INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 4, p. 34-46, 2014

REVIEW PAPER OPEN ACCESS

Genomics of lactic acid bacteria: features, function, and comparative genomics: a review

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Key words: Genomics, lactic acid bacteria, fermentation, food.

http://dx.doi.org/10.12692/ijb/5.4.34-46 Article published on August 18, 2014

Abstract

Lactic acid bacteria (LAB) are a heterogeneous family and they are associated with various plant and animal niches and play a key role in the production of fermented foods and beverages The lactic acid bacteria used in a variety of ways, including food production, health improvement and production of macromolecules, enzymes and metabolites. Species-to-species variation in the number of pseudogenes as well as genes directing nutrient uptake and metabolism reflects the adaptation of LAB to food matrices and the gastrointestinal tract. Genomic analyses of multiple members of the lactic acid bacteria, at the genus, species, and strain level, have now elucidated many genetic features that direct their fermentative and probiotic roles. This review is based on the genomic content of LAB that is responsible for the functional and ecological diversity of these bacteria and highlights some of the findings from these analyses in the context of the numerous roles the LAB play.

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Introduction

The lactic acid bacteria (LAB) might be the most numerous group of bacteria linked to humans. They are naturally associated with mucosal surfaces, particularly the gastrointestinal tract, and are also indigenous to food-related habitats, including plant (fruits, vegetables, and cereal grains), wine, milk, and meat environments (Kira *et al*., 2007; Wood and Warner; 2003). Lactic acid bacteria (LAB) are historically defined as a group of microaerophilic, Gram-positive organisms that ferment hexose sugars to produce primarily lactic acid (Miller and Wetterstrom, 2000). We are exposed to a huge variety of microorganisms on a daily basis; one group of bacteria that humans have developed a particularly intimate relationship with are the lactic acid bacteria (LAB). The LAB group is composed of microaerophilic, nonsporulating rods and cocci that are functionally linked by their common capacity to produce primarily lactic acid from hexose sugars (Makarova and Koonin 2007; Joel and Todd 2009).The functional classification includes a variety of industrially important genera, including *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc*, and *Lactobacillus* species. The seemingly simplistic metabolism of LAB has been exploited throughout history for the preservation of foods and beverages in nearly all societies dating back to the origins of agriculture (Miller and Wetterstrom, 2000). Domestication of LAB strains passed down through various culinary traditions and continuous passage on food stuffs has resulted in modern-day cultures able to carry out these fermentations. Today, LAB play a prominent role in the world food supply, performing the main bioconversions in fermented dairy products, meats, and vegetables. LAB also are critical for the production of wine, coffee, silage, cocoa, sourdough, and numerous indigenous food fermentations (Wood 1998; Klaenhammer 2006). Complete genome sequences have been published for eight fermentative and commensal LAB species: *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus johnsonii*, *Lactobacillus acidophilus*, *Lactobacillus sakei*, *Lactobacillus bulgaricus*, *Lactobacillus salivarius*, and *Streptococcus thermophilus* (Claesson, 2006; Altermann, 2005; Bolotin, 2004; Chaillou, 2005; Pridmore, 2004). Lactic acid bacteria (LAB) are found to occupy a variety of ecological niches including fermented foods as well as mucosal surfaces of humans and other vertebrates (Todd, 2005). The metabolic characteristics of LAB have been exploited for the preservation of foods and have been passed down from generation to generation through food 'traditions' that continue to flourish in many cultures to this day. Foods fermented using LAB are still widely consumed, the sales of fermented foods reaching tens of billions of dollars per year, worldwide. Recently, commensal LAB have been given increased attention due to evidence suggesting their important roles in the maintenance of health and the prevention of infection (Joel Schroeter and Todd, 2009).

Makarova *et al*., (2006) report nine genome sequences representing the phylogenetic and functional diversity of these bacteria They said lactic acid-producing bacteria are associated with various plant and animal niches and play a key role in the production of fermented foods and beverages.The small genomes of lactic acid bacteria encode a broad repertoire of transporters for efficient carbon and nitrogen acquisition from the nutritionally rich environments they inhabit and reflect a limited range of biosynthetic capabilities that indicate both prototrophic and auxotrophic strains. Phylogenetic analyses, comparison of gene content across the group, and reconstruction of ancestral gene sets indicate a combination of extensive gene loss and key gene acquisitions via horizontal gene transfer during the coevolution of lactic acid bacteria with their habitats.

Klaenhammer *et al*., (2002) summarized a collection of lactic acid bacteria that are undergoing genomic sequencing and analysis. Summaries are presented on twenty different species, with each overview discussing the organisms fundamental and practical significance, environmental habitat, and its role in fermentation, bioprocessing, ormprobiotics. For those

projects where genome sequence data were available by March 2002, summaries include 30 a listing of key statistics and interesting genomic features. These efforts will revolutionize their molecular view of Gram-positive bacteria, as up to 15 genomes from the low GC content lactic acid bacteria are expected to be available in the public domain by the end of 2003. their collective view of the lactic acid bacteria will be fundamentally changed as they rediscover the relationships and capabilities of these organisms through genomics.

Todd (2005) selected members of the lactic acid bacteria have been implicated in a number of probiotic roles that impact general health and well-being. Genomic analyses of multiple members of the lactic acid bacteria, at the genus, species, and strain level, have now elucidated many genetic features that direct their fermentative and probiotic roles. This information is providing an important platform for understanding core mechanisms that control and regulate bacterial growth, survival, signaling, and fermentative processes and, in some cases, potentially underlying probiotic activities within complex microbial and host ecosystems.

Erika *et al*., (2007) concluded the lactic acid bacteria (LAB) are one of the most industrially important groups of bacteria. These organisms are used in a variety of ways, including food production, health improvement and production of macromolecules, enzymes and metabolites. The genome sequencing of 20 LAB provides an expanded view of their genetic and metabolic capacities and enables researchers to perform functional and comparative genomic studies. They highlights some of the findings from these analyses in the context of the numerous roles the LAB play.

This review is based on the genomic content of LAB that is responsible for the functional and ecological diversity of these bacteria and highlights some of the findings from these analyses in the context of the numerous roles the LAB play.

Genomic history and general genome features of lactic acid bacteria

Sequencing the genomes of many species in a class of bacteria enables the examination of their evolution and divergence. Divergence of Lactobacillales from their ancestor in the Bacilli was marked by the loss of 600–1200 genes, including many genes encoding biosynthetic enzymes (Makarova and Koonin, 2007). Other losses include genes related to sporulation, a function that is seemingly unnecessary in nutrient-rich food environments (Makarova. and Koonin 2007). Besides gene losses occurring early in the lineage of the LAB, more recent events have contributed to shaping these species, including parallel losses in genes involved in various metabolic processes. The most notable example of gene loss occurred in Streptococcus thermophilus, which diverged from pathogenic Streptococcus species through the loss and decay of virulence-associated genes, such as those involved in antibiotic resistance and adhesion. This genomic record has thus far provided solid evidence supporting the 'generally recognized as safe' status for use of S. thermophilus in foods (Bolotin, 2004). Gene gains in the LAB also reflected a shift toward a nutrient-rich lifestyle during specific niche adaptations.Soon after the divergence of the Lactobacillales, there occurred duplications of genes involved in the transport and metabolism of carbohydrates, including genes for enolases and phosphotransferase (PTS). Genes involved in amino acid transport and peptidases were also duplicated, further enhancing the ability of these species to exploit protein-rich environments systems (Makarova and Koonin, 2007). Horizontal gene transfer (HGT) has also shaped these genomes. For example, many sugar transport and metabolism genes in Lactobacillus plantarum are clustered in a lower GC content area of the genome, and it is possible that many of these genes were acquired as a result of HGT (Kleerebezem *et al*., 2003). HGT has also shaped the genome of S. thermophilus, which possesses a 17-kb region that contains extensive identity with genes in *L. lactis* and *L. bulgaricus subsp.* Delbrueckii (hereafter *L. bulgaricus*), two species that are also associated with growth in milk. The genes from L.

bulgaricus enable S. thermophilus to synthesize methionine, which is rare in milk (Bolotin . 2004). Other LAB genomes exhibit a high incidence of HGT, especially in genes involving sugar metabolism (Makarova and Koonin 2007).

From birth, we are exposed to these species through our food and environment. Species of LAB are so diverse that they occupy many niches, including milk, plants, meats, grains and the gastrointestinal (GI) tract of vertebrates, yet because of their similarities they create the common metabolic end product, lactic acid. LAB are Gram-positive, nonsporulating bacteria. The term 'lactic acid bacteria' does not reflect a phyletic class, but rather the metabolic capabilities of these species. This group encompasses several species from the order Lactobacillales. A list of species and the general features of their genomes is shown in Table 1.

Table 1. Features of sequenced LAB genomes (Erika ., 2007).

Species	Primary application	NCBI accession number	Genome size	Plasmids	Pseudogenes	Prophages (complete)	Protein
Lactobacillus acidophilus NCFM	Probiotic	NC_006814	1.9 Mb	0	0	0	1864
Lactobacillus brevis ATCC 367	Starter culture	NC_008497	2.3 Mb	2	49	1	2221
Lactobacillus casei ATCC 334	Starter culture, probiotic	NC_008526	2.9 Mb	1	82	2	2776
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 11842	Starter culture	NC_008054	1.9 Mb	0	533	0	1562
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC BAA-365	Starter culture	NC_008529	1.9 Mb	0	192	0	1725
Lactobacillus gasseri ATCC 33323	Probiotic	NC_008530	1.9 Mb	0	48	1	1763
Lactobacillus johnsonii NCC 533	Probiotic	NC_005362	1.9 Mb	0	0	2	1821
Lactobacillus plantarum WCFS1	Vegetable fermentation, probiotic	NC_004567	3.3 Mb	3	42	2	3009
Lactobacillus reuteri F275	Probiotic	NC_009513	2.0 Mb	0	39		1900
Lactobacillus sakei subsp. *sakei* 23k	Starter culture	NC_007576	1.9 Mb	0	0	1	1879
Lactobacillus salivarius subsp. *salivarius* UCC118	Probiotic	NC_007929	1.8 Mb	3	49	2	1717
Lactococcus lactis subsp. *cremoris* MG1363	Starter culture/type strain	NC_09004	2.5 Mb	0	82	2	2434
Lactococcus lactis subsp. *cremoris* SK11	Cheese production	NC_008527	2.4 Mb	5	144	4	2509
Lactococcus lactis subsp. *lactis* IL1403	Milk fermentation	NC_002662	2.3 Mb	0	1	3	2321
Leuconostoc mesenteroides subsp. *mesenteroides* ATCC 8293	Starter culture	NC_008531	2.0 Mb	1	19	1	2009
Oenococcus oeni PSU-1	Secondary wine fermentation	NC_008528	1.8 Mb	0	122	0	1701
Pediococcus pentosaceus ATCC 25745	Starter culture	NC_008525	1.8 Mb	0	20	2	1757
Streptococcus thermophilus CNRZ1066	Starter culture	NC_006449	1.8 Mb	0	0	1	1915
Streptococcus thermophilus LMD-9	Starter culture	NC_008532	1.8 Mb	2	206	1	1710
Streptococcus thermophilus LMG 18311	Starter culture	NC_006448	1.8 Mb	0	0	0	1889

These genomes have low GC contents and range in size from 1.8 Mb for Oenococcus oeni to 3.3 Mb for Lactobacillus plantarum. Many of the LAB genomes have reduced biosynthetic capacities resulting from the genome degradation events that reflect their adaptation to nutrient-rich environments, such as milk and the GI tract (Erika and Todd, 2007).

The specialized adaptation to milk is particularly interesting because this fermentation environment would not exist without human intervention. The selective pressure came not only from the natural environment, but also from anthropogenic environments created by humans, which essentially domesticated these organisms over the last 5000

years through repeated transfer of LAB cultures for production of fermented dairy products (Joel and Todd 2009). The availability of sequenced genomes has allowed for a deeper understanding of the evolutionary divergence of the LAB, and reveals a trend of relatively recent and ongoing reduction in genome size (van de Guchte, 2006). The last common ancestor of Lactobacillales appears to have lost c. 600–1200 genes and gained <100 during its divergence from the Bacilli ancestor (Makarova and Koonin, 2007). The extent of genome reduction varies greatly among LAB with Oenococcus oeni having only c. 1700 predicted ORFs compared with the c. 3000 of Lactobacillus plantarum (Pfeiler and Klaenhammer, 2007). Analysis of the available genomes of LAB suggests that the bulk of the genes lost were due to adaptation to nutrient-rich food environments, particularly those organisms that have adapted to milk and other food environments rich in protein and carbohydrates. The yogurt bacterium Lactobacillus delbrueckii ssp. bulgaricus shows a large difference in G–C% content (49.7%) from the closely related species Lactobacillus acidophilus (34.7%), a gastrointestinal commensal organism. Interestingly, the difference was primarily in the less conserved third codon position, which had a 65% G–C content implying rapid ongoing evolution to a higher G–C content. Furthermore, the number of rRNA and tRNA genes in L. bulgaricus is c. 50% higher than the average for a genome of its size. These numbers would correspond to a genome of 3–4Mb, significantly larger than its actual size of 1.8 Mbp (van de Guchte, 2006; Joel and Todd, 2009).

Table 2. Components of the Lactobacillus helveticus CNRZ 32 proteolytic enzyme system: A look before and after genome sequence determination (Cogan . 2007).

Genes identified and characterized before sequencing project	Genes identified upon completion of the draft genome sequence
Proteinases *prt*H	*prt*H2 plus 9 additional proteases
Endopeptidases *pep*E, *pep*O, *pep*O2 endopeptidases	*pep*E2, *pep*F, *pep*O3, plus 2 glycoproteins
Aminopeptidases *pep*C, *pep*N, *pep*X	*pep*C2 plus 7 additional aminopeptidases
Di-Tripeptidases *pep*D, *pep*I, *pep*Q, *pep*R	*pep*D2, *pep*D3, *pep*D4, *pep*Q2, *pep*T1, and *pep*T2
Other	Oligo- and di-tripeptide transport systems: *opp*A, *opp*A2, *opp*B-D, *opp*F, and *dtp*A, *dtp*A2, and *dtp*T Multiple amino acid transporters

Probiotic Features

Probiotic strains, notably lactobacilli, possess unique features that enable them to survive the rigors of the GI tract, including the presence of acid and bile, and competition from other microorganisms.The majority of the probiotic features are related with the survival of the strain in the GI tract. The intestinal environment results from three main factors: dietary intake, bacterial ecology, and host physiology, including factors such as peristalsis and glandular secretions. Several factors restrict bacterial cell growth including gastric acidity, oxidative stress, digestive enzymes, bile salts, peristalsis, mucus, the resident commensal microfl ora, exfoliation of enterocytes during epithelial renewal, epithelial translocation of secretory IgA, $CD8^+$ intraepithelial T lymphocytes, and innate host defense mechanisms mediated by gene – encoded antimicrobial peptides (Andrea, 2010).

LAB - encoding genes involved in both oxidative stress resistance and regulatory mechanisms have

been identified and oxidative resistance genes, such as antioxidant enzymes such as superoxide dismutases SOD and catalase, have been expressed in probiotic strains such as Lact. gasseri and technologically relevant organisms like Lact. delbrueckii subsp. Bulgaricu (Andrea, 2010).

Some species of LAB are added to foods not to drive the fermernation, but because of health benefits associated with their consumption. Sequencing the genomes of L. acidophilus, L. casei, L. johnsonii, L. plantarum and L. salivarius led to the elucidation of many genes that underlie these characteristics. These species are remarkably deficient in their biosynthetic capacities, which are compensated for by their abundant proteolytic systems and extensive capacity for uptake of macromolecules (Altermann, 2005).

One important attribute of probiotic LAB is their ability to resist and thrive in acidic environments like the stomach of mammals and fermented low - pH foods. Several mechanisms of acid resistance have been described in LAB. The most important and universally present in the group, the multisubunit F1 F0 ATPase, which links the production of ATP molecules to the transmembrane proton motive force (PMF), can either generate ATP at the expense of PMF or produce PMF – consuming ATP. The PMF facilitates the extrusion of protons from the cytoplasm. The F 0 complex has proton translocating activity, while the peripherally bound F1 complex has ATPase activity (Andrea , 2010).

L. acidophilus and *L. plantarum* ferment different types of fructo-oligosaccharides (FOS), and that their FOS metabolism operons possess different architectures (Saulnier, 2007). suggesting that these genes were acquired after evolutionary divergence of the species. Acid tolerance, which affects survival both in the stomach and in fermented foods, has been explored extensively in L. acidophilus using microarrays and a directed gene knockout system. A two-component regulatory system (2CRS) was identified that has a role in both acid tolerance and proteolytic activity, suggesting an evolutionary link between growth in milk and acidification by lactic acid (Azcarate, 2005).

The elucidation of cell surface proteins putatively involved in adhesion was of particular interest in LAB genome sequencing projects. The L. acidophilus genome contains 26 genes encoding proteins predicted to anchor at the cell surface, including those that might bind mucus and fibrinogen (Altermann, 2005).

By screening 14 L. plantarum strains for their mannose adherence capabilities and examining their genotypes using DNA microarrays, twocandidate genes involved in adhesion were identified in the WCFS1 strain. Subsequent gene mutations and adhesion analysis confirmed that one protein has a role in mannose adhesion (Pretzer, 2005). This study highlights the power of genotype and phenotype analysis in comparing the capabilities of unsequenced and sequenced genomes. The sequenced genomes of intestinal lactobacilli have illustrated the mechanisms by which these species have adapted to life in the GI tract, including stress tolerance, uptake of carbohydrates, and adhesion to intestinal cells and mucus (Erika and Todd, 2007).

Food fermentations (*LAB Evolution in Dairy Environment*)

Genomic and metabolic simplification through gene loss or degradation is a recurring theme following the adaptation to milk, a nutritious environment that provides lactose as the main carbohydrate source, casein as the main source of proteins, and most vitamins and minerals. One of the most prominent examples of the adaptation of a microorganism is illustrated by the evolution of some LAB to the dairy environment. Yogurt production relies upon fermentation of milk by two LAB species, *L. bulgaricus* and *S. thermophilus*. Through protocooperation, these organisms lower the pH of milk more quickly than either can alone. Genome sequencing has shown that the metabolic capabilities of these two organisms make them reliant on each other for maximum growth. For example, L.

bulgaricus encodes a complete folate biosynthesis pathway, but lacks the ability to produce p-aminobenzoic acid (PABA), a key intermediate that is supplied by S. thermophilus (van de Guchte, 2006). In addition, an exchange of polyamines might occur between these organisms that could have a role in their oxidative stress tolerance. A major difference in the genomes of L. bulgaricus and S. thermophilus is reflected in their biosynthetic capabilities. The presence of an extracellular protease in L. bulgaricus, but the absence of many amino acid biosynthesis pathways, reflects the adaptation of this species to the protein-rich milk environment.By contrast, S. thermophiles has retained the pathways to synthesize all amino acids except histidine. It is unclear if S. thermophilus exploits the proteolytic capabilities of L. bulgaricus or retains some advantage in synthesizing its own amino acids thermophilus (van de Guchte, 2006).

As an example, the most important characteristics of the genome sequence of *Lactobacillus helveticus* DPC4571 (Callanan, 2008), a Swiss cheese isolate commonly used as starter and adjunct culture in cheese manufacture, are a predicted dependency on external supplies of amino acids and cofactors similar to that described for closely related gastrointestinal (GI) tract isolates, *Lactobacillus acidophilus* (Altermann, 2005) and *Lactobacillus johnsonii* (Pridmore, 2004), a high peptidolytic activity, and the ability to lyse rapidly in the cheese matrix, features that play a critical part in cheese ripening. Also an unusually high number of insertion sequence (IS) elements suggests that horizontal gene transfer may have played a very important role in the origin of LAB that are specialized for growth in milk (Makarova, 2006).

The draft sequence of another strain of *Lact. helveticus,* CNRZ32, characterized more essential components of the proteolytic enzyme system that confirmed a expanded the knowledge of this system in this bacterium (Table 2; Cogan, 2007). Transcriptome profiling tools have recently begun to define the relationship between LAB and the milk environment. Microarray technology confirmed the overexpression of several members of the proteolytic system in cultures of *L . helveticus* CNRZ32 growing in milk versus a complex medium, including previously characterized genes (*pep* E, *pep* N, *pep* R, *pep* O2, *pep* O, *pep* X) and genes identified by comparative genomics (*prt* H2 and the *opp* operon (Smeianov, 2007).

In *L. acidophilus* NCFM, transcriptome analysis during growth in milk identified similar members of the proteolytic system as well as a two - component regulatory system involved in the regulation of oligopeptide transport in this probiotic bacteria (Andrea ., 2010; Azcarate – Peril, 2009).

LAB Evolution in Vegetable and Meat Environments

Although fermented dairy products are the most recognizable to consumers, LAB are also used to preserve meat, grain and vegetable products. Knowledge of the biosynthetic capacities of the LAB that will carry out the fermentation can assist in strain selection and defining optimal fermentation conditions to minimize growth of the undesirable microorganisms that are naturally present on the raw materials. Some features in the genome of Lactobacillussakei, a meat starter culture, reflect the sausage environment from which it was first isolated. Open reading frames (ORFs) encoding several putative osmoprotectant and psychroprotectant proteins were identified, in addition to proteins putatively involved in heme usage and oxidative stress resistance (Chaillou 2005). Transcriptional and functional examination of this strain in a meat matrix indicates that stress tolerance genes are induced to support the ability of this organism to compete and grow in this harsh fermentation environment (Hufner, 2007).

Strain improvement is an ongoing objective for fermentation technologists, who seek strains that ferment quickly and create a consistent product of high quality. With sequenced and annotated bacterial genomes, the full biosynthetic capabilities of a strain are made evident. One example of how genomics can

improve strain selection is the elucidation of the proteolytic system in L. *helveticus CNRZ32*. Proteolysis has an important role in cheese ripening, and several years were spent examining this system in L. helveticus, culminating in the discovery of 12 genes encoding proteolytic enzymes. Genome sequencing of this strain (which is currently in progress) has already identified many other genes involved in proteol- ysis, illustrating how genomic information can identify metabolic capabilities more rapidly than previous methods (Cogan, 2007).

The microbiota of fresh vegetables normally consists of Gram (–) aerobic bacteria, yeasts, and a lower number of LAB (Harris 1998). However, varying conditions of anaerobiosis, moisture levels, concentration of salt, and temperature result in changes in the population balance and select for spontaneous fermentation by LAB. The same process occurs in meat products where LAB are intially present at low numbers (10^2 – 10^3 colony forming units, [cfu]/g), but they rapidly dominate the fermentation due to favorable conditions (Andrea, 2010; Rantsiou and Cocolin, 2006). In addition to their ability to produce lactic acid and to reduce pH, LAB are also competitive in vegetable and meat fermentations because of their ability to produce bacteriocins. Bacteriocins are small peptides (30 – 60 aa) with antimicrobial properties against bacteria usually of the same or closely related species (narrow spectrum), and occasionally against a broader spectrum of species (Rantsiou, 2006; Settanni and Corsetti 2008).

Genetic engineering can also be employed to improve or expand fermentation abilities. L. bulgaricus produces hydrogen peroxide in the presence of oxygen, but cannot detoxify this compound. As a result, the growth of L. bulgaricus is slowed after being exposed to oxygen in a fermentation setting. Cloning and expression of the L. plantarum manganese-dependent catalase in *L. casei* protected *L. bulgaricus* from hydrogen peroxide toxicity when grown in coculture with the modified *L. casei* strain (Rochat, 2006). Although genetically modified organisms (GMOs) are not widely accepted for food use, genetic modification of this type could be used to improve strains used in the manufacture of enzymes, pharmaceuticals and industrial compounds.

The genomics of flavor

One of the prominent use of the LAB has been to develop flavor in fermented foods (Erika and Todd 2007; Smit, 2005). Complex flavors are often difficult to attribute to a specific compound or metabolic process, but genomics has enabled researchers to explore which genes are important in flavor development.

Malolactic fermentation in wine is carried out widely by LAB, most importantly by Oenococcus oeni. This organism can survive in the harsh wine environment where stresses include alcohol, low pH, nutritional starvation and the presence of sulfites. Genes involved in the stress tolerance of this species have been identified (Beltramo, 2006). The most important role of O. oeni in winemaking involves flavor development and deacidification through the conversion of malate to lactate and carbon dioxide. In addition to a complete malate decarboxylase pathway, genomic analysis revealed the presence of a pathway for citrate metabolism, which can lead to the production of many compounds associated with wine aroma, including diacetyl. Further characterization of metabolic pathways will help in understanding how compounds that affect flavor are produced and potentially improve control of fermentations to produce desired attributes.

Cheese production relies on LAB for acid production, proteolytic activity and creation of flavor compounds. An application of genomics in this area includes prediction of flavor-related compounds that could be generated based on the pathways predicted from annotated genomes. In addition, probes can be constructed to identify important sequences rapidly using high-throughput strain screening and selection (Erika and Todd 2007; Smit, 2005). Using a bioinformatics approach, the citrate catabolic pathway of L. casei in a cheese matrix was identified

(Diaz, 2006). To date, other studies have identified genes responsible for decarboxylation of branched-chain a-keto acids in L. lactis (Erika and Todd 2007), a reaction that correlates with malty flavors in cheese (Erika and Todd 2007; Smit, 2005). Another study identified a proteinase associated with bitterness in Cheddar cheese and suggested that the deletion of the prt gene could eliminate this defect from the finished product (Broadbent, 2002). In many types of cheeses, the activity of LAB peptidases to hydrolyze milk proteins is the most important step in flavor formation (Erika and Todd 2007; Smit, 2005). Transcriptional and proteomic profiles have been created for L. lactis, S. thermophilus and L. helveticus that indicate upregulation of proteolysis genes during growth in milk (Erika and Todd 2007; Smeianov, 2007).

Comparative genomics of lactobacilli

Comparison of the similarities and differences within these groups is expected to provide an important view of gene content, organization, and regulation that contributes to both gut and probiotic functionality (Todd, 2005). A recent comparative analysis between the complete genomes of L. plantarum and L. johnsonii revealed striking differences in gene content and synteny in the genome, prompting a conclusion that these two species are only marginally more related to each other than to other Gram-positive bacteria (Boekhorst, 2004). Nevertheless, 70% of the proteins in L. johnsonii still had homologs in the larger *L. plantarum* genome. Unique proteins found in these two genomes, when compared against the published and draft genomes of the LAB, were primarily unknown proteins and prophage-related ORFs (Boekhorst *et al*., 2004). Complete genomes are now published or available for four Lactobacillus species (*acidophilus, gasseri, johnsonii and plantarum*). Whole genome comparison between *L. acidophilus, L. gasseri* and *L. johnsonii* revealed a highly conserved region harboring a cluster of genes predicted to encode a cell surface exopolysaccharide (EPS). The cluster of genes was oriented similarly in *L. acidophilus* and *L. johnsonii*, and inverted in the *L. gasseri* genome as a result of the chromosomal rearrangement. Transcriptional analysis revealed that the eps genes were expressed by L. acidophilus during log phase growth on most of the eight carbohydrates examined (Barrangou *et al*., 2003; Todd *et al*., 2005).

Conclusions

Currently, the LAB are receiving significant attention as vehicles for delivery of biotherapeutics because of their ability to reach the gastrointestinal tract and interact with the host immune system. Key features of the genomes of LAB continue to be discovered as genome sequence and functional genomic information continues to explode. The role of LAB in food and health continues to expand and evolve as new discoveries are made. Potential biotherapeutic applications being explored currently include using LAB for drug and vaccine delivery vehicles, where the drug is produced directly within the gastrointestinal tract in a proximal position to the immune cells present in the human gut (Delcenserie *et al*., 2008). The improved understanding of the genomics of LAB not only answers many questions but also raises many new ones, helping to expand our knowledge of their relationship with mankind. LAB genomics has contributed significantly to our understanding of the relationships between genotype and phenotype, which will have important ramifycations in controlling biotechnologically relevant processes, in developing novel vaccines, and in improving diagnostics. Furthermore, we can envision that strain sequencing will become routine with the development of next generation, massively parallel ultra - high throughput sequencing methods like Solexa (Illumina), SOLiD (Applied Biosystems), 454 (Roche) Heliscope (Helicos BioSciences Corp.), and Polonator G.007 (Danaher Motion and George Church). Genome sequences alone, however, do not provide a full understanding of a microorganism. In the post-sequencing era, scientists are taking the first steps to integrating sequence data with transcriptional and functional studies so as to better define complex traits. New methods of analysis, such as metabolomics and metagenomics, can also aid in characterization and should be added to the

repertoire of tools for investigation of complex microbial ecosystems.

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