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MINI-REVIEW

Understanding the industrial application potential of lactic acid bacteria through genomics

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Abstract Lactic acid bacteria (LAB) are a heterogeneous group of bacteria contributing to various industrial applications, ranging from food and beverage fermentation, bulk and fine chemicals production to pharmaceuticals manufacturing. Genome sequencing is booming; hitherto, 25 genomes of LAB have been published and many more are in progress. Based on genomic content of LAB, this review highlights some findings related to applications revealed by genomics and functional genomics analyses. Finally, this review summarizes mathematical modeling strategies of LAB in the context of genomics, to further our understanding of industrial related features.

Keywords Genomics · Lactic acid bacteria · Application

Introduction

Lactic acid bacteria (LAB) are a nontaxonomic group of Gram-positive, low GC content, nonmotile bacteria characterized by their capability to ferment sugars to lactic acid. In food industry, LAB are used for food and beverage fermentation, flavor forming (Urbach 1995), preservation (Stiles 1996), production of add-in ingredients (Hugenholtz et al. 2002), bacteriocins (De Vuyst and Leroy 2007), and exopolysaccharides (Cerning 1990; Welman and Maddox 2003). LAB can also be used to produce bulk and fine chemicals, including lactic acid (Kwon et al. 2001), polyols (Wisselink et al. 2002), and B vitamins (Burgess et al. 2004; Taranto et al. 2003). The rest of the potential applications of LAB are summarized in Table 1. In 2001, the first genome of LAB (*Lactococcus lactis* ssp. *lactis* IL1403) was sequenced and published (Bolotin et al. 2001). To date, 25 LAB genomes (15 *Lactobacillus*, three *Lactococcus*, three *Streptococcus*, two *Leuconostoc*, one *Pediococcus*, and one *Oenococcus*) have been sequenced and published (Table 2). LAB have many traits of industrial importance. Over years, the molecular mechanisms underlying these features have been elucidated by molecular microbiologists using single-gene approaches. Although these findings greatly increased our knowledge on LAB, they did not give an overall picture on the functionality. Genomics and functional genomics provide us an unprecedented opportunity to take a global insight into physiological and metabolic capabilities of LAB. The massive new knowledge generated through genomics can help to discover novel application potentials of LAB worthy of future exploration.

This review aims to address how genomics helps to increase our understanding to the application potential of LAB. To this end, general features of LAB genomes were summarized, followed by illustrating how to identify and characterize gene functions through genomics and functional genomics. We further extend the genomics approach to genome-scale stoichiometric modeling strategies, presenting the industrial relevant features of LAB revealed by modeling approach.

Overall industrial relevant features revealed by genomics

The most updated genome sequencing information (GOLD, genome online database, http://www.genomesonline.org/) shows that 25 LAB genomes have been sequenced and

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Table 1 Primary applications of LAB

Strain name	Primary applications	References
Lactobacillus acidophilus NCFM	Probiotics	Altermann et al. (2005)
Lactobacillus brevis ATCC 367	Starter culture of silage, sourdough, beer fermentation	Dubchak et al. (2006a)
Lactobacillus casei ATCC 334	Probiotics, milk fermentation, and flavor development of cheese	Dubchak et al. (2006b)
Lactobacillus casei BL23	Probiotics, milk fermentation, and flavor development of cheese	Kandler and Weiss (1986)
Lactobacillus delbrueckii ssp. *bulgaricus* ATCC 11842	Yogurt fermentation	Tamime and Robinson (1999)
Lactobacillus delbrueckii ssp. *bulgaricus* ATCC BAA-365	Yogurt fermentation	Tamime and Robinson (1999)
Lactobacillus fermentum IFO 3956	Probiotics	Morita et al. (2008)
Lactobacillus gasseri ATCC 33323	Probiotics	Azcarate-Peril et al. (2008)
Lactobacillus helveticus DPC 4571	Cheese manufacture	Callanan et al. (2008)
Lactobacillus johnsonii NCC 533	Probiotics	Pridmore et al. (2004)
Lactobacillus plantarum WCFS1	Food preservation, feed raw materials such as milk, meat, vegetables, probiotics	Kleerebezem et al. (2003)
Lactobacillus reuteri F275	Probiotics	Morita et al. (2008)
Lactobacillus reuteri JCM 1112	Probiotics	Morita et al. (2008)
Lactobacillus sakei ssp. *sakei* 23K	Food preservation, meat fermentation	Chaillou et al. (2005)
Lactobacillus salivarius ssp. *salivarius* UCC118	Probiotics	Claesson et al. (2006)
Lactococcus lactis ssp. *cremoris* MG1363	Dairy food fermentation, model strain	Wegmann et al. (2007)
Lactococcus lactis ssp. *cremoris* SK11	Dairy food fermentation, model strain	Lawerence et al. (1976)
Lactococcus lactis ssp. *lactis* IL1403	Dairy food fermentation, model strain	Bolotin et al. (2001)
Leuconostoc citreum KM20	Kimchi fermentation	Kim et al. (2008)
Leuconostoc mesenteroides ssp. *mesenteroides* ATCC 8293	Food fermentation, such as sauerkraut, vegetables, and some bread sourdough; dextran production	Dubchak et al. (2006c)
Oenococcus oeni PSU-1	Wine making	Mills et al. (2005)
Pediococcus pentosaceus ATCC 25745	Dairy fermentations, sausage fermentations, cucumber and green bean fermentations, and silage fermentations	Dubchak et al. (2006d)
Streptococcus thermophilus CNRZ1066	Yogurt and cheese fermentation	Bolotin et al. (2004)
Streptococcus thermophilus LMD-9	Yogurt and cheese fermentation	Dubchak et al. (2006e)
Streptococcus thermophilus LMG 18311	Yogurt and cheese fermentation	Bolotin et al. (2004)

annotated, while 67 projects are in progress (59 *Lactobacillus*, three *Lactococcus*, three *Leuconostoc*, one *Oenococcus*, and one *Streptococcus*). Most LAB genomes are relatively small (1.8–3.3 Mb; Table 2). The numbers of protein-encoding genes differ from 1,700 to 3,200, indicating substantial gene loss or gene gain events during evolution. Comparative genomic analysis revealed that many biosynthesis related genes were lost and external nutrients utilization abilities were enhanced by acquiring genes through horizontal gene transfer or gene duplication, due to the prevailing reductive evolution trend driven by adaptation to the nutrient-rich niches (Makarova et al. 2006). In the genomes of dairy LAB *Streptococcus thermophilus* (Bolotin et al. 2004), *Lactobacillus delbrueckii* ssp. *bulgaricus* (van de Guchte et al. 2006), and *Lactobacillus helveticus* (Callanan et al. 2008), more than 10% coding genes lost their functions and present as pseudogenes. Especially in *S. thermophilus*, degeneration of virulence-associated genes, such as those related with antibiotic resistance and adhesion function, diverges it from its pathogenic *Streptococci* neighbors. This ongoing genome decay process indicates the genome content of LAB could be easily changed and thus might benefit engineering, such as genome shuffling (Patnaik et al. 2002; Stephanopoulos 2002) or domestication under controlled industrial conditions.

Various mobile genetic elements (MGEs) were found in LAB genomes, including plasmids, prophages (Desiere et al. 2002), insertion sequence elements, transposons, and group II introns (Shearman et al. 1996). MGEs contribute to genome plasticity, host competitiveness, and environmental adaptation (Frost et al. 2005; Top and Springael 2003). One of the most important newly discovered MGEs in LAB is the 242-kb plasmid pMP118 present in *Lactobacillus salivarius* UCC118 (Claesson et al. 2006; Li et al. 2007). This led to the discovery of the widespread of megaplasmids with similar replication origin to pMP118

Table 2 General features of the sequenced LAB genomes (data were collected from genome database of the National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome)

Strain names	Origin/usage	NCBI access no.	GC content (%)	Length (Kb)	Genome size (Mb)	No. of genes	No. of proteins	No. of pseudogenes	References
Lactobacillus acidophilus NCFM[a]	Probiotics	NC_006814	34.7	1,993	1.99	1,936	1,862	0	Altermann et al. (2005)
Lactobacillus brevis ATCC 367[b]	Beer fermentation, sourdough starter culture	NC_008497	46.2	2,291	2.34	2,314	2,218	52	Makarova et al. (2006)
		NC_008498 (pLVIS1)	38.6	13		12	11	1	
		NC_008499 (pLVIS2)	38.5	36		25	22	3	
Lactobacillus casei ATCC 334[a]	Swiss cheese, probiotics	NC_008526	46.6	2,895	2.92	2,909	2,751	82	Makarova et al. (2006)
		NC_008502 (pLSEI1)	42.2	29		20	20	0	
Lactobacillus casei BL23[b]	Starter culture, probiotics	NC_010999	46.3	3,079	3.08	3,044	3,044	0	
Lactobacillus delbrueckii ssp. *bulgaricus* ATCC 11842[c]	Yogurt fermentation	NC_008529	49.7	1,864	1.86	2,217	1,562	533	van de Guchte et al. (2006)
Lactobacillus delbrueckii ssp. *bulgaricus* ATCC BAA-365[c]	Yogurt fermentation	NC_008529	49.7	1,857	1.86	2,040	1,721	192	Makarova et al. (2006)
Lactobacillus fermentum IFO 3956[b]	Probiotics, animal & plant material	NC_010610	51.5	2,098	2.10	1,912	1,843	0	Morita et al. (2008)
Lactobacillus gasseri ATCC 33323[c]	Probiotics	NC_008530	35.3	1,894	1.89	1,898	1,755	43	Azcarate-Peril et al. (2008); Makarova et al. (2006)
Lactobacillus helveticus DPC 4571[c]	Cheese flavor development	NC_010080	37.1	2,080	2.08	1,838	1,610	155	Callanan et al. (2008)
Lactobacillus johnsonii NCC 533[c]	Probiotics	NC_005362	34.6	1,992	1.99	1,918	1,821	0	Pridmore et al. (2004)
Lactobacillus plantarum WCFS1[b]	Vegetable fermentation, probiotics	NC_004567	44.5	3,308	3.35	3,135	3,007	42	Kleerebezem et al. (2003); van Kranenburg et al. (2005)
		NC_006375 (pWCFS101)	39.5	2		3	3	0	
		NC_006376 (pWCFS102)	34.3	2		4	4	0	
		NC_006377 (pWCFS103)	40.8	36		43	43	0	
Lactobacillus reuteri F275[b]	Probiotics	NC_009513	38.9	2,000	2.00	2,027	1,900	39	
Lactobacillus reuteri JCM 1112[b]	Probiotics	NC_010609	38.9	2,039	2.04	1,901	1,820	0	Morita et al. (2008)
Lactobacillus sakei ssp. *sakei* 23K[b]	Meat fermentation	NC_007576	41.3	1,884	1.88	1,963	1,879	0	Chaillou et al. (2005)
Lactobacillus salivarius ssp. *salivarius* UCC118[b]	Probiotics	NC_007929	32.9	1,827	2.13	1,864	1,717	49	Claesson et al. (2006)
		NC_006529 (pSF118-20)	39.1	20		27	27	0	
		NC_006530 (pSF118-44)	39.6	44		49	47	2	

Table 2 (continued)

Strain names	Origin/usage	NCBI access no.	GC content (%)	Length (Kb)	Genome size (Mb)	No. of genes	No. of proteins	No. of pseudogenes	References
		NC_007930 (pMP118)	32.1	242		242	222	21	
Lactococcus lactis ssp. *cremoris* MG1363[c]	dairy/model strain	NC_009004	35.7	2,529	2.53	2,597	2,434	82	Wegmann et al. (2007)
Lactococcus lactis ssp. *cremoris* SK11[c]	Cheese production, flavor formation	NC_008527,	35.9	2,438	2.59	2,610	2,384	144	Makarova et al. (2006)
		NC_008503 (pLACR1)	34.4	14		11	10	1	
		NC_008504 (pLACR2)	30.4	9		8	6	2	
		NC_008505 (pLACR3)	35.4	74		66	61	3	
		NC_008506 (pLACR4)	34.8	47		39	35	4	
		NC_008507 (pLACR5)	33.5	14		8	8	0	
Lactococcus lactis ssp. *lactis* IL1403[c]	Milk fermentation soft cheese	NC_002662	35.3	2,365	2.37	2,425	2,321	1	Bolotin et al. (2001)
Leuconostoc citreum KM20[b]	Kimchi fermentation	NC_010471	39	1,796	1.90	1,785	1,702	1	Kim et al. (2008)
		NC_010470 (pLCK1)	37.4	39		49	49	0	
		NC_010466 (pLCK2)	38	31		36	36	0	
		NC_010467 (pLCK3)	33	18		20	20	0	
		NC_010479 (pLCK4)	36.9	12		13	13	0	
Leuconostoc mesenteroides ssp. *mesenteroides* ATCC 8293[b]	Vegetable fermentation, polymer production	NC_008531	37.7	2,038	2.08	2,073	1,970	19	Makarova et al. (2006)
		NC_008496 (pLEUM1)	35.4	37		35	35	0	
Oenococcus oeni PSU-1[b]	Wine making	NC_008528	37.9	1,780	1.78	1,864	1,691	122	Mills et al. (2005)
Pediococcus pentosaceus ATCC 25745[b]	Fermentation starter culture, preservation	NC_008525	37.4	1,832	1.83	1,847	1,755	20	Makarova et al. (2006)
Streptococcus thermophilus CNRZ1066[b]	Yogurt	NC_006449	39.1	1,796	1.80	2,000	1,915	0	Bolotin et al. (2004)
Streptococcus thermophilus LMD-9[b]	Yogurt	NC_008532	39.1	1,856	1.86	2,003	1,710	206	Makarova et al. (2006)
		NC_008500 (pSTER1)	37	4		4	4	0	
		NC_008501 (pSTER2)	35.1	3		2	2	0	
Streptococcus thermophilus LMG 18311[b]	Yogurt	NC_006448	39.1	1,796	1.80	1,974	1,889	0	Bolotin et al. (2004)

[a] Facultative heterolactic fermentative strain

[b] Heterolactic fermentative strain

[c] Homolactic fermentative strain

in 33 strains of *L. salivarius* (Li et al. 2007). In addition, megaplasmids of sizes ranging from 120 to 490 kb were found in six other species of *Lactobacillus*: *Lactobacillus hamsteri*, *Lactobacillus intestinalis*, *Lactobacillus kalixensis*, *Lactobacillus ingluviei*, *Lactobacillus acidophilus*, and *Lactobacillus equi* (Li et al. 2007). Interestingly, none of the megaplasmids present in these six *Lactobacillus* species shares a similar replication origin to pMP118 (Li et al. 2007).

Little is known about the biology of the megaplasmids in LAB. pMP118 is the largest sequenced plasmid in LAB, while the chromosome of *L. salivarius* UCC118 is the smallest one in sequenced *Lactobacillus* genomes to date. *L. salivarius* was traditionally classified as homofermentative bacterium. Genome sequencing discovered that the two key enzymes for completing pentose phosphate pathway, transketolase and transaldolase, are pMP118-encoded. Further experiments showed that some *L. salivarius* strains can indeed ferment xylose (Li et al. 2006). This led to a reclassification of *L. salivarius* to facultative heterofermentative bacterium (Li et al. 2006). In addition, it gives an elegant example showing how to ferment pentose by the cooperation of megaplasmid and chromosome encoded genes. Plasmids are common vehicles for rapid genetic transfer. In last decade, food-grade gene cloning and expression systems for LAB were successfully developed (Table 3), such as the nisin-controlled gene expression system in *L. lactis* (Mierau and Kleerebezem 2005). The megaplasmids mentioned above can potentially be modified to novel genetic tools to be used in Gram-positive bacteria, like BAC vector in *Escherichia coli*, to clone large DNA fragments.

Understanding application-related physiological features through genomics

LAB are exploited for many industrial applications because of their related physiological features, which include substrate utilization, stress response, metabolic capabilities, population interaction, and probiotic properties. The mechanisms underlying these features are rather complex. Genomics and functional genomics approaches, characterized by high throughput, large scale, combination of both experimental and computational methodologies, are used to discover novel genes, signaling pathways, metabolic routes, and regulatory circuits. Here, we show how "omics" analysis increase our knowledge of the industrial application-related physiological features.

Substrate utilization

LAB are generally considered as nutrients fastidious, which means they need more nutrients to grow. This is due to that LAB are usually isolated from the nutrient-rich niches such as plants, fermented foods, and gastrointestinal (GI) tract (Holzapfel and Wood 1998). Genome sequencing and annotation revealed that most LAB lack biosynthetic pathways for essential amino acids, nucleotides, and vitamins (Altermann et al. 2005; Azcarate-Peril et al. 2008; Bolotin et al. 2004, 2001; Callanan et al. 2008; Makarova et al. 2006; Pridmore et al. 2004; van de Guchte et al. 2006). Through genomics analysis, many saccharides uptake systems, proteolysis system, and amino acid transporters were found encoded on the genomes to help the host to take up nutrients from milk, plant, or mammalian GI tract.

Genomics revealed that the improved substrate utilization ability of LAB was basically achieved by either gene duplication or horizontal gene transfer (Kleerebezem et al. 2003; Makarova et al. 2006). In Lactobacillales, gene duplication event occurred in phosphotransferase system, amino acid transporters, and peptidases after divergence from the common ancestor, which increases adaptation to nutrients-rich environment (Makarova et al. 2006). *Lactobacillus plantarum* (3.3 Mb) is the most versatile and flexible species of LAB. The diverse sugar utilization of *L. plantarum* was achieved by clustering related transporters, metabolic enzymes, and other regulatory proteins on a lower GC content region, named "lifestyle adaptation island". Because genes on this island were acquired by horizontal gene transfer (Kleerebezem et al. 2003), it is considered as a region with high plasticity, evidenced by DNA microarray-based comparison of 20 strains of *L. plantarum* (Molenaar et al. 2005). It would therefore be desirable if industrial nutrition fastidious strain can have the free living lifestyle by acquiring these islands through genome shuffling.

LAB can take use of a variety of carbon sources, but a comprehensive understanding on how carbon sources are taken up and metabolized has not yet been achieved. Whole-genome transcriptome profiling and comparative analysis for growth of probiotic *L. acidophilus* NCFM on different sugars identified genetic elements responsible for carbohydrate metabolism (Barrangou et al. 2006). Three classes of transporters (ATP-binding cassette, phosphoenolpyruvate phosphotransferase system, and galactoside pentose hexuronide permease) and related hydrolyases were found specifically induced by their substrates but repressed by glucose, suggesting the sugar metabolism of *L. acidophilus* is subjected to carbon catabolite repression regulation (Barrangou et al. 2006). Very often, sugar availability is limited during biopreservation and meat fermentation process. Alternative carbon source utilization capabilities are therefore required to cope with glucose starvation. An example is *Lactobacillus sakei* 23K which could catabolize external ATP breakdown intermediates for energy production, through a predicted purine nucleoside scavenging pathway revealed by genome sequencing (Chaillou et al.

Table 3 Genetic tools for LAB

Host	Vector	Composition	References	Type
L. sakei	pRV566	Original putative *ori* and *rep*, ColE1 *ori*, Ery^R, Ap^R	Alpert et al. (2003)	NI
E. coli–Lactobacillus	pJLE4942	Original *ori* and *repA* from *L. casei* plasmid pLC494, ColE1 *ori*, Cm^R	An and Miyamoto (2006)	NI
Lactobacillus sakei	pKRV3	sakacin A inducible gene expression system, P*sapIP*, *sapIP*, *sapKR*, *lacZ*, Ap^R	Axelsson et al. (2003)	I
E. coli–G+ bacteria	pMSP3535	pAMβ1 and ColE1 replicons, *nisRK*, P*nisA*, Ery^R	Bryan et al. (2000)	I
E. coli–G+ bacteria	pMSP3535VA	pAMβ1 and ColE1 replicons, *nisRK*, P*nisA*, Kan^R	Bryan et al. (2000)	I
E. coli–Lactobacillus	pLE16	pBR328 and pLB10 (plasmid from *L. bulgaricus* 10) replicons, Cm^R	Chagnaud et al. (1992)	NI
L. sakei	pRV610	pRV500 (plasmid from *L. sakei*) and pBluescript replicons with a MCS-α*lacZ*, Ery^R, Ap^R	Crutz-Le Coq and Zagorec (2008)	NI
L. lactis	pNZ8008 and derivatives	Promoterless *gusA* gene fused to the P*nisA*, used for study of the nisin-controlled expression in *L. lactis*, Cm^R	de Ruyter et al. (1996b)	NI
L. lactis	pNZ9572 and pNZ9573	Plasmid for integration of *nisRK* into the *pepN* locus, Cm^R, Ery^R, p15A *ori* of *E. coli*	de Ruyter et al. (1996a)	I
L. lactis	pNZ8018	*lytH*, *lytA* from *L. lactis* phage φUS3	de Ruyter et al. (1997)	I
L. lactis	pVEC1 and pCOM1	pCD4 replication *ori*, a two-component cloning system, Ap^R, Ery^R	Emond et al. (2001)	I
E. coli–Lactobacillus–L. lactis	pLS203 and pLS208	pSF 118-20 replication region (or with additional putative mobilization locus), ColE1 *ori*, *lacZ*, Ery^R	Fang et al. (2008)	NI
L. helveticus ssp. *jugurti* SBT2161C	pLHR	pBR329 and pLJ1 (plasmid from *L. helveticus* ssp. *jugurti* SBT2161C) replicons, Ap^R, Cm^R	Hashiba et al. (1990)	NI
E. coli–L. lactis	pCI372 and pCI3340	pBR322 and pCI305 (plasmid of *L. lactis* subsp. *lactis* UC317) replicons, Cm^R	Hayes et al. (1990)	NI
L. johnsonii FI9785	pFI2431	p9785S (plasmid from *L. johnsonii* FI9785), Cm^R	Horn et al. (2005)	NI
L. mesenteroides ssp. *mesenteroides* Y110.	pSJ33E	pFMBL1 (plasmid from *Leuconostoc mesenteroides* SY2) replicon, Ery^R, Ap^R	Jeong et al. (2007)	NI
L. lactis	pMSP3535H2	High copy variant of pMSP3535 with modified *nisA*, transcription terminator	Kim and Mills (2007)	I
L. lactis	pNZ9520	*nisRK* genes on plasmid pIL253 (high copy number), expression of *nisRK* under control of *rep* promoter of the plasmid, Ery^R	Kleerebezem et al. (1997)	I
L. lactis	pNZ9530	*nisRK* genes on plasmid pIL252 (low copy number), expression of *nisRK* under control of *rep* promoter of the plasmid, Ery^R	Kleerebezem et al. (1997)	I
L. lactis	pNZ8048	NcoI site used for translational fusions, standard vector, Cm^R	Kuipers et al. (1998)	I
E. coli–Lactobacillus	pDOJ4	p15A and pDOJ1 (plasmid from *L. delbrueckii*), Cm^R, *lacZ*	Lee et al. (2007)	NI
E. coli–L. reuteri	pUE80	pTE80 *ori*, *lacZ*′, Ery^R	Lin and Chung (1999)	NI
L. lactis	pNZ8148	NcoI site used for translational fusions, deletion of 60 bp residual DNA from *Bacillus subtilis*, standard vector, Cm^R	Mierau and Kleerebezem (2005)	NI
L. lactis	pNZ8150	ScaI site used for translational fusions, standard vector, Cm^R	Mierau and Kleerebezem (2005)	I
L. lactis	pNZ8021	Vector for transcriptional fusions, Cm^R	Mierau and Kleerebezem (2005)	I
L. lactis	pNZ8110	Vector for protein secretion using the signal sequence of Usp45 of *L. lactis*, translational fusion via NaeI site, Cm^R	Mierau and Kleerebezem (2005)	I
L. gasseri UKLbg1	pTN1	*rep* from cryptic plasmid of *L. curvatus*, Ery^R	Neu and Henrich (2003)	NI
L. lactis	pMSP3535H3	*nisI* insertion of pMSP3535H2	Oddone et al. (2009)	I
E. coli–Leuconostoc–Lactobacillus	pCW4	ColE1 and pC7 *ori*, Ap^R, Ery^R	Park et al. (2005)	NI
L. fermentum KC5b	pSP1	pVA891 (*streptococcal* integration plasmid) containing pKC5b (plasmid from *L. fermentum* KC5b) *repA* and *repB*, Ery^R, Cm^R	Pavlova et al. (2002)	NI
L. lactis	pNZ2104, pNZ2105,	pSH71 *ori*, *lacF*/*gusA* as selection marker	Platteeuw et al. (1996)	I

Table 3 (continued)

Host	Vector	Composition	References	Type
	pNZ2120/ pNZ2121, pNZ2122/2123			
L. lactis	pFG200	pFG1 *ori*, *repB*, *supD* as a selectable marker by suppressing pyrimidine auxotrophs ability	Dickely et al. (1995); Sorensen et al. (2000)	NI
E. coli–L. plantarum–L. sakei	pLV111	p256 (plasmid from *L. plantarum* NC7) and pGEM-7Zf(+) replicons, Ery^R, *lacZ*	Sorvig et al. (2005)	NI
E. coli–L. plantarum–L. sakei	pSIP301, pSIP401, pSIP501	*E. coli ori*, p256 replicon, Ery^R, *SapKR* (or *sppKR*, *nisKR*)	Sorvig et al. (2003)	I
L. casei A23	pSMA23-based shuttle-vectors	pBlueScript SK+ and pMSA23 replicons, Ery^R, Cm^R	Sudhamani et al. (2008)	NI
L. lactis and *L. plantarum*	pLEB590	pSH71 replicon, P*45-nisI*, Nis^R, *nisI* as a selection marker	Takala and Saris (2002)	I
E. coli–Enterococcus faecalis–Lactobacillus–Pediococcus	pRS5C	pRS5 (plasmid from *Pediococcus pentosaceus* RS4) and *E. coli* plasmid replicons, Cm^R	Teresa Alegre et al. (2009)	NI
E. coli–L. reuteri	pNICE	*E. coli–L. reuteri* shuttle vector pSTE32 derivative, nisin-controlled expression vector containing P*nisA*, and *nisRK* regulatory genes	Wu et al. (2006)	I
Lactococci	pRAF800	pSRQ800 (from *L. lactis*) replicon, *aga* gene (from *Lactobacillus raffinolactis*) as selection marker	Boucher et al. (2002)	NI
L. casei EM76::cI	pEM76-based delivery system	*E. coli ori*, Ap^R, Ery^R, integration region *int-attP* (phage A2), two directly oriented copies of the β-recombinase binding site (*six*)	Martin et al. (2000)	RI
L. casei CECT 5276 with a frameshift in *lacF*	pIlac	*E. coli ori*, Ery^R, Ap^R, *lacG* 3′ end and *lacF* gene	Gosalbes et al. (2000)	R
L. acidophilus ADH	pMYL1-*βgal*	*β-gal*	Lin et al. (1996)	R
L. casei Shirota	pMSK761	Integration–excision vector, *cat* gene, Ery^R, applying *L. casei* phage φFSW integration system	Shimizu-Kadota (2001)	RI
L. lactis	M1107s-opt, M1083s-opt, and T942a	P*nisA*, Ll.ltrB, for gene inactivation	Frazier et al. (2003)	RI
L. acidophilus, *L. gasseri*	pTRK669	pWV01 *ori*, Cm^R, $RepA^+$	Russell and Klaenhammer (2001)	R
L. plantarum WCFS1	pNZ5319 (general mutagenesis vector)	*Cre-lox* system for multiple gene knockout	Lambert et al. (2007)	RI

NI noninducible expression system, *I* inducible expression system, *R rec*-dependent recombination system, *RI rec*-independent recombination system

2005). Another example is the metabolism of casein, which can be used by many LAB as nitrogen and carbon sources. Upregulation of proteolysis genes during growth in milk were found by transcriptome and proteome profiling for *L. lactis*, *S. thermophilus*, and *L. helveticus* (Derzelle et al. 2005; Gitton et al. 2005; Smeianov et al. 2007). These understandings can assist strain selection and defining optimal growth conditions to increase the growth yield of LAB strains.

Stress response

Adverse conditions are often encountered during food fermentation processes, including extreme temperature, acid, oxygen, and osmotic stresses. An ideal LAB strain with industrial potential should resist to these harsh conditions; for example, the LAB strains used as starter culture or probiotics are expected to survive spray drying process (Mauriello et al. 1999; van de Guchte et al. 2002). Response and defense mechanisms in LAB have been studies for years (Rallu et al. 1996; van de Guchte et al. 2002). With the assistance of genomics, the complicated molecular mechanism of stress responses can be understood at a global level (Bron et al. 2006; Budin-Verneuil et al. 2005; Serrano et al. 2007; Xie et al. 2004). This can possibly result in designing interesting strategies to improve the robustness of LAB for application purpose.

Acid stress response is important for LAB as lactic acid is the main catabolism product, which acidifies the media and arrests cell multiplication. In *L. plantarum*, comparative transcriptome profiling analysis revealed that a novel group of cell surface proteins were specifically induced by the lactic acid stress (Pieterse et al. 2005). Scanning electron microscope revealed that stressed cell surface became unevenly rougher than unstressed one. The authors speculated that it is caused by the induced expression of those surface proteins (Pieterse et al. 2005). In terms of oxidative stress, LAB are facultative anaerobic microorganisms and reactive oxygen species like superoxide and hydroxyl radical could attack cell components. In the past, one report suggested *L. lactis* could respire when heme was present in the culture (Sijpesteijn 1970). Genomic analysis revealed that *L. lactis* has the genes necessary for respiration but lacks full heme synthesis pathway and citric acid cycle (Bolotin et al. 2001). When heme and oxygen are available, the sugar metabolism of *L. lactis* can shift to respiration, which remarkably reduces the effect of oxidative and acid stress and leads to an improved long-term survival of *L. lactis*. A series of genome-scale analysis confirmed this alternation, and some respiration regulatory mechanisms were also discovered (Gaudu et al. 2002; Pedersen et al. 2008; Vido et al. 2004). Engineered LAB strains with aerobic respiration ability are expected to have an increased growth yield.

Metabolites of industrial potential

LAB could be used as cell factory for production of bulk and fine chemicals, including pyruvate-dissipating end products, exopolysaccharides, bacteriocins, vitamins, low-calorie sugars, complex flavor compounds, and polylactic acid or polylactide (Taguchi et al. 2008). Sorbitol is a popular low-calorie sweetener for its health-promoting properties. Genome sequencing of *L. plantarum* WCFS1 revealed that it has two putative sorbitol 6-phosphate dehydrogenase genes (*srlD1* and *srlD2*). By reverting the sorbitol catabolic pathway by overexpressing *srlD* genes in mutant deficient in both L- and D-lactate dehydrogenase activities, high yield of sorbitol from fructose-6-phosphate was achieved (Ladero et al. 2007). Another example on enhanced folate biosynthesis ability of *L. lactis* ssp. *cremoris* MG1363 demonstrated that secondary metabolism pathway of LAB can be engineered, with assistance from genome annotation information of *L. lactis* ssp. *lactis* IL1403 (Sybesma et al. 2003). Flavor forming is an important trait for industrial application of LAB. The improved gene annotation leads to a better prediction of the flavor-forming pathways. With comparison of published LAB genomes, original annotations were improved and thus certain flavor-forming genes were identified in various LAB strains, providing a starting point for direct selection of potential strains for flavor production (Liu et al. 2008). Compared with genome of closely related strain *Lactobacillus fermentum* IFO 3956, *Lactobacillus reuteri* JCM 1112^T was found harboring a unique genome island of bacteriocin biosynthesis, indicating its health-promoting role in GI tract (Morita et al. 2008). These studies suggest that comparative genomics approach would be an efficient tool to discover novel biochemical pathways for product with industrial potential (Piskur et al. 2007).

Population interactions and probiotic properties

Natural food processing are typically mixed-culture fermentations thus species live in such an environment become interdependent. For example, yogurt is made by fermenting milk with two LAB species, *L. bulgaricus* and *S. thermophilus* (Tamime and Robinson 1999). Molecular mechanisms of inner- or inter-interactions are difficult to understand. By using fluorescence in situ hybridization analysis, researchers could speculate interaction between strains by analyzing population dynamics (Sakai and Ezaki 2006). The availability of genome sequences of both species shed light on an integral analysis of the interactions and metabolic activity in milk. Genome sequences of both species showed a rapid adaptation to the milk environment, evidenced by the numbers of inactivated genes (Bolotin et al. 2004; van de Guchte et al. 2006). Interestingly, the inactivated metabolic function in *L. bulgaricus* can be complemented by *S. thermophilus* and vice versa. First, proteolytic *L. bulgaricus* provides nonproteolytic *S. thermophilus* with amino acids and peptides produced by cell-wall anchored extracellular protease. Second, *L. bulgaricus* lacks pyruvate-formate lyase and folate biosynthesis ability, while *S. thermophilus* can provide formic acid, folic acid, and carbon dioxide to *L. bulgaricus*. Third, other compounds such as long-chain fatty acid, putrescine, and ornithine could also contribute to mutualistic interaction. On one hand, *S. thermophilus* may supply *L. bulgaricus* with several unsaturated long-chain fatty acid, as the *de novo* biosynthesis pathways of long-chain fatty acid are incomplete in the latter one. On the other hand, ingredients such as putrescine and ornithine could be produced by both species, and the exchange of them mutually increases the resistance of both species to oxidative stress (Sieuwerts et al. 2008). Recently, *S. thermophilus* cultured with *L. bulgaricus* in milk was characterized with proteomics and transcriptomics methods. Besides confirming the existence of mutual benefiting hypothesis, other effects for *S. thermophilus* such as obtaining amino acids and purine from *L. bulgaricus*, adaptation to hydrogen peroxide (H_2O_2) were discovered, which provides more clues for

understanding their interactions in dairy environment (Herve-Jimenez et al. 2009). The big challenge in mix culture biotechnology is to determine the species composition. Genomics and high-throughput technology will accelerate the innovation process by uncovering intra- or interspecies interactions, regulatory responses to different substrates and processing conditions, as these reviews indicated (Pastink et al. 2008; Sieuwerts et al. 2008).

Some LAB species are known for manufacturing probiotic foods. Other than food fermentation, probiotic functionalities include mainly host–microbial interactions. Probiotic LAB have to survive GI tract, by processing unique features such as acid and bile resistance, adherence, bacteriocin production capability, and growth on prebiotics (Klaenhammer 2000). Some of these features were validated at the genetic level with the aid of genomics and functional genomics analysis (Klaenhammer et al. 2008). One common feature of probiotics is bile tolerance. Comparative transcriptome profiling revealed the responses of *L. acidophilus* and *L. reuteri* to bile stress shared common mechanisms, including cell-envelope reorganization, denatured protein degradation, and DNA damage repairing (Pfeiler et al. 2007; Whitehead et al. 2008). Probiotic LAB are capable of utilizing complex carbohydrates that are indigestible by human and other microbiota and these sugars could selectively stimulate growth of probiotic LAB (Gibson and Roberfroid 1995). Characterization of the metabolism of these carbohydrates in *L. acidophilus* and *L. plantarum* has identified specific transporters and hydrolases for fructooligosaccharides (Barrangou et al. 2003, 2006; Saulnier et al. 2007). Adhesion properties of probiotic LAB result in a markedly prolonged duration within GI tract and pathogen inhibition, because glycosyl residues on intestinal cell surface could be competitively bound by LAB surface proteins (Pretzer et al. 2005). Aiming to identify mannose-specific adhesin genes in *L. plantarum*, *L. plantarum* strains were screened for adhesion ability and their genotypes were identified through DNA microarrays. The candidate genes were thus selected and only one was identified to be adhesin-encoding gene, through mutation verification (Pretzer et al. 2005). This study provides a useful "matching" strategy to identify related genes for certain functions through comparative genomics analysis.

Genome-scale modeling strategies

Mathematical models for improving fermentation were widely used in process design and control, but they are mostly empirical-based and scale-limited (Hoefnagel et al. 2002). Changing of energy charge, ratio of NAD^+ to NADH (or ratio of $NADP^+$ to NADPH), and concentration of coenzymes will dramatically affect bacterial metabolic network, since those metabolites participate many biochemical reactions and are always highly connected hubs in the network (Jeong et al. 2000). These biochemical reactions could be predicted by genome annotation. Recently, genome-scale stoichiometric modeling techniques were developed. After reconstruction of the metabolic network from annotation results, a genome-scale stoichiometric model can be built to investigate cellular metabolic capacities, either by calculating metabolic flux distribution or by analyzing metabolic network topological features (Borodina and Nielsen 2005; Feist et al. 2009; Teusink and Smid 2006; Trinh et al. 2009). However, within the 30 genome-scale models (26 species) available so far (Feist et al. 2009), there are only two genome-scale stoichiometric models for LAB strains (Oliveira et al. 2005b; Teusink et al. 2006).

One genome-scale model is for *L. lactis* ssp. *lactis* IL1403, with 621 reactions and 509 metabolites (Oliveira et al. 2005a). With appropriate constraints, the model was tested useful for predicting gene essentiality and substrates preferability. Metabolic engineering strategies for improving diacetyl yield were also predicted. There are three genes targeted to be knocked out, which had never been reported before (Oliveira et al. 2005a). Another genome-scale stoichiometric model for *L. plantarum* WCFS1 was developed after reconstruction of metabolic network and extensive curation (Teusink et al. 2006). Interestingly, the amino acid catabolism pathways related with flavor-forming characteristics were also predicted as ATP producers in this model (Teusink et al. 2006). Many futile cycles and parallel pathways were found, which remarkably increase metabolic flexibility of *L. plantarum*. However, their regulation mechanism on metabolic level is still unknown and experimental validations are needed (Teusink et al. 2006).

In general, the stoichiometric modeling is still in its infancy. First, based on steady-state hypothesis, continuous culture is needed, while batch culture is more popular in industrial application. Scientists tried to describe a serial steady state at successive time points (Luo et al. 2006; Mahadevan et al. 2002). To this end, novel global kinetic modeling theories and techniques for continuous variation are needed, because the cellular metabolism in most situations is not at a steady state (Jamshidi and Palsson 2008). As most kinetic parameters in enzymatic dynamic equations are difficult to obtain, a trade-off between calculability and complexity should be made (Bulik et al. 2009; Hoppe et al. 2007; Nikerel et al. 2006; Smallbone et al. 2007; Voit 2008). Second, the objective function could be altered, according to network features. For example, in modeling solventogenesis phase of *Clostridium acetobutylicum* ATCC 824, a nonlinear objective function

was successfully introduced to avoid multiple-solution problem due to cyclic pathway and to better characterize stationary features of cellular growth (Lee et al. 2008). In the model of *L. plantarum*, flux balance analysis (FBA) was tested failure, and the authors suggested the overflow feature (i.e., energetically inefficient metabolic behavior) should be viewed in whatever novel objective functions (Teusink et al. 2006). Third, constraints information of specific fluxes has to be set manually, to restrict the solution space, and it only provides static presentation of cellular metabolism. Recently, integration of transcriptomic data with metabolic modeling was described, both by examples and methods (Covert and Palsson 2002, 2003; Cox et al. 2005). Gene regulatory mechanisms are described as a binary system, such as if-then rules using Boolean logic on FBA to make more accurately predictions, for additional constraints could reduce the solution space (Covert and Palsson 2003). Thus, it is expected to take use of multiple omics data, to build an integrated model that incorporate multiple cellular processes at the genome-scale for LAB similar to what has been done for *E. coli* and *Saccharomyces cerevisiae* (Covert et al. 2008; Min Lee et al. 2008).

Concluding remarks and perspectives

In the near future, the number of the available LAB genomes will be approaching 100. This represents a large group of bacteria in microbial genomics, as compared to the total number of the sequenced bacteria (792 finished, 2,392 ongoing). With the rapid development of next generation sequencing techniques, it is not surprising that LAB researchers will be all working with their pet bacterium with genome available. Genomics have made significant advances on the genetics, physiology, and application of LAB. Deep insights into complex physiological features, including substrate uptake and utilization, stress responses, and metabolite biosynthesis, are obtained. Systems biology approaches, which integrate transcriptomics, proteomics, metabolomics, and modeling techniques, are emerging to interpret the "dark" part of sequence. Based on the "dry" genome annotation and "wet" high-throughput analyses, global networks will be developed to quantitatively predict the systems behavior, in a real fermentation situation, for example, complex food matrix. Understanding the interaction among each individual LAB strains is extremely important to explore the application of LAB populations in a form of mixed culture. This can be seen from a simple model system present in yogurt fermentation, the population interaction between *L. bulgaricus* and *S. thermophilus*. Quorum sensing is believed to be one of approaches for LAB communications. Future genomics study is expected to reveal the mechanism for communication within LAB, which will further improve the functionalities of LAB and its industrial application.

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References

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

Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, Souther N, Dobson A, Duong T, Callanan M et al (2005) Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. Proc Natl Acad Sci U S A 102:3906–3912

An HY, Miyamoto T (2006) Cloning and sequencing of plasmid pLC494 isolated from human intestinal *Lactobacillus casei*: construction of an *Escherichia coli–Lactobacillus* shuttle vector. Plasmid 55:128–134

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

Azcarate-Peril MA, Altermann E, Goh YJ, Tallon R, Sanozky-Dawes RB, Pfeiler EA, O'Flaherty S, Buck BL, Dobson A, Duong T et al (2008) Analysis of the genome sequence of *Lactobacillus gasseri* ATCC 33323 reveals the molecular basis of an autochthonous intestinal organism. Appl Environ Microbiol 74:4610–4625

Barrangou R, Altermann E, Hutkins R, Cano R, Klaenhammer TR (2003) Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. Proc Natl Acad Sci U S A 100:8957–8962

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

Bolotin A, Wincker P, Mauger S, Jaillon O, Malarme K, Weissenbach J, Ehrlich SD, Sorokin A (2001) The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. Genome Res 11:731–753

Bolotin A, Quinquis B, Renault P, Sorokin A, Ehrlich SD, Kulakauskas S, Lapidus A, Goltsman E, Mazur M, Pusch GD et al (2004) Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. Nat Biotechnol 22:1554–1558

Borodina I, Nielsen J (2005) From genomes to in silico cells via metabolic networks. Curr Opin Biotechnol 16:350–355

Boucher I, Parrot M, Gaudreau H, Champagne CP, Vadeboncoeur C, Moineau S (2002) Novel food-grade plasmid vector based on melibiose fermentation for the genetic engineering of *Lactococcus lactis*. Appl Environ Microbiol 68:6152–6161

Bron PA, Molenaar D, de Vos WM, Kleerebezem M (2006) DNA micro-array-based identification of bile-responsive genes in *Lactobacillus plantarum*. J Appl Microbiol 100:728–738

Bryan EM, Bae T, Kleerebezem M, Dunny GM (2000) Improved vectors for nisin-controlled expression in gram-positive bacteria. Plasmid 44:183–190

Budin-Verneuil A, Pichereau V, Auffray Y, Ehrlich DS, Maguin E (2005) Proteomic characterization of the acid tolerance response in *Lactococcus lactis* MG1363. Proteomics 5:4794–4807

Bulik S, Grimbs S, Huthmacher C, Selbig J, Holzhutter HG (2009) Kinetic hybrid models composed of mechanistic and simplified enzymatic rate laws—a promising method for speeding up the kinetic modelling of complex metabolic networks. FEBS J 276:410–424

Burgess C, O'Connell-Motherway M, Sybesma W, Hugenholtz J, van Sinderen D (2004) Riboflavin production in *Lactococcus lactis*: potential for in situ production of vitamin-enriched foods. Appl Environ Microbiol 70:5769–5777

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

Cerning J (1990) Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol Rev 87:113–130

Chagnaud P, Chan Kwo Chion CK, Duran R, Naouri P, Arnaud A, Galzy P (1992) Construction of a new shuttle vector for *Lactobacillus*. Can J Microbiol 38:69–74

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

Claesson MJ, Li Y, Leahy S, Canchaya C, van Pijkeren JP, Cerdeno-Tarraga AM, Parkhill J, Flynn S, O'Sullivan GC, Collins JK et al (2006) Multireplicon genome architecture of *Lactobacillus salivarius*. Proc Natl Acad Sci U S A 103:6718–6723

Covert MW, Palsson BO (2002) Transcriptional regulation in constraints-based metabolic models of *Escherichia coli*. J Biol Chem 277:28058–28064

Covert MW, Palsson BO (2003) Constraints-based models: regulation of gene expression reduces the steady-state solution space. J Theor Biol 221:309–325

Covert MW, Xiao N, Chen TJ, Karr JR (2008) Integrating metabolic, transcriptional regulatory and signal transduction models in *Escherichia coli*. Bioinformatics 24:2044–2050

Cox SJ, Shalel Levanon S, Bennett GN, San KY (2005) Genetically constrained metabolic flux analysis. Metab Eng 7:445–456

Crutz-Le Coq AM, Zagorec M (2008) Vectors for lactobacilli and other Gram-positive bacteria based on the minimal replicon of pRV500 from *Lactobacillus sakei*. Plasmid 60:212–220

de Ruyter PG, Kuipers OP, Beerthuyzen MM, van Alen-Boerrigter I, de Vos WM (1996a) Functional analysis of promoters in the nisin gene cluster of *Lactococcus lactis*. J Bacteriol 178:3434–3439

de Ruyter PG, Kuipers OP, de Vos WM (1996b) Controlled gene expression systems for *Lactococcus lactis* with the food-grade inducer nisin. Appl Environ Microbiol 62:3662–3667

de Ruyter PG, Kuipers OP, Meijer WC, de Vos WM (1997) Food-grade controlled lysis of *Lactococcus lactis* for accelerated cheese ripening. Nat Biotechnol 15:976–979

De Vuyst L, Leroy F (2007) Bacteriocins from lactic acid bacteria: production, purification, and food applications. J Mol Microbiol Biotechnol 13:194–199

Derzelle S, Bolotin A, Mistou MY, Rul F (2005) Proteome analysis of *Streptococcus thermophilus* grown in milk reveals pyruvate formate-lyase as the major upregulated protein. Appl Environ Microbiol 71:8597–8605

Desiere F, Lucchini S, Canchaya C, Ventura M, Brussow H (2002) Comparative genomics of phages and prophages in lactic acid bacteria. Antonie Van Leeuwenhoek 82:73–91

Dickely F, Nilsson D, Hansen EB, Johansen E (1995) Isolation of *Lactococcus lactis* nonsense suppressors and construction of a food-grade cloning vector. Mol Microbiol 15:839–847

Dubchak I, Grigoriev I, Shabalov I, Cantor MN, Dusheyko S, Hornick L, Hugenholtz P, Korzeniewski F, Minovitsky S, Nikitin R and others (2006a) *Lactobacillus brevis* ATCC 367. In: JGI. Available via DIALOG. http://genome.jgi-psf.org/finished_microbes/lacbr/lacbr.home.html. Accessed 28 Apr 2009

Dubchak I, Grigoriev I, Shabalov I, Cantor MN, Dusheyko S, Hornick L, Hugenholtz P, Korzeniewski F, Minovitsky S, Nikitin R and others (2006b) *Lactobacillus casei* ATCC 334. In: JGI. Available via DIALOG. http://genome.jgi-psf.org/draft_microbes/lacca/lacca.home.html. Accessed 28 Apr 2009

Dubchak I, Grigoriev I, Shabalov I, Cantor MN, Dusheyko S, Hornick L, Hugenholtz P, Korzeniewski F, Minovitsky S, Nikitin R and others (2006c) *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293. In: JGI. Available via DIALOG. http://genome.jgi-psf.org/finished_microbes/leume/leume.home.html. Accessed 28 Apr 2009

Dubchak I, Grigoriev I, Shabalov I, Cantor MN, Dusheyko S, Hornick L, Hugenholtz P, Korzeniewski F, Minovitsky S, Nikitin R and others (2006d) *Pediococcus pentosaceus* ATCC 25745. In: JGI. Available via DIALOG. http://genome.jgi-psf.org/finished_microbes/leume/pedpe.home.html. Accessed 28 Apr 2009

Dubchak I, Grigoriev I, Shabalov I, Cantor MN, Dusheyko S, Hornick L, Hugenholtz P, Korzeniewski F, Minovitsky S, Nikitin R and others (2006e) *Streptococcus thermophilus* LMD-9. In: JGI. Available via DIALOG. http://genome.jgi-psf.org/finished_microbes/strth/strth.home.html. Accessed 28 Apr 2009

Emond E, Lavallee R, Drolet G, Moineau S, LaPointe G (2001) Molecular characterization of a theta replication plasmid and its use for development of a two-component food-grade cloning system for *Lactococcus lactis*. Appl Environ Microbiol 67:1700–1709

Fang F, Flynn S, Li Y, Claesson MJ, van Pijkeren JP, Collins JK, van Sinderen D, O'Toole PW (2008) Characterization of endogenous plasmids from *Lactobacillus salivarius* UCC118. Appl Environ Microbiol 74:3216–3228

Feist AM, Herrgard MJ, Thiele I, Reed JL, Palsson BO (2009) Reconstruction of biochemical networks in microorganisms. Nat Rev Microbiol 7:129–143

Frazier CL, Filippo JS, Lambowitz AM, Mills DA (2003) Genetic manipulation of *Lactococcus lactis* by using targeted group II introns: Generation of stable insertions without selection. Appl Environ Microbiol 69:1121–1128

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3:722–732

Gaudu P, Vido K, Cesselin B, Kulakauskas S, Tremblay J, Rezaiki L, Lamberret G, Sourice S, Duwat P, Gruss A (2002) Respiration capacity and consequences in *Lactococcus lactis*. Antonie Van Leeuwenhoek 82:263–269

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Gitton C, Meyrand M, Wang J, Caron C, Trubuil A, Guillot A, Mistou MY (2005) Proteomic signature of *Lactococcus lactis* NCDO763 cultivated in milk. Appl Environ Microbiol 71:7152–7163

Gosalbes MJ, Esteban CD, Galan JL, Perez-Martinez G (2000) Integrative food-grade expression system based on the lactose regulon of *Lactobacillus casei*. Appl Environ Microbiol 66:4822–4828

Hashiba H, Takiguchi R, Ishii S, Aoyama K (1990) Transformation of *Lactobacillus helveticus* subsp. *jugurti* with plasmid pLHR by electroporation. Agric Biol Chem 54:1537–1541

Hayes F, Daly C, Fitzgerald GF (1990) Identification of the minimal replicon of *Lactococcus lactis* subsp. *lactis* UC317 Plasmid pCI305. Appl Environ Microbiol 56:202–209

Herve-Jimenez L, Guillouard I, Guedon E, Boudebbouze S, Hols P, Monnet V, Maguin ERul F (2009) Postgenomic analysis of *Streptococcus thermophilus* cocultivated in milk with *Lactobacillus delbrueckii* subsp. *bulgaricus*: involvement of nitrogen, purine, and iron metabolism. Appl Environ Microbiol 75:2062–2073

Hoefnagel MH, Starrenburg MJ, Martens DE, Hugenholtz J, Kleerebezem M, Van S II, Bongers R, Westerhoff HV, Snoep JL (2002) Metabolic engineering of lactic acid bacteria, the combined approach: kinetic modelling, metabolic control and experimental analysis. Microbiology 148:1003–1013

Holzapfel WHN, Wood BJ (1998) The genera of lactic acid bacteria. Blackie Academic & Professional, London

Hoppe A, Hoffmann S, Holzhutter HG (2007) Including metabolite concentrations into flux balance analysis: thermodynamic realizability as a constraint on flux distributions in metabolic networks. BMC Syst Biol 1:23

Horn N, Wegmann U, Narbad A, Gasson MJ (2005) Characterisation of a novel plasmid p9785S from *Lactobacillus johnsonii* FI9785. Plasmid 54:176–183

Hugenholtz J, Sybesma W, Groot MN, Wisselink W, Ladero V, Burgess K, van Sinderen D, Piard JC, Eggink G, Smid EJ et al (2002) Metabolic engineering of lactic acid bacteria for the production of nutraceuticals. Antonie Van Leeuwenhoek 82:217–235

Jamshidi N, Palsson BO (2008) Formulating genome-scale kinetic models in the post-genome era. Mol Syst Biol 4:171

Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL (2000) The large-scale organization of metabolic networks. Nature 407:651–654

Jeong SJ, Park JY, Lee HJ, Kim JH (2007) Characterization of pFMBL1, a small cryptic plasmid isolated from *Leuconostoc mesenteroides* SY2. Plasmid 57:314–323

Kandler O, Weiss N (1986) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore

Kim JH, Mills DA (2007) Improvement of a nisin-inducible expression vector for use in lactic acid bacteria. Plasmid 58:275–283

Kim JF, Jeong H, Lee JS, Choi SH, Ha M, Hur CG, Kim JS, Lee S, Park HS, Park YH et al (2008) Complete genome sequence of *Leuconostoc citreum* KM20. J Bacteriol 190:3093–3094

Klaenhammer TR (2000) Probiotic bacteria: today and tomorrow. J Nutr 130:415S–416S

Klaenhammer TR, Altermann E, Pfeiler E, Buck BL, Goh YJ, O'Flaherty S, Barrangou R, Duong T (2008) Functional genomics of probiotic Lactobacilli. J Clin Gastroenterol 42(Suppl 3 Pt 2): S160–S162

Kleerebezem M, Beerthuyzen MM, Vaughan EE, de Vos WM, Kuipers OP (1997) Controlled gene expression systems for lactic acid bacteria: transferable nisin-inducible expression cassettes for *Lactococcus*, *Leuconostoc*, and *Lactobacillus* spp. Appl Environ Microbiol 63:4581–4584

Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R, Tarchini R, Peters SA, Sandbrink HM, Fiers MWEJ et al (2003) Complete genome sequence of *Lactobacillus plantarum* WCFS1. Proc Natl Acad Sci U S A 100:1990–1995

Kuipers OP, de Ruyter PGGA, Kleerebezem M, de Vos WM (1998) Quorum sensing-controlled gene expression in lactic acid bacteria. J Biotechnol 64:15–21

Kwon S, Yoo IK, Lee WG, Chang HN, Chang YK (2001) High-rate continuous production of lactic acid by *Lactobacillus rhamnosus* in a two-stage membrane cell-recycle bioreactor. Biotechnol Bioeng 73:25–34

Lee JH, Halgerson JS, Kim JH, O'Sullivan DJ (2007) Comparative sequence analysis of plasmids from *Lactobacillus delbrueckii* and construction of a shuttle cloning vector. Appl Environ Microbiol 73:4417–4424

Lambert JM, Bongers RS, Kleerebezem M (2007) Cre-lox-based system for multiple gene deletions and selectable-marker removal in *Lactobacillus plantarum*. Appl Environ Microbiol 73:1126–1135

Lawerence RC, Thomas TD, Terzaghi BE (1976) Reviews of the progress of dairy science: cheese starters. J Dairy Res 43:141–193

Lee J, Yun H, Feist AM, Palsson BO, Lee SY (2008) Genome-scale reconstruction and in silico analysis of the *Clostridium acetobutylicum* ATCC 824 metabolic network. Appl Microbiol Biotechnol 80:849–862

Li Y, Raftis E, Canchaya C, Fitzgerald GF, van Sinderen D, O'Toole PW (2006) Polyphasic analysis indicates that *Lactobacillus salivarius* subsp *salivarius* and *Lactobacillus salivarius* subsp *salicinius* do not merit separate subspecies status. Int J Syst Evol Microbiol 56:2397–2403

Li Y, Canchaya C, Fang F, Raftis E, Ryan KA, van Pijkeren JP, van Sinderen D, O'Toole PW (2007) Distribution of megaplasmids in *Lactobacillus salivarius* and other lactobacilli. J Bacteriol 189:6128–6139

Lin CF, Chung TC (1999) Cloning of erythromycin-resistance determinants and replication origins from indigenous plasmids of *Lactobacillus reuteri* for potential use in construction of cloning vectors. Plasmid 42:31–41

Lin MY, Harlander S, Savaiano D (1996) Construction of an integrative food-grade cloning vector for *Lactobacillus acidophilus*. Appl Microbiol Biotechnol 45:484–489

Liu M, Nauta A, Francke C, Siezen RJ (2008) Comparative genomics of enzymes in flavor-forming pathways from amino acids in lactic acid bacteria. Appl Environ Microbiol 74:4590–4600

Luo RY, Liao S, Tao GY, Li YY, Zeng S, Li YX, Luo Q (2006) Dynamic analysis of optimality in myocardial energy metabolism under normal and ischemic conditions. Mol Syst Biol 2:2006.0031

Mahadevan R, Edwards JS, Doyle FJ III (2002) Dynamic flux balance analysis of diauxic growth in *Escherichia coli*. Biophys J 83:1331–1340

Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, Pavlov A, Pavlova N, Karamychev V, Polouchine N et al (2006) Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci U S A 103:15611–15616

Martin MC, Alonso JC, Suarez JE, Alvarez MA (2000) Generation of food-grade recombinant lactic acid bacterium strains by site-specific recombination. Appl Environ Microbiol 66:2599–2604

Mauriello G, Aponte M, Andolfi R, Moschetti G, Villani F (1999) Spray-drying of bacteriocin-producing lactic acid bacteria. J Food Prot 62:773–777

Mierau I, Kleerebezem M (2005) 10 years of the nisin-controlled gene expression system (NICE) in *Lactococcus lactis*. Appl Microbiol Biotechnol 68:705–717

Mills DA, Rawsthorne H, Parker C, Tamir D, Makarova K (2005) Genomic analysis of *Oenococcus oeni* PSU-1 and its relevance to winemaking. FEMS Microbiol Rev 29:465–475

Min Lee J, Gianchandani EP, Eddy JA, Papin JA (2008) Dynamic analysis of integrated signaling, metabolic, and regulatory networks. PLoS Comput Biol 4:e1000086

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

Morita H, Toh H, Fukuda S, Horikawa H, Oshima K, Suzuki T, Murakami M, Hisamatsu S, Kato Y, Takizawa T et al (2008)

Comparative genome analysis of *Lactobacillus reuteri* and *Lactobacillus fermentum* reveal a genomic island for reuterin and cobalamin production. DNA Res 15:151–161

Neu T, Henrich B (2003) New thermosensitive delivery vector and its use to enable nisin-controlled gene expression in *Lactobacillus gasseri*. Appl Environ Microbiol 69:1377–1382

Nikerel IE, van Winden WA, van Gulik WM, Heijnen JJ (2006) A method for estimation of elasticities in metabolic networks using steady state and dynamic metabolomics data and linlog kinetics. BMC Bioinformatics 7:540

Oddone GM, Mills DA, Block DE (2009) Incorporation of nisI-mediated nisin immunity improves vector-based nisin-controlled gene expression in lactic acid bacteria. Plasmid 61(3):151–158

Oliveira A, Nielsen J, Forster J (2005a) Modeling *Lactococcus lactis* using a genome-scale flux model. BMC Microbiol 5:39

Oliveira AP, Nielsen J, Forster J (2005b) Modeling *Lactococcus lactis* using a genome-scale flux model. BMC Microbiol 5:39

Park J, Lee M, Jung J, Kim J (2005) pIH01, a small cryptic plasmid from *Leuconostoc citreum* IH3. Plasmid 54:184–189

Pastink MI, Sieuwerts S, de Bok FAM, Janssen PWM, Teusink B, Vlieg JETV, Hugenholtz J (2008) Genomics and high-throughput screening approaches for optimal flavour production in dairy fermentation. Int Dairy J 18:781–789

Patnaik R, Louie S, Gavrilovic V, Perry K, Stemmer WP, Ryan CM, del Cardayre S (2002) Genome shuffling of *Lactobacillus* for improved acid tolerance. Nat Biotechnol 20:707–712

Pavlova SI, Kilic AO, Topisirovic L, Miladinov N, Hatzos C, Tao L (2002) Characterization of a cryptic plasmid from *Lactobacillus fermentum* KC5b and its use for constructing a stable *Lactobacillus* cloning vector. Plasmid 47:182–192

Pedersen MB, Garrigues C, Tuphile K, Brun C, Vido K, Bennedsen M, Mollgaard H, Gaudu P, Gruss A (2008) Impact of aeration and heme-activated respiration on *Lactococcus lactis* gene expression: identification of a heme-responsive operon. J Bacteriol 190:4903–4911

Pfeiler EA, Azcarate-Peril MA, Klaenhammer TR (2007) Characterization of a novel bile-inducible operon encoding a two-component regulatory system in *Lactobacillus acidophilus*. J Bacteriol 189:4624–4634

Pieterse B, Leer RJ, Schuren FH, van der Werf MJ (2005) Unravelling the multiple effects of lactic acid stress on *Lactobacillus plantarum* by transcription profiling. Microbiology 151:3881–3894

Piskur J, Schnackerz KD, Andersen G, Bjornberg O (2007) Comparative genomics reveals novel biochemical pathways. Trends Genet 23:369–372

Platteeuw C, van Alen-Boerrigter I, van Schalkwijk S, de Vos WM (1996) Food-grade cloning and expression system for *Lactococcus lactis*. Appl Environ Microbiol 62:1008–1013

Pretzer G, Snel J, Molenaar D, Wiersma A, Bron PA, Lambert J, de Vos WM, van der Meer R, Smits MA, Kleerebezem M (2005) Biodiversity-based identification and functional characterization of the mannose-specific adhesin of *Lactobacillus plantarum*. J Bacteriol 187:6128–6136

Pridmore RD, Berger B, Desiere F, Vilanova D, Barretto C, Pittet AC, Zwahlen MC, Rouvet M, Altermann E, Barrangou R et al (2004) The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. Proc Natl Acad Sci U S A 101:2512–2517

Rallu F, Gruss A, Maguin E (1996) *Lactococcus lactis* and stress. Antonie Van Leeuwenhoek 70:243–251

Russell WM, Klaenhammer TR (2001) Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes via homologous recombination. Appl Environ Microbiol 67:4361–4364

Sakai K, Ezaki Y (2006) Open L-lactic acid fermentation of food refuse using thermophilic *Bacillus coagulans* and fluorescence in situ hybridization analysis of microflora. J Biosci Bioeng 101:457–463

Saulnier DM, Molenaar D, de Vos WM, Gibson GR, Kolida S (2007) Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum* WCFS1 through microarrays. Appl Environ Microbiol 73:1753–1765

Serrano LM, Molenaar D, Wels M, Teusink B, Bron P, de Vos W, Smid E (2007) Thioredoxin reductase is a key factor in the oxidative stress response of *Lactobacillus plantarum* WCFS1. Microb Cell Fact 6:29

Shearman C, Godon JJ, Gasson M (1996) Splicing of a group II intron in a functional transfer gene of *Lactococcus lactis*. Mol Microbiol 21:45–53

Shimizu-Kadota M (2001) A method to maintain introduced DNA sequences stably and safely on the bacterial chromosome: application of prophage integration and subsequent designed excision. J Biotechnol 89:73–79

Sieuwerts S, de Bok FA, Hugenholtz J, van Hylckama Vlieg JE (2008) Unraveling microbial interactions in food fermentations: from classical to genomics approaches. Appl Environ Microbiol 74:4997–5007

Sijpesteijn A (1970) Induction of cytochrome formation and stimulation of oxidative dissimilation by hemin in *Streptococcus lactis* and *Leuconostoc mesenteroides*. Antonie Van Leeuwenhoek 36 (348):335–348

Smallbone K, Simeonidis E, Broomhead DS, Kell DB (2007) Something from nothing: bridging the gap between constraint-based and kinetic modelling. FEBS J 274:5576–5585

Smeianov VV, Wechter P, Broadbent JR, Hughes JE, Rodriguez BT, Christensen TK, Ardo Y, Steele JL (2007) Comparative high-density microarray analysis of gene expression during growth of *Lactobacillus helveticus* in milk versus rich culture medium. Appl Environ Microbiol 73:2661–2672

Sorensen KI, Larsen R, Kibenich A, Junge MP, Johansen E (2000) A food-grade cloning system for industrial strains of *Lactococcus lactis*. Appl Environ Microbiol 66:1253–1258

Sorvig E, Gronqvist S, Naterstad K, Mathiesen G, Eijsink VG, Axelsson L (2003) Construction of vectors for inducible gene expression in *Lactobacillus sakei* and *L plantarum*. FEMS Microbiol Lett 229:119–126

Sorvig E, Skaugen M, Naterstad K, Eijsink VG, Axelsson L (2005) Plasmid p256 from *Lactobacillus plantarum* represents a new type of replicon in lactic acid bacteria, and contains a toxin-antitoxin-like plasmid maintenance system. Microbiology 151:421–431

Stephanopoulos G (2002) Metabolic engineering by genome shuffling. Nat Biotechnol 20:666–668

Stiles ME (1996) Biopreservation by lactic acid bacteria. Antonie Van Leeuwenhoek 70:331–345

Sudhamani M, Ismaiel E, Geis A, Batish V, Heller KJ (2008) Characterisation of pSMA23, a 3.5 kbp plasmid of *Lactobacillus casei*, and application for heterologous expression in *Lactobacillus*. Plasmid 59:11–19

Sybesma W, Starrenburg M, Kleerebezem M, Mierau I, de Vos WM, Hugenholtz J (2003) Increased production of folate by metabolic engineering of *Lactococcus lactis*. Appl Environ Microbiol 69:3069–3076

Taguchi S, Yamada M, Matsumoto K, Tajima K, Satoh Y, Munekata M, Ohno K, Kohda K, Shimamura T, Kambe H et al (2008) A microbial factory for lactate-based polyesters using a lactate-polymerizing enzyme. Proc Natl Acad Sci U S A 105:17323–17327

Takala TM, Saris PE (2002) A food-grade cloning vector for lactic acid bacteria based on the nisin immunity gene nisI. Appl Microbiol Biotechnol 59:467–471

Tamime AY, Robinson RK (1999) Yoghurt: science and technology. Woodhead, Cambridge

Taranto MP, Vera JL, Hugenholtz J, De Valdez GF, Sesma F (2003) *Lactobacillus reuteri* CRL1098 produces cobalamin. J Bacteriol 185:5643–5647

Teresa Alegre M, Rodriguez MC, Mesas JM (2009) Characterization of pRS5: a theta-type plasmid found in a strain of *Pediococcus pentosaceus* isolated from wine that can be used to generate cloning vectors for lactic acid bacteria. Plasmid 61:130–134

Teusink B, Smid EJ (2006) Modelling strategies for the industrial exploitation of lactic acid bacteria. Nat Rev Microbiol 4:46–56

Teusink B, Wiersma A, Molenaar D, Francke C, de Vos WM, Siezen RJ, Smid EJ (2006) Analysis of growth of *Lactobacillus plantarum* WCFS1 on a complex medium using a genome-scale metabolic model. J Biol Chem 281:40041–40048

Top EM, Springael D (2003) The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. Curr Opin Biotechnol 14:262–269

Trinh CT, Wlaschin A, Srienc F (2009) Elementary mode analysis: a useful metabolic pathway analysis tool for characterizing cellular metabolism. Appl Microbiol Biotechnol 81:813–826

Urbach G (1995) Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int Dairy J 5:877–903

van de Guchte M, Serror P, Chervaux C, Smokvina T, Ehrlich SD, Maguin E (2002) Stress responses in lactic acid bacteria. Antonie Van Leeuwenhoek 82:187–216

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

van Kranenburg R, Golic N, Bongers R, Leer RJ, de Vos WM, Siezen RJ, Kleerebezem M (2005) Functional analysis of three plasmids from *Lactobacillus plantarum*. Appl Environ Microbiol 71: 1223–1230

Vido K, Le Bars D, Mistou MY, Anglade P, Gruss A, Gaudu P (2004) Proteome analyses of heme-dependent respiration in *Lactococcus lactis*: involvement of the proteolytic system. J Bacteriol 186:1648–1657

Voit EO (2008) Modelling metabolic networks using power-laws and S-systems. Essays Biochem 45:29–40

Wegmann U, O'Connell-Motherway M, Zomer A, Buist G, Shearman C, Canchaya C, Ventura M, Goesmann A, Gasson MJ, Kuipers OP et al (2007) Complete genome sequence of the prototype lactic acid bacterium *Lactococcus lactis* subsp. cremoris MG1363. J Bacteriol 189:3256–3270

Welman AD, Maddox IS (2003) Exopolysaccharides from lactic acid bacteria: perspectives and challenges. Trends Biotechnol 21:269–274

Whitehead K, Versalovic J, Roos S, Britton RA (2008) Genomic and genetic characterization of the bile stress response of probiotic *Lactobacillus reuteri* ATCC 55730. Appl Environ Microbiol 74:1812–1819

Wisselink HW, Weusthuis RA, Eggink G, Hugenholtz J, Grobben GJ (2002) Mannitol production by lactic acid bacteria: a review. Int Dairy J 12:151–161

Wu CM, Lin CF, Chang YC, Chung TC (2006) Construction and characterization of nisin-controlled expression vectors for use in *Lactobacillus reuteri*. Biosci Biotechnol Biochem Biosci Biotechnol Biochem 70:757–767

Xie Y, Chou LS, Cutler A, Weimer B (2004) DNA Macroarray profiling of *Lactococcus lactis* subsp. *lactis* IL1403 gene expression during environmental stresses. Appl Environ Microbiol 70:6738–6747