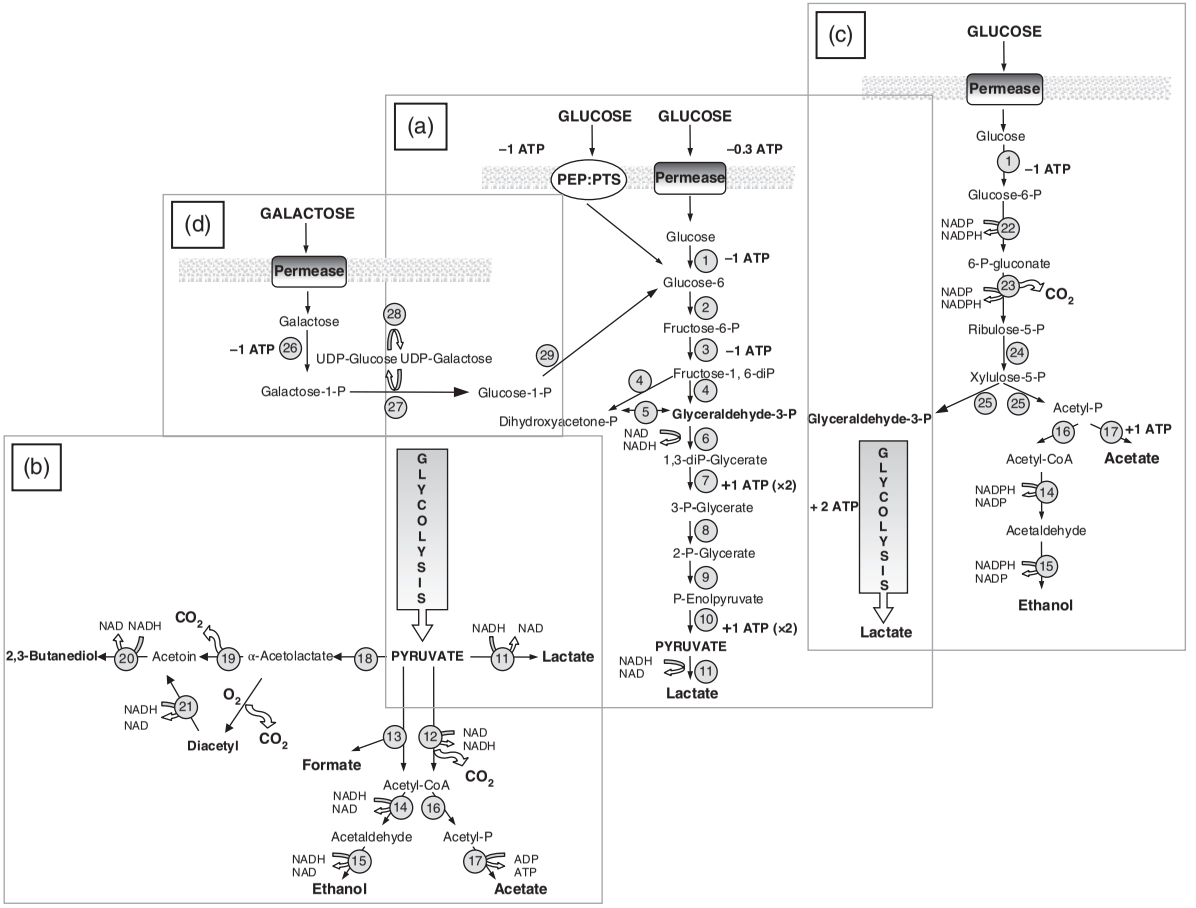

Figure 1.1. Pathways of glucose metabolism. (a) Homofermentative pathway. (b) Mixed-acid metabolism. (c) Heterofermentative pathway. (d) Leloir pathway. Reactions are catalyzed by the following enzymes: 1, glucokinase (GLK); 2, glucose-phosphate isomerase (GPI); 3, phosphofructokinase (PFK); 4, fructose-bisphosphate aldolase (FBPA); 5, triose-phosphate isomerase (TPI); 6, glyceraldehyde-phosphate dehydrogenase (GAPDH); 7, phosphoglycerate kinase (PGK); 8, phosphoglycerate mutase (PMG); 9, enolase (ENO); 10, pyruvate kinase (PK); 11, lactate dehydrogenase (LDH); 12, pyruvate dehydrogenase (PDH); 13, pyruvate formate lyase (PFL); 14, acetaldehyde dehydrogenase (ACDH); 15, alcohol dehydrogenase (ADHE); 16, phosphotransacetylase (PTA); 17, acetate kinase (ACK); 18, α-acetolactate synthase (ALS); 19, α-acetolactate decarboxylase (ALD); 20, 2,3-butanediol dehydrogenase (BDH); 21, diacetyl reductase (DR); 22, glucose-6-P dehydrogenase (G6PDH); 23, 6-P-gluconate dehydrogenase (6PGDH); 24, ribulose-5-P-3-epimerase (RPPE); 25, D-xylulose-5P phosphoketolase (XPK); 26, galactokinase (GK); 27, galactose-1-P-uridylyltransferase (GPUDLT); 28, UDP-galactose-1-epimerase (UDPE); 29, phosphoglucomutase (PGM).

galactose PTS, followed by Gal6P dephosphorylation to galactose, which is further metabolized via the Leloir pathway (Neves et al. 2010). This knowledge has been used to genetically engineer strains that enhance galactose consumption rates by up to 50% (Neves et al. 2010).

Sugar fermentation leads to the formation of lactic acid alone or in combination with other organic acids and ethanol. Variations in the metabolic products of LAB have yielded three categories of fermentation: homofermentation, mixed-acid metabolism, and heterofermentation (Figure 1.1). These three types of fermentation and their regulatory mechanisms have been comprehensively reviewed in the first edition of this book (Mayo et al. 2010). Currently, the control and regulation of glycolytic flux in LAB are not fully understood (Martinussen et al. 2013). The control of glycolytic flux in *L. lactis* is not due to the actions of a single enzyme, sugar transport, or ATP-dependent mechanism. However, a combination of these mechanisms cannot be ruled out as a possible explanation (Martinussen et al. 2013).

In recent years, studies have shifted from digestible disaccharides to indigestible higher oligosaccharides as interest in intestinal microbial ecology and the commercial use of prebiotic oligosaccharides has emerged. Mono- and disaccharide metabolism is well understood; however, few data are available on the metabolism of higher oligosaccharides, which are abundant in cereals, milk, fruits, and the upper intestinal tract of animals. The metabolism of four major oligosaccharide groups have been examined in detail: (i) starch, maltodextrins, and isomalto-oligosaccharides (IMO); (ii) fructo-oligosaccharides (FOS); (iii) β-galacto-oligosaccharides (βGOS); and (iv) raffinose-family oligosaccharides and α-galacto-oligosaccharides (ROF and αGOS, respectively) (Gänzle and Follador 2012).

In addition to metabolizing sugar, LAB are able to direct sugar towards exopolysaccharide (EPS) biosynthesis. These long-chain saccharides are loosely attached to the cell surface to form capsule-like structures or are secreted into the environment. EPS production by lactobacilli has been previously discussed in a comprehensive review (Badel et al. 2011). Several studies of biopolymer diversity in LAB from cereal (Bounaix et al. 2009, 2010; Palomba et al. 2012) and in intestinal LAB (Salazar et al. 2009; Górska-Frączek et al. 2011, 2013; Sims et al. 2011) have also been published since the first edition of this book.

EPSs come in many different structures, sizes, and sugar compositions and are classified into two groups: homopolysaccharides (HoPS), which consist of one type of monosacharide (α-D-glucan, β-D-glucan, fructan, or a polygalactan); and heteropolysaccharides (HePS), which consist of different types of monosaccharides (D-glucose, D-galactose, L-rhamnose, and their derivatives). HoPS are synthesized extracellularly by highly specific glycosyltransferase enzymes as well as glucan- or fructan-sucrases. HoPS synthesis specifically requires sucrose as a substrate and the energy generated by its hydrolysis. The crystal structures, reaction and product specificities of glucansucrases as well as structural analyses of α-glucan polymers have been recently reviewed (Leemhuis et al. 2013).

HePS are synthesized from glucose, galactose, or other monosaccharides by the combined actions of several types of glycosyltransferases. HePS biosynthesis involves four major consecutive steps: (i) sugar transport into the cytoplasm, (ii) sugar-1P synthesis, (iii) polymerization of repeating unit precursors, and (iv) EPS export outside the cell. The synthesis of two EPSs in *Lactobacillus johnsonii* FI9785 is dependent on the 14-kb *eps* gene cluster; however, the precise regulation of EPS biosynthesis has yet to be identified (Dertli et al. 2013). It is thought that EPS production can be regulated at each of these four steps. Genome sequencing of EPS-related genes and their organization (Koryszewska-Baginska et al. 2014) may provide additional insight into whether this is the case.

1.1.1. Practical Aspects of Sugar Catabolism

The ability of LAB to ferment sugars has been widely utilized in various foods' production. However, LAB also have the potential for increasing the production value of biofuels and biochemical products due to their robustness and tolerance for ethanol, low pH, and high temperatures (Martinussen et al. 2013). Focus has increased on optimizing lactate production from natural substrates such as starchy

or lignocellulosic materials from agricultural, agro-industrial, and forestry sources due to their abundance, low price, high polysaccharide content, and renewability (Okano et al. 2009; Abdel-Rahman et al. 2011; Castillo Martinez et al. 2013). In addition, EPS from LAB can play an important role in the food industry as an emulsifier, thickener, viscosifier, and stabilizer. EPS has been used in the rheology and texture of fermented milks (Ramchandran and Shah 2009) and other fermented products, such as sourdough (Katina et al. 2009; Galle et al. 2010) and cereal-based beverages (Zannini et al. 2013). It may also improve the quality, safety, and acceptability of gluten-free bread (Moroni et al. 2009) and replace hydrocolloids in sorghum sourdough (Galle et al. 2011). EPSs from LAB are also of great interest to agro-food industries since their vast structural diversity may lead to innovative applications. However, the majority of LAB only produce low levels of polysaccharides; therefore, optimized methodologies for increased EPS production and recovery are still required (Notararigo et al. 2013).

EPS from LAB also has beneficial physiological properties for humans (Patel et al. 2011). Cell-bound EPSs from *Lactobacillus acidophilus* 606 (Kim et al. 2010) and *Lactobacillus plantarum* 70810 (Wang et al. 2014) have been shown to have antitumor properties. EPSs have also been shown to exhibit immunomodulatory activity for macrophages (Liu et al. 2011; Ciszek-Lenda et al. 2011) as well as intestinal epithelial cells (Patten et al. 2014). In addition, EPSs produced by *Lactobacillus reuteri* can inhibit enterotoxigenic *Escherichia coli*-induced hemagglutination of porcine erythrocytes, which further indicates that EPS has therapeutic potential (Wang et al. 2010). EPS from *Lact. plantarum* 70810 has a metal binding capacity and could be used as a potential biosorbent for lead removal from the environment (Feng et al. 2012). LAB can also produce a variety of functional oligosaccharides that can be used as prebiotics (Pepe et al. 2013), nutraceuticals, sweeteners, humectants, drugs against colon cancer, and immune stimulators (Patel et al. 2011). Some probiotic LAB can also utilize prebiotic compounds, including non-digestible FOSs, inulin-type fructans, or β-glucans (Russo et al. 2012), which stimulate the growth of beneficial commensals in the gastrointestinal tract.

1.2. Citrate Metabolism and Formation of Aroma Compounds

In addition to sugars, several LAB species can metabolize citrate. Citrate fermentation in LAB leads to the production of volatile compounds. In fermented dairy products, these compounds are C_4 compounds, such as diacetyl, acetoin, and butanediol, which are responsible for the typical aroma of many fermented dairy products. Therefore, citrate metabolizing LAB, such as *L. lactis* subsp. *lactis* biovar. diacetylactis (*L. diacetylactis*) and some *Leuconostoc* and *Weissella* species, are currently used as starter and adjunct cultures for the production of these C_4 compounds. However, in other fermented products, such as wine, beer, and sausages, the volatile compounds produced from the fermentation of citrate by LAB are considered off-flavors, and their presence should be avoided. Citrate utilization by LAB has been previously described in detail (Quintans et al. 2008) and summarized in the first edition of this book (Mayo et al. 2010). Therefore, in this chapter, we are presenting only citrate metabolism in LAB in the context of recent achievements.

1.2.1. Citrate Transport

Citrate transport is a limiting step for citrate utilization and is performed by a variety of membrane-associated permeases. In contrast, volatile compounds formed in the cytoplasm are secreted without requiring specific transporters. Most LAB species internalize citrate using a 2-hydroxycarboxylate (2-HCT) transporter, which can transport dicarboxylic and tricarboxylic acids. The 2-HCT family of transporters includes CitP from *Lactococcus*, *Leuconostoc*, and *Weissella* (Pudlik and Lolkema 2010). CitP is an antiport transporter that exchanges H-citrate^{2-} and lactate^{1-} to generate a membrane potential (Figure 1.2). In *L. diacetylactis*, CitP is encoded by the *citQRP* operon located on the "citrate

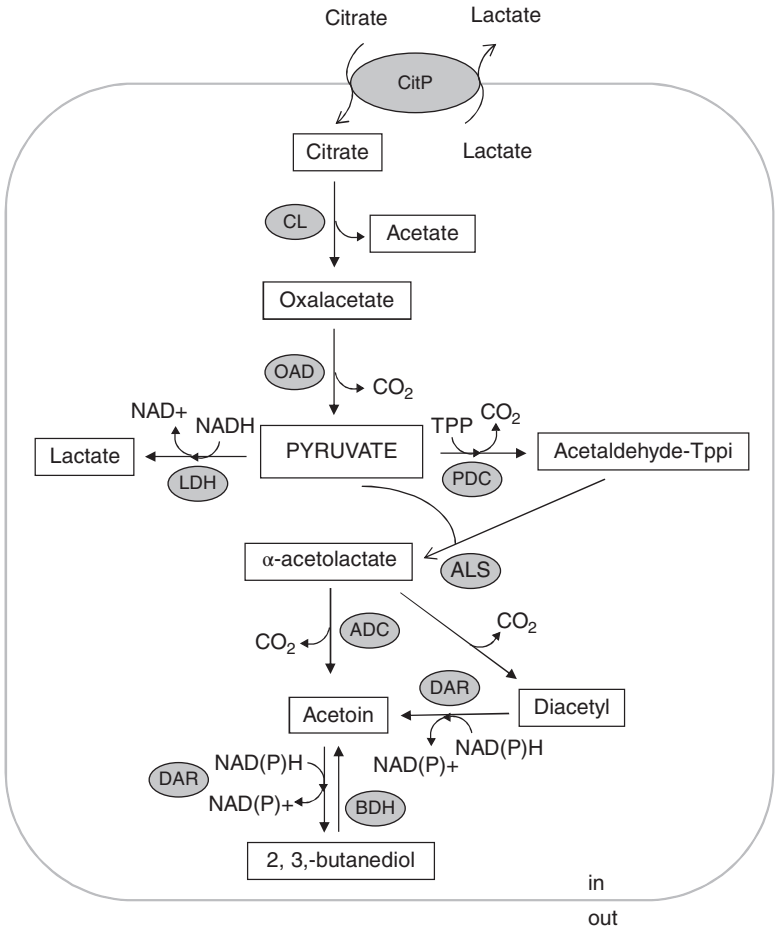

Figure 1.2. Citrate metabolism in *Lactococcus*, *Leuconostoc*, and *Weissella* species. Key for the enzymes: CL, citrate lyase; OAD, oxaloacetate decarboxylase; LDH, lactate dehydrogenase; PDC, pyruvate decarboxylase; ALS, α-acetolactate synthase; ADC, α-acetolactate decarboxylase; DAR, diacetyl acetoin reductase; BDH, 2,3-butanediol dehydrogenase; Tppi, thiamine pyrophosphate.

plasmid" (Drider et al. 2004; Kelly et al. 2010). In *L. diacetylactis*, transcription of the promoters that control *cit* operons are specifically activated by low pH environments as an adaptive response to acid stress. This has been confirmed by transcriptomics analysis of *L. diacetylactis* in milk (Raynaud et al. 2005) and cheese (Cretenet et al. 2010). In *Weissella paramesenteroides* and *Leuconostoc mesenteroides* subsp. *cremoris*, the *citP* genes (*citMCDEFGRP*) are located in a plasmidic or chromosomal *citI* cluster, respectively (Martín et al. 1999, 2000; Bekal et al. 1998). Enlarged citrate plasmids (15 to 23 kbp) have also been observed in wild (non-starter) strains of *L. diacetylactis* (Drici et al. 2010; Kelly et al. 2010).

In *Enterococcus faecalis* and *Lactobacillus casei*, citrate is transported by CitM and CitH transporters, respectively, both belonging to the family of citrate-metal symporters (CitMHS) (for a review see Lensbouer and Doyle 2010). CitMHS transporters transport citrate in cation (Ca^{2+}, Mn^{2+}, or Fe^{3+}) complexes. Recently, CitH in *Lact. casei* has been shown to be a proton symporter that transports a Ca^{2+}-citrate complex and uses Ca^{2+} as a substrate (Mortera et al. 2013). Other types of citrate

transporters have also been identified in *Lact. plantarum*, *Oenococcus oeni* (for a review see Mayo et al. 2010), and the atypical citrate-fermenting wild *L. diacetylactis* strain (Passerini et al. 2013b).

1.2.2. Conversion of Citrate into Pyruvate and Production of Aroma Compounds

Once inside the cell, citrate is converted into acetate and oxaloacetate in a reaction catalyzed by the citrate lyase (CL) enzyme complex (Figure 1.2). Next, oxaloacetate is decarboxylated by oxaloacetate decarboxylase (OAD), which generates pyruvate and CO_2 (Figure 1.2). Analysis of various LAB genomes have identified genes encoding the α-, β-, and δ-subunits of OAD (Makarova et al. 2006). However, the physiological role of OAD remains poorly understood in LAB species; only enzymes from *E. faecalis* (Repizo et al. 2013) and *Lact. casei* (Mortera et al. 2013) have been recently investigated. In other LAB species, including *L. diacetylactis*, *W. paramesenteroides*, *Leuc. mesenteroides*, *Lact. plantarum*, and *O. oeni*, oxaloacetate is decarboxylated by the soluble and cytoplasmic malic enzyme (ME) (Sender et al. 2004). Surprisingly, the *cit* locus of *E. faecalis* has been recently shown to contain genes that encode both OAD and ME (Espariz et al. 2011).

Pyruvate metabolism in LAB can yield different end products, including lactate, formate, acetate, and ethanol as well as the important aroma compounds diacetyl, acetoin, and butanediol (Neves et al. 2005).

1.2.3. Conversion of Citrate into Succinate

Some LAB species cannot truly convert citrate into pyruvate. Instead, the CitT transporter generates succinate via malate and fumarate. Furthermore, the complete tricarboxylic acids (TCA) pathway has recently been identified in the *Lact. casei* genome using *in silico* analysis (Díaz-Muñiz et al. 2006). In this LAB species, the dominant end-products of citrate metabolism were acetic acid and L-lactic acid at both excess and limiting amounts of carbohydrates. Trace amounts of D-lactic acid, acetoin, formic acid, ethanol, and diacetyl confirm OAD activity; however, succinic acid, malic acid, and butanendiol were not observed (Díaz-Muñiz et al. 2006; Mortera el al. 2013).

1.2.4. Bioenergetics of Citrate Metabolism

The co-metabolism of glucose and citrate produces different physiological effects in homofermentative and heterofermentative LAB. In homofermentative LAB, citrate utilization has a protective effect against acid stress. In heterofermentative LAB, "citrolactic" fermentation generates one extra mol of ATP per mol of citrate. In milk, *L. diacetylactis* metabolizes lactose and produces lactic acid, which is exchanged by the antiporter CitP for citrate during excretion. *L. diacetylactis* is homofermentative and converts glucose into lactate, generating two moles of ATP per mol of glucose. The NAD^+ consumed in the first steps of this pathway is regenerated during the transformation of pyruvate into lactate, thereby maintaining the redox potential. In the presence of glucose and citrate, each mol of citrate produces one mol of pyruvate without generating NADH. This excess of pyruvate is diverted to α-acetolactate synthesis and the subsequent production of aroma compounds. Similar to *L. diacetylactis*, the higher biomass of *Lact. casei* during Ca^{2+}-citrate and carbohydrate co-metabolism has been attributed to counteracting the growth inhibition of carbohydrate metabolism that is induced by acidification in its final stages (Mortera et al. 2013).

1.3. The Proteolytic System of Lactic Acid Bacteria

L. lactis strains isolated from dairy products are characterized by a high number of amino acid auxotrophies and the ability to utilize milk proteins as an amino acid source. These properties are common to dairy strains even from distant geographic locations in Asia, Europe, North America, and

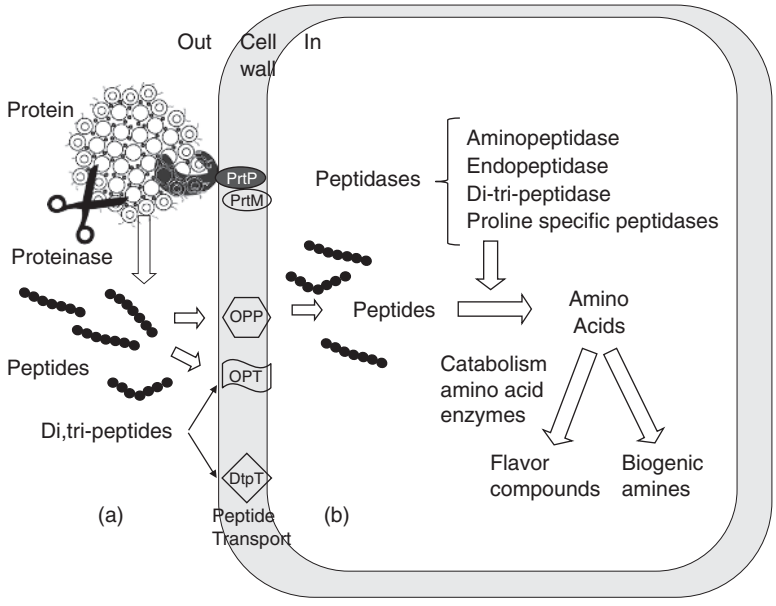

Figure 1.3. Diagram of the proteolytic systems of lactic acid bacteria. (a) Extracellular components: PrtP, cell-envelope proteinase; PrtM, proteinase maduration protein; Opp, oligopetide permease; DtpT, the ion linked trasnsporter for di-and tripeptides; and Opt, the ABC transporter for peptides. (b) Intracelullar components: pool of about 20–25 peptidases, including general (PepN, PepC) and specific (PepX, PepQ) peptidases, and amino acid catabolic enzymes (carboxylases, aminotransferases, etc.).

New Zealand (Rademaker et al. 2007; Kelly et al. 2010). The use of environmental proteins as an amino acid source in LAB explains the importance of their proteolytic system. LAB depend on this system to obtain the essential amino acids that are then used as precursors for not only peptides and proteins but also for many other biomolecules. Amino acids are the precursors of aromatic compounds, which are important to the final flavor of food products. Proteolytic activity also generates other molecules, such as bioactive peptides, that have functions related to the probiotic properties of LAB.

The proteolytic system in LAB can be divided into several steps: protein degradation, peptide transport, peptide degradation, and amino acid catabolism (Figure 1.3).

1.3.1. Protein Degradation

Studies of protein degradation in LAB initially focused on casein degradation using *L. lactis* as a model organism. Casein hydrolysis in LAB is initiated by a cell-envelope proteinase (CEP), which degrades proteins into oligopeptides, and gene deletion studies have shown that LAB are unable to grow in milk in the absence of a functional CEP. However, since CEP is extracellular, peptides produced by CEP can also be consumed by protease-negative variants, allowing them to survive in culture (Bachmann et al. 2012).

Lactocepins are a diverse group of CEPs that belong to the subtilisin protein family of serine proteases. CEPs are anchored to the cell wall via sortase A (SrtA) (Dandoy et al. 2011). LAB lactocepins are encoded by *prtP*, *prtB*, *prtS*, and/or *prtH*, which differ in their number of functional domains. CEP distribution varies widely among strains. Overall, the most abundant paralog among LAB is *prtH3*, which is carried by over 80% of strains tested, followed by paralogs of *prtH* and *prtH4* (Broadbent et al. 2011). Most LAB possess only one CEP. However, four CEP genes (*prtH*,

prtH2, *prtH3*, and *prtH4*) have been identified in *Lactobacillus helveticus* CNRZ32 (Jensen et al. 2009). The presence of several protease genes in *Lact. helveticus* could explain its high proteolytic efficiency. Only *prtH2* is common to all characterized *Lact. helveticus* strains (Genay et al. 2009). However, analysis of the proteinase in BGRA43 showed that the only active gene was *prtH*. CEP activation requires the maturation proteinase PrtM. Two PrtMs (PrtM1 and PrtM2) have been identified in *Lact. helveticus* CNRZ32 (Savijoki et al. 2006). Additional studies (Genay et al. 2009; Broadbent et al. 2011) have reported that PrtM1 is required for PrtH activation, while PrtM2 plays a role in the activation of other CEP paralogs.

As mentioned above, CEP activity was initially evaluated using casein as a substrate. However, LAB strains isolated from non-milk or non-fermentation environments have also exhibited casein hydrolysis. For instance, *Lact. helveticus* BGRA43, which is isolated from human feces, has strong proteolytic activity and is able to completely hydrolyze α_{s1}-, β-, and κ-caseins (Strahinic et al. 2013). In addition, the lactocepin secreted by *Lactobacillus paracasei* VSL#3 can selectively degrade cell-associated and tissue-distributed IP-10 and other proinflammatory chemokines *in vitro* (von Schillde et al. 2012). These findings indicate that lactocepin, which is encoded by *prtP*, is highly selective despite targeting a broad spectrum of cleavage sites. Therefore, additional protein characteristics, such as surface charge and/or three-dimensional structure, determine whether a protein is cleaved or not. The selective degradation of pro-inflammatory chemokines could be also related to differences in the regulation of *prtP* expression.

In *Streptococcus thermophilus*, the cell wall associated proteinase PrtS is highly conserved (95% identity) with the PrtS of *Streptococcus suis*. Although recent studies have suggested that PrtS contributes to the virulence of *Strep. suis* (Bonifait el al. 2010), the primary role of PrtS in *Strep. thermophilus* strains is cleaving casein to oligopeptides. This function is clearly related to the adaptation of *Strep. thermophilus* to dairy environments; analyses of *prtS* distribution in *Strep. thermophilus* found that it occurs infrequently in historical specimens but frequently in more recent industrial ones. Furthermore, this "ecological" island confers an important metabolic trait for milk adaptation and appears to be laterally transferred in *Strep. thermophilus*. Together, these data suggest that *Strep. thermophilus* evolved via gene acquisition and selection as the result of the environmental pressures of food production (Delorme et al. 2010).

The second stage of protein degradation is the transport of di-, tri-, and oligo-peptides into the cell. Three oligo-, di-, and tri-peptide transport systems (Opp, Dpp and DtpT, respectively) have been described in LAB. *Lact. acidophilus*, *Lactobacillus brevis*, *Lact. casei*, *Lactobacillus rhamnosus*, and *L. lactis*, possess all three of these peptide transport systems. Some *Lact. helveticus* strains, such as DPC4571, also have three peptide transport systems, while others, such as H10, only have two (Opp and DtpT). These results indicate that the proteolytic systems differ between different strains of even the same species. Finally, *Lact. reuteri* only has one functional peptide transport system (DtpT) (Liu et al. 2010).

1.3.2. Peptidases

Once casein-derived peptides are taken up by LAB cells, they are degraded via the concerted action of peptidases with differing and partially overlapping specificities. Peptidases are a very important part of the proteolytic system in LAB and are involved in peptide hydrolysis and the release of essential amino acids. Peptidases can be divided into two main groups: endopeptidases and exopeptidases. Endopeptidases hydrolyze the internal peptide bonds of oligopeptides to generate peptides that can be further degraded by exopeptidases. Exopeptidases act on the terminal ends of oligopeptides to generate smaller peptides or amino acids.

The main LAB endopeptidases that have been characterized are PepO, PepF, PepG, and PepE, all of which act on NH_2-X_n↓X_n-COOH substrates. PepO is encoded by three paralogous genes (*pepO*,

pepO2, and *pepO3*). In *Lact. helveticus* strains, these genes are identical; however, strain heterogeneity may be observed due to the loss of gene function or sequence polymorphisms, which may affect the specificity or relative activity of the individual peptidase enzymes (Broadbent et al. 2011).

Three paralogous genes also encode PepF (*pepF*, *pepF1*, and *pepF2*). In *L. lactis*, *pepF1* is located on the chromosome, while *pepF2* is located on a plasmid. The location of *pepF2* explains the variability in its absence or presence among different *Lactococcus* strains (Liu et al. 2010).

The PepE and PepG proteins are absent in lactococci and streptococci. In *Lactobacillus*, two paralogous genes have been described for PepE (*pepE* and *pepE2*). In *Lact*. *helveticus*, these genes are almost universally conserved among strains from different origins, including cheese, whey, whiskey malt, and commercial cultures. PepE also plays an important role in the debitterness function of *Lact*. *helveticus* (Broadbent et al. 2011).

Exopeptidases are traditionally classified by their specificity, and four exopeptidase groups have been identified in LAB: aminopeptidases, dipeptidases, tripeptidases, and proline-specific peptidases. Aminopeptidases hydrolyze a single amino acid from the *N*-terminal oligopeptide NH_2–X↓Xn–COOH. They can be divided into either general or specific aminopeptidases. General aminopeptidases (PepN and PepC) are present in all genomes, usually as one gene per genome. Some LAB genomes have two peptidase homologs that potentially have the same function; for example, there are two PepC homologs in *Lact. johnsonii* (Liu et al. 2010). Specific aminopeptidases are categorized according to the type of residue they hydrolyze. PepS, which has only been described in *Strep. thermophilus*, is specific for aromatic residues. PepA is specific for Glu and Asp residues; it is found in streptococci and some *Lactobacillus* and *Lactococcus* strains but is absent in *Pediococcus* and *Oenococcus*. PepM is specific for methionine residues and is present in *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, and *Oenococcus* strains (Liu et al. 2010).

Dipeptidases are exopeptidases specific for NH2–X↓X–COOH dipeptides. The PepD dipeptidase family has a broad specificity for various dipeptides. The *pepD* genes are distributed heterogeneously in LAB genomes and can vary in number from 0 to 6 paralogs. PepV is also encoded by multiple paralogous genes. It is present in all LAB and also has a broad specificity.

Tripeptidases release the amino acid from the *N*-terminal position of NH2–X↓X–X–COOH tripeptides. They have a broad specificity but preferentially target hydrophobic peptides and do not hydrolyze proline residues. The only tripeptidase currently identified in LAB is PepT. The *pepT* gene is present in all LAB genomes and can occur as two paralogous genes in some LAB (*Lact. acidophilus*, *Lact. johnsonii*, and *Lactobacillus gasseri*).

Proline-specific peptidases can hydrolyze proline residues from the *N*-terminal position of peptides. Proline iminopeptidase (PepI) has aminopeptidase activity towards *N*-terminal proline peptides and prefers tri-peptides (NH2–Pro↓Xn–COOH). The prolinase PepR has a broad specificity for dipeptides (NH2–Pro↓X–COOH). These proline-specific peptidases are absent from all *L. lactis* strains. The activity of cell extract from *Lact. helveticus* and *Lact. rhamnosus* for several proline dipeptides was significantly reduced in PepR-deletion mutant. Those observations suggest that PepI and PepR may contribute to the specific proteolytic capacity for breaking down peptides containing proline in *Lactobacillus* strains (Liu et al. 2010).

PepP is a proline peptidase that can cleave any *N*-terminal amino acid linked to proline in an oligopeptide (NH2–X↓Pro–Xn–COOH). PepQ is also a proline peptidase but is specific for proline dipeptides (NH2–X↓Pro–COOH). One *pepP* gene is found in each LAB genome, except in *Lactobacillus sakei* and *Pediococcus pentosaceus*. The absence of *pepP* in both these genomes is likely due to gene loss. PepQ genes are equally distributed in all LAB genomes as one copy per genome. However, the *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lact. bulgaricus*) strains have two *pepQ* paralogs; one paralog is clustered with other orthologs, while the second is located in a separate cluster. This may be the result of an ancient duplication or horizontal gene transfer (HGT) event. PepX is a proline-specific endopeptidase (NH2–X–Pro↓Xn–COOH) that is present in all LAB

genomes as one gene per genome. However, some LAB genomes have two peptidase homologs, and these are normally the genomes of strains from dairy environments. PepX2 is a PepX homolog of *L. lactis* subsp. *lactis* IL1403. This putative *pepX2* gene was originally annotated as a hypothetical protein named *ymgC*. The only members of the PepX2 (YmgC) group are from *L. lactis* subsp. *lactis* IL1403 and *Pediococcus* (Liu et al. 2010).

1.3.3. Technological Applications of the Proteolytic System

The proteolytic system provides LAB with the amino acids they require to grow. For instance, *Lact. bulgaricus* and *Lact. helveticus* have a very extensive set of proteolytic enzymes, which is consistent with previous findings that *Lact. bulgaricus*, rather than *Strep. thermophilus*, serves as the proteolytic organism in yogurt. *Lact. helveticus* is a proteolytic adjunct culture of cheese that is used to degrade bitter peptides. Interestingly, *Lact. bulgaricus* encodes the Dpp system, which prefers the uptake of hydrophobic di/tripeptides. This complements the presence of *Strep. thermophilus*, which encodes the general di/tripeptide transporter DtpT and suggests that more peptides can be utilized by both bacteria when they are grown together. LAB from plants, such as *Lact. plantarum*, *O. oeni*, and *Leuc. mesenteroides*, encode fewer proteolytic enzymes, which is consistent with their fiber-rich ecological niche that contains less proteins. Milk proteins have also been used as raw material for obtaining bioactive peptides. Various peptides with immunostimulating, antimicrobial, opioid, anticancer, mineral binding, and antihypertensive properties have been isolated as proteolytic products in LAB (Griffiths and Tellez, 2013).

1.3.4. Amino Acid Catabolism

The catabolism of amino acids has quality (flavor compound formation) and safety (biogenic amine synthesis) implications for fermented foods. Amino acid catabolism is also thought to have an important role in the abilities of LAB to obtain energy in nutrient-limited conditions. Furthermore, amino acid catabolism has also been identified as a mechanism of pH control.

Amino acid degradation is important for the synthesis of volatile compounds and the transamination of some amino acids: methionine, branched-chain, and aromatic amino acids. Transamination is the main degradation pathway that leads to the formation of α-keto acids, which are then degraded into various aromatic compounds. The conversion of amino acids to keto- and hydroxyl acids is initiated by lactobacilli, and *Lactococcus* strains further convert these products to carboxylic acid. This cooperation between LAB and non-starter LAB can enhance cheese flavor.

Leucine, valine, and isoleucine catabolism can be divided into two parts. The first comprises the main degradation pathway; an aminotransferase reaction converts the amino acid to α-keto acid or, in the case of leucine, α-keto isocaproate (KICA). The resulting compounds are then converted to aldehyde, alcohol, or carboxylic acid via three different pathways: α-keto acid decarboxylation, oxidative decarboxylation, or an alternative dehydrogenation route that generates α-hydroxy-isocaproate (HICA) (Smit et al. 2005). Aromatic amino acids are also degraded by the amino transferase AraT. Transamination generates indole pyruvate, phenyl pyruvate, and p-hydroxy-phenyl pyruvate from tryptophan, phenylalanine, and tyrosine, respectively. Alpha-ketoacids generated by aromatic amino acid transamination are further degraded to various compounds by enzymatic (dehydrogenation, decarboxylation, or oxidative decarboxylation) or chemical reactions.

Sulfur compounds are generated by sulfur amino acid catabolism and are potent odorants that contribute flavor to many fermented foods. Methionine catabolism produces various volatile sulfur compounds (VSCs) such as H_2S, methanethiol, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) (Fernández et al. 2000). The enzymes in LAB strains from raw goats' milk cheeses crucial for VSC formation from L-methionine have very diverse enzyme capabilities.

Lactococci had higher C-S lyase and aminotransferase activities specific for sulfur-containing compounds than *Lactobacillus* and *Leuconostoc* (Hanniffy et al. 2009).

Amino acid catabolism also generates biogenic amines (BA). BA are basic nitrogenous organic compounds that are primarily formed by amino acid decarboxylation. BA are present in a wide range of foods, including dairy products, and can accumulate to elevated concentrations. Consuming foods with high amounts of these amines has toxicological effects. Tyrosine, histidine, lysine, ornithine, and arginine can all be decarboxylated into tyramine, histamine, cadaverine, putrescine, and agmatine, respectively. Agmatine can be further deaminated to produce putrescine. Amino acid decarboxylation pathways involve the transport of the amino acid into the cell, decarboxylation, and transport of the resulting BA out of the cell. The amino acid/BA interchange is performed by a transporter protein, and the tyramine, histamine, and putrescine biosynthesis pathways have been characterized in LAB. The genes encoding the decarboxylase and transporter proteins are located on the chromosomes for most LAB strains. Although BA production in LAB has been proposed as strain specific, genomic analysis of *E. faecalis*, *Enterococcus durans*, and *Enterococcus faecium* have shown that tyramine production is species specific (Ladero et al. 2012). Tyramine biosynthesis requires tyrosine, the decarboxylase (TDC), and a transporter protein (TyrP). The genes that encode TDC and TyrP form a chromosomal cluster, and a third open reading frame that encodes a protein homologous to tyrosyl tRNA synthase is always present in this cluster, upstream of the *tdc* gene (Linares et al. 2012). In cheese, *Lactobacillus* and *Enterococcus* genera are the main producers of tyramine.

In dairy products, the agmatine pathway (AgDI) has been proposed as the main route for putrescine synthesis since the ornithine decarboxylase pathway is not present in dairy LAB. These bacteria make putrescine from agmatine in three steps that are catalyzed by agmatine deiminase (AgDI), putrescine transcarbamylase (PTC), and carbamate kinase (CK). An antiporter then exchanges putrescine for agmatine. In addition to putrescine, ATP and NH_3 are also products of this set of reactions. The agmatine pathway has been identified in *E. faecium*, *E. faecalis*, *Lact. brevis*, and *Lactobacillus curvatus*. In the last two species, both the tyramine and putrescine pathways are linked on the chromosome. Some *Lactococcus* strains are also able to produce putrescine from agmatine (Ladero et al. 2012). In *Lactococcus*, some strains carry the gene cluster and some have an insertion element that renders the cluster non-functional. These combinations suggest that these strains putatively evolved from an ancestral strain that carried the gene cluster; the gene cluster could then accumulate mutations or even be lost if agmatine was not present in the strain's environment.

The agmatine pathway is similar to the arginine deiminase pathway (ADI), which is comprised of three reactions catalyzed by arginine deiminase, ornithine carbamoyl-transferase, and CK. The ADI pathway is widely distributed among LAB and has been described in strains from the *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Streptococcus*, and *Weissella* genera.

Histamine is another BA that occurs frequently in fermented products. It is produced from histidine in a reaction catalyzed by histidine decarboxylase (HdcA). Histamine synthesis also requires an antiporter protein that interchanges histidine and histamine. The histamine gene cluster has a gene encoding for the HdcB protein, which is cotranscribed with *hdcA* and is necessary for HdcA maturation (Trip et al. 2011). A gene encoding a protein homologous to histidyl tRNA synthetase is also found in this cluster, although its position varies from one LAB species to another (Calles-Enriquez et al. 2010). The HDC cluster has been characterized in different species of *Lactobacillus*, *O. oeni*, *Strep. thermophilus*, *Tetragenococcus muriaticus*, and *Tetragenococcus halophilus* and is located on the chromosome, except for *Lactobacillus hilgardii* (Lucas et al. 2005).

BA synthesis always requires the presence of amino acids; however, other parameters such as pH, carbon source, or temperature differ from one BA to another (Linares et al. 2012).

1.4. LAB Metabolism in Light of Genomics, Comparative Genomics, and Metagenomics

The use of next generation sequencing technology revolutionized microbial science by rapidly increasing the number of publically available genomes. The first LAB genome was published in 2001 (Bolotin et al. 2001) and, since then, nearly 100 complete genomes from typical LAB species (excluding enterococci) have been deposited at NCBI, and more than 600 whole genome sequencing projects with a permanent draft or incomplete status have been reported, according to GOLD (as of 20 May 2015). Among draft genome sequences, a dozen *Lactobacillus* strains have been generated as part of the Human Microbiome Project (The Human Microbiome Jumpstart Reference Strains Consortium et al. 2010). Furthermore, the technological advances and cost reductions of sequencing have allowed the resequencing of the *L. lactis* MG1363 genome and its comparison to the genome of its derivative *L. lactis* NZ9000 (Linares et al. 2010). This comparison identified six differences that were not attributable to known errors in the published sequence. Two specific mutations were found in the *ptcC* promoter, which plays a key role in regulating cellobiose and glucose uptake. A comparative analysis of the genomes of six *Lactobacillus* and several other LAB has also identified nine niche-specific genes (O'Sullivan et al. 2009). A comparison of 20 complete *Lactobacillus* genomes showed that their bacterial pan-genome consists of approximately 14,000 genes, including a core genome of 383 orthologous genes (Kant et al. 2011).

The analysis of mixed cultures, which is very important in industrial fermentations, has also become possible using metagenomic approaches. Novel molecular technologies such as metagenomics can be used to analyze the metabolic potentials of LAB. In one study, LAB isolates from African pearl millet slurries and the metagenomes of amylaceous fermented foods were screened for genes involved in probiotic functions and starch metabolism (Turpin et al. 2011). Metagenomic approaches were used to monitor changes in bacterial populations, metabolic potential, and the overall genetic features of the microbial community during a 29-day fermentation process of the traditional Korean food kimchi (Jung et al. 2011). The resulting metagenomic sequences were categorized by their functions. Sequences in the carbohydrate category indicated that the kimchi microbiome was enriched with genes associated with mono-, di-, and oligosaccharide fermentation. Similarly, sequences in the fermentation category were enriched for various genes involved in lactate fermentation and acetoin and butanediol metabolism. The number of metabolic genes involved in carbohydrate metabolism and fermentation generally increased as kimchi fermentation progressed.

In addition to fermented food products, the human gastrointestinal tract and its complex microbial community have also garnered considerable interest. More than three million bacterial genes in the human gastrointestinal tract have been characterized (Qin et al. 2010; Methé et al. 2012).

1.5. Novel Aspects of Metabolism Regulation in the Post-genomic Age

Transcriptional regulation is essential for microorganism adaptations to changes in external or intracellular conditions. In bacteria, transcription can be activated or inhibited by various transcription factors (TFs) that recognize specific *cis*-regulatory DNA elements and TF-binding sites (TFBSs) in gene promoter regions. The set of genes or operons under direct control of the same TF is known as a regulon, and all the regulons in an organism form a transcriptional regulatory network.

In recent years, the number of dairy and non-dairy LAB genomic sequences has dramatically increased. A study of 30 LAB representatives found that 3.5% (*Strep. thermophillus, Lact. delbrueckii, Lact. helveticus*) to 7.5% (*Lact. plantarum*) of the proteome helped regulate transcription (Figure 1.4; Ravcheev et al. 2013). In addition, the total amount of TFs varied significantly between species, ranging from 63–64 (*Lact. helveticus* and *Strep. thermophilus*, respectively) to 240 (*Lact. plantarum*). Among 30 *Lactobacillales* genomes, putative TFs were identified in 49 protein

families (average of 36 TFs per genome), and approximately 90% belonged to 24 major families (at least two TFs per genome) (Ravcheev et al. 2013). The most represented TF family was the Xre family (298 TFs in total), and a dozen or even several dozen Xre members were identified in each genome (average of 19 per genome). Other TF families were considerably less represented; those that had at least four representatives per genome included TetR, GntR, MarR, OmpR, LacI, LysR, MerR, and AraC (Figure 1.4) (Ravcheev et al. 2013).

Other systematic genome-wide analyses of transcriptional regulatory networks have been performed for single LAB species, including *Lact. plantarum* (Wels et al. 2011) and *L. lactis* (de Jong et al. 2012, 2013). These sequence- and motif-based reconstructions of gene regulatory networks have led to the development of several analysis tools, such as PEPPER, RegTransBase, PRODORIC, RegPrecise, FITBAR, RegAnalyst, and MGcV (Overmars et al. 2013). Tools for discovering new motifs have also been developed, including MEME (Bailey et al. 2009), Tmod (Sun et al. 2009), GIMSAN (Ng and Keich 2008), and EXTREME (Quang and Xie 2014).

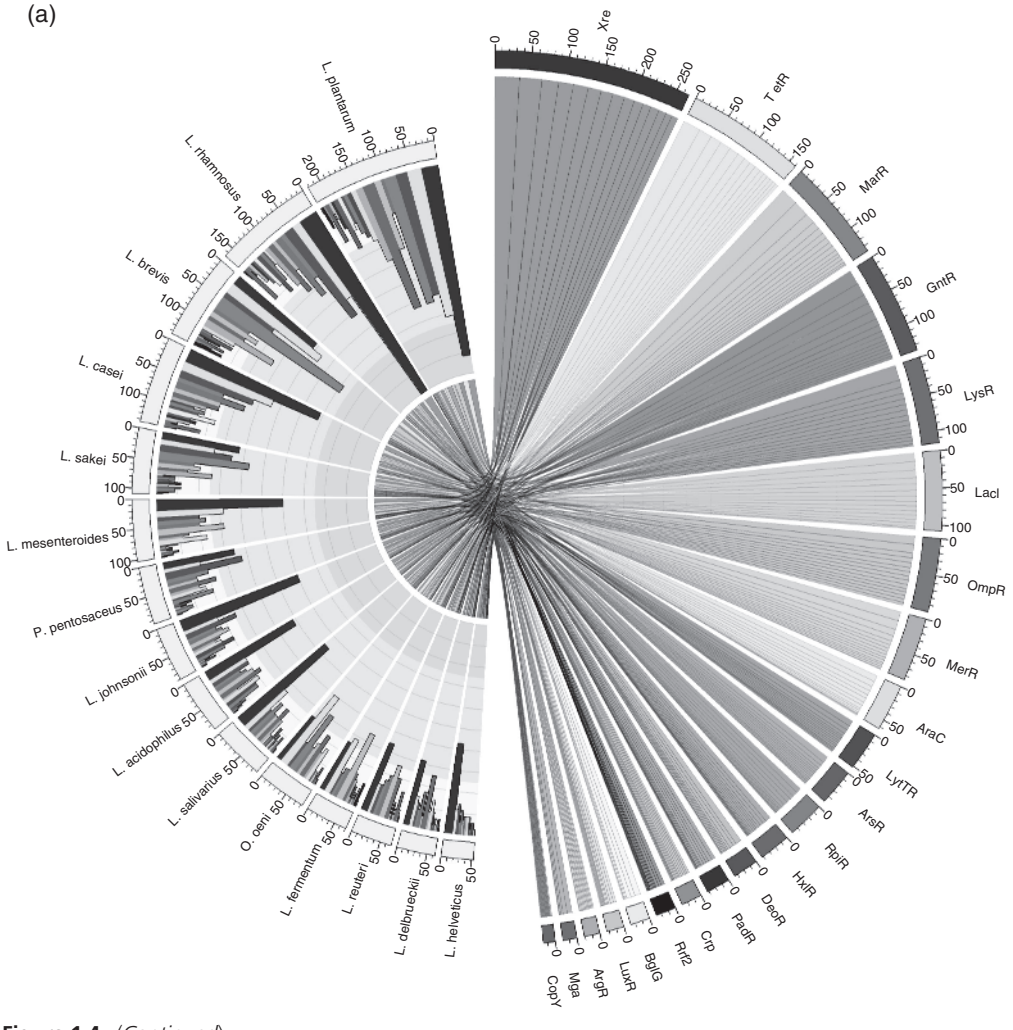

Figure 1.4. (*Continued*)

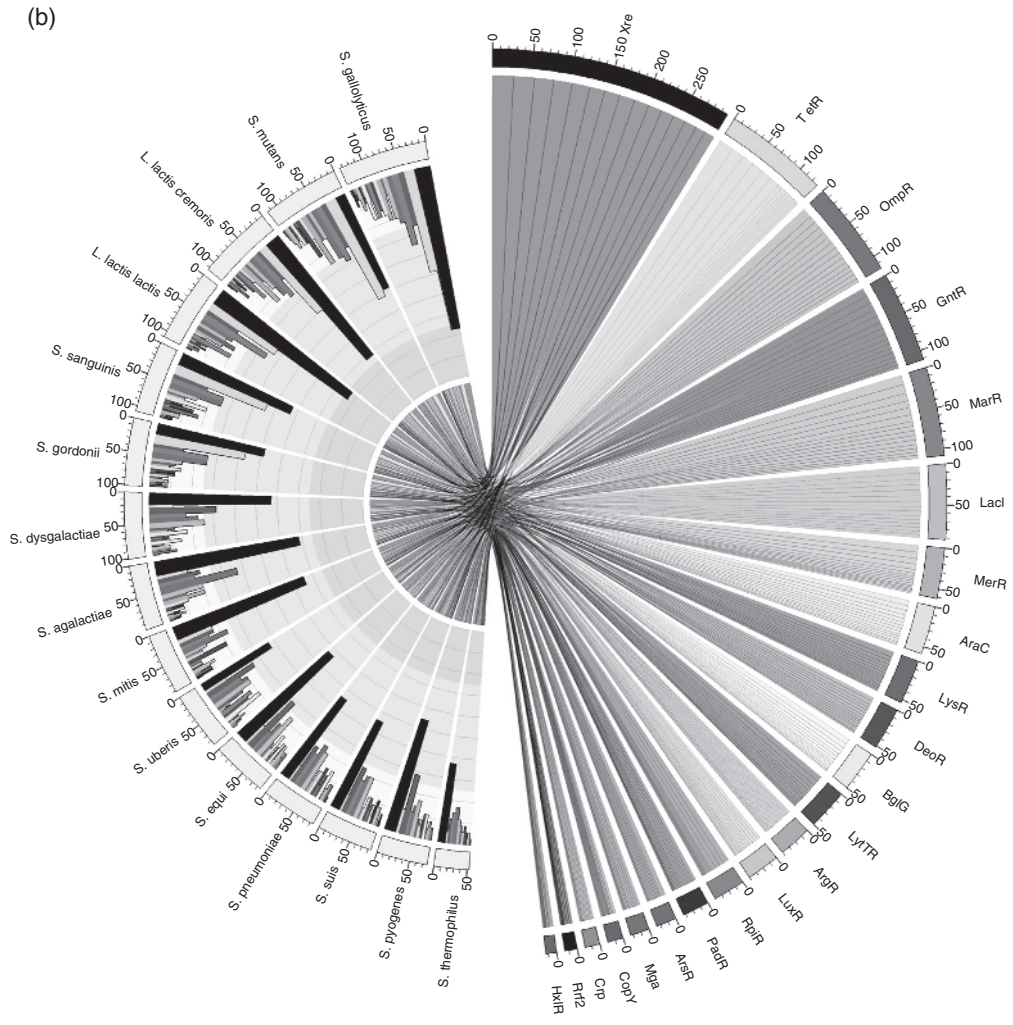

Figure 1.4. Distribution of predicted transcription factors (TFs) in selected Lactobacillaceae (a) and Streptococcaceae (b) genomes. The least-represented TFs families (AbrB, AsnC, BirA, CcpN, CodY, ComK, CtsR, DtxR, Fis, Fur, GlnL, GutM, HrcA, HTH_11, IclR, LexA, NiaR, NrdR, NrtR, PF04394, PurR, Rex, ROK, SdaR, SfsA, SorC, YobV) are not presented on the ideograms. Row data derive from (Ravcheev et al. 2013). Graphical presentation of these data has been developed with Circos (v0.64) (Krzywinski et al. 2009). Strains of presented *Lactobacillales* species: *L. lactis cremoris* (*Lactococcus lactis* subsp. *cremoris* SK11), *L. lactis lactis* (*Lactococcus lactis* subsp. *lactis* IL1403), *S. thermophilus* (*Streptococcus thermophilus* CNRZ1066), *S. agalactiae* (*Streptococcus agalactiae* 2603V/R) *S. uberis* (*Streptococcus uberis* 0140J), *S. equi* (*Streptococcus equi* MGCS10565), *S. dysgalactiae* (*Streptococcus dysgalactiae* GGS_124), *S. pyogenes* (*Streptococcus pyogenes* M1 GAS), *S. gallolyticus* (*Streptococcus gallolyticus* UCN34), *S. mutans* (*Streptococcus mutans* UA159), *S. suis* (*Streptococcus suis* 05ZYH33), *S. mitis* (*Streptococcus mitis* B6), *S. pneumoniae* (*Streptococcus pneumoniae* TIGR4), *S. gordonii* (*Streptococcus gordonii* CH1), *S. sanguinis* (*Streptococcus sanguinis* SK36), *L. sakei* (*Lactobacillus sakei* 23K), *L. casei* (*Lactobacillus casei* ATCC 334), *L. rhamnosus* (*Lactobacillus rhamnosus* GG), *L. delbrueckii* (*Lactobacillus delbrueckii* ATCC BAA-365), *L. acidophilus* (*Lactobacillus acidophilus* NCFM), *L. helveticus* (*Lactobacillus helveticus* DPC 4571), *L. johnsonii* (*Lactobacillus johnsonii* NCC 533), *P. pentosaceus* (*Pediococcus pentosaceus* ATCC 25745), *L. brevis* (*Lactobacillus brevis* ATCC 367), *L. plantarum* (*Lactobacillus plantarum* WCFS1), *L. fermentum* (*Lactobacillus fermentum* IFO 3956), *L. reuteri* (*Lactobacillus reuteri* JCM 1112), *O. oeni* (*Oenococcus oeni* PSU-1), *L. mesenteroides* (*Leuconostoc mesenteroides* ATCC 8293), *L. salivarius* (*Lactobacillus salivarius* UCC118).

Despite extensive analyses of the available genomes, comprehensive, experimental studies of LAB regulatory networks remain lacking. Most gene regulation studies in LAB focus on individual regulons in primarily model species, such as *L. lactis* subsp. *cremoris* MG1363 or *L. lactis* subsp. *lactis* IL1403. These studies have identified both global (CcpA – catabolite control protein A) and specific (ClaR, FruR, MalR, and XylR from the RpiR, DeoR, LacI, and AraC families, respectively) TFs for different α- or β-glucosides, fructose, galactose, lactose, maltose, sorbose, or xylose assimilation genes (reviewed by Aleksandrzak-Piekarczyk 2013; de Jong et al. 2012). CcpA is a component of carbon catabolite repression (CCR), which also includes HPr, HPr kinase, and the glycolytic enzymes fructose 1,6-bisphosphate and glucose-6-phosphate (Deutscher 2008; Görke and Stülke 2008). Large-scale approaches, such as transcriptomics and proteomics, have shown that CcpA is also involved in the regulation of tens of genes associated with membrane transport, nucleotide and nitrogen metabolism, protein biosynthesis, and folding (Mazzeo et al. 2012; Zomer et al. 2006). In addition, CcpA-dependent CCR has been shown to impede the expression of two operons involved in citrate metabolism in *E. faecalis* (Suárez et al. 2011).

Many genes undergo diverse regulation by CcpA or other TFs. In milk, transcriptomics has identified several regulatory networks in *L. lactis* (de Jong et al. 2013). A comparative systems biology approach revealed a crucial role for phosphate in regulating central metabolism and glucose uptake in *L. lactis* and *Streptococcus pyogenes* (Levering et al. 2012). Comparing kinetics models in both strains revealed variations that can be explained by differences in phosphate levels in their natural environments.

Recent studies have sought to determine the scope of proteolytic systems and amino acid biosynthesis regulation in *L. lactis* cells. Previous studies identified a significant role for CodY, a global transcriptional regulator, in negatively regulating components of the proteolytic system, which are stimulated by the intracellular pool of branched chain amino acids (BCAAs) (den Hengst et al. 2005). Subsequent studies have reported on the CodY-dependant regulation of several proteolytic system components in other LAB, including *Strep. thermophilus* (Liu et al. 2009), *Streptococcus pneumoniae* (Hendriksen et al. 2008), *Streptococcus mutans* (Lemos et al. 2008), and *O. oeni* (Ritt et al. 2009) but not in lactobacilli. The latest studies have indicated that a proteolysis regulator separate from CodY exists in lactobacillaceae, enterococcaceae, leuconostocaceae, carnobacteriaceae, listeriaceae, exiguobacteria, and bacillaceae. This has been thoroughly examined in *Lact. helveticus*, and a novel BCARR protein has been identified that represses proteolysis gene expression in response to BCAAs (Wakai and Yamamoto 2013). No BCARR orthologs are present in streptococcaceae, including lactococci, which have a CodY homolog.

Amino acid assimilation is crucial for LAB that are generally auxotrophic for amino acids. In addition to RNA structural switches, regulating sulfur amino acid metabolism is also dependent on LysR-family transcriptional regulators, including CmbR in *L. lactis* and CysR, HomR, and MetR in *Strep. mutans* (Liu et al. 2012 and references within). Global ArgR and AhrC regulons have been established by transcriptome analyses, which indicate that both regulators are dedicated to controlling arginine metabolism in *L. lactis* (Larsen et al. 2008). Interestingly, pneumococcal ArgR and AhrC are not involved in regulating arginine biosynthesis and breakdown, as is the case in other bacteria, and instead control the expression of genes associated with arginine and peptide uptake (Kloosterman and Kuipers 2011). AhrC has also recently been reported to have a role in *Strep. pneumoniae* (Kloosterman and Kuipers 2011) and *E. faecalis* (Frank et al. 2013) virulence. Very few global studies of modifications in LAB cell metabolism and the various regulatory mechanisms involved with amino acid shortages have been performed. One study analyzed the global transcriptome and proteomic response of *L. lactis* during progressive isoleucine starvation. CodY appeared to be specifically dedicated to regulating the supply of isoleucine, while the other regulators identified were associated with growth rate and stringent response (Dressaire et al. 2011).

1.6. Functional Genomics and Metabolism

1.6.1. Transcriptomics, Proteomics, and Metabolomics

One of the greatest achievements of recent molecular biology is the development of high-throughput functional genomics approaches, such as transcriptomics, proteomics, or metabolomics. Transcriptomics have been used to analyze the LAB response to different growth conditions, culturing regimens, and various stresses (de Vos 2011). A variety of studies, including attempts at functional genomics, have contributed to the discovery and further characterization of respiratory metabolism in many LAB species (for recent reviews see Lechardeur et al. 2011a; Pedersen et al. 2012). Respiration was found to be activated by cofactors such as exogenous heme or heme and menaquinone. Some LAB species were also found to use nitrate as a terminal electron acceptor (Brooijmans et al. 2009). Furthermore, activating electron transport chains leads to higher biomass production and increased robustness, which is beneficial for industrial applications (Pedersen et al. 2012).

Transcriptional analysis identified genes involved in oxygen respiration. For example, the *ygfCBA* operon from *L. lactis*, which encodes a putative transport system and regulator, is strongly induced by heme and is involved in heme tolerance and homeostasis (Pedersen et al. 2008). YgfC (or HrtR4) is a heme-responsive repressor that regulates *hrtRBA* (formerly *ygfCBA*) operon expression in response to free heme molecules (Lechardeur et al. 2011b). Recently, the crystal structures of HrtR have confirmed that it senses and binds a heme molecule to regulate expression of the heme-efflux system that is responsible for heme homeostasis in *L. lactis* (Sawai et al. 2012). A community transcriptomic approach revealed huge differences in gene expression profiles between *Lact. plantarum* from the human intestine and that from laboratory media (Marco et al. 2010). Moreover, similar expression profiles were observed in mice and humans, which suggests that there are some common intestinal adaptation mechanisms. The metabolic capacity of *Lact. plantarum* is specifically adapted in the intestine for carbohydrate acquisition and the expression of exopolysaccharide and proteinaceous cell surface compounds. The transcriptome response in complex food ecosystems has also been analyzed in bacteria in yogurt fermentation. In milk, *Strep. thermophilus* LMD-9 in the presence of *Lact. bulgaricus* overexpresses genes involved in amino acid transport and metabolism as well as DNA replication (Goh et al. 2011). The metatranscriptomic approach in yogurt showed that specific compounds and metabolic pathways are involved in interactions between *Strep. thermophilus* and *Lact. bulgaricus* strains (Sieuwerts et al. 2010). Similar approaches have been applied to complex communities in fermented foods, such as kimchi (Nam et al. 2009) or sourdough (Weckx et al. 2011).

Proteomic tools can also be used to improve the understanding of metabolism in food microorganisms. Recently, the proteomic approach was used to quantitatively analyze the bacterial enzymes released in Swiss-type cheese during ripening (Jardin et al. 2012). This approach has also been used to investigate stress responses in LAB to acidic growth conditions (Nezhad 2010) or low temperatures (Garnier et al. 2010). Recent modeling approaches of protein and mRNA stability based on transcriptome and proteome *L. lactis* data have also increased the quantitative abilities of functional genomics (Dressaire et al. 2009; Picard et al. 2009).

Global metabolomics studies of LAB have not been frequently reported (de Vos 2011). One study of folate-overproducing *Lact. plantarum* cells (Wegkamp et al. 2010) found that reductions in growth rate occurred when the folate gene cluster was overexpressed. In another study, the response of *L. lactis* to acid stress was analyzed at the metabolite level and complemented by performing a genome-wide transcriptome analysis (Carvalho et al. 2013). The H+/lactate stoichiometry of lactic acid export was determined, and the study findings were used to propose a metabolic model that accounted for the molecular mechanisms of the *L. lactis* response to acid stress.

1.6.2. Global Phenotypic Characterization of Microbial Cells

Phenotype MicroArray™ (PM) technology (Biolog Inc.) is a system of cellular assays designed for the simultaneous and high-throughput screening of a large variety of phenotypes. This technology can be used to complement traditional genomic, transcriptomic, and proteomic approaches. PM can test for nearly 2000 microbial phenotypes (C, N, P, and S metabolism; pH growth range; pH control; sensitivity to NaCl and other ions; and sensitivity to chemical agents) to gain a comprehensive overview of pathway functions in a single experiment (Bochner 2009). In recent years, several studies have presented the results of PM analysis in LAB. PM is most often used to analyze gene function by comparing LAB cell lines that differ by a single gene mutation. For example, the role of the lactate dehydrogenase enzyme (LDH) in *L. lactis*, *E. faecalis*, and *Strep. pyogenes* was identified by screening a large variety of carbon sources using PM arrays. The *ldh* deletion mutants were not able to utilize all carbon sources as efficiently as their corresponding wild-types, which explained why their growth was impaired in a rich but not chemically-defined medium (Fiedler et al. 2010). In another study, the *liaS* gene in *Strep. mutants* was found to encode a cell-envelope stress-sensing histidine kinase, which is thought to be important for the expression of virulence factors (Zhang and Biswas 2009). The *liaS* mutant was more tolerant to various inhibitors that targeted protein synthesis, DNA synthesis, and cell-wall biosynthesis.

PM can also be used to analyze naturally occurring microbial strains, the biological properties of these strains, the effects of chemicals on their cells, and as a tool in bioprocess optimization. For instance, PM was used to understand the phenotypic manifestations of bacterial environmental adaptation (Di Cagno et al. 2010). In this study, the metabolisms of 72 *Lact. plantarum* isolates from raw vegetables and fruits were compared on a variety of carbon sources. Eight clusters of *Lact. plantarum* isolates were categorized based on their metabolism on 27 carbon sources, which interestingly, was not associated with their original habitats (Di Cagno et al. 2010). In another study, combined genomic, transcriptomic, and PM analyses were used to understand the survival capacity of microbes in sourdough (Passerini et al. 2013a). Bacteria that could effectively utilize five-carbon (C5) and six-carbon (C6) sugars from lignocellulose would significantly improve the economic conversion of lignocellulose to biofuels and bioproducts. PM analyses indicated that the *Lactobacillus buchneri* strain can metabolize a broad spectrum of carbon sources, including various C5 and C6 monosaccharides, disaccharides, and oligosaccharides. Furthermore, *Lact. buchneri* had better metabolic rates under anaerobic conditions, high tolerance to ethanol and other stresses, and therefore was an attractive candidate for biomass conversion to various bioproducts (Liu et al. 2007).

1.7. Systems Biology of LAB

Different modeling approaches have been successfully applied to LAB monocultures (Santos et al. 2013). Kinetics models are used to integrate experimental data from biochemical studies of transport, enzyme kinetics, as well as flux and metabolites measurements (Teusink et al. 2011). In *L. lactis*, kinetics modeling has been primarily used to model glycolysis (Hoefnagel et al. 2002a, 2002b; Voit et al. 2006; Andersen et al. 2009; Oh 2011; Levering et al. 2012). Genome sequencing, genome analysis, and omic techniques (proteomics, transcriptomics, metabolomics, etc.) have been used to construct genome-scale metabolic models (Teusink et al. 2006; Pastink et al. 2009; Santos et al. 2009; Flahaut et al. 2013), which can help elucidate many cellular biochemical pathways. Coupling genomic data with metabolic fingerprinting using high-throughput techniques can further facilitate the extraction of relevant biological information. Such techniques have been used to characterize citrate utilization and the subsequent generation of flavor compounds (Bachmann et al. 2009; de Bok et al. 2011). The latest developments in and future of modeling lie in mixed culture and complex ecosystem applications (for a review, see Santos et al. 2013). These analyses have been

applied to food consortia, such as *Strep. thermophilus* and *Lact. bulgaricus* in yogurt (Sieuwerts 2009) as well as the gut microbiota of the human gastrointestinal tract (de Graaf et al. 2010). Unravelling the physiology of multi-species microbial communities is a great challenge and will require the combination of phylogenetic, metagenomic, and meta-functional genomics data (Martins dos Santos et al. 2010).

Furthermore, systems biology is another powerful tool that can be used to decipher metabolism in LAB. Systems biology studies microorganisms as integrated and interacting networks of genes, proteins, and biochemical reactions (Teusink et al. 2011; de Vos 2011).

Acknowledgments

Work in the authors' laboratories has been supported by the Applied Research Program (PBS) of the National Centre for Research and Development (NCBR) funds (PBS2/B8/12/2014) and the National Science Centre (NCN) (grant UMO-2011/01/B/NZ2/05377). Projects were also funded by the Spanish Ministry of Economy and Competitiveness and Innovation (Ref. AGL2011-24300) and INIA (Ref. RM2010-00017-00-00 and RM2011-00005-00-00).

References

Abdel-Rahman, M.A., Tashiro, Y., and Sonomoto, K. (2011) Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: Overview and limits. *J Biotechnol* **156**, 286–301.

Aleksandrzak-Piekarczyk, T. (2013) Lactose and β-glucosides metabolism and its regulation in *Lactococcus lactis*: A review. In: *Lactic Acid Bacteria - R& D for Food, Health and Livestock Purposes*. ed Marcelino Kongo, J. InTech. Available at http://cdn.intechopen.com/pdfs-wm/42338.pdf [accessed 30 January 2013].

Andersen, A.Z., Carvalho, A.L., Neves, A.R., et al. (2009) The metabolic pH response in *Lactococcus lactis*: An integrative experimental and modelling approach. *Comput Biol Chem* **33**, 71–83.

Bachmann, H., Kruijswijk, Z., Molenaar, D., et al. (2009) A high-throughput cheese manufacturing model for effective cheese starter culture screening. *J Dairy Sci* **92**, 5868–5882.

Bachmann, H., Starrenburg, M.J.C., Molenaar, D., et al. J.E.T. (2012) Microbial domestication signatures of *Lactococcus lactis* can be reproduced by experimental evolution. *Genome Res* **22**, 115–124.

Badel, S., Bernardi, T., and Michaud, P. (2011) New perspectives for Lactobacilli exopolysaccharides. *Biotechnol Adv* **29**, 54–66.

Bailey, T.L., Boden, M., Buske, F.A., et al. (2009) MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* **37** (Web Server), W202–W208.

Bekal, S., Van Beeumen, J., Samyn, B., et al. (1998) Purification of *Leuconostoc mesenteroides* citrate lyase and cloning and characterization of the *citCDEFG* gene cluster. *J Bacteriol* **180**, 647–654.

Bochner, B.R. (2009) Global phenotypic characterization of bacteria. *FEMS Microbiol Rev* **33**, 191–205.

De Bok, F.A.M., Janssen, P.W.M., Bayjanov, J.R., et al. (2011) Volatile compound fingerprinting of mixed-culture fermentations. *Appl Environ Microbiol* **77**, 6233–6239.

Bolotin, A., Wincker, P., Mauger, S., et al. (2001) The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res* **11**, 731–753.

Bonifait, L., de la Cruz Dominguez-Punaro, M., Vaillancourt, K., et al. (2010) The cell envelope subtilisin-like proteinase is a virulence determinant for *Streptococcus suis*. *BMC Microbiol* **10**, 42.

Bounaix, M.-S., Gabriel, V., Morel, S., et al. (2009) Biodiversity of exopolysaccharides produced from sucrose by sourdough Lactic Acid Bacteria. *J Agric Food Chem* **57**, 10889–10897.

Bounaix, M.-S., Gabriel, V., Robert, H., et al. (2010) Characterization of glucan-producing *Leuconostoc* strains isolated from sourdough. *Int J Food Microbiol* **144**, 1–9.

Broadbent, J.R., Cai, H., Larsen, R.L., et al. (2011) Genetic diversity in proteolytic enzymes and amino acid metabolism among *Lactobacillus helveticus* strains. *J Dairy Sci* **94**, 4313–4328.

Brooijmans, R., de Vos, W.M., and Hugenholtz, J. (2009) Electron transport chains of lactic acid bacteria-walking on crutches is part of their lifestyle. *F1000 Biol Rep* **1**. Available at http://F1000.com/Reports/Biology/content/1/34 [accessed 29 April 2009].

Di Cagno, R., Minervini, G., Sgarbi, E., et al. (2010) Comparison of phenotypic (Biolog System) and genotypic (random amplified polymorphic DNA-polymerase chain reaction, RAPD-PCR, and amplified fragment length polymorphism, AFLP) methods for typing *Lactobacillus plantarum* isolates from raw vegetables and fruits. *Int J Food Microbiol* **143**, 246–253.

Calles-Enriquez, M., Eriksen, B.H., Andersen, P.S., et al. (2010) Sequencing and transcriptional analysis of the *Streptococcus thermophilus* histamine biosynthesis gene cluster: factors that affect differential *hdcA* expression. *Appl Environ Microbiol* **76**, 6231–6238.

Carvalho, A.L., Turner, D.L., Fonseca, L.L., et al. (2013) Metabolic and transcriptional analysis of acid stress in *Lactococcus lactis*, with a focus on the kinetics of lactic acid pools. *PLoS One* **8**, e68470.

Castillo Martinez, F.A., Balciunas, E.M., Salgado, J.M., et al. (2013) Lactic acid properties, applications and production: A review. *Trends Food Sci Technol* **30**, 70–83.

Castro, R., Neves, A.R., Fonseca, L.L., et al. (2009) Characterization of the individual glucose uptake systems of *Lactococcus lactis*: mannose-PTS, cellobiose-PTS and the novel GlcU permease. *Mol Microbiol* **71**, 795–806.

Ciszek-Lenda, M., Nowak, B., Śróttek, M., et al. (2011) Immunoregulatory potential of exopolysaccharide from *Lactobacillus rhamnosus* KL37. Effects on the production of inflammatory mediators by mouse macrophages: *L. rhamnosus* exopolysaccharide and inflammation. *Int J Exp Pathol* **92**, 382–391.

Cretenet, M., Laroute, V., Ulve, V., et al. (2010) Dynamic analysis of the *Lactococcus lactis* transcriptome in cheeses made from milk concentrated by ultrafiltration reveals multiple strategies of adaptation to stresses. *Appl Environ Microbiol* **77**, 247–257.

Dandoy, D., Fremaux, C., de Frahan, M.H., et al. (2011) The fast milk acidifying phenotype of *Streptococcus thermophilus* can be acquired by natural transformation of the genomic island encoding the cell-envelope proteinase PrtS. *Microb Cell Fact* **10** (Suppl 1), S21.

Delorme, C., Bartholini, C., Bolotine, A., et al. (2010) Emergence of a cell wall protease in the *Streptococcus thermophilus* population. *Appl Environ Microbiol* **76**, 451–460.

Dertli, E., Colquhoun, I.J., Gunning, A.P., et al. (2013) Structure and biosynthesis of two exopolysaccharides produced by *Lactobacillus johnsonii* FI9785. *J Biol Chem* **288**, 31938–31951.

Deutscher, J. (2008) The mechanisms of carbon catabolite repression in bacteria. *Curr Opin Microbiol* **11**, 87–93.

Díaz-Muñiz, I., Banavara, D.S., Budinich, M.F., et al. (2006) *Lactobacillus casei* metabolic potential to utilize citrate as an energy source in ripening cheese: a bioinformatics approach. *J Appl Microbiol* **101**, 872–882.

Dressaire, C., Gitton, C., Loubière, P., et al. (2009) Transcriptome and proteome exploration to model translation efficiency and protein stability in *Lactococcus lactis*. *PLoS Comput Biol* **5**, e1000606.

Dressaire, C., Redon, E., Gitton, C., et al. (2011) Investigation of the adaptation of *Lactococcus lactis* to isoleucine starvation integrating dynamic transcriptome and proteome information. *Microb Cell Fact* **10** (Suppl 1), S18.

Drici, H., Gilbert, C., Kihal, M., and Atlan, D. (2010) Atypical citrate-fermenting *Lactococcus lactis* strains isolated from dromedary's milk. *J Appl Microbiol* **108**, 647–657.

Drider, D., Bekal, S., and Prévost, H. (2004) Genetic organization and expression of citrate permease in lactic acid bacteria. *Genet Mol Res* **3**, 271–281.

Espariz, M., Repizo, G., Blancato, V., et al. (2011) Identification of malic and soluble oxaloacetate decarboxylase enzymes in *Enterococcus faecalis*: *E. faecalis* malic enzyme family proteins. *FEBS J* **278**, 2140–2151.

Feng, M., Chen, X., Li, C., et al. (2012) Isolation and identification of an exopolysaccharide-producing lactic acid bacterium strain from chinese paocai and biosorption of Pb(II) by its exopolysaccharide. *J Food Sci* **77**, T111–T117.

Fernandez, E., Alegria, A., Delgado, S., et al. (2011) Comparative phenotypic and molecular genetic profiling of wild *Lactococcus lactis* subsp. *lactis* strains of the *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* genotypes, isolated from starter-free cheeses made of raw milk. *Appl Environ Microbiol* **77**, 5324–5335.

Fernández, M., van Doesburg, W., Rutten, G.A., et al. (2000) Molecular and functional analyses of the *metC* gene of *Lactococcus lactis*, encoding cystathionine β-lyase. *Appl Environ Microbiol* **66**, 42–48.

Fiedler, T., Bekker, M., Jonsson, M., et al. (2010) Characterization of three lactic acid bacteria and their isogenic *ldh* deletion mutants shows optimization for YATP (cell mass produced per mole of ATP) at their physiological pHs. *Appl Environ Microbiol* **77**, 612–617.

Flahaut, N.A.L., Wiersma, A., van de Bunt, B., et al. (2013) Genome-scale metabolic model for *Lactococcus lactis* MG1363 and its application to the analysis of flavor formation - Springer. *Appl Microbiol Biotechnol* **97**, 8729–8739.

Frank, K.L., Guiton, P.S., Barnes, A.M.T., et al. (2013) AhrC and Eep are biofilm infection-associated virulence factors in *Enterococcus faecalis*. *Infect Immun* **81**, 1696–1708.

Galle, S., Schwab, C., Arendt, E., and Gänzle, M. (2010) Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. *J Agric Food Chem* **58**, 5834–5841.

Galle, S., Schwab, C., Arendt, E.K., and Gänzle, M.G. (2011) Structural and rheological characterisation of heteropolysaccharides produced by lactic acid bacteria in wheat and sorghum sourdough. *Food Microbiol* **28**, 547–553.

Gänzle, M.G. and Follador, R. (2012) Metabolism of oligosaccharides and starch in lactobacilli: A review. *Front Microbiol* **3**. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3458588/pdf/fmicb-03-00340.pdf [accessed 26 September 2012].

Garnier, M., Matamoros, S., Chevret, D., et al. (2010) Adaptation to cold and proteomic responses of the psychrotrophic biopreservative *Lactococcus piscium* strain CNCM I-4031. *Appl Environ Microbiol* **76**, 8011–8018.

Genay, M., Sadat, L., Gagnaire, V., and Lortal, S. (2009) *PrtH2*, not *prtH*, is the ubiquitous cell wall proteinase gene in *Lactobacillus helveticus*. *Appl Environ Microbiol* **75**, 3238–3249.

Goh, Y.J., Goin, C., O'Flaherty, S., et al. (2011) Specialized adaptation of a lactic acid bacterium to the milk environment: the comparative genomics of *Streptococcus thermophilus* LMD-9. *Microb Cell Fact* **10** (Suppl 1), S22.

Görke, B. and Stülke, J. (2008) Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nat Rev Microbiol* **6**, 613–624.

Górska-Frączek, S., Sandström, C., Kenne, L., et al. (2013) The structure and immunoreactivity of exopolysaccharide isolated from *Lactobacillus johnsonii* strain 151. *Carbohydr Res* **378**, 148–153.

Górska-Frączek, S., Sandström, C., Kenne, L., et al. (2011) Structural studies of the exopolysaccharide consisting of a nonasaccharide repeating unit isolated from *Lactobacillus rhamnosus* KL37B. *Carbohydr Res* **346**, 2926–2932.

De Graaf, A.A., Maathuis, A., de Waard, P., et al. (2010) Profiling human gut bacterial metabolism and its kinetics using [U-13C] glucose and NMR. *NMR Biomed* **23**, 2–12.

Griffiths, M.W. and Tellez, A.M. (2013) *Lactobacillus helveticus*: the proteolytic system. *Front Microbiol* **4**. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587842/pdf/fmicb-04-00030.pdf [accessed 5 March 2013].

Hanniffy, S.B., Pelaez, C., Martinez-Bartolome, M.A., et al. (2009) Key enzymes involved in methionine catabolism by cheese lactic acid bacteria. *Int J Food Microbiol* **135**, 223–230.

Hendriksen, W.T., Bootsma, H.J., Estevao, S., et al. (2008) CodY of *Streptococcus pneumoniae*: link between nutritional gene regulation and colonization. *J Bacteriol* **190**, 590–601.

Hoefnagel, M.H., van der Burgt, A., Martens, D.E., et al. (2002a) Time dependent responses of glycolytic intermediates in a detailed glycolytic model of *Lactococcus lactis* during glucose run-out experiments. *Mol Biol Rep* **29**, 157–161.

Hoefnagel, M.H., Starrenburg, M.J., Martens, D.E., et al. (2002b) Metabolic engineering of lactic acid bacteria, the combined approach: kinetic modelling, metabolic control and experimental analysis. *Microbiology* **148**, 1003–1013.

Jardin, J., Mollé, D., Piot, M., et al. (2012) Quantitative proteomic analysis of bacterial enzymes released in cheese during ripening. *Int J Food Microbiol* **155**, 19–28.

Jensen, M.P., Ardö, Y., and Vogensen, F.K. (2009) Isolation of cultivable thermophilic lactic acid bacteria from cheeses made with mesophilic starter and molecular comparison with dairy-related *Lactobacillus helveticus* strains. *Lett Appl Microbiol* **49**, 396–402.

De Jong, A., Hansen, M.E., Kuipers, O.P., et al. (2013) The transcriptional and gene regulatory network of *Lactococcus lactis* MG1363 during Growth in Milk. *PLoS One* **8**, e53085.

De Jong, A., Pietersma, H., Cordes, M., et al. (2012) PePPER: a webserver for prediction of prokaryote promoter elements and regulons. *BMC Genomics* **13**, 299.

den Hengst, C.D., van Hijum, S.A., Geurts, J.M., et al. (2005) The Lactococcus lactis CodY regulon: identification of a conserved cis-regulatory element. *J Biol Chem* **7**, 343323–34342.

Jung, J.Y., Lee, S.H., Kim, J.M., et al. (2011) Metagenomic analysis of kimchi, a traditional korean fermented food. *Appl Environ Microbiol* **77**, 2264–2274.

Kant, R., Blom, J., Palva, A., Siezen, R.J., and de Vos, W.M. (2011) Comparative genomics of *Lactobacillus*: Comparative analysis of 20 complete *Lactobacillus* genomes. *Microb Biotechnol* **4**, 323–332.

Katina, K., Maina, N.H., Juvonen, R., et al. (2009) *In situ* production and analysis of *Weissella confusa* dextran in wheat sourdough. *Food Microbiol* **26**, 734–743.

Kelly, W.J., Ward, L.J.H., and Leahy, S.C. (2010) Chromosomal diversity in *Lactococcus lactis* and the origin of dairy starter cultures. *Genome Biol Evol* **2**, 729-744.

Kim, Y., Oh, S., Yun, H.S., Oh, S., and Kim, S.H. (2010) Cell-bound exopolysaccharide from probiotic bacteria induces autophagic cell death of tumour cells. *Lett Appl Microbiol* **51**, 123-130.

Kloosterman, T.G. and Kuipers, O.P. (2011) Regulation of arginine acquisition and virulence gene expression in the human pathogen *Streptococcus pneumoniae* by transcription regulators ArgR1 and AhrC. *J Biol Chem* **286**, 44594–44605.

Koryszewska-Baginska, A., Bardowski, J., and Aleksandrzak-Piekarczyk, T. (2014) Genome sequence of the probiotic strain *Lactobacillus rhamnosus* (Formerly *Lactobacillus casei*) LOCK908. *Genome Announc* **2**, e00120–14–e00120–14.

Krzywinski, M., Schein, J., Birol, I., et al. (2009) Circos: An information aesthetic for comparative genomics. *Genome Res* **19**, 1639–1645.

Ladero, V., Fernández, M., Calles-Enríquez, M., et al. (2012) Is the production of the biogenic amines tyramine and putrescine a species-level trait in enterococci? *Food Microbiol* **30**, 132–138.

Larsen, R., van Hijum, S.A.F.T., Martinussen, J., et al. (2008) Transcriptome analysis of the *Lactococcus lactis* ArgR and AhrC regulons. *Appl Environ Microbiol* **74**, 4768–4771.

Lechardeur, D., Cesselin, B., Fernandez, A., et al. (2011a) Using heme as an energy boost for lactic acid bacteria. *Curr Opin Biotechnol* **22**, 143–149.

Lechardeur, D., Cesselin, B., Liebl, U., et al. (2011b) Discovery of intracellular heme-binding protein HrtR, which controls heme efflux by the conserved HrtB-HrtA transporter in *Lactococcus lactis*. *J Biol Chem* **287**, 4752–4758.

Leemhuis, H., Pijning, T., Dobruchowska, J.M., et al. (2013) Glucansucrases: Three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *J Biotechnol* **163**, 250–272.

Lemos, J.A., Nascimento, M.M., Lin, V.K., et al. (2008) Global regulation by (p)ppGpp and CodY in *Streptococcus mutans*. *J Bacteriol* **190**, 5291–5299.

Lensbouer, J.J. and Doyle, R.P. (2010) Secondary transport of metal–citrate complexes: the CitMHS family. *Crit Rev Biochem Mol Biol* **45**, 453–462.

Levering, J., Musters, M.W.J.M., Bekker, M., et al. (2012) Role of phosphate in the central metabolism of two lactic acid bacteria - a comparative systems biology approach: Modelling glycolysis of lactic acid bacteria. *FEBS J* **279**, 1274–1290.

Linares, D.M., Kok, J., and Poolman, B. (2010) Genome sequences of *Lactococcus lactis* MG1363 (Revised) and NZ9000 and comparative physiological studies. *J Bacteriol* **192**, 5806–5812.

Linares, D.M., del Río, B., Ladero, V., et al. (2012) Factors influencing biogenic amines accumulation in dairy products. *Front Microbiol* **3**. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3390585/pdf/fmicb-03-00180.pdf [accessed 28 May 2012].

Liu, C.-F., Tseng, K.-C., Chiang, S.-S., et al. (2011) Immunomodulatory and antioxidant potential of *Lactobacillus* exopolysaccharides. *J Sci Food Agric* **91**, 2284-2291.

Liu, F., Du, L., Du, P., and Huo, G. (2009) Possible promoter regions within the proteolytic system in *Streptococcus thermophilus* and their interaction with the CodY homolog. *FEMS Microbiol Lett* **297**, 164–172.

Liu, M., Bayjanov, J.R., Renckens, B., et al. (2010) The proteolytic system of lactic acid bacteria revisited: a genomic comparison. *BMC Genomics* **11**, 36.

Liu, M., Prakash, C., Nauta, A., et al. (2012) Computational analysis of cysteine and methionine metabolism and its regulation in dairy starter and related bacteria. *J Bacteriol* **194**, 3522–3533.

Liu, S., Skinner-Nemec, K.A., and Leathers, T.D. (2007) *Lactobacillus buchneri* strain NRRL B-30929 converts a concentrated mixture of xylose and glucose into ethanol and other products. *J Ind Microbiol Biotechnol* **35**, 75–81.

Lucas, P.M., Wolken, W.A.M., Claisse, O., et al. (2005) Histamine-producing pathway encoded on an unstable plasmid in *Lactobacillus hilgardii* 0006. *Appl Environ Microbiol* **71**, 1417–1424.

Makarova, K., Slesarev, A., Wolf, Y., et al. (2006) Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci U S A* **103**, 15611–15616.

Marco, M.L., de Vries, M.C., Wels, M., et al. (2010) Convergence in probiotic *Lactobacillus* gut-adaptive responses in humans and mice. *ISME J* **4**, 1481–1484.

Martín, M., Corrales, M.A., Mendoza, D., et al. (1999) Cloning and molecular characterization of the citrate utilization *citMCDEFGRP* cluster of *Leuconostoc paramesenteroides*. *FEMS Microbiol Lett* **174**, 231–238.

Martín, M., Magni, C., López, P., and de Mendoza, D. (2000) Transcriptional control of the citrate-inducible *citMCDEFGRP* operon, encoding genes involved in citrate fermentation in *Leuconostoc paramesenteroides*. *J Bacteriol* **182**, 3904–3912.

Martins dos Santos, V.A., Müller, M., and De Vos, W.M. (2010) Systems biology of the gut: the interplay of food, microbiota and host at the mucosal interface. *Curr Opin Biotechnol* **21**, 539–550.

Martinussen, J., Solem, C., Holm, A.K., and Jensen, P.R. (2013) Engineering strategies aimed at control of acidification rate of lactic acid bacteria. *Curr Opin Biotechnol* **24**, 124–129.

Mayo, B., Aleksandrzak-Piekarczyk, T., Fernández, M., et al. (2010) Updates in the metabolism of lactic acid bacteria. In: *Biotechnology of Lactic Acid Bacteria* ((eds) Mozzi, F., Raya, R.R., Vignolo, G.M.) pp. 3–33. Wiley-Blackwell. Available at http://dx.doi.org/10.1002/9780813820866.ch1 [accessed 25 March 2010].

Mazzeo, M.F., Cacace, G., Peluso, A., et al. (2012) Effect of inactivation of *ccpA* and aerobic growth in *Lactobacillus plantarum*: A proteomic perspective. *J Proteomics* **75**, 4050–4061.

Methé, B.A., Nelson, K.E., Pop, M., et al. (2012) A framework for human microbiome research. *Nature* **486**, 215–221.

Moroni, A.V., Dal Bello, F., and Arendt, E.K. (2009) Sourdough in gluten-free bread-making: An ancient technology to solve a novel issue? *Food Microbiol* **26**, 676–684.

Mortera, P., Pudlik, A., Magni, C., et al. (2013) Ca2+-citrate uptake and metabolism in *Lactobacillus casei* ATCC 334. *Appl Environ Microbiol* **79**, 4603–4612.

Nam, Y.D., Chang, H.W., Kim, K.H., et al. (2009) Metatranscriptome analysis of lactic acid bacteria during kimchi fermentation with genome probing microarrays. *Int J Food Microbiol* **130**, 140–146.

Neves, A., Pool, W., Kok, J., et al. (2005) Overview on sugar metabolism and its control in – The input from *in vivo* NMR. *FEMS Microbiol Rev* **29**, 531–554.

Neves, A.R., Pool, W.A., Solopova, A., et al. (2010) Towards enhanced galactose utilization by *Lactococcus lactis*. *Appl Environ Microbiol* **76**, 7048–7060.

Nezhad, Mh. (2010) Effect of growth at low pH on the cell surface properties of a typical strain of *Lactobacillus casei* group. *Iran J Microbiol* **2**. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279780/pdf/IJM-2-147.pdf [accessed July 2010].

Ng, P. and Keich, U. (2008) GIMSAN: a Gibbs motif finder with significance analysis. *Bioinformatics* **24**, 2256–2257.

Notararigo, S., Nácher-Vázquez, M., Ibarburu, I., et al. (2013) Comparative analysis of production and purification of homo- and hetero-polysaccharides produced by lactic acid bacteria. *Carbohydr Polym* **93**, 57–64.

O'Sullivan, O., O'Callaghan, J., Sangrador-Vegas, A., et al. (2009) Comparative genomics of lactic acid bacteria reveals a niche-specific gene set. *BMC Microbiol* **9**, 50.

Oh, E. (2011) Dynamic modeling of lactic acid fermentation metabolism with *Lactococcus lactis*. *J Microbiol Biotechnol* **21**, 162–169.

Okano, K., Tanaka, T., Ogino, C., et al. (2009) Biotechnological production of enantiomeric pure lactic acid from renewable resources: recent achievements, perspectives, and limits. *Appl Microbiol Biotechnol* **85**, 413–423.

Overmars, L., Kerkhoven, R., Siezen, R.J., and Francke, C. (2013) MGcV: the microbial genomic context viewer for comparative genome analysis. *BMC Genomics* **14**, 209.

Palomba, S., Cavella, S., Torrieri, E., et al. (2012) Polyphasic screening, homopolysaccharide composition, and viscoelastic behavior of wheat sourdough from a *Leuconostoc lactis* and *Lactobacillus curvatus* exopolysaccharide-producing starter culture. *Appl Environ Microbiol* **78**, 2737–2747.

Passerini, D., Coddeville, M., Le Bourgeois, P., et al. (2013a) The carbohydrate metabolism signature of *Lactococcus lactis* strain A12 reveals its sourdough ecosystem origin. *Appl Environ Microbiol* **79**, 5844–5852.

Passerini, D., Laroute, V., Coddeville, M., et al. (2013b) New insights into *Lactococcus lactis* diacetyl- and acetoin-producing strains isolated from diverse origins. *Int J Food Microbiol* **160**, 329–336.

Pastink, M.I., Teusink, B., Hols, P., et al. (2009) Genome-scale model of *Streptococcus thermophilus* LMG18311 for metabolic comparison of lactic acid bacteria. *Appl Environ Microbiol* **75**, 3627–3633.

Patel, S., Majumder, A., and Goyal, A. (2011) Potentials of Exopolysaccharides from lactic acid bacteria. *Indian J Microbiol* **52**, 3–12.

Patten, D.A., Leivers, S., Chadha, M.J., et al. (2014) The structure and immunomodulatory activity on intestinal epithelial cells of the EPSs isolated from *Lactobacillus helveticus* sp. *Rosyjski* and *Lactobacillus acidophilus* sp. 5e2. *Carbohydr Res* **384**, 119–127.

Pedersen, M.B., Garrigues, C., Tuphile, K., et al. (2008) Impact of aeration and heme-activated respiration on *Lactococcus lactis* gene expression: identification of a heme-responsive operon. *J Bacteriol* **190**, 4903–4911.

Pedersen, M.B., Gaudu, P., Lechardeur, D., et al. (2012) Aerobic respiration metabolism in lactic acid bacteria and uses in biotechnology. *Annu Rev Food Sci Technol* **3**, 37–358.

Pepe, O., Ventorino, V., Cavella, S., et al. (2013) Prebiotic content of bread prepared with flour from immature wheat grain and selected dextran-producing lactic acid bacteria. *Appl Environ Microbiol* **79**, 3779–3785.

Picard, F., Dressaire, C., Girbal, L., and Cocaign-Bousquet, M. (2009) Examination of post-transcriptional regulations in prokaryotes by integrative biology. *C R Biol* **332**, 958–973.

Pudlik, A.M. and Lolkema, J.S. (2010) Citrate uptake in exchange with intermediates in the citrate metabolic pathway in *Lactococcus lactis* IL1403. *J Bacteriol* **193**, 706–714.

Qin, J., Li, R., Raes, J., Arumugam, M., et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65.

Quang, D. and Xie, X. (2014) EXTREME: An online EM algorithm for motif discovery. *Bioinformatics* btu093.

Quintans, G., Nieves, Blancato, V., Repizo, G., et al. (2008) Citrate metabolism and aroma compound production in lactic acid bacteria. In: *Molecular Aspects of Lactic Acid Bacteria for Traditional and New Applications* ((eds) Mayo, B. López, P. and Pérez-Martínez, G.) pp. 65–88. Kerala: Research Signpost. Available at http://www.cib.csic.es/repositorio_bd/publicacion/1773/urls_documento/chapter_3_publicado.pdf.

Rademaker, J.L.W., Herbet, H., Starrenburg, M.J.C., et al. (2007) Diversity analysis of dairy and nondairy *Lactococcus lactis* isolates, using a novel multilocus sequence analysis scheme and (GTG)5-PCR fingerprinting. *Appl Environ Microbiol* **73**, 7128–7137.

Ramchandran, L. and Shah, N.P. (2009) Effect of exopolysaccharides on the proteolytic and angiotensin-I converting enzyme-inhibitory activities and textural and rheological properties of low-fat yogurt during refrigerated storage. *J Dairy Sci* **92**, 895–906.

Ravcheev, D.A., Best, A.A., Sernova, N.V., et al. (2013) Genomic reconstruction of transcriptional regulatory networks in lactic acid bacteria. *BMC Genomics* **14**, 94.

Raynaud, S., Perrin, R., Cocaign-Bousquet, M., and Loubiere, P. (2005) Metabolic and transcriptomic adaptation of *Lactococcus lactis* subsp. *lactis* Biovar *diacetylactis* in response to autoacidification and temperature downshift in skim milk. *Appl Environ Microbiol* **71**, 8016–8023.

Repizo, G.D., Blancato, V.S., Mortera, P., et al. (2013) Biochemical and genetic characterization of the *Enterococcus faecalis* oxaloacetate decarboxylase complex. *Appl Environ Microbiol* **79**, 2882–2890.

Ritt, J.-F., Remize, F., Grandvalet, C., et al. (2009) Peptidases specific for proline-containing peptides and their unusual peptide-dependent regulation in *Oenococcus oeni*. *J Appl Microbiol* **106**, 801–813.

Russo, P., López, P., Capozzi, V., et al. (2012) Beta-glucans improve growth, viability and colonization of probiotic microorganisms. *Int J Mol Sci* **13**, 6026–6039.

Salazar, N., Prieto, A., Leal, J.A., et al. (2009) Production of exopolysaccharides by *Lactobacillus* and *Bifidobacterium* strains of human origin, and metabolic activity of the producing bacteria in milk. *J Dairy Sci* **92**, 4158–4168.

Santos, F., Teusink, B., Molenaar, D., et al. (2009) Effect of amino acid availability on vitamin B12 production in *Lactobacillus reuteri*. *Appl Environ Microbiol* **75**, 3930–3936.

Santos, F., de Vos, W.M., and Teusink, B. (2013) Towards metagenome-scale models for industrial applications - the case of Lactic Acid Bacteria. *Curr Opin Biotechnol* **24**, 200–206.

Savijoki, K., Ingmer, H., and Varmanen, P. (2006) Proteolytic systems of lactic acid bacteria. *Appl Microbiol Biotechnol* **71**, 394–406.

Sawai, H., Yamanaka, M., Sugimoto, H., et al. (2012) Structural basis for the transcriptional regulation of heme homeostasis in *Lactococcus lactis*. *J Biol Chem* **287**, 30755–30768.

Von Schillde, M.-A., Hörmannsperger, G., Weiher, M., et al. (2012) Lactocepin secreted by *Lactobacillus* exerts anti-inflammatory effects by selectively degrading proinflammatory chemokines. *Cell Host Microbe* **11**, 387–396.

Sender, P.D., Martín, M.G., Peirú, S., and Magni, C. (2004) Characterization of an oxaloacetate decarboxylase that belongs to the malic enzyme family. *FEBS Lett* **570**, 217–222.

Sieuwerts, S. (2009) Analysis of molecular interactions between yoghurt bacteria by an integrated genomics approach. PhD thesis Wageningen University, the Netherlands.

Sieuwerts, S., Molenaar, D., van Hijum, S.A.F.T., et al. (2010) Mixed-culture transcriptome analysis reveals the molecular basis of mixed-culture growth in *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Appl Environ Microbiol* **76**, 7775–7784.

Sims, I.M., Frese, S.A., Walter, J., et al. (2011) Structure and functions of exopolysaccharide produced by gut commensal *Lactobacillus reuteri* 100-23. *ISME J* **5**, 1115–1124.

Smit, G., Smit, B., and Engels, W. (2005) Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiol Rev* **29**, 591–610.

Strahinic, I., Lozo, J., Terzic-Vidojevic, A., et al. (2013) Technological and probiotic potential of BGRA43 a natural isolate of *Lactobacillus helveticus*. *Front Microbiol* **4**. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3552238/pdf/fmicb-04-00002.pdf [accessed 23 January 2013].

Suárez, C.A., Blancato, V.S., Poncet, S., et al. (2011) CcpA represses the expression of the divergent cit operons of *Enterococcus faecalis* through multiple *cre* sites. *BMC Microbiol* **11**, 227.

Sun, H., Yuan, Y., Wu, Y., et al. (2009) Tmod: toolbox of motif discovery. *Bioinformatics* **26**, 405–407.

Teusink, B., Bachmann, H., and Molenaar, D. (2011) Systems biology of lactic acid bacteria: a critical review. *Microb Cell Fact* **10** (suppl 1): S11. Available at http://www.microbialcellfactories.com/content/10/S1/S11 [accessed 30 August 2011].

Teusink, B., Wiersma, A., Molenaar, D., et al. (2006) Analysis of growth of *Lactobacillus plantarum* WCFS1 on a complex medium using a genome-scale metabolic model. *J Biol Chem* **281**, 40041–40048.

The Human Microbiome Jumpstart Reference Strains Consortium, Nelson, K.E., Weinstock, G.M., et al. (2010) A catalog of reference genomes from the human microbiome. *Science* **328**, 994–999.

Trip, H., Mulder, N.L., Rattray, F.P., and Lolkema, J.S. (2011) HdcB, a novel enzyme catalysing maturation of pyruvoyl-dependent histidine decarboxylase: HdcB catalyses maturation of histidine decarboxylase. *Mol Microbiol* **79**, 861–871.

Turpin, W., Humblot, C., and Guyot, J.-P. (2011) Genetic screening of functional properties of lactic acid bacteria in a fermented pearl millet slurry and in the metagenome of fermented starchy foods. *Appl Environ Microbiol* **77**, 8722–8734.

Voit, E.O., Neves, A.R., and Santos, H. (2006) The intricate side of systems biology. *Proceedings of the National Academy of Sciences* **103**, 9452–9457.

De Vos, W.M. (2011) Systems solutions by lactic acid bacteria: from paradigms to practice. *Microb Cell Fact* **10** (Suppl 1), S2. Available at http://www.microbialcellfactories.com/content/10/S1/S2 [accessed 30 August 2011].

Wakai, T. and Yamamoto, N. (2013) A novel branched chain amino acids responsive transcriptional regulator, BCARR, negatively acts on the proteolytic system in *Lactobacillus helveticus*. *PLoS One* **8**, e75976.

Wang, K., Li, W., Rui, X., et al. (2014) Characterization of a novel exopolysaccharide with antitumor activity from *Lactobacillus plantarum* 70810. *Int J Biol Macromol* **63**, 133–139.

Wang, Y., Ganzle, M.G., and Schwab, C. (2010) Exopolysaccharide synthesized by *Lactobacillus reuteri* decreases the ability of enterotoxigenic *Escherichia coli* to bind to porcine erythrocytes. *Appl Environ Microbiol* **76**, 4863–4866.

Weckx, S., Allemeersch, J., Van der Meulen, R., et al. (2011) Metatranscriptome analysis for insight into whole-ecosystem gene expression during spontaneous wheat and spelt sourdough fermentations. *Appl Environ Microbiol* **77**, 618–626.

Wegkamp, A., Mars, A.E., Faijes, M., et al. (2010) Physiological responses to folate overproduction in *Lactobacillus plantarum* WCFS1. *Microb Cell Fact* **9**, 100–106.

Wels, M., Overmars, L., Francke, C., et al. (2011) Reconstruction of the regulatory network of *Lactobacillus plantarum* WCFS1 on basis of correlated gene expression and conserved regulatory motifs: Reconstruction of the *L. plantarum* regulatory network. *Microb Biotechnol* **4**, 333–344.

Zannini, E., Mauch, A., Galle, S., et al. (2013) Barley malt wort fermentation by exopolysaccharide-forming *Weissella cibaria* MG1 for the production of a novel beverage. *J Appl Microbiol* **115**, 1379–1387.

Zhang, J. and Biswas, I. (2009) A phenotypic microarray analysis of a *Streptococcus mutans liaS* mutant. *Microbiology* **155**, 61–68.

Zomer, A.L., Buist, G., Larsen, R., et al. (2006) Time-resolved determination of the CcpA regulon of *Lactococcus lactis* subsp. *cremoris* MG1363. *J Bacteriol* **189**, 1366–1381.