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PAN-GENOME CHARACTERISTICS OF *LACTOCOCCUS LACTIS*: A GENOMIC AND TRANSCRIPTOMIC DIVERSITY TECHNIQUES

Puntip Tan-a-ram

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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PAN-GENOME CHARACTERISTICS OF *LACTOCOCCUS LACTIS*: A GENOMIC AND TRANSCRIPTOMIC DIVERSITY TECHNIQUES

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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L. lactis subsp. *lactis* จำนวน 6 สายพันธุ์ถูกเลือกจากแหล่งเก็บสะสมเชื้อจุลินทรีย์ Génoferment culture เมื่อนำจุลินทรีย์นี้มาตรวจหาความหลากหลายของชนิดย่อยของจุลินทรีย์ในกลุ่มเดียวกันที่ระดับฟีโนไทป์ด้วยการนำไปเพาะเลี้ยงในน้ำนมดิบที่กรองเอาส่วนไขมันออก และในอาหารเลี้ยงเชื้อชนิดต่างๆ เช่น อาหารสังเคราะห์ (chemically defined medium; CDM) อาหาร complex medium (M17) และอาหารสังเคราะห์ดัดแปลง (M13) จากการทดลอง พบว่า เชื้อจุลินทรีย์ทั้ง 6 สายพันธุ์เจริญเติบโตได้ดีใน UF-cheese model โดยมีอัตราการเจริญเติบโตอยู่ระหว่าง 0.78 และ 0.88 ต่อชั่วโมง และมีค่าความเป็นกรดสูง นอกจากนี้ ยังพบว่าแบคทีเรียทุกสายพันธุ์มีอัตราการเจริญจำเพาะในอาหารสังเคราะห์ CDM สูงกว่าในอาหาร M17 เช่นเดียวกับที่ไม่พบการเจริญเติบโตของแบคทีเรียในอาหาร M13 จากนั้นแบคทีเรียทั้ง 6 สายพันธุ์ ถูกนำไปตรวจหาแกนของจีโนมด้วยวิธี array-based comparative genomic hybridization (CGH) และคำนวณหาค่าอัตราส่วนของความหนาแน่นระหว่างแบคทีเรียที่ทดสอบกับแบคทีเรียแลคติก สายพันธุ์ IL1403 เพื่อหาความแตกต่างของจีโนมในระหว่างสายพันธุ์ที่ทดสอบ จากการทดลองแสดงให้เห็นว่า *L. lactis* subsp. *lactis* ทั้ง 6 สายพันธุ์ มีความเหมือนกันมากในระหว่างลำดับเบสโดยมีส่วนของแกนจีโนม (dairy core genome) ใหญ่ที่มีจำนวนจีนมากถึง 1,915 จีนเหมือนกันในทุกสายพันธุ์ อย่างไรก็ตาม มีเพียงลำดับเบสของแบคทีเรียสายพันธุ์ LD61 ที่มีความใกล้ชิดกันกับลำดับเบสของแบคทีเรียสายพันธุ์ IL1403 มากกว่าสายพันธุ์อื่น นอกจากนั้นแกนจีโนมของแบคทีเรียทั้งหมดถูกนำมาทดสอบความแตกต่างของการแสดงออกของจีนด้วยไมโครแอเรย์ของดีเอ็นเอ และนำผลการทดสอบที่ได้มาจัดกลุ่มด้วยวิธีการ hierarchical clustering เพื่อหาความสัมพันธ์กันของแบคทีเรียสายพันธุ์ต่างๆ ซึ่งจากภาพแสดงความสัมพันธ์ของแบคทีเรียแต่ละสายพันธุ์แสดงให้เห็นว่าแบคทีเรียสายพันธุ์ LD55 มีความใกล้ชิดกันกับแบคทีเรียสายพันธุ์ UCMA571 ในทางตรงกันข้ามแบคทีเรียสายพันธุ์ LL08 มีความแตกต่างจากแบคทีเรียสายพันธุ์อื่นๆ มากที่สุด และเมื่อตรวจหาจำนวนของจีนที่แสดงออกแตกต่างกันในแต่ละสายพันธุ์ พบว่ามีจีนที่แตกต่างกันมากถึง 968 จีนซึ่งถูกควบคุมอย่างน้อยในสายพันธุ์ใดสายพันธุ์หนึ่งในห้าสายพันธุ์โดยเปรียบเทียบกับสายพันธุ์ LD61 และความแตกต่างของจีนที่พบมากในแบคทีเรียทั้ง 5 สายพันธุ์นั้นพบว่าเกี่ยวข้องกับเมตาบอลิซึม

นอกจากนี้แบคทีเรียถูกนำมาตรวจสอบคุณลักษณะทางกายภาพจากการเปรียบเทียบความต้องการไนโตรเจน เบส และอะมิโนแอซิดที่มีกิ่งก้าน จากการทดลอง พบว่า แบคทีเรียทุกสายพันธุ์เจริญได้เล็กน้อยในอาหารเลี้ยงเชื้อ CDM ที่มีการดัดแปลงสูตรของไนโตรเจน เบส และอะมิโนแอซิดที่มีกิ่งก้าน เมื่อเปรียบเทียบกับอาหารเลี้ยงเชื้อ CDM ที่ไม่มีการดัดแปลงสูตรอาหาร

PUNTIP TAN-A-RAM : PAN-GENOME CHARACTERISTICS OF

สาขาวิชาจุลชีววิทยา ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2554 ลายมือชื่ออาจารย์ที่ปรึกษา_____

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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

LACTOCOCCUS LACTIS: A GENOMIC AND TRANSCRIPTOMIC DIVERSITY TECHNIQUES. THESIS ADVISOR : ASST. PROF. SUNTHORN KANCHANATAWEE, Ph.D. 238 PP.

LACTOCOCCUS LACTIS/GENOMIC/TRANSCRIPTOMIC APPROACH

Six strains of *L. lactis* subsp. *lactis* were selected from the Génoferment culture collection. Intra-subspecies diversity at the phenotypic levels was detected by growing these strains in skimmed raw milk ultrafiltration (UF) retentate and in different media: the synthetic chemically defined medium (CDM), complex medium (M17), and modified synthetic medium (M13). It was found that all the six strains were highly grown in the UF-cheese model with values of the growth rate ranging between 0.78 and 0.88 h^{-1} and were also grown under highly acid condition. The higher specific growth rate was also detected in all the strains on CDM medium than in M17 medium whereas none of these strains were able to grow on M13 medium. The core genome of these strains was determined by using arrary-based comparative genomic hybridization (CGH). Afterwards, the ratios of intensities were calculated between the tested strain and the IL1403 for the genomic divergences between the strains. It revealed that the six *L. lactis* subsp. *lactis* strains had strong similarities between the sequences by sharing of a large dairy core genome of 1,915 genes. However, the sequence of LD61 strain was very close to the sequence of the IL1403 strain compared with the other five strains. The differential transcriptomic analysis for the core genome of these strains was examined with DNA arrays and was then classified by a hierarchical clustering for the relationships of the different strains. The

strain dendrogram showed the strains grouping between the LD55 and UCMA571 strains. In contrast, LL08 strain was the most divergent strain compared with the other four strains. The number of genes differentially expressed was determined for each strain. It was found that the total of 968 different genes was differentially regulated in at least one of the five strains compared with the LD61 strain. The strong divergence of the five strains compared with LD61 strain was observed within many regulated categories distributed all over the metabolism. In addition, the physiological characteristics of these strains were also determined by comparing the nitrogenous bases and the branched chain amino acids (BCAAs) requirements. The slightly grow of these strains was observed in different kinds of CDM medium with the nitrogenous base and the BCAAs compared with the classic CDM medium.

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LIST OF ABBREVIATIONS

bp	=	Base pair
^{o}C	=	Degree celsius
CFU	=	Colony forming unit
dATP	=	Deoxyadenosine triphosphate
dCTP	=	Deoxycytidine triphosphate
dGTP	=	Deoxyguanosine triphosphate
dNTP	=	Deoxynucleoside triphosphate
dTTP	=	Deoxythymidine triphosphate
DNA	=	Deoxyribonucleic acid
et al.	=	el alia (and others)
e.g.	=	For example
etc.	=	et cetera, and others
EDTA	=	Ethylenediaminetetraacetic acid
g	=	Gram
h	=	Hour
kb	=	Kilo base
M	=	Molar
Mbp	=	Mega base pairs
µg	=	Microgram
µL	=	Microliter

LIST OF ABBREVIATIONS (Continued)

mL	=	Milliliter
mM	=	Millimolar
ng	=	Nanogram
nm	=	Nanometer
%	=	Percentage
PCR	=	Polymerase chain reaction
rpm	=	Round per minute
RNA	=	Ribonucleic acid
SDS	=	Sodium dodecyl sulphate
U	=	Unit

CHAPTER I

INTRODUCTION

Lactococcus lactis is a member of the lactic acid bacteria and produces lactic acid from sugars. It is found in environment including plant and animal habitats (Teuber *et al*., 2006). *L. lactis* is one of the main microbes in the food application and widely used as a starter cultures for the production of cheese products and milk fermentations. Owing to several metabolic properties such as degradation of casein, acidification by lactic acid synthesis, and production of flavor compounds and exopolysaccharides (EPS) which are used *in situ* to improve the textural characteristics of fermented dairy products (Dabour *et al*., 2005), these microbes are promising candidates for innovative applications. Many important functions in fermentation are encoded on many conjugative plasmids contained in these bacteria (Mills *et al*., 2006).

Microbial genomes are highly flexible and genetic adaptation through gene (in)-activation or DNA exchange as adaptive responses to challenge environments is important sources of intraspecies diversity (Van Hylckama Vlieg *et al*., 2006). Generally, *Lactococcus lactis* is continuously exposed to stress conditions generated by either in natural ecological niches including plants, animals, gastrointestinal tracts (Drouault *et al*., 1999) or during industrial processes. It encounters a wide range of different conditions such as extremes in temperature, pH, acid or osmotic pressure, and suboptimal growth. The bacterial response to stress conditionsis very complex and

can be regulated at all steps of gene expression such as transcription and translation efficiency, or the stability of mRNAs (Redon *et al*., 2005) or the allosteric regulation of the enzymes. Hence, it is important to know how the observed phenotype is related to the global regulation of gene expression during growth.

The development of microarray, enables the whole transcripts profile, *i.e.* the expression levels of thousands of genes simultaneously in different samples or conditions, to be examined. The coordinately regulated genes are also identified. Transcriptomic analysis can also be used for strain comparison at the functional level. Recently, the complete genome sequence of *L. lactis* subsp. *lactis* IL1403 has become available (Bolotin *et al*., 2001) and lead to intraspecies comparative genomic as well as functional genomics studies. There are gained a lot of understanding in physiological processes and regulatory networks operating of lactococci. Moreover, for a deeper understanding, these genomics studies can be correlated with the phenotypic behavior of the strains (Klaenhammer *et al*., 2007). Recently, genome-wide transcriptomic analysis of Lactic Acid Bacteria (LAB) was performed during the cell response and adaptation of *L. lactis* strains which led all the gene expression changes due to various stresses to be quantified.

Hence, this study was carried out to determine the biodiversity of dairy strains of *Lactococcus lactis* subsp. *lactis* using comparative genomic and transcriptomic approaches with microarrays. In our experiments, six strains of *L. lactis* subsp. *lactis* were studied for phenotypic aspects, in which the influence of different compounds within the media on growth was determined by comparative genome hybridization (CGH) for genomic characteristics and transcriptomic approach for gene expression.

Research objectives

1. To determine the physiological characteristics of six strains of *Lactococcus lactis* subsp. *lactis*.

2. To determine the genomic characteristics of the *L. lactis* strains by using comparative genomic hybridization (CGH).

3. To determine the correlation between genes expression and its function of *L. lactis* strains using transcriptomic analysis.

CHAPTER II

LITERATURE REVIEW

2.1 Lactic acid bacteria

The lactic acid bacteria (LAB) are a group of Gram-positive, facultative anaerobes, non-spore-forming bacteria, which produce lactic acid as the major end-product during the fermentation of carbohydrates. They have a cocci or rod shape and generally lack catalase activity. Cultures of LAB can be either mesophilic with optimal growth at approximately 26-30 ^{o}C or thermophilic (optimal growth at approximately 42 ^{o}C) (Marth and Steele, 2001). LAB can be sub-classified into 7 phylogenetic classes: *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc* and *Oenococcus* (óSullivan *et al*., 2009). Generally, LAB are associated with nutrient-rich habitats containing simple sugars. They are found naturally in a variety of environmental habitats, including dairy, meat, vegetable, cereal and plant environments, where fermentation can occur (Klaenhammer *et al*, 2005) but some are also members of the normal flora of the mouth, intestine and vagina of mammals (Salminen and Wright, 1998).

2.1.1 Type of lactic acid fermentation

Lactic acid fermentation can be divided into two major types (Salminen and Wright, 1998).

2.1.1.1 Homolactic fermentation

Homolactic fermentation is a metabolic process in which glycolysis (Embden-Meyerhof-Parnas pathway; EMP pathway) occurs as the major pathway for hexose fermentation (Figure 2.1A). All LAB usually use this pathway except *Leuconostoc*, group III *Lactobacilli*, *Oenococci* and *Weissella* (Salminen and Wright, 1998). Under standard conditions such as non-limiting concentrations of glucose and growth factors, glucose can be converted to be more than 90% lactic acid by the homolactic acid bacterium *Lactococcus lactis* (Garrigues, Loubiere, Lindley and Cocaign-Bousquet, 1997).

2.1.1.2 Heterolactic fermentation

Heterolactic fermentation is a metabolic process which is other main fermentation pathway rather than EMP pathway. It is performed such as the 6-phosphogluconate/phosphoketolase pathway, for hexose fermentation (Figure 2.1B). This metabolism leads to significant amounts of other end-products such as ethanol, acetic acid, and CO_2 in addition to lactic acid.

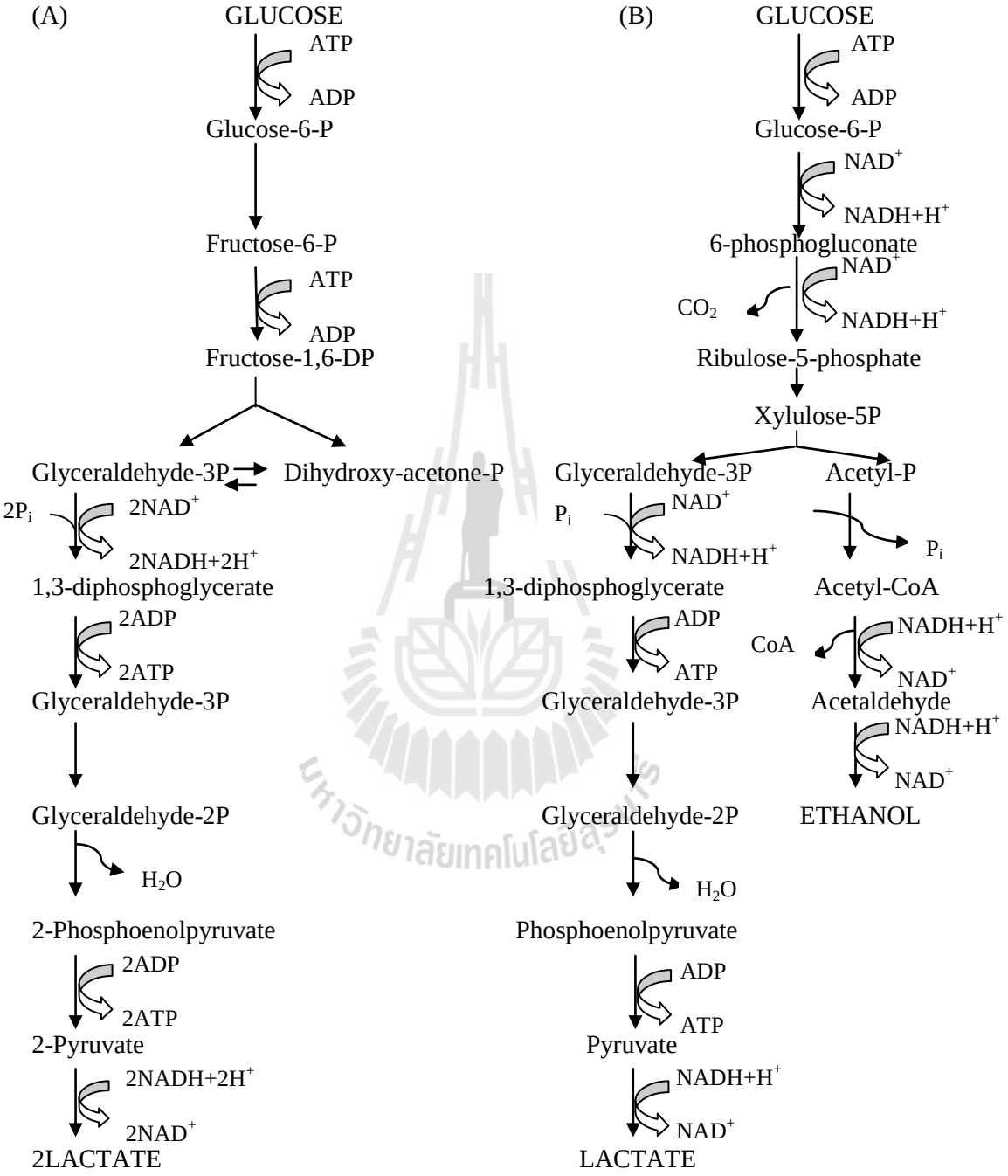

Figure 2.1 Pathways of hexose fermentation (A) Homolactic fermentation (glycolysis, Embden-Meyerhof pathway); (B) Heterolactic fermentation (6-phospho-gluconate/ phosphoketolase pathway) (Salminen and Wright., 1998).

2.1.2 Applications of LAB

LAB are used for food and beverage fermentation (Table 2.1), mainly for acidification, flavor forming (Ayad *et al*., 1999), preservation, bacteriocins (Garcia-Almendárez, Cann, Martin, Guerrero-Legarreta, and Regalado, 2008), and exopolysaccharides production in food (Ayala-Herna'ndez, Hassan, Goff, and Corredig, 2009). In addition, there are application areas for use of LAB as probiotics since they are defined as live micro-organisms which confer a health benefit on the host when administered in adequate amounts (Picard *et al*., 2005). The most commonly used strains are members of the heterogeneous group of lactic acid bacteria; lactobacilli, enterococci and bifidobacteria. In particular, lactobacilli and bifidobacteria are widely used as probiotics primarily in dairy products and dietary supplements (Cebeci and Gürakan, 2003; Ouwehand, Salminen, and Isolauri, 2007; Picard *et al*., 2005). Moreover, LAB can also be used to produce bulk and fine chemicals, including lactic acid, polyols, and vitamins B (Hugenholtz, 2008).

Table 2.1 The potential applications of LAB.

Type of an application	LAB	References
Cheese manufacture	*L. lactis* subsp. *cremoris*	Dabour *et al*. (2005)
	Leuconostoc spp.	Bonetta *et al*. (2008)
	Lactococcus lactis subsp. *lactis* biovar *diacetylactis*	Bissonnette *et al*. (2000)
	Brevibacterium linens	Bockelmann *et al*. (2005)
	Lactobacillus helveticus	Helinck *et al*. (2004)
	Lactobacillus delbrueckii subsp. *lactis*	De Angleis *et al*. (2008)
	Streptococcus thermophilus	Liggett *et al*. (2008)
Food fermentation	*L. lactis*	Zamfir *et al*. (2006)
	Leuconostoc spp.	
	Enterococcus spp.	
	Lactobacillus plantarum IB2	Tamang *et al*. (2009)

Table 2.1 (Continued).

Type of an application	LAB	References
Food preservation	*L. lactis* W8	Mitra *et al*. (2010)
Meat fermentation	*L. lactis* subsp. *cremoris* NCDO763	Herranz *et al*. (2003)
	Lactobacillus sakei *Lactobacillus plantarum* *Leuconostoc carnosum* *Leuconostoc gelidum* *Leuconostoc pseudomesenteroides*	Parente (2001)
Probiotics	*Bifidobacterium* sp.	Picard *et al*. (2005)
	Lactobacillus casei Shirota	Ouwehand *et al*. (2002)
	Lactobacillus plantarum	Cebeci and GÜrakan (2003)
Wine making	*Lactobacillus hilgardii* X_1B	Arena *et al*. (2002)
	Oenococcus oeni *L. sakei*	Leroy and De Vuyst (2004)

2.2 The genus *Lactococcus*

The genus *Lactococcus* was reclassified from some species of the genera *Streptococcus* and *Lactobacillus* in 1985 by Schleifer and co-worker (Teuber and Geis, 2006). It includes five species, *Lactococcus garvieae*, *Lactococcus piscium*, *Lactococcus plantarum*, *Lactococcus raffinolactis*, and *Lactococcus lactis*. *L. lactis* is differentiated into subspecies *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *hordniae* and *L. lactis* subsp. *lactis* (Casalta and Montel, 2008). Lactococci are Gram-positive, homofermentative microaerophilic cocci which lack the cytochromes of the respiration chain.

2.2.1 Characterization of Lactococci

2.2.1.1 Physiological characteristics

The lactococci are usually found in the various niches of the dairy industry environment. They are characterized by spheres of ovoid cells occurring

single, in pairs or in chains, and being often elongated in the direction of the chain (Wood and Holzapfel, 1995). *L. lactis* does not possess flagella and does not create endospores (Wood and Holzapfel, 1995), while some of their strains are capable of excreting extracellular polysaccharide substances (EPS). Looijesteijn and coworkers (2001) studied the physiological function of EPS in *L. lactis* and found that the presence of cell associated EPS was shown to increase tolerance to copper and nisin and to protect the bacteria against bacteriophages and the cell wall degrading enzyme lysozyme. In addition, Van Casteren *et al*. (1998) reported that exopolysaccharide from *L. lactis* subsp. *cremoris* B40 consists of rhamnose, galactose and glucose in the ratio of 0.9:1.2:2.0 and the molar ratio of carbohydrate and phosphorus is 4.7:1. *L. lactis* used in dairy production, are subdivided into *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*. *L. lactis* subsp. *cremoris* is generally distinguished from subsp. *lactis* by a few phenotypic characteristics, including lack of growth at 40 ^{o}C, in 4% NaCl or at pH 9.2 (Table 2.2) (Kim, 1999) and inability to hydrolyse arginine. In addition, it cannot decarboxylate glutamate to γ-aminobutyric acid (GABA), while subsp. *lactis* displays this activity (Nomura *et al*., 1999). Among the lactococci, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* differs from *L. lactis* subsp. *lactis* and subsp. *cremoris* in their ability to utilize citrate with production of diacetyl. These strains possess the citrate permease (CitP) that enables them to transport citrate into a cell (Samarzija *et al*., 2001). The mechanism of citrate uptake in resting cell of *L. lactis* subsp. *lactis* biovar *diacetylactis* has been described by Magni *et al*. (1996). The pH gradient (ΔpH) and the membrane potential (ΔΨ) are driving forces for citrate uptake. Moreover, it seems that CitP activity requires neither Na^{+} nor Mg^{+} cations. In addition, *Lactococci* have no catalase or functional electron-transfer chain, therefore, they do

not require oxygen for growth and in fact, a negative effect of oxygen on the growth of these bacteria has often been observed. However, Duwat *et al*. (1995) and Gaudu *et al*. (2002) found that the respiration, in the presence of heme, resulted in the growth of *L. lactis* by inducing the changing of metabolism and improving oxygen tolerance and long-term survival. Moreover, *L. lactis* has been reported that it possesses oxygen metabolizing enzymes like superoxide dismutase (Sanders *et al*., 1995) or NADH oxidases.

Table 2.2 Physiological and other properties of dairy Lactococci used for identification and differentiation.

Properties	*L. lactis* subsp. *lactis*	*L. lactis* subsp. *cremoris*	*L. lactis* subsp. *lactis* biovar. *diacetylactis*
Growth at 10 ^{o}C	+	+	+
Growth at 40 ^{o}C	+	_	+
Growth at 45 ^{o}C	_	_	_
Growth in 4% NaCl	+	_	+
Growth in 6.5% NaCl	_	_	_
Growth at pH 9.2	+	_	+
NH_3 from arginine	+	_	+
CO_2 from citrate	_	_	+
Diacetyl and acetoin	_	_	+

2.2.1.2 Genomic characteristics

1) Plasmid

Between 1 and 12 plasmids of different sizes can be found in different strains of *L. lactis* (Wood and Holzapfel, 1995) and sizes are varied from about 2 to more than 100 kb (Teuber and Geis., 2006). The complete sequences of four plasmids of *L. lactis* subsp. *cremoris* NIZO B697, a derivative of strain SK11, have been reported by Siezen *et al*. (2005). This strain was found to contain the plasmids pSK11A, pSK11B, pSK11L and pSK11P which have sizes about 10,372, 13,332, 47,165 and 75,814 bp, respectively. Recently, the complete 42,180-bp nucleotide sequence of the mobilization plasmid pNZ4000, encoding for exopolysaccharide (EPS) production in *Lactococcus lactis* NZ4010, has been reported by Van Kranenburg *et al*. (2000).

As plasmids are mobile elements, they can be lost by growth without lactose or casein, by growth at high sublethal temperatures (about 38-42 ^{o}C), by ethidium bromide treatment, by freezing and thawing or by freeze-drying, and acquired by natural conjugation or transduction at reasonable rates (Mills *et al*., 2006). The lactose plasmid from *L. lactis* subsp. *cremoris* SK11, pSK11L, is extremely stable in *L. lactis* subsp. *cremoris* strains. However, it has been shown in the laboratory that pSK11L displays a number of phenotypes in *L. lactis* LM0230 which are not observed in *L. lactis* subsp. *cremoris* SK11 or EB_5. Among these are plasmid instability and temperature-sensitive plasmid maintenance. The stabilities of plasmids under these different treatments are strain dependent. Kim *et al*. (2001) demonstrated the survival response and rearrangement of plasmid DNA of *L. lactis* LL41-1 during long-term

starvation and found that the original plasmids presenting in the parent were rearranged in a certain way, and an entirely new plasmid was generated.

In addition, many of the functions encoded on plasmids turned out to be related to or necessary for growth of lactococci in milk. According to Wood and Holzapfel (1995), the following functions possibilities exist for the identification of plasmids encoded in *L. lactis*.

(i) Lactose transport and metabolism

(ii) Casein degradation by cell wall protease

(iii) Citrate and oligopeptide transport (permease)

(iv) Bacteriophage protection by restriction/ modification and abortive infection

(v) Formation of extracellular polysaccharides (slime)

(vi) Bacteriocin production and immunity

(vii) Insertion (IS) element dependent recombination and cointegrate formation

(viii) Antibiotic resistance

(ix) Conjugal transfer and mobilization of plasmids

(x) Plasmid replication

These properties can contribute to the desired flavor and texture of the product and optimal growth on the milk components lactose and casein, as well as stability and survival.

2) Chromosomal DNA

The genome of *L. lactis* is AT-rich and consists of a circular chromosome of about 2.0 to 2.7 Mbp (Davidson *et al*., 1995). Because of the closely

relatedness in phenotype and genotype between *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, Le Borgeois *et al*. (1992; 1995) and Tulloch *et al*. (1991) performed study on the combination between physical and genetic map of the chromosome in independent lactococcal strains, IL1403, DL11 and MG1363 which belong to subspecies *lactis* for the two first and *cremoris* for the last one, by using pulsed-field gel electrophoresis (PFGE). They revealed that the chromosome size in the strains IL1403 and DL11 are 2,420 kb and 2,580 Kb while the MG1363 chromosome appeared to be 2,560 kb long. The comparison of physical maps of the three strains showed an overall conservation of restriction site locations for the *L. lactis* subsp. *lactis* strains but not for the *L. lactis* subsp. *cremoris* strain. At the genetic organization level, different kinds of rearrangements were observed. A large inversion convering almost half of the chromosome (Le Borgeois *et al*., 1995) was identified by comparing strains of the two different subspecies. In addition, the divergences of certain regions of chromosome between these two subspecies, *latis* and *cremoris*, have been described by Delorme *et al*. (1994). The results indicated that the conserved regions differ by less than 20%, whilst variable regions differ by more than 60%. Moreover, Davidson *et al*. (1995) reported that the genetic maps of the chromosomes in the different *L. lactis* subsp. *cremoris* strains, MG1363 and FG2, have an inversion of approximately 40% of the chromosome when compared with the maps of two *L. lactis* subsp. *lactis* strains, DL11 and IL1403, and the translocation or inversion of four discrete regions had occurred between the two *L. lactis* subsp. *cremoris* strains (Daveran-Mingot *et al*., 1998).

3) IS element and Bacteriophage

Insertion sequence elements of the genus *Lactococcus* commonly present in both the chromosome and the plasmids. The sequencing data allowed the prediction of some very interesting features of the lactococcal genome. Bolotin and coworkers (2001) performed sequencing the genome of the laboratory strain IL1403, using a novel two-step strategy that comprises diagnostic sequencing of the entire genome and a shortgun polishing step. It was found that there are six different IS elements in the IL1403 chromosome. Non-random distribution of IS elements indicates that the chromosome of the sequenced strain may be a product of recent recombination between two closely related genomes. Recently, the genome of *L. lactis* MG1363 has been sequenced and it was found that it carries 11 different IS elements involving a total of 67 kb of DNA (Wegmann *et al*., 2007). Two unique ISs which are IS*712* and IS*1675*, are found in *L. lactis* MG1363 and are not presented in *L. lactis* IL1403 and *L. lactis* SK11 whereas IS*981* and IS*982* are contained in the SK11 strain (Table 2.3).

Table 2.3 IS elements in different *Lactococcus lactis* strains (From Wegmann *et al*., 2007).

IS elements	*L. lactis* strain[a]		
	MG1363	IL1403	SK11
IS*904*	9(1)	9	7
IS*1077*	9(9)	7	10(1)
IS*905*	14(8)	1	13
IS*981*	16(1)	10	30(1)
IS*982*	2(1)	1	55(1)
IS*983*	0	15	0

Table 2.3 (Continued).

IS elements	*L. lactis* strain[a]		
	MG1363	IL1403	SK11
IS712	8(1)	0	0
IS-LL6	9(9)	0	3
IS946	1	0	2
IS1216	1	0	3
IS1297	1	0	7
IS1675	1	0	0
Total	71	43	130

[a] Number in parentheses indicates pseudogenes.

Lactococcal phages are classified into 12 species based on morphology and DNA homologies (Durmaz and Klaenhammer, 2000). The c2 (prolate-headed) species and the 936 and P335 (small isometric-headed) species are the most important taxa, since these are the major organisms that disrupt dairy fermentations worldwide. While the 936 species is composed of only lytic phages, P335 species exhibits high level of DNA homology between temperate and lytic members. P335 phages have been appearing in cheese plants and are now considered members of an important new phage species. The sequencing of the *L. lactis* MG1363 genome has shown that the *L. lactis* MG1363 chromosome harbors six regions that represent bacteriophage-related sequence (Wegmann *et al*., 2007). Two sites appear to contain complete prophage genomes, designated phiT712 (42,085 bp) and MG-3 (44,200 bp). The remaining bacteriophage sequences, designated MG-1 (19,053 bp), MG-2 (6,019), MG-4 (18,029 bp) and MG-5 (10,598 bp), appear to represent remnant

or satellite phages. The bacteriophage sequences encompass approximately 5.5% of the *L. lactis* MG1363 genome, representing a large portion of the observed genomic differences between *L. lactis* MG1363, *L. lactis* SK11 and *L. lactis* IL1403. Furthermore, it results that lysogenic bacteriophages significantly contribute to genome variability within this species. In addition, comparing the six prophages present in *L. lactis* IL1403 and *L. lactis* MG1363 revealed only one common integration site, and showed that two phages, MG-1 and bIL310, displayed the highest level of homology and synteny whereas a similar phage is not presented in *L. lactis* SK11 (Wegmann *et al*., 2007). The phage genomes in *L. lactis* SK11 and *L. lactis* IL1403 are not interrupted by IS elements, except for phage bIL311 in *L. lactis* IL1403, which contains two IS983 elements (Chopin *et al*., 2001). However, Deveau and coworkers (2006) have performed the classification of *L. lactis* phages by stringent DNA-DNA hybridization studies, electron microscopy observation, and sequence analyses. A new classification scheme for lactococcal phages is proposed that the groups of phase be reduced for 12 to 8. Recently, the genetic organization of six prophages presenting in the genome of *L. lactis* IL1403 has been reported by Chopin *et al*. (2001) who found that the three larger prophages (36-42 kb) belong to those already described P335 group of temperate phages, whereas the three smaller ones (13-15 kb) are most probably satellites relying on helper phages for multiplication. P335 temperate phages have variable genomes, sharing homology over only 10-33% of their length. In contrast, virulent phages have highly similar genomes sharing homology over >90% of their length. Ventura and coworkers (2007) have also described the genetic organization of six and five apparent prophage-like elements present in the genomes of the *Lactococcus lactis* subsp. *cremoris* MG1363 and SK11,

respectively. The phylogenetic investigation as well as bioinformatic analyses indicated that all 11 prophages belong to subdivision of the lactococcal P335 group of temperate bacteriophages.

Table 2.4 Features of sequenced *L. lactis* genomes.

Strains	Size (Mbp)	%GC	plasmids	Pseudo-genes	Pro-phages	Proteins	Ref.
L. lactis subsp. *lactis* IL1403	2.3	35.4	0	1	3	2321	Bolotin *et al*. (2001)
L. lactis subsp. cremoris SK11	2.4	30.9	5	144	4	2509	Pfeiler *et al*. (2007)
L. lactis subsp. *cremoris* MG1363	2.5	37.1	0	81	2	2436	Wegman *et al*. (2007)

2.2.2 Natural diversity of Lactococci

Lactococcus is viable in a number of diverse environments such as plant origin or in the gastrointestinal tracts of animals, insects, and humans, and also in fermented foods mostly from dairy origin. They are generally into close proximity to a wide variety of other microorganisms with a large reservoir of gene transfer (Wegmann *et al*., 2007). The presence of lactococci in raw milk is due to contamination from forage during milking. The two lactococci most commonly found in raw milk, cheese and other dairy products are *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* as well as *Lactococcus raffinolactic* and *Lactococcus garvieae* which have also occasionally been found in raw milk and cheese (Casalta and Montel, 2008).

Plant environment is a second natural niche for lactococci. Most plant-associated strains belong to *L. lactis* subsp. *lactis* and have the *lactis* phenotype (Van Hylckama Vlieg *et al*., 2006). Owing to the highly variable niches with respect to chemical composition, for instance, the availability of carbohydrates other than lactose as growth substrates, plant-associated strains should possess a metabolic potential which helps them to survive and to live in this habitat. One could expect that lactococci might degrade plant sugars in order to use them as energy and carbon sources. Generally, the most abundant sugars in plants are cellulose, sucrose and starch. Despite the lack of cellulose degrading enzymes in lactococci, these bacteria were shown to produce those, splitting the other two sugars, disaccharide sucrose, and most recently, a polysaccharide starch (Okano *et al*., 2007). Doman and coworkers (2000) studied the production and genetic regulation of an amylase in *L. lactis* and found that two of the tested strains were shown to produce an amylase. One of them, *L. lactis* IBB500 started to produce the extracellular amylases in the BHI broth at the end of the logarithmic phase of growth and the maximal amount of enzyme was detected at the early stationary phase. These experiments showed that various sugars modulated the production of the enzyme secreted to the medium; starch was found to be the best inducer, while glucose strongly repressed amylolytic activity. In addition, Petrov *et al*. (2008) reported the ability of the natural strain *L. lactis* subsp. *lactis* B84, isolated from spontaneously fermented rye sour dough, to utilize starch as a sole carbon source and to produce L(+)-lactate. In addition, the gene expression of the key enzymes, *amyl* and *amyY* involved in starch degradation, was observed in B84 genome by using the methods of reverse transcription polymerase chain reaction (RT-PCR).

In addition, lactococci are capable of assimilating β-glucosides, such as cellobiose, salicin, arbutin and esculin, which also belong to plant sugars. Interestingly, it seems that some of the β-glucosides, e.g. cellobiose, can modulate the environment adaptation of these bacteria and prompt them to grow in milk, another habitat of lactococci. Aleksandrzak *et al*. (2000) studied the regulation of carbon catabolism in *L. lactis*. The results showed that low concentrations of cellobiose induce a β-glycosidase activity in plasmid-free, lactose-negative *Lactococcus lactis* IL1403 cells, enabling them to hydrolyze lactose, the main carbohydrate present in milk. These observations suggest that there is a kind of coupling between cellobiose and lactose assimilations in *L. lactis*, and it was proposed that the cellobiose and lactose are transported by a phosphotransferase system (PTS) that is negatively controlled by the CcpA regulator (Kowalczyk *et al*., 2008).

Furthermore, the adaptation to growth on substrates derived from plant cell wall is evident from the presence of gene sets for the degradation of complex plant polymers. Doman-Pytka *et al*. (2004) demonstrated that the 11-kb plasmid DNA fragment representing a gene-cassette, is important for the wild-type *L. lactis* IBB500 strain for its adaptation to the plant environment. Similarly, the capability of *L. lactis* strains isolated from vegetable products to transfer the ability to ferment raffinose and sucrose, was observed in conjugation experiments with the recipient strain *L. lactis* MG1614. Pulsed-field gel electrophoresis analysis showed that all transconjugants had acquired large chromosomal insertions at two main sites. Nisin-sucrose transconjugants had gained inserts of 70 kb, while those that fermented sucrose without nisin production contained inserts of between 50 and 110 kb (Kelly *et al*., 1998; 2000).

Moreover, the strains isolated from fermenting plant material do not harvest amino acids through proteolysis but depend on amino acid biosynthesis (Ayad *et al*., 1999). Therefore, it can be anticipated that strains adapted to the plant ecological niche will exhibit large metabolic differences and their metabolic diversity will most certainly exceed that of dairy strains (Siezen *et al*., 2008). Plant-derived lactic acid bacteria are expected to show additional capabilities compared with milk-derived strains, for instance, plant-derived strains demonstrate greater tolerance to stress than milk-derived strains. Therefore, the use of plant-derived strains could result in dairy products with improved taste, and these strains might also be used to perform additional functions (Nomura *et al*., 2006). Recently it has been shown that strains isolated from a nondairy environment, KF147 and KF282, exhibited tolerance to high salt concentration and high pH value, and fermented more kinds of carbohydrates than the milk-derived strains (Nomura *et al*., 2006). In addition, some nondairy strains showed the ability to produce the key flavor aldehydes as a result of a unique α-keto acid decarboxylase and glutamate dehydrogenase activities which convert glutamate to α-ketoglutarate. These two enzymes involve in the first step in the production of flavor compounds from amino acid, and present at rate-limiting concentrations in cheese (Van Hylckama Vlieg *et al*., 2006; Tanous *et al*., 2002).

2.2.3 Adaptive response to environment

L. lactis is widely used as a starter in dairy technology. During the food process as well as in its natural environment or in the intestinal tract, it is subject to a variety of adverse conditions, including acids, oxidation, heating and cooling, high osmolarity such as dehydration, bile salts, ethanol and starvation. In response to these stress conditions, bacterial cells are able to rapidly and transiently induce specific or

general protection mechanisms (Sanders *et al*., 1999) such as enzymes involved in particular chaperone proteins and proteolysis, which act in the cytoplasm or in the cell envelope to repair or degrade abnormal proteins.

Stress factors induce cellular responses that vary with the type, magnitude, and method of stress application. Factors that cause the stress response during dairy starter-culture production and cheese manufacture include the follows:

2.2.3.1 Changes in temperature

1) Heat shock

The major problem encountered by cells at high temperature is the denaturation of proteins and their subsequent aggregation. In addition, destabilization of macromolecules such as ribosome and RNA, and alterations of membrane fluidity were also described (Van de Guchte *et al*., 2002). The heat-shock (HS) response to increase in temperature causes the induction of a small group of proteins, the heat shock proteins. Heat shock proteins such as DnaK, DnaJ, GroEL, GroES and GrpE, play essential physiological roles as molecular chaperones in protecting cells against damage due to thermal stress by binding to cellular proteins in a manner that maintains their native conformation and minimizes denaturation (Yousef and Juneja, 2003). Whitaker and Batt (1991) have characterized the heat shock response in *L. lactis* subsp. *lactis* and found that a shift in temperature from 30 to 42 ^{o}C was sufficient to arrest the growth of *L. lactis* subsp. *lactis*, but growth resumed after a shift in temperature back to 30 ^{o}C. At 42 ^{o}C, the two heat shock-induced proteins appeared to be homolog to GroEL an DnaK, based on their molecular weights and reactivity with antiserum against the corresponding *Escherichia coli* proteins.

2) Low temperature (cold stress)

Starter LAB is exposed to low temperatures during frozen storage, as well as during low-temperature fermentation. Whereas growth at high temperature is harmful to a cell, growth at low temperatures merely slows down biological processes. Several bacteria respond to a decrease in temperature by inducing a set of proteins, called cold shock proteins (CSPs) (Van De Guchte *et al*., 2002). These proteins can function as RNA chaperones, transcriptional activators, freeze-protective compounds that are also found in *E. coli* and *Bacillus subtilis* (Bae *et al*., 2005; Graumann and Marahiel, 1998; Wouters *et al*., 2000). Wouters *et al*. (1999) have studied the low-temperature responses of *L. lactis* MG1363 and characterized the effects of multiple *csp* gene disruptions on adaptation to cold and gene regulation of *L. lactis* (Wouters *et al*., 2001). It resulted in the identification of a group of 7-kDa proteins that appear to represent the most highly induced proteins upon a rapid downshift in temperature and it was also found to have five members in the CSP family (Wouters *et al*., 1998). In addition, they found that the deletion of *csp* genes affected freeze survival of *L. lactis*, the remaining counterparts of the lactococcal CSP family and the several cold-induced proteins (CIPs) production. Recently, Yinghua *et al*. (2008) demonstrated that CspC, a 6.2 kDa cold-shock protein, improved the recovery of cells, and a 7 kDa cold-shock protein, CspD, increased the viability after freezing (30-40 folds). Furthermore, factors affecting the survival of LAB during freezing-thawing cycles have been reported by Lee (2004), in a study including different diluents, growth phases, and cold temperatures. Viability experiments showed that this strain displaying cold shock cryotolerance had an improved survival capacity in stationary phase. Similarly, the survival capacity of *L. lactis* subsp. *lactis*

IL1403 to cold temperatures had also been improved in stationary phase (Panoff *et al*., 1994). Panoff *et al*. (1995) demonstrated the physiology of the cold-shock response in *L. lactis* subsp. *lactis* IL1403 at a subzero temperature and found that pre-incubation of cells at 8 ^{o}C led to an enhanced capacity to survive exposure to freezing temperature (-20 ^{o}C). Moreover, the effects of low temperature stress on the glycolytic activity of *L. lactis* were studied (Wouters *et al*., 2000). The maximal glycolytic activity increased approximately 2.5-fold at 10 ^{o}C for 4 h compared with at 30 ^{o}C. Analysis of cold adaptation of strains with disrupted genes involved in sugar metabolism showed that both the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) subunit HPr and catabolite control protein (CcpA) are involved in the increased acidification at low temperatures, and the protein analysis showed that the production of both HPr and CcpA was induced up to two- to three-fold upon exposure to low temperature.

2.2.3.2 pH

Milk fermentations by lactic acid bacteria (LAB) are extensively used to produce cheeses and other dairy products. Generally, in milk fermentations, LAB are able to degrade lactose resulting in lactate accumulation and consequently acidification of the media to as low as pH 4.0 (Rallu *et al*., 1996). The further growth and metabolism may be inhibited either by acidification or lactate even if nutrients are still available. Lactic acid is a weak organic acid that it is not charged at low pH and can easily pass the cell membrane in the protonated form. Budin-Verneuil *et al*. (2005) and O'Sullivan and Codon (1999) showed that *L. lactis* strains, including MG1363, display an inducible acid tolerance response (ATR) when they encounter a moderate acid pH during logarithmic growth. ATR improves the cell survival in normally lethal

acid conditions. Furthermore, in proteomic characterization of acid tolerance response of *L. lactis* MG1363 in different media, Budin-Verneuil *et al*. (2005) showed that the development of ATR was fully dependent on *de novo* protein synthesis in chemically defined culture media and only partly dependent in M17. Bacteria are equipped with a number of mechanisms that confer acid tolerance. Among these mechanisms are proton translocation, arginine deaminase (ADI) pathway, amino acid decarboxylation-antiporter reactions, and the citrate transport system (Yousef and Juneja, 2003).

1) Proton movement: H^+-ATPase

The maintenance of the cytoplasmic pH (pH_{in}) which is more alkaline than the extracellular pH (pH_{out}), is directly required for the survival of the lactic acid bacteria, because many cytoplasmic enzymes have their pH optima in a neutral range (Amachi *et al*., 1998). The primary mechanism of *L. lactis* for surviving at low pH is the membrane-bound F_oF_1 ATPase. It functions to maintain a favorable intracellular pH and protect cells during exposure to acidic environments by translocating protons to the environment at the expense of ATP hydrolysis (Yousef and Juneja, 2003). O' Sullivan and Condon (1999) demonstrated that the intracellular pH (pH_i) plays a major role in the induction of this multistress resistance response. The pH_i was dependent on the extracellular pH and on the specific acid used to reduce the extracellular pH (pH_o). Siegumfeldt *et al*. (2000) described the dynamics of changes in the pH_i values of a number of LAB in response to a rapid drop in the extracellular pH by using method based on fluorescence ratio imaging of single cells. It was found that the pH_{in} decreased as the pH_{out} decreased in order to maintain a constant transmembrane pH gradient rather than a constant pH. Genes encoding F_oF_1 ATPase in *L. lactis* subsp. *cremoris* MG1363 have also been cloned and sequenced.

Afterward, a mutant strain was constructed in which the original *atp* promoter on the chromosome was replaced with an inducible nisin promoter. It was shown that a mutant strain in which expression of H^+-ATPase on the chromosome was completely dependent on the presence of nisin for growth. It is indicated that the H^+-ATPase is essential for growth of *L. lactis* under these conditions (Koebmann *et al*., 2000). Moreover, the major role of this enzyme in regulation of the cytoplasmic pH has been confirmed with the acid sensitivity of a mutant of *L. lactis* subsp. *lactis* C2 with a reduced membrane-bound ATPase activity (Amachi *et al*., 1998).

2) Arginine deiminase (ADI) pathway

Most of LAB metabolize arginine by the arginine deiminase (ADI) pathway (Figure 2.2). This pathway consists of three enzymes such as arginine deiminase, ornithine carbamoyltransferase and carbamate kinase. A fourth component, identified in *L. lactis*, is a membrane-bound antiport protein that catalyzes the exchange between arginine and ornithine. These enzymes catalyze the conversion of arginine to ornithine, ammonia, and carbon dioxide and generate 1 mol of ATP per mole of arginine consumed. The ADI pathway is a mechanism for survival in acidic environments by generating ammonia. The development of acid tolerance depends on the rise in pH associated with ammonia production (Marquis *et al*., 1987). The enzymatic properties of the ADI pathway are well documented in a variety of bacteria. The enzymes in the ADI pathway are inherently acid tolerant and are activated in response to low pH in several species of *Streptococcus* (Cotter and Hill, 2003). As such, these enzymes allow bacteria to recover from acid stress severe enough to prevent the cell membrane from functioning normally. In most LAB, the ADI pathway is repressed by glucose and induced by arginine. The ADI pathway imparts LAB with

enhanced tolerance to acid, primarily through the continuous production of acid-neutralizing ammonia from arginine.

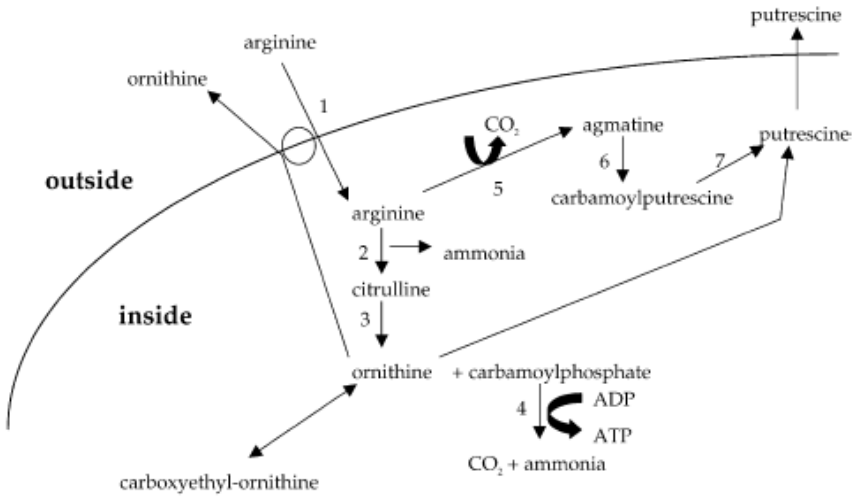

Figure 2.2 Metabolic fate of arginine in bacteria by arginine deiminase (ADI) or arginine decarboxylase pathway (Chou, Weimer and Cutler, 2001).

The arginine deiminase system provides a source of ATP derived from catabolism of arginine to ornithine, CO_2, and ammonia in a variety of organism, including many streptococci and members of the genus *Pseudomonas* (Marquis *et al*., 1987). The system is generally inducible and under the control of catabolite repression. Larsen *et al*. (2004) showed that ArgR and AhrC, which are transcrtiptional regulators, are both required for regulation of arginine metabolism in *L. lactis*. As one of the end products of this pathway is ammonia, ADI activity results in an increase in the extracellular pH (pH_o) and, therefore, in an enhanced survival in low pH conditions.

3) Degradative amino acid decarboxylases

Since *L. lactis* increases the medium acidity through its anaerobic fermentation, it is likely to have acid-resistance mechanism to maintain viability under low pH conditions. Amino acid decarboxylation involves transporting an amino acid into the cell where it is decarboxylated. A proton is consumed in the reaction, and the product is exported from the cell via an antiporter. The result of this reaction is a decrease in intracellular acidity. Glutamate decarboxylase (GAD) constitutes a glutamate-dependent acid resistance mechanism with a glutamate-GABA (γ-aminobutyrate) antiporter (Nomura *et al*., 2000). Sanders *et al*. (1998) sequenced the *L. lactis gadCB* genes and suggested that it encoded a glutamate-dependent acid-resistance mechanism comprised of glutamate-GABA antiporter and GAD. In addition, it was shown that *L. lactis gadB* mutant and a strain unable to express both *gadB* and *gadC* encoding GAD and the glutamate-GABA antiporter, respectively, was more sensitive to low pH than the wild type when NaCl and glutamate were present. Furthermore, the biochemical characteristics of GAD indicated that there was only one GAD structural gene in *L. lactis* (Nomura *et al*., 1999). Nomura *et al*. (2000) studied the sequence of *gadB* gene in *L. lactis* subsp. *lactis* and in *L. lactis* subsp. *cremoris* and found that *L. lactis* subsp. *lactis* strains show glutamate decarboxylase activity, whereas *L. lactis* subsp. *cremoris* strains do not. The *gadB* gene encoding glutamate decarboxylase was detected in the *L. lactis* subsp. *cremoris* genome but was poorly expressed.

4) Citrate transport system

Citrate is present in milk at low concentrations and is co-metabolized with glucose to yield aroma compounds through the diacetyl/acetoin

biosynthetic pathway (Curic *et al*., 1999) (Figure 2.3). Citrate fermentation results in the formation of an electrochemical proton gradient across the cell membrane (proton motive force) by a secondary mechanism in which the CitP plays a crucial role (Bandell *et al*., 1998). Mechanism of citrate metabolism in *L. lactis* CRL264 resistance against lactate toxicity at low pH has been reported by Magni *et al*. (1999). Measurement of the flux through the citrate fermentation pathway showed that the pathway was constitutively expressed, but its activity was significantly enhanced at low pH and the flux was correlated with the magnitude of the membrane potential and pH gradient that were generated when citrate was added to the cells. Garcia-Quintans *et al*. (1998) studied the influence of the extracellular pH on the expression of *citP* and found that in lactococci both transcription of *citP* and citrate uptake increased when cells were grown at low pH. This increase in citrate transport leads to more efficient glucose utilization, which results in a growth advantage for *L. lactis* subsp. *lactis* biovar *diacetylactis* at acid pH. In addition, acid growth can enable to trigger the conversion of citrate into α-acetolactate via pyruvate, by induction at the transcriptional level of diacetyl/acetoin biosynthetic pathway but no influence on levels of lactate dehydrogenase and pyruvate dehydrogenase, presumably contributing to lactococcal pH homeostasis by synthesis of neutral compounds and by decreasing levels of pyruvate (Garcia-Quintans *et al*., 2008). This is correlated with the results of Sánchez *et al*. (2008), showing that the beneficial effect of citrate on growth of *L. lactis* CRL264 under acid stress conditions (pH 4.5) is not primarily due to the concomitant alkalinization of the medium but it is caused by less expenditure of ATP, derived from glucose catabolism, to achieve pH homeostasis. Frees and coworker (2003) have also investigated the proteins which are induced when *L. lactis* is exposed

to condition of low pH (pH 4.5) by using two-dimensional gel electrophoresis. It was found that reducing the pH of the growth medium with hydrochloric acid induced the synthesis of a small subset of proteins such as the oxidative stress proteins superoxide dismutase, alkylhydroperoxidase and the autoinducer synthesis protein, LuxS, as well. When the extracellular pH is reduced to 4.5, the intracellular pH is reduced to 5.0-5.5 within a few minutes (Siegumfeldt *et al*., 2000) and thus, protein denaturation is expected to take place leading to an induction of both HrcA- and CtsR-controlled genes. A differential induction of heat shock proteins as members of the CtsR regulon, ClpE and ClpP was also observed at pH 5.5, and it is indicated that CtsR responds either to a signal different from misfolded proteins or to lower concentrations of denatured proteins than HrcA or another regulatory element is controlling *clpE* and *clpP* expression.

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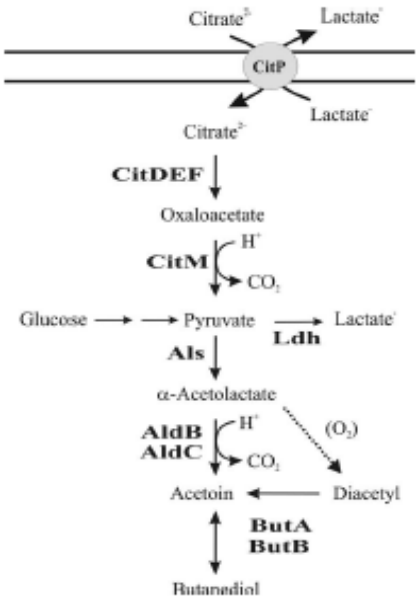

Figure 2.3 Acid induction of citrate transport, citrate metabolism, and diacetyl/acetoin pathways in *L. lactis* (Garcia-Quintans, Repizo, Martin, Magni, and Lopez, 2008).

2.2.3.3 Oxidative stress and DNA damage

Oxidative stress can be defined as an excess of reactive oxygen species (ROS) that have strong oxidizing potential for cells (Rochat *et al*., 2005). During the cellular processes, oxygen is partially reduced to water, leading to the formation of reactive oxygen species, which are the superoxide anion (O_2^-), the hydroxyl radical (OH^*), and hydrogen peroxide (H_2O_2). These intermediates have a high oxidizing potential and thus are responsible for cellular oxygen toxicity (Miyoshi *et al*., 2003). At the molecular level, O_2^-, OH^* and H_2O_2 can react with cellular targets such as proteins, lipids and nucleic acids (Rochat *et al*., 2005) as well as many effects of O_2 are also observed at the metabolic level such as the formation of H_2O_2 which causes a reduction of the growth rate of *L. lactis*, and even its death. The most common oxidative stress resistance mechanism found in *L. lactis* is performed by a coupled NADH oxidase/NADH peroxidase system. Recently, Jiang and Bommarius (2004) have successfully applied the sequence comparison-based approach to develop a novel hydrogen peroxide-forming NADH oxidase (nox-1) from *L. lactis* that reduces oxygen directly to hydrogen peroxide. A second common resistance mechanism is provided by the action of a superoxide dismutase, SOD, which removes O_2^- anion. Most LAB have SOD activity. A unique manganese-containing SOD (MnSOD) has been identified in *L. lactis*, during an analysis of acid stress-induced protein expression (Sanders *et al*., 1995). *L. lactis sodA* has a low initial expression under anaerobic condition which is shifted to a high gene expression level under aerated conditions. An alternative mechanism to eliminate O_2^- anion, which could compensate the low SOD activity, can be provided by high levels of intracellular glutathione (Li *et al*., 2003). In addition, Woo-Suk and So (1999) characterized the superoxide dismutase in *L. lactis*

and found that the SOD activity was found to be growth-phase dependent by increasing the activity until the late stationary phase in aerobically grown cells. In addition, it was found that *L. lactis* possessed a single manganese-containing SOD (MnSOD) after the activity of SOD also increased when the concentration of manganese in the medium increased. Another oxidative stress resistance mechanism is supported by RecA activity. This protein plays a key role in the SOS response and homologous recombination. Duwat *et al*. (1995) constructed a *L. lactis recA* mutant strain, and showed that RecA was involved in resistance to oxidative and thermal stresses. They observed that during exponential and stationary growth phase, *recA* mutants were highly sensitive to aeration, resulting in a reduction in growth rate and viability. After adding an iron chelator to aerated cultures of *L. lactis recA* mutants, bacterial doubling time was restored close to that of anaerobic conditions, leading to the observation that ROS are responsible for oxygen toxicity and that RecA plays an essential role in the repair of DNA damage caused by these compounds. Moreover, from the study of the function of RecA under thermal stress, it was found that activation of *recA* by oxidative stress could confer cross-protection against thermal stress. A single stimulus can activate various stress resistant mechanisms such formamidopyrimidine DNA glycosylase (*fpg*), another DNA repair gene, conferring protection against various types of stress in *L. lactis.* Because of the overlapping of the stress resistant mechanisms in *L. lactis*, cells under one specific stress condition could trigger different stress responses. Control of stress response could also be achieved by cellular systems that sense and transmit environmental signals into the cell, thereby modulating gene expression and physiological changes. Two-component regulatory systems are composed of a membrane-anchored sensor protein, usually a histidine

protein kinase, and an intracellular response regulator. In *L. lactis*, six of these systems have been identified and it was found that three of these systems (systems B, D and F) were involved in specific susceptibility to acid, osmotic, and oxidative stress (O'Connell-Motherway *et al*., 2000), respectively. The system F mutant strain obtained by insertional mutagenesis of *L. lactis*, has greater H_2O_2 sensitivity than that of the wild-type strain.

2.2.3.4 Starvation

The depletion of essential nutrients from the growth medium can lead to growth arrest of the cells and entry into stationary phase. During stationary phase, most of the LAB seem to have the capacity to maintain an active metabolic state (Van de Guchte *et al*., 2002), with cell adaptation such as the changing in cell size and fatty acid composition, decreasing in the overall rate of protein synthesis, and induction of distinct sets of proteins (Hartke *et al*., 1994). In particular, carbohydrate (sugar) starvation is important to understand the starvation response because it leads to cell energy depletion (Van de Guchte *et al*., 2002). Amino acid catabolism plays a role in survival of *L. lactis*, therefore, survival capacity of lactococci upon nitrogen starvation is related to the protein synthesis (Van de Guchte *et al*., 2002). Recently, Ganesan *et al*. (2007) have characterized the ability of lactococci to become nonculturable under carbohydrate starvation while maintaining metabolic activity. They found that the cells contained at least 100 pM of intracellular ATP after 6 months of starvation and amino peptidase and lipase/esterase activities decreased below detection limits during the nonculturable phase. In addition, the cells retained the ability to transport amino acids via proton motive force and peptides via ATP-driven translocation during sugar exhaustion and entry into nonculturability. Similarly, the influence of carbohydrate

and arginine starvation on culturability and amino acid utilization of *Lactococcus lactis* subsp. *lactis* has been studied by Stuart *et al*. (1999). Results indicated that lactococci remain viable in the absence of lactose or arginine. The cells were able to use other amino acids to survive, produce ATP, and maintain cellular integrity without being culturable on agar. In addition, Hartke *et al*. (1994) have reported that carbohydrate-starved cultures of *L. lactis* subsp. *lactis* IL1403 showed enhanced resistance to heat, ethanol, acid, osmotic and oxidative stresses. This cross-protection seems to be established progressively during the transitional growth phase, with maximum resistance occurring when cells enter the stationary phase. Moreover, the survival capacity of lactococci upon carbohydrate starvation is related to the maintenance of glycolytic capacity. Kunji *et al*. (1993) have studied the physiological responses of *L. lactis* ML3 to alternating conditions of growth and carbohydrate starvation and found that loss of glycolytic activity is associated with loss of glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase and pyruvate kinase activities. However, the glycolytic activities can be restored to 100% values with addition of sugar to starved cultures. Moreover, the breakdown of proteins during starvation appears to be largely nonspecific and the rate of synthesis of protein decreases rapidly in the first hour of starvation. From the onset of starvation, at least 45 proteins are no longer synthesized. During starvation, relative induction of fourteen to fifteen proteins can be observed. Recently, the stability of mRNA was investigated at the genomic scale during carbon starvation adaptation of *L. lactis* IL1403. Gene expression was mostly controlled by altered transcription prior to carbon source exhaustion, while the influence of mRNA stability increased during the starvation phase (Redon *et al*., 2005).

In addition to carbohydrate, phosphate is an essential component of bacterial cells because phosphate starvation can be deleterious for both energy supply and DNA/RNA synthesis (Van de Guchte *et al*., 2002). Phosphate starvation induces many genes that code for proteins transporting phosphate into the cells and enzymes that release phosphate from organic compounds. The phosphate starvation-induced Pho regulons of the Gram-negative bacterium *E. coli* and of the Gram-positive bacterium *Bacillus subtilis* have been well characterized. The *pho* genes are regulated by a two-component signal transduction system in these bacteria. In the genomic sequence of *L. lactis*, a putative *pst* operon, the first gene of which codes for a putative phosphate transport substrate-binding protein, can be found. It has been suggested that also the transcription of the *L. lactis pst* operon is regulated by a histidine kinase sensor and a response regulator. Sirén *et al*. (2008) have isolated the *pst* promoter of *L. lactis* and used it for the efficient heterologous production of two industrially interesting enzymes: α-amylase and β-galactosidase.

2.2.3.5 Osmotic stress

In various applications in the food and feed industry, lactic acid bacteria can be exposed to osmotic stress when important quantities of salt or sugar are added to the product. In response to high osmolality, bacteria accumulate compatible solutes (betaine, proline), which restore turgor pressure and stimulate growth (Guillot *et al*., 2000). O'Callaghan and Condon (2000) have investigated differences between certain strains of *L. lactis* in their tolerance to low water activity. A strong correlation was observed between usage of the compatible solute glycine betaine and tolerance to sodium chloride. All the NaCl tolerant strains were stimulated by the presence of glycine betaine. Glycine betaine accumulation occurs either as a result of transport or

by synthesis from choline. Molenaar *et al*. (1993) have studied the accumulation of glycine betaine in *L. lactis* ML3 and found that glycine betaine was created via a constitutively expressed high affinity transport system whereas proline was transported via an inducible transport system. However, the capacity to accumulate betaine is extremely variable among lactococci strains. *L. lactis* subsp. *cremoris* was described as more sensitive to osmotic stress than subsp. *lactis* strains (Obis *et al*., 2001). Obis and coworkers (1999) have characterized the osmoadaptative capacity of *L. lactis* and reported that the betaine transport capacity of *L. lactis* NCDO763 is linked to a single high-affinity ABC transporter, encoded by *busA*, which is in an operon composed of only two genes. The betaine transport capacity of *L. lactis* was found to be under osmotic control at both the genetic and biochemical levels. Moreover, the lacking or a low activity of the betaine transporter BusA is also associated with an osmosensitive phenotype (Obis *et al*., 2001). Furthermore, the effects of culture conditions on osmodependent betaine transport in *L. lactis* subsp. *cremoris* NCDO763 have been studied by Guillot *et al*. (2000) who found that sodium chloride, temperature and Tween-80 alter fatty acid membrane composition and modify the osmotic activation of betaine transport activity. The main modification in *L. lactis* membrane fatty acid composition in response to high osmolality is the increase of cyclopropane fatty acid (CFA), ΔC19:0, whereas unsaturated/saturated ratio remains unchanged.

2.3 Metabolism of Lactococci

2.3.1 Carbohydrate metabolism

The two major pathways for hexose (glucose) fermentation occuring within LAB, are homolactic fermentation and heterolactic fermentation. Homolactic fermentation follows the familiar Embden-Meyerhof-Parnas (EMP) pathway for glycolysis. Lactic acid is virtually the only end-product. The other fermentation pathway, the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway, leads to significant amounts of other end-products such as CO_2 and ethanol in addition to lactic acid, and is referred to as a heterolactic fermentation.

The metabolism of milk ingredients such as lactose, caseins, citrate, and other compounds by the two subspecies *lactis* and *cremoris* of *L. lactis*, provides the basis for the spontaneous and industrial fermentations of milk into sour milk, sour cream and many different types of cheeses. The production of acid from carbohydrates is an important and indispensable property used in the identification and differentiation of individual *Lactococcus* species (Wood and Holzapfel, 1995). The most important function of lactococci in industrial dairy fermentations is the fermentative conversion of lactose into lactic acid. The metabolic conversions included the lactose phosphotransferase system (PTS), the tagatose-6-phosphate pathway and the glycolytic pathway (Figure 2.4). The key enzymes in the utilization of lactose are induced during growth on lactose and often are located on plasmids (Akçelik, 2001). Both fermentative pathways lead to the formation of L (+)-lactic acid which is excreted into the medium. A key compound in the intermediate metabolism in lactococci is pyruvate. Under normal anaerobic conditions of glycolysis, pyruvate is reduced to lactate by lactate dehydrogenase in order to regenerate NAD^+, and serve as

an electron acceptor for the substrate level phosphorylation. The typical butter-flavor compound diacetyl is produced by the metabolism of citrate in *L. lactis* subsp. *latis* biovar. *diacetylactis.* This biovar is differing from normal *L. lactis* subsp. *latis* in that it possesses a plasmid encoded citrate permease. In citrate-utilizing *L. lactis*, citrate is converted initially into oxaloacetate and acetate by the enzyme citrate lyase. Acetate is a good indicator in citrate-containing cultures of homofermentative lactic acid bacteria that citrate is metabolized. Fermentation of citrate generally leads to a mixture of products including lactate, CO_2, acetate, formate and C4-compounds (acetoin, diacetyl, and butanediol).

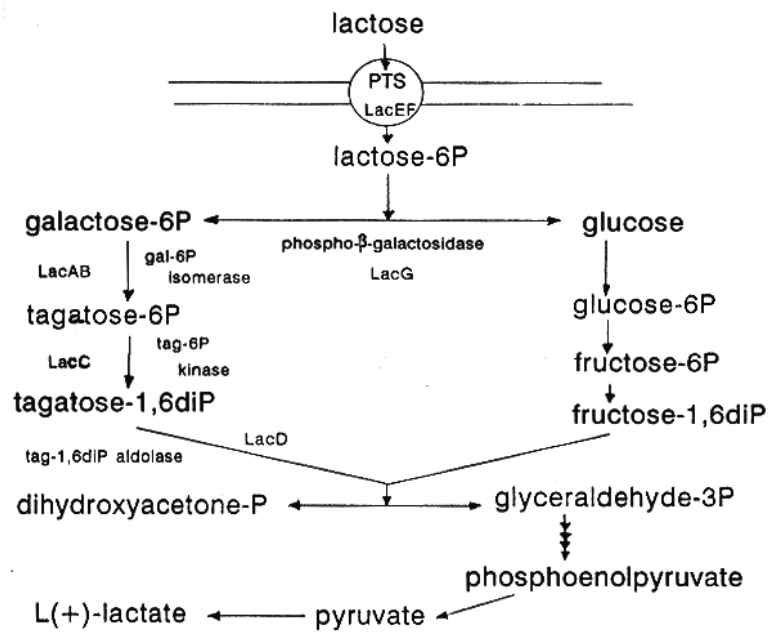

Figure 2.4 Pathway of lactose utilization by *L. lactis* strains (Wood and Holzapfel, 1995).

2.3.2 Nitrogen metabolism

Generally, LAB has a limited capacity to synthesize amino acids using inorganic nitrogen sources. They are dependent on preformed amino acids being

present in the growth medium as nitrogen source. However, the requirement for amino acids differs among the species and strain variations exist within species. Cocaign-Bousquet *et al.* (1995) studied the rational development of a simple synthetic medium for the sustained growth of *L. lactis* and found that the sustained growth of the vegetable strain, was only possible in minimal media supplemented with six amino acids (Glu, Met, Ile, Leu, Val, Ser), indicating that the definition of prototrophy/auxotrophy is partly dependent upon the medium composition. Growth of LAB on chemically defined minimal media is generally slow. In order to achieve a sufficient lactic fermentation, the dairy lactococci have developed a proteolytic system. All dairy lactococci used for acidification of milk (cheese manufacture) have proteolytic acitivity. An extracellular, membrane-anchored serine proteinase (PrtP) was identified as being essential for this activity. Several peptidases with different specificities have been identified in lactococci, but to date, all peptidases have been found to be intracellular. There are amino acids transport systems, two di- and tripeptide transport systems (DtpT and DtpP) and an oligopeptide transport system (Opp) accepting four to eight residue peptides. The transcription of 16 genes encoding 12 peptidases, P_I and P_{III} proteinases, and three transport systems of *L. lactis* MG1363 in response to different environmental factors has been described by Guédon *et al.* (2001). Elevated temperature had no significant effect on the level of transcription of these genes *PrtP1*, *prtP3*, *pepC*, *pepN*, *pepX* while the *opp-pepO1* operon was the most highly expressed genes in chemically defined medium, and their expression was repressed 5- to 150-fold by addition of peptide sources.

2.3.3 Other metabolism

For optimum growth, *L. lactis* requires biotin, pyridoxal, folic acid, riboflavin, niacinamid, thiamine, and pantothenate. Niacin, pantothenate and biotin are essential. Micronutrients should include molybdate, borate, cobalt, copper, manganese and zinc.

2.4 Gene expression and gene regulation in LAB

Gene expression in LAB has received much attention during the last decade. These studies have addressed transcription initiation and termination as well as translation initiation and codon usage (Kok, 1996). As with all organisms, gene expression systems in LAB may be divided into two categories, constitutive expression and controlled expression system (De Vos, 1999). Most of controlled expression systems were developed in *L. lactis* such as those based on promoters controlled by sugar (lactose operon promoter), by salt (*gadC* promoter), by temperature upshift (*tec* phage promoter), pH decrease (P170), and phage infection (phi31-promoter) (Renault, 2002; Grath *et al*., 2002). All of these systems are inducible expression systems. In addition, another controlled expression system is hierarchical control of carbohydrate utilization, which includes carbon catabolite repression (CCR) and integrates carbon-regulatory signals by using the HPr protein of the PTS system.

2.4.1 Inducible expression systems

Sugar utilization has been widely studied in LAB because of its important role in the industrial fermentations. It is specifically controlled by a dedicated regulator (De Vos, 1999). The first system developed for controlled gene expression in lactic acid bacteria was based on components of the *L. lactis* lactose operon

(Djordjevic and Klaenhammer, 1998). They are organized in a 7.8 kb operon with the gene order *lacABCDFEGX* (De Vos and Gasson, 1989). Expression of the *lac* operon is regulated by the product of the *lacR* gene, which acts as a transcriptional repressor (Kok, 1996). The transcription of the *lac* operon is induced up to ten-fold during growth on lactose. Conversely, transcription of the *lacR* gene is similarly induced during growth on glucose (Van Rooijen *et al*., 1992). In addition, other sugar-inducible expression systems are also used for study of gene expression in *L. lactis*. For example, during the utilization of sucrose by *L. lactis*, two divergently transcribed operons are involved, *sacBK* and *sacAR*. The expression of *sac* genes is repressed by the product of the *sacR* gene exhibiting homology to the LacI/GalR family of bacterial regulators (Luesink *et al*., 1999). In the absence of sucrose, the SacR binds to the operator sites of the sucrose operon leading to the transcriptional repression. The observed substrate induction and negative autoregulation of *sacR* by its gene product result in efficient transcriptional control of the *sac* genes in response to variations in extracellular sucrose concentrations. A new controlled production system to target heterologous proteins to cytoplasm or extracellular medium was also studied in *L. lactis*. In *Lactococcus lactis* NCDO2118, Miyoshi *et al*. (2004) demonstrated that a xylose-inducible *nuc* expression is tightly controlled and resulted in high-level and long-term protein production, and correct targeting either to the cytoplasm or to the extracellular medium. Furthermore, this expression system is versatile and can be switched on or off easily by adding either xylose or glucose, respectively.

Generally, proteolysis in bacteria plays a central role in turnover, maturation, and regulation of proteins and in assimilation of extracellular proteins and peptides. The proteolytic system of lactococci is composed of an extracellular

proteinase, peptide transport systems, and intracellular peptidases. The regulation of the plasmid-encoded cell wall proteinase PrtP is one of the best known among the components of the proteolytic pathway in lactococci (Guédon *et al*., 2001). In several strains of *L. lactis*, the synthesis of the cell wall proteinase is reduced during growth in rich media compared with milk medium (Kok, 1996). Transcription regulation of the extracellular proteinase gene and the divergently transcribed genes required for proteinase production (*prtP* and *prtM*) of *L. lactis* SK11 has been investigated by Marugg *et al*. (1995; 1996). The results showed that a 10-fold repression of initiation of transcription was observed by adding a complex peptide mixture to the medium. A high-level production of *prtP*- or *prtM*- specific mRNAs was found in media with low peptide concentrations, while increasing of peptide concentrations resulted in an approximately eightfold decreasing in mRNA production. Furthermore, peptide-dependent regulation of *prt* was examined by adding specific peptides to the growth medium. Out of 12 di- and tripeptides tested, only leucylproline and prolylleucine repressed the transcription of the *prtP-gusA* fusion (Marugg *et al*., 1995). Moreover, a systematic study of the transcription of 16 genes involved in the proteolytic system of *L. lactis* has been reported by Guédon *et al*. (2001). The transcription of several genes was found to be regulated by the peptide supply. Among these peptides, five promoters are repressed by specific dipeptides. On the other hand, *pepP* transcription is regulated by the carbon source.

Amino acid biosynthesis results in a substantial energy demand and should be repressed when unnecessary. Conversely, in the absence of sufficient exogenous supply, biosynthesis should be rapidly induced and coordinated to achieve optimized growth of the cells (Chopin, 1993). The operons for three amino acid biosynthetic

pathways from *L. lactis* have been cloned, sequenced, and analyzed (Bardowski *et al*., 1992, Delorme *et al*., 1992; 1993, Godon *et al*., 1992; 1993). Trytophan biosynthesis operon of *L. lactis* IL1403 contains seven structural genes in the order *trpEGDCFBA* and is preceded by a leader region containing a putative transcription terminator (Bardowski *et al*., 1992). The transcription pattern of the *trp* operon of *L. lactis* and also three parameters controlling transcription have been described by Raya *et al*. (1998). These parameters were shown to differentially affect the amount of the transcripts, among which tryptophan depletion. Depletion in any amino acids increase transcription initiation about four-fold, and the amount of the *trp* transcript decreases abruptly upon entry of the cells into stationary phase. The branch chain amino acid (BCAA) pathway by which leucine, isoleucine, and valine are synthesized, has been widely studied in bacteria, fungi, and plants (Godon *et al*., 1992). In *L. lactis* subsp. *lactis*, the structural genes for BCAA synthesis are present in a single operon that also contains three additional genes, *leu-ilv-ald* operon (Goupil-feuillerat *et al*., 2000). The characterization of genes for the branched chain amino acids biosynthesis in *L. lactis* subsp. *lactis* NCDO2118 which is prototrophic strain, has been reported by Godon *et al*. (1992). The results showed that nine structural genes are clustered on a 12-kb DNA fragment in the order *leuABCD ilvDBNCA*. Upstream of these genes, the nucleotide sequence suggests the existence of regulation by transcriptional attenuation. In addition, *leu* genes from an auxotrophic dairy strain, IL1403, have also been cloned and sequenced (Godon *et al*., 1993). The results showed that the sequence is 99% homologous to the prototroph NCDO2118. Two nonsense mutations and two small deletions were found in the auxotroph sequence. Nevertheless, the *leu* genes from the auxotroph appear to be transcribed and regulated similarly to those from the

prototroph. The histidine operon of *L. lactis* NCDO2118 has the gene order *hisCGDBHAFIE* (Kok, 1996). Transciption of *L. lactis his* gene is controlled by the presence of histidine. The presence of histidine prevents the initiation of the transcription from the promoter upstream of the *his* operon. In addition, the transcription of the downstream genes may be controlled by transcription attenuation (Chopin, 1993). The organization of a cluster of *L. lactis* subsp. *lactis* NCDO2118 genes, encoding eight of the nine histidine biosynthesis enzymes as well as six other genes of unknown function has been reported by Delorme *et al*. (1992). Complementation studies in *B. subtilis* and *E. coli* indicated at least seven *his* genes (*hisC, -G, -D, -B, -A, -F* and *-IE*) present within an 11-kb region. In addition, the histidine requirement of *L. lactis* dairy strains compared with nondairy strains has been described by Delorme *et al*. (1993). The results showed that among 60 dairy strains tested, 56 required histidine, whereas only 1 of 11 nondairy strains had this requirement. Moreover, 10 of 56 auxotrophic strains were able to grow in the presence of histidinol (Hol^+), the immediate histidine precursor. This indicates that adaptation to milk often results in histidine auxotrophy.

Environmental factors and growth condition such as pH, and temperature also affect gene expression through transcription and translation efficiency or stability of mRNAs. The pH of the medium plays an important role in gene expression by *L. lactis*. Sanders *et al*. (1998) demonstrated the nucleotide sequence and functional analysis of two genes transcribed from a chloride-dependent chromosomal promoter of *L. lactis*, *gadC* and *gadB*, and found that the expression of *gadCB* in *L. lactis* in the presence of chloride was increased when the culture pH was allowed to decrease to low levels by omitting buffer from the medium. Similarly, the presence of 0.5M NaCl

could induce that expression more than 1000-fold (Sanders *et al*., 1997). In addition, a chromosomal *citM-citCDEFXG* operon of *L. lactis* CRL264 has been described by Martín *et al*. (2004). This operon contains the genes encoding the three subunits of the citrate lyase. The increase of *citM-citCDEFXG* operon expression as well as the citrate lyase activity was observed when cells were grown under acidic pH conditions. Recently, Raynaud *et al*. (2005) studied the metabolic and transcriptomic adaptation of *L. lactis* subsp. *lactis* biovar *diacetylactis* in response to autoacidification and temperature downshift in skim milk. The induction of gene involved in alternative metabolic pathways derived from some glycolytic metabolites was observed just upstream of the postulated glycolytic bottlenecks, as a consequence of accumulation of these metabolites. Other transcriptional responses to autoacidification and to a decrease in temperature were induced at the end of the growth phase and were partially maintained during the stationary phase.

2.4.2 Hierarchical control of carbohydrate utilization

Catabolic repression of operons is one of the important regulatory mechanisms observed for genes encoding enzymes that are involved in transport and metabolism of less preferred carbohydrates (Kowalczyk and Bardowski, 2007). It permits bacteria to select and utilize a carbon source in order to provide the fastest growth rate (Loll *et al*., 2007). The presence of a rapidly metabolizable carbon source in the growth medium of bacteria reduces the expression of genes involved in the utilization of other carbon sources. In Gram-positive bacteria, it has been established that carbon catabolite repression (CR) is mediated via a negative regulatory mechanism. Carbon catabolite control protein A (CcpA) is the main regulator involved in carbon catabolite repression and highly conserved in Gram-positive bacteria (Gaudu

et al., 2003 and Zomer *et al*., 2007). Recently, a comparison of the transcriptome data with putative CcpA binding site (*cre* site) in promoter sequences in the genome of *L. lactis* has been described by Zomer *et al*. (2007). The main differences in time-dependent expression of CcpA-regulated genes were observed between the exponential and transition growth phases. Large effects of carbon and nitrogen metabolic genes were obtained in the exponential phase. Effects on nucleotide metabolism genes were observed primarily in the transition phase. Furthermore, gel retardation experiment, northern blotting, and enzyme assays showed that CcpA is subject to autoregulation in *L. lactis*. In addition, Luesink *et al*. (1998) has described the *L. lactis ccpA* gene and also the effects of its disruption on the catabolite repression of the *galAMKTE* genes involved in galactose catabolism. The result showed that CcpA can act as an activator of transcription of the *las* operon, containing the *pfk*, *pyk* and *ldh* genes encoding the key glycolytic enzymes phosphofructokinase, pyruvate kinase and L-lactate dehydrogenase, respectively, involved in energy production and lactic acid formation by *L. lactis.* Moreover, the involvement of HPr in the catabolite repression of galactose metabolism has been described by Luesink *et al*. (1999). The expression of the *L. lactis ptsHI* operon is regulated at the transcriptional level in response to the carbon source, since transcription levels were at least 10-fold higher in glucose-grown cells than in galactose-grown cells.

2.5 Trancriptome analysis

2.5.1 Purpose of transcriptome analysis

Many cellular processes are regulated at the transcriptional level. For this reason the transcriptome of a cell, the total set of RNAs under a specific condition, contains information on the biological state of the cell and the genes that play a role under specific circumstances. Transcriptomics is the research method that studies the effect of specific conditions on alterations in the expression levels of complete sets of genes. A certain number of classical analyses allow the study of gene transcription. The technique of Northern blotting can be used to identify the tissues or stages wherein a gene is transcribed, and the size of the messenger (Bernot, 2001). In Northern blot analyses or multiple Northern blots, it is only possible to determine the expression of one or a few genes (David *et al*., 2005). The challenges of the global approach are being overcome with the development of omic tools, such as those provided by microarray technology, which can enable the analysis of thousands of genes in parallel by specific hybridization to a miniaturized, orderly array of DNA fragments. Microarrays contain grids of up to tens of thousands of array elements presented in a miniaturized format (Sensen, 2005). For DNA microarrays, those array elements or spots comprise minute amounts of DNA that have been either laid down robotically or synthesized *in situ* at precise locations on a solid support. These arrays are interrogated by allowing their immobilized sequences to hybridize by Watson-Crick base-pairing with labeled nucleic acids derived from the samples of study. The intensity of hybridization over individual spots is a measure of the amount of homologous sequence in the sample and an array can be made to cover the entire known expressed gene content of an organism. Unlike the genome, the transcriptome

is highly dynamic and changes rapidly and dramatically in response to perturbations or even during normal cellular events such as DNA replication and cell division (Lockhart and Winzeler, 2000). This has allowed intraspecies comparative genomics studies as well as functional genomics studies aimed at a better understanding of physiological processes and regulatory networks operating in lactococci. Kuipers *et al*. (2002) described the initial set-up of a DNA microarray to enable transcriptome analysis of various Gram-positive bacteria, including a ssp. *lactis* and a ssp. *cremoris* strain of *L. lactis*.

2.5.2 Application of transcriptome analysis in *Lactococci*

The availability of genomic information on lactococcal and other bacterial species has opened the way for a number of analytical and experimental approaches, which were impossible to perform without this data. Genome mining and comparison studies yield valuable information on the presence or absence of certain features among lactococci, as well as on evolutionary phenomena. Currently, their physiological features, which include substrate utilization, stress response, metabolic capabilities, population interaction, and probiotic properties (Zhu *et al*., 2009) and molecular biology, give a particular characterization to LAB, and as a result there are many applications in a broad range of studies. There are three major types of applications of transcriptome anlaysis in lactococci as follows:

2.5.2.1 Transcriptomics to study diversity and evolution

Members of the LAB group have close phylogenetic relationships largely due to their sharing relatively small, AT-rich genomes (~2.4 Mb) and common metabolic pathways (Ósullivan *et al*., 2009). Despite their phylogenetic closeness, the LAB occupy a diverse set of ecological niches including fermenting plants, milk,

wine, sour-dough, the human and animal GI tract and the oral cavities of vertebrates. Such niche diversity among closely-related species suggests considerable genetic adaptation during their evolution. Comparative genome hybridization (CGH) has been used to assess the plasticity of bacterial genome structures in closely related microorganism to deduce the evolutionary relatedness of LAB or to understand the genetic diversity (Van Hijum *et al*., 2008). Recently, an extensive whole-genome diversity analysis on 39 of *L. lactis* strains, isolated from dairy and plant sources has described by Siezen *et al*. (2010). Comparative genome hybridization analysis with multi-strain microarrays was used to assess presence or absence of genes and gene clusters in these strains, relative to all *L. lactis* sequences in public databases, whereby chromosomal and plasmid-encoded genes were computationally analyzed separately.

2.5.2.2 Transcriptomics to study adaptation, regulation and stress response

LAB is widely found in different environments where they have adapted to varying conditions, such as extreme pH and changing availability of nutrients. Functional analysis of gene expression using metabolic and transcriptomic could provide insight into adaptation for autoacidification, temperature downshift and also regulation mechanism of *L. lactis* subsp. *lactis* biovar *diacetylactis* strain in skim milk (Raynaud *et al*., 2005) as well as in *L. lactis* IL1403 (Redon *et al*., 2005) during carbon starvation. Moreover, transcriptomics analysis has given the possibility to look at gene regulatory networks that are operative in the organism under study. Guédon *et al*. (2002) reported potential regulators in *Lactococcus lactis* IL1403. Among these regulators, most could have a direct role as transcriptional regulators, while the others may have less well defined functions in transcriptional regulation or more general

functions, such as the GTP binding protein family. Moreover, many *L. lactis* regulators have functions that could not be proposed by transposition of the knowledge currently available in other bacteria.

2.5.2.3 Transcriptomics to study the analysis of cell-cell interaction

Concern about growth and survival of LAB in several complex ecosystems, including starter and non-starter cultures in fermented foods as well as the GI tract have prompted interaction with other LAB, the natural microbiota and sometimes spoilage bacteria within the fermented product (Siezen *et al*., 2004). While Nouaille *et al*. (2009) were investigating the transcriptomic response of a dairy strain of *L. lactis* in mixed culture with the food pathogen, *Staphylococcus aureus*, they observed an advanced decrease of the growth rate regulon in the earlier carbon limitation due to consumption of glucose by both species. In addition, Maligoy *et al*. (2008) performed extensive studies on the transcriptome analysis of *L. lactis* in co-culture with *Saccharomyces cerevisiae.* They revealed that the mRNA levels were significantly modified between the pure and the mixed cultures of *L. lactis*. These changes in transcript abundance were demonstrated to be regulated by the ethanol produced by the yeast.

CHAPTER III

MATERIALS AND METHODS

3.1 Microorganisms

The six strains of *L. lactis* subsp. *lactis* UCMA5713, LD55, LD61, LL08, LL5-2 and S86, isolated from milk have been selected for their genetic diversity in a national research program (ANR Ge´noferment). These strains were isolated from various origins (places and sources). These strains were kept and obtained from the Laboratory of Institut National des Sciences Appliquées, Toulouse, France. These strains were maintained on CDM medium supplemented with 20% glycerol and were stored at -80 ^{o}C until used.

3.2 Materials, Media and Chemicals

3.2.1 Materials

3.2.1.1 *L. lactis* IL-1403-specific PCR product was provided by Eurogentec and spotted in duplicate on positively charged nylon membrane (Roche) by the biochips platform (Four deposits per spot; Toulouse Genopole, France). A total of 1,948 Open Reading Frames (ORFs) identified in the genome were effectively available on these membranes (Bolotin *et al*., 2001).

3.2.1.2 Primers used for real-time PCR were purchased from Eurogen-tec as shown in Table C1 (Appendix C).

3.2.2 Microbiological media

The main media of microbiological preparation, CDM medium, are given in Appendix A as described by Otto *et al*. (1983) and modified by Poolman and Konings (1988) as well as MS13R preparation was shown in the Appendix A as described by Novak *et al*. (1997). M17 broth was purchased from Difco and was kept at 4 ^{o}C until used.

3.2.3 Chemicals

3.2.3.1 Chemicals for CDM preparation

Glucose, sodium acetate, KH_2PO_4, K_2HPO_4, $MgCl_2.6H_2O$, $FeSO_2.7H_2O$, $CoCl_2$. $6H_2O$ were purchased from Prolabo. Ammonium citrate was purchased from Sigma. $CaCl_2$. $2H_2O$ was purchased from Panreac. $ZnSO_4.7H_2O$ was purchased from Carlo erba. For the amino acids such as alanine, asparagines, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophane, valine were purchased from Sigma whereas arginine was purchased from Fluka. Phenylalanine was purchased from Fluka whereas tyrosine was purchased from sigma. For all bases includes adenine, guanine, urcile, xanthine, inosine, and thymidine were purchased from Sigma as well as all vitamins such as P-aminobenzoic acid, biotine, cyano-cobalamine (B12), folic acid, nicotinic acid, orotic acid, calcium-pantothenate, pyridoxamine, pyridoxine, riboflavine, thiamine, DL-6,8-thioctic acid were obtained from Sigma. Cysteine was purchased from Fluka.

3.2.3.2 Chemicals for chromosomal DNA extraction

Tris-(hydroxymethyl) aminomethane (Tris) was purchased from Prolabo. Sucrose was purchased from Merck. Proteinase K was purchased from Euromedex. Lysozyme was purchased from Sigma. Rnase A was purchased from Qiagen.

3.2.3.3 Chemicals for transcriptome analysis (membrane)

Superscript II Rnase H^- reverse transcriptase, dNTP, randome primer, RnaseH were purchased from Invitrogen Life Technologies. dCTP [α-P^{33}] was purchased from Perkin whereas specific primer was purchased from Eurogentec. Microspin G25 columns was purchased from Amersham Biosciences. $NaH_2PO_4.2H_2O$, $Na_2HPO_4.12H_2O$, sodium chloride, EDTA, and Tris were purchased from Prolabo whereas Ficoll 400 and 20% SDS were purchased from Eurobio. Polyvinylpyrrolidone and BSA were purchased from Sigma.

3.3 Instrumentation

Instruments required for analysis the biodiversity of *Lactococcus lactis* strains by transcriptomic approach such as the cultivation of microorganisms in different media, genomic DNA extraction, cDNA preparation, reverse transcription and labeling were mainly located at the Laboratoire Biotechnologie Bioprocédés, Institut National des Sciences Appliquées, Toulouse, France. Some instruments required for assessment of the quantity and purity of the total RNA, assessing of the quantity of DNA, detection of the hybridization signals located at Instrument Buildings of Plateforme Génomique, Toulouse, France.

3.4 Cultivation of the strains in UF-cheese model

Strains were cultured on skimmed raw milk ultrafiltration (UF) retentate. The UF retentate was pre-incubated overnight at 4 ^{o}C, then 45 minutes at 50 ^{o}C and homogenized during 1.5 minutes at 24,000 rpm with an ultra-turrax (Imlab, France). After addition of rennet (0.3 µl ml^{-1}), 400 g of UF retentate was inoculated at 2 x 10^{6} CFU/g with *L. lactis* subsp. *lactis* strains. After incubation for 8 hours at 30 ^{o}C, the cheeses were transferred at 12 ^{o}C during 7 days for simulation of ripening. Their ability to grow and acidify was compared all along the cheese repining.

3.5 Cultivation of the microorganisms

3.5.1 Cell pre-culture

The six strains of *Lactococcus lactis* subsp. *lactis* (0.3 ml from cryotube) were inoculated into six bottle containing 10 ml of the Chemical Defined Medium (CDM) medium, described by Otto *et al* (1983) and modified by Poolman & Konings (1988), and then incubated overnight at 30 ^{o}C under a nitrogen atmosphere with an agitation speed of 250 rpm and used as first pre-culture. The cultured broth was then measured for optical density at 600 nm to obtain an initial OD_{600} of 0.1 as described in the formular (Appendix B). Then, it was transferred to the test tube containing 10 ml of CDM. Growth of the tested microorganisms was measured by spectrophotometer at the wavelength of 600 nm and subsequently used as an inoculum.

3.5.2 Cell cultivation

After growth cell entered to the logarithmic phase, the suspensions were then inoculated to test tubes under controlled gas environment by flushing with nitrogen. These tubes containing 10 ml of CDM and then incubated without shaking at

the same condition as described above. These tubes were prepared in duplicate. Bacterial growth was estimated by spectrophotometer at 600 nm. Afterwards, cultures were measured for pH and cells were collected by centrifugation at 5,000 rpm for 10 min. Supernatant and cell pellet were kept at -20 ^{o}C for further study.

3.6 Measurement of pH

All pH measurements were determined using a Metrohohm pH meter which was calibrated prior to use by using pH 4.0 and 7.0 buffers.

3.7 Growth ability of the microorganism in different media

The six strains of *Lactococcus lactis* subsp. *lactis* were grown in different media. These media included M13 and M17.

3.7.1 Cultivation of the strains in the M13 medium

3.7.1.1 Cell pre-culture

These strains from the cryotube (0.3 ml) were inoculated into the anaerobic test tubes which flushing with nitrogen gas, containing 20 ml of CDM medium supplemented with 5% glucose. The control contained no strain. They were incubated in the rotary shaker under the condition as described above for overnight. The cultured broth was then measured for optical density at 600 nm until an initial OD_{600} of 0.1 was obtained and subsequently used as an inoculum.

3.7.1.2 Cell cultivation

Five milliliters of culture broth were added to the 15 ml of Falcon tube, and then the cells were harvested by centrifugation at 5,000 rpm for 10 min at 4 ^{o}C. The cell pellet was washed with a 0.9% NaCl solution twice and collected cell by re-centrifugation in the same condition. The pellets were then re-suspended in 5 ml of NaCl, and mixed well with pipette. The cell suspensions were subsequently

transferred to 10 ml of M13R medium. These tubes were prepared in duplicate. The growth culture was measured with spectrophotometer at the wavelength of 600 nm. Cell suspension was also measured for pH and harvested by centrifugation at the same condition as described above. The supernatant and the cell pellet were kept at -20 ^{o}C for further study.

3.7.2 Cultivation of the strains in the M17 medium

3.7.2.1 Cell pre-culture

The strains (0.3ml) from cryo-tube were inoculated into six bottle containing 20 ml of M17 medium supplemented with 1% of glucose under controlled gas environment by flushing with nitrogen gas. These bottles were incubated in a rotary shaker, 250 rpm, at 30 ^{o}C overnight. They were then measured by spectrophotometer at the wavelength of 600 nm to obtain an initial OD_{600} of 0.1 as described in the formular (Appendix B). Then, the cultured broth was inoculated into six bottle containing 25 ml of M17 medium supplemented with 1% glucose under the same control condition as described above. They were incubated in a rotary shaker, 250 rpm, at 30 ^{o}C. The growth culture was measured by spectrophotometer at the wavelength of 600 nm until an initial OD_{600} of 0.1 was obtained and subsequently used as an inoculum.

3.7.2.2 Cell cultivation

The culture broth was inoculated into test tube containing of 10 ml of the same medium containing 1% of glucose. These tubes were prepared in duplicate and they were incubated at 30 ^{o}C without shaking. The growth was measured by spectrophotometer at the wavelength of 600 nm. Cell suspension was measured for pH and harvested by centrifugation at the same condition as described above. In

addition, the culture broth was also inoculated in bottle containing 50 ml of the same medium supplemented with 1% glucose (3 bottles/ strain)**.** The bottles were incubated and the cultures were measured for growth at the same condition as mentioned above. Then, the cells of the tested microorganism were harvested by centrifugation at 5,000 rpm at 4 ^{o}C for 10 min. The supernatant and the cell pellet were kept at -20 ^{o}C for further study.

3.8 Transcriptomic analysis of *Lactococcus lactis* subsp. *lactis* strains

3.8.1 RNA extraction

Total RNA was extracted from cells grown for 24 h in UF-cheese (Ulve *et al*., 2008).

3.8.2 Assessing the quantity and purity of total RNA

RNA was spectophotometrically quantified (at 260 and 280 nm) by using the NanoDrop (ND1000) spectrophotometer and the integrity of total RNA was assessed on Agilent 2100 bioanalyzer (Nanopuce).

3.8.3 cDNA preparation

cDNA was synthesized from RNA in the mixture containing 10 µg of total RNA and mixed with 1 µl of random hexamer primers (500 ng/µl), and 1 µl of *L. lactis* specific primers (500 ng/µl). Then, the sterile water was added to a final volume of 24 µl. The mixture was incubated at 70 ^{o}C for 5 min in the water bath and then cooled down rapidly on ice. Reverse transcription was performed for 1 h at 42 ^{o}C with 1.5 µl of SuperScriptII reverse transcriptase (200 U/µl), 5 µl of dithiothreitol (0.1M), 1.5 µl of each dATP, dGTP, dTTP (10 mM), 5 µl of [α-^{33} P]-dCTP (10µCi/µl) and 10 µl of 5x first strand buffer. After one hour, 0.5 µl of unlabeled dCTP (10mM) and 0.5 µl of SuperScriptII were added and then the reaction was carried out for one more

hour at 42 ^{o}C. The reaction was stopped by heating at 70 ^{o}C for 15 min and the remaining of the RNA was hydrolyzed by RNaseH (2 U) at 37 ^{o} C for 20 min and then stored in ice until cDNAs purification. Before hybridization, labeled cDNA was purified by using Microspin G25 columns (Amersham Biosciences) according to the manufacturer's instructions.

3.8.4 Hybridization and detection

Prior to hybridization, membranes were washed for 5 min at room temperature in 50 ml of 2X SSPE (1X SSPE; 0.18 mM NaCl, 1 mM EDTA and 10 mM phosphate buffer, pH 7.7) and prehybridized for 2 to 4 h at 68 ^{o}C in 5 ml of hybridization buffer (5X SSPE, 2% SDS, and 1X Denhardt's reagent). Labeled cDNA was heated for 10 min at 95 ^{o}C and then cooled down rapidly to 4 ^{o}C for 5 min. The whole sample was collected by centrifugation. Afterwards, membrane hybridization was carried out for 14 to 16 h at 68 ^{o}C with 5 ml of hybridization buffer containing labeled and denatured cDNA. Membranes were washed three times with 50 ml of washing solution (0.5X SSPE and 0.2% SDS) for 5 min at room temperature and three times with 50 ml of preheated washing solution for 20 min at 68 ^{o}C. After hybridization, dried membranes was exposed to a phosphorimaging screen for 3 days and scanned with a phosphofluoroimager (Storm scanner; Amersham Biosciences).

3.9 Comparative genomic hybridization (CGH)

3.9.1 Genomic DNA extraction

Pellet of the six strains were resuspended with TE buffer (Tris 10 mM, EDTA 10 mM), whereas two hundred microliters of an aliquote of *L. lactis* subsp. *lactis* IL1403 were inoculated in M17 medium supplement with 1% glucose and used as reference strain. The cultured broth of IL1403 strain was incubated at 37.5 ^{o}C,

overnight. Two milliliters of the suspension were pipetted into the eppendrof tube (3 repetition/strain) as well as four milliliters of IL1403 suspension were taken to eppendrof tube. Afterwards, cells were collected by centrifugation at 14,000 rpm for 3 min. The pellet was then suspended in 250 µl of Solution C1(set-lysozyme-RNase: 20% Saccharose, 50 mM Tris, pH7.5, 5 mM EDTA, 1 mg/mL of Lysozyme and 10 Unit/mL of RNase) and incubated in the water bath at 37 ^{o}C for 1.5 h. The cell suspensions were subsequently suspended with solution C2 (Tris-EDTA-SDS: 10 mM Tris, pH 8, 5 mM EDTA, 1% SDS) and then vigorously mixed. One hundred microliters of Proteinase K (concentration 4 mg/mL of TE) were added to the suspension and incubated in the water bath at 55 ^{o}C for overnight. Phenol/chloroform/isoamyl alcohol (500 µl), pH 7.8 were added to the suspension and then vigorously mixed by hand and vortex for 1 min. Afterwards, the cell suspensions were harvested by centrifugation at 14,000 rpm for 10 min. The aqueous phase at the top was transfered to the new eppendrof and then re-extract with 500 µl of phenol/chloroform/isoamyl alcohol for 2 to 3 times. Furthermore, five hundred microliters of isopropanol were added and collected by centrifugation again at the same condition as described above for 3 min at room temperature. The pellet was suspended in 150 µl of LiCl (0.4M) and then mixed with pipette. The cell pellet was subsequently added with ethyl alcohol (70%) and harvested by centrifugation at 14,000 rpm for 3 min at 4 ^{o}C. Pellet was mixed with 100 µl of TE buffer, and quantified the DNA by loading on the gel agarose.

3.9.2 Genomic DNA fragmentation

Eighty micrograms per milliliter of genomic DNA was diluted with sterile water in the eppendrof. The diluted sample DNA was sonicated at 20 units

speed for 5 min. The sonicate probe was dipped into the DNA solution for a minute and then left for 5 min. DNA solution was stored on ice during the time of operation. This method was performed to obtain 500 bp genomic DNA in length in all strains. The size of fragmented genomic DNA was confirmed by 1.5% of agarose gel electrophoresis.

3.9.3 Assessing the quantity of DNA

DNA was quantified by using the NanoDrop (ND1000) spectrophotometer at 260 and 280 nm.

3.9.4 DNA labeling

Two micrograms of diluted DNA were pipetted into 31 µl of sterile water in microtube. They were heated in the boiling water for 5 min and then were rapidly placed on ice at 4 ^{o}C for 5 min. The suspension was collected as briefly at 4 ^{o}C. Reaction was performed by adding 1 µl of random primers (500ng/ µl), 1 µl of specific primers of *L. lactis* (500ng/ µl), 5 µl of Ecopol buffer, 1.5 µl of each dATP, dTTP and dGTP (1mM), 5 µl of [α^{33}P] dCTP and 1 µl of Klenow fragment of DNA polymerase I. The reaction was incubated at 37 ^{o}C for 1 h. The reaction was stopped by putting on ice and spinned the sample at 3,800 rpm for 2 min. Afterwards, the labeled DNA was purified by using Microspin G25 columns (Amersham Biosciences) according to the manufacturer's instructions.

3.9.5 Hybridization and detection

Prior to hybridization, membranes was washed for 5 min at room temperature in 50 ml of 2X SSPE (1X SSPE; 0.18 mM NaCl, 1 mM EDTA and 10 mM phosphate buffer, pH 7.7) and prehybridized for 2 to 4 h at 68 ^{o}C in 5 ml of hybridization buffer (5X SSPE, 2% SDS, and 1X Denhardt's reagent). Labeled cDNA

was heated for 10 min at 95 ^{o}C and then cooled down rapidly to 4 ^{o}C for 5 min. The whole sample was collected by centrifugation. Afterwards, membrane hybridization was carried out for 14 to 16 h at 68 ^{o}C with 5 ml of hybridization buffer containing labeled and denatured cDNA. Membranes were washed three times in 50 ml of washing solution (0.5X SSPE and 0.2% SDS) for 5 min at room temperature and three times in 50 ml of preheated washing solution for 20 min at 68 ^{o}C. After hybridization, dried membranes was exposed to a phosphorimaging screen for 3 days and scanned with a phosphofluoroimager (Storm scanner; Amersham Biosciences).

3.10 Data analysis

Hybridization signals were quantified and assigned to gene names with the Bioplot software developed by Sokol, S. in Plateforme Génomique, Toulouse. Three individual repetitions were made for experiment. Local background was removed from all spot intensities.

For transcriptomic experiments, signals were normalized by the mean intensity of the corresponding membrane. Expression ratios were calculated between the strain of interest and strain LD61 which was considered as the reference for expression studies in milk (Raynaud *et al.*, 2005). In order to be compared, transcriptomic analysis were performed in parallel for all the strains in a same series and repeated three times independently. The statistical significance of expression ratios was evaluated using False Discovery Rate (FDR) calculations and a statistical threshold of 7%. In order to determine expression changes at the level of the functional (sub)categories (global tendencies), over- and under-expressed gene enrichments in the different groups were calculated with the Wilcoxon test. This test was performed without any *a priori* selection of expression data and we have considered a significant

p-value of 5% (Dressaire *et al*., 2008). The classification of Bolotin and co-worker was used (Bolotin *et al*., 2001).

For CGH experiments, genes were declared as absent or highly divergent if they were detected as absent by all the three statistical methods (a, b and c). In the statistical method a, genes corresponding to spot intensities less than a cut-off value in at least two repetitions out of three were declared as absent. For each membrane, a cut-off value was defined as the detection limit plus two times its standard deviation. The detection limit of each membrane was calculated as the mean intensity of the 178 empty spots (spots containing no probe). The b and c statistical treatments were performed on whole detected spots without any previous selection. In this case the ratios of the signal intensities were calculated between the studied strain and the sequenced reference strain, IL1403 (Bolotin *et al*., 2001), after subtraction of the local background and signal normalisation. Two normalisation methods were used: either by the mean intensity of the corresponding membrane (method b) or by the intensities of a subset of conserved genes with minimal sequence divergence between the samples analyzed (method c) (Van Hijum *et al*., 2008). We chose the subset of 57 conserved genes previously identified for *L. lactis* strains by van Hijum and co-workers using three *Streptococcus* strains as reporter genomes, and we removed four genes (*atpA*, *rplW*, *rpmC* and *ychH*) from this subset because they were missing on our membranes. Absent genes were those corresponding to ratios of normalized intensities (strain of interest compared with IL1403) lower than 0.5. This threshold of 0.5 was chosen because it was previously used in CGH data analyses (Fukiya *et al*., 2004; Taibi *et al*., 2010).

Genes were declared to be divergent when the ratios of their normalized intensities (compared to IL1403) by method c were lower than 0.76. The 0.76 criterion was chosen after estimation of the average technical variation of the signal ratios using three independent repetitions of each tested strain. However, it should be kept in mind that the number of divergent genes could be biased, since low CGH ratios could also result from artifacts (cross-hybridization with another gene in the genome, small GC-rich region).

ORFs detected as absent with all statistical approaches (low spot intensity and low intensities ratios with both normalisations) were subjected to PCR-amplification using the genomic DNA preparation already used for CGH experiments. Primers were those previously designed by Eurogentec. *Taq* polymerase (NewEngland BioLabs) and Phusion polymerase (Finnzymes) were used, and the hybridization temperature was ranging between 42 and 55 ^{o}C.

Clustering analysis was performed with the R free statistical software (http://www.r-project.org/) to find the clusters of genes that have similar expression profiles compared with the reference strains.

3.11 Real time PCR

Samples of 10 µg of total mRNA were retrotranscribed by using 2 µl of random primer, each deoxynucleoside triphosphate (0.3 mM), and superscript II transcriptase (6U· µl^{-1}; Invitrogen) (Maligoy *et al.*, 2008) and incubated for 1 h at 42 ^{o}C. The reaction was stopped by incubation for 15 min at 70 ^{o}C. RNaseH (0.05U; Invitrogen) was added before purification of cDNA by Microspin G25 columns (Amersham Biosciences). Primers for real-time PCR (Appendix C) were designed with Bio-Rad Beacon Designer software to have lengths of 20 to 25 bases with GC contents of more

than 50%, and melting temperatures of about 60 °C. The primer used for amplifying the PCR products of 88 to 145 bases long. The specificities of the primers for the genes of interest were controlled by using the *L. lactis* IL1403 genome with Vector NTI software (Invitrogen). Real-time PCR was carried out on a MyIQ unit with Sybr green supermix (BioRad). After cDNA was diluted, 5 µl was added to 20 µl of PCR mixture (12.5 µl Sybr green supermix, 4 µl of primer at 10 µM, and 3.5 µl of RNase-free water). Three or four dilutions of cDNA were performed to determine the efficiencies of real-time PCR. A negative control was replaced by water. Thermal cycling conditions were denaturation at 95 ^{o}C for 3 min and 40 repeats of 95 ^{o}C for 15 s and 60 ^{o}C for 45 s and annealing step where fluorescence measurements were recorded. The *pbp2A* and *smc* genes were chosen as internal normalization controls, since they did not show significant expression variation in these experiments. The Pfaffl analysis method (Pfaffl, 2001) was used to calculate the change in transcript levels between strains UCMA5713, LD55, LL08, LL52, and S86 and the reference strain LD61. Four genes *(yaiA*, *feoA*, *ipd* and *pyrR*, coding for hypothetical protein, ferrous ion transport protein, indole-3-pyruvate decarboxylase and bifunctional pyrimidine regulatory protein PyrR uracil phosphoribosyltransferase, respectively) were tested, and three independent measurements were performed for each genes between the interesting strain and the reference strain (LD61). For direct comparison with transcriptomic data, quantitative reverse transcription-PCR results were expressed as ratios in transcript concentrations between the strain of interest and the reference strain, corrected by using the *pbp2A* or *smc* normalization ratios.

3.12 Physiological characteristics analysis of *L. lactis* subsp. *lactis*

3.12.1 Cultivation of microorganisms

3.12.1.1 Cell pre-culture

The six strains of *Lactococcus lactis* subsp. *lactis* (0.15 ml) from stock solution in the cryotube were inoculated in bottle containing 10 ml of CDM in anaerobic conditions under the nitrogen atmosphere and were incubated in a rotary shaker, 250 rpm, at 30 ^{o}C overnight. The growth culture was measured by spectrophotometer at the wavelength of 600 nm until the initial OD_{600} of 0.1 was obtained. Then, it was transferred to the anaerobic tubes containing 10 ml of CDM under the nitrogen atmosphere. The culture was incubated at the temperature of 30 ^{o}C with agitation speed of 250 rpm overnight. Growth of the tested microorganism was measured by spectrophotometer at the wavelength of 600 nm and subsequently used as an inoculum.

3.12.1.2 Cell cultivation

The suspensions were inoculated to test tubes which controlled gas environment by flushing with nitrogen gas. These tubes containing 10 ml of CDM and then incubated without shaking at the same condition as described above. These tubes were carried out in duplicate. The growth culture was measured by spectrophotometer at the wavelength of 600 nm. Afterwards, cells were measured for pH and collected by centrifugation at 5,000 rpm for 10 min. Supernatant and cell pellet were kept at -20 ^{o}C.

3.12.2 Test for purine biosynthesis

The six strains of *L. lactis* subsp. *lactis* were determined for growth ability in CDM medium under the different conditions as following; without purine

base or without pyrimidine base or without both nitrogenous bases. The control contained only bases in the medium. Afterwards, the cell culture was incubated at the same condition as described above. The culture was carried out in duplicate. The growth culture was measured by spectrophotometer at the wavelength of 600 nm. Cells were measured for pH and collected by centrifugation at 5,000 rpm for 10 min. Supernatant and cell pellet were kept at -20 ^{o}C.

3.12.3 Test for branch chain amino acid (BCAA) requirement

L. lactis subsp. *Lactis* for the six strains were determined for amino acid requirement by growing the strains in the CDM medium under various conditions as following; without isoleucine, without leucine, without valine, and without all BCAA. The control contained only branch chain amino acids. The culture was carried out in duplicate. The growth culture was measured by spectrophotometer at the wavelength of 600 nm. Cells were measured for pH and collected by centrifugation at 5,000 rpm for 10 min. Supernatant and cell pellet were kept at -20 ^{o}C.

CHAPTER IV

RESULTS

4.1 Phenotypic analysis of the strains in different media

Six strains of *L. lactis* subsp. *lactis*, namely UCMA5713, LD55, LD61, LL08, LL52 and S86 were selected from the Génoferment culture collection to assess the intra-subspecies diversity at the phenotypic levels. Five strains (LD55, LD61, LL08, LL52 and S86) were typically found in dairy fermentations but one, strain UCMA5713 was a plant-associated strain isolated from the French Normand meadow (Table 4.1). Strain LD61 is the reference strain usually used in dairy industry.

Table 4.1 Strain origins, genomic characteristics and taxonomy.

Strains	Origins	Eco R1 Ribotype	Chromosome size (Mb)	Biovar *diacetylactis*[1]
S86	Starter (France)	1797-S-1	2.482 ± 35	No
LD55	Camembert (Normandy, France)	1797-S-4	2.595 ± 93	No
LD61	Soft cheese starter (France)	58-S-6	2.579 ± 59	Yes
LL08	Raw milk (Normandy, France)	199-S-7	2.545 ± 33	No
UCMA5713	Grassland (Normandy, France)	1797-S-1	2.362 ± 14	No
LL52	Soft cheese stater (France)	199-S-7	2.677 ± 27	No

[1]: results from Passerini *et al*. (2010) who revisited the definition of the biovar *diacetylactis* and positioned the six strains in this taxonomy.

4.1.1 Phenotypic analysis of the strains in UF-cheese model

The present study was performed to see ability of these strains to grow and acidify in UF-cheese model. Strains were cultured on skimmed raw milk ultrafiltration (UF) retentate. After pre-incubated overnight at 4 ^{o}C, then 45 minutes at 50 ^{o}C, the cultures were homogenized during 1.5 minutes at 24,000 rpm with an ultra-turrax (Imlab, France). The homogenized-cultures were subsequently added with rennet, then UF retentate was inoculated at 2 x 10^6 CFU/g with *L. lactis* subsp. *lactis* strains. After incubation for 8 hours at 30 ^{o}C, the cheeses were transferred at 12 ^{o}C during 7 days for simulation of ripening as described above. Their ability to grow and acidify this medium was compared all along with the cheese repining (Figure 4.1).

Results shown in Figure 4.1 revealed that the cell population profiles were similar for all strains, leading to a constant value ranging between 1.9 and 2.3 x 10^9 CFU/g of cheese at the end of the cultivation (after 7 days). The growth rate, calculated from the growth phase between UF-cheese inoculation and 8 hours, confirmed the large growth similarities for all the six strains with values ranging between 0.78 and 0.88 h^{-1} (data not shown). However, acidification properties of the six strains differed in UF-cheese model, with end pH values between 4.58 (UCMA5713) and 4.85 (LD61). This end pH variation was considered to be significant in dairy industry for cheese quality.

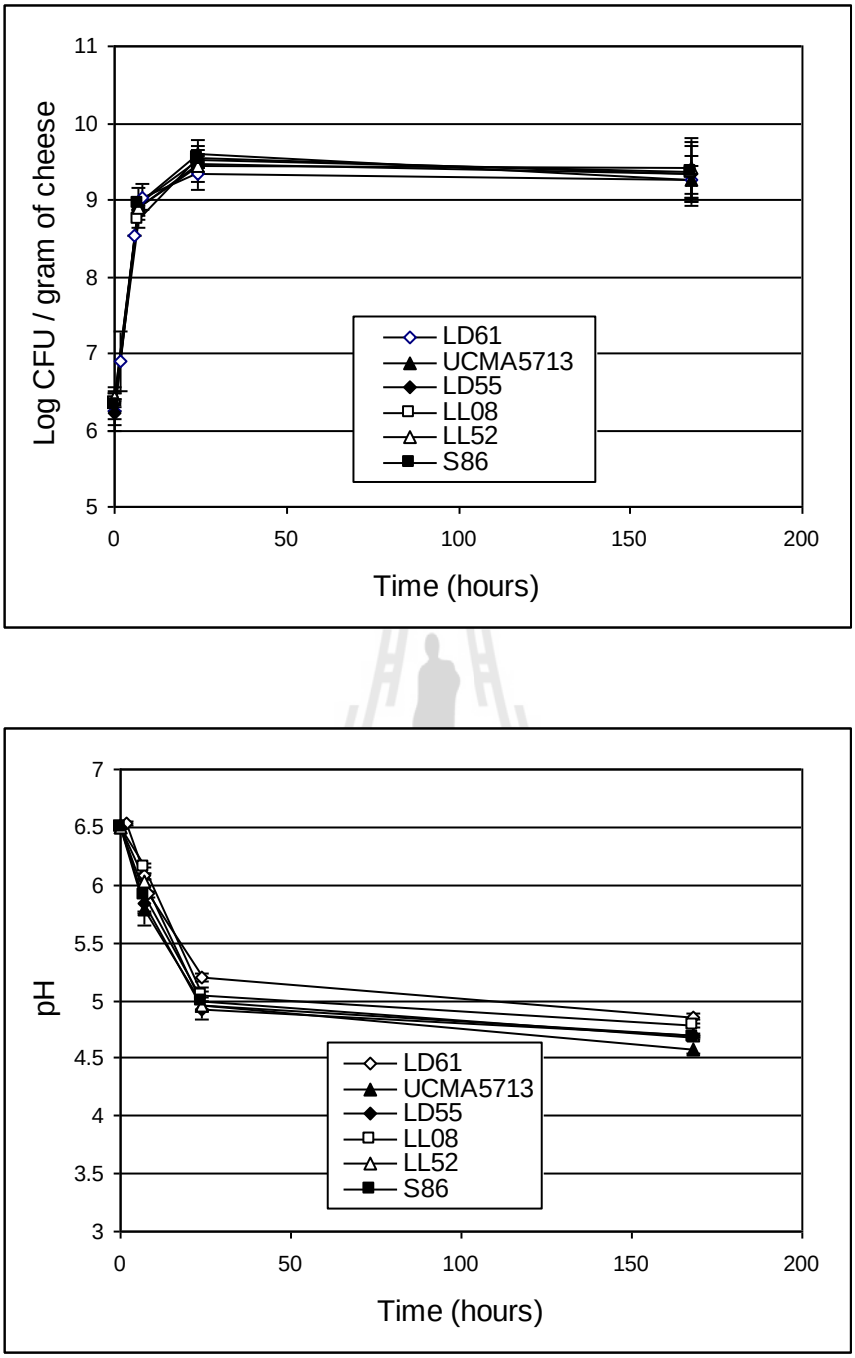

Figure 4.1 Growth of the six *L. lactis* strains and pH profile during UF-cheese ripening (average of at least three independent experiments with standard deviation).

4.1.2 Phenotypic analysis of the six strains in synthetic CDM medium

The further study was carried out by using other sugar, like glucose as carbon source on media commonly used in laboratories, *i.e.* the synthetic CDM medium. The CDM was a synthetic medium that contained 18 amino acids, 12 vitamins and 6 bases. Cultures were cultivated in anaerobic tubes at 30 ^{o}C under a nitrogen atmosphere and agitation speed of 250 rpm. The growth of strains was examined by measuring the optical density of the incubated culture broth at 600 nm. The final pH values were measured after 24 hours of growth and the results of these studies were shown in Table 4.2. Results showed that the strains exhibited the similarity of final cell population in all strains with a values ranging between 1.21 and 1.83 x 10^{9} CFU/g after 24 hours of growth on CDM. However, end pH value was around 4.5 in all five strains except in the LL52 strain which reached the low final pH value of 4.12 on CDM medium.

Table 4.2 Final cell population and pH for the six strains after 24 hours of growth on CDM medium.

Strains	Final cell population ($x10^{9}$ CFU/g)	Final pH
UCMA5713	1.64 ± 0.01	4.54 ± 0.01
LD55	1.57 ± 0.10	4.57 ± 0.01
LD61	1.83 ± 0.27	4.46 ± 0.00
LL08	1.61 ± 0.01	4.57 ± 0.01

Table 4.2 (Continued).

Strains	Final cell population ($x10^9$ CFU/g)	Final pH
LL52	1.21 ± 0.00	4.12 ± 0.01
S86	1.57 ± 0.06	4.55 ± 0.01

4.1.3 Phenotypic analysis of the six strains on M17 medium

After the study of growth ability on CDM medium, M17 medium, which was the complex medium for isolating and enumerating lactic streptococci in yogurt, cheese starters and other dairy products, was used to determine the growth ability with glucose as carbon source. The cultures of the tested strains were cultivated in anaerobic tubes at 30 ^{o}C under a nitrogen atmosphere and agitation speed of 250 rpm as described above. Afterwards, the growth of strains was examined by measuring the optical density of the incubated culture broth at 600 nm. The final pH values were measured after 24 hours of growth. The results of these studies were shown in Table 4.3. Results showed that the final cell population was very similar for all the strains, leading to a constant value ranging between 2.18 and 2.50 x 10^9 CFU/g after 24 hours of growth on M17 medium. In addition, end pH value was observed to be around 4.5, more precisely at 4.54, in all six strains.

Table 4.3 Final cell population and pH for the six strains after 24 hours of growth on M17 medium.

Strains	Final cell population ($x10^9$ CFU/g)	Final pH
UCMA5713	2.18 ± 0.05	4.53 ± 0.02
LD55	2.35 ± 0.01	4.55 ± 0.01
LD61	2.39 ± 0.01	4.51 ± 0.00
LL08	2.38 ± 0.02	4.57 ± 0.02
LL52	2.25 ± 0.04	4.55 ± 0.00
S86	2.50 ± 0.04	4.53 ± 0.01

4.1.4 Phenotypic analysis of the six strains on M13 medium

The further study was carried out by using M13 medium which differed from CDM medium by lacking 12 amino acids, 7 vitamins and all bases. Cultures were cultivated in anaerobic tubes at 30 ^{o}C under a nitrogen atmosphere and agitation speed of 250 rpm. The growth of strains was examined by measuring the optical density of the incubated culture broth at 600 nm. The final pH values were measured after 24 hours of growth. However, results showed that none of the six strains were able to grow on M13 medium.

4.2 Comparative hybridization analysis

Array-based comparative genome hybridization (CGH) was used to determine the core genome (similar genomic content) of the six strains of *L. lactis* subsp. *lactis* exhibiting a dairy phenotype. We have also included in these experiments the genome of the sequenced strain *L. lactis* subsp. *lactis* IL1403, which was used for microarray platform design. Genomic DNA in each strain was extracted from cells grown overnight on M17, then it was sonicated and labelled by random priming at the conditions as described above. Afterwards, the purified labeled DNA was hybridized on nylon membrane containing the PCR fragments of *L. lactis* IL1403. Membranes were exposed to a phosphoimager screen for three days and scanned with a phosphofluoroimager. Signal intensities were quantified, tested statistically, and assigned to gene names with the Bioplot software. Local background was removed from all spot intensities. Genes were declared as absence or highly divergence if they were detected as absence by all the three statistical methods (a, b and c) as described in Materials and Methods Section. Using the IL1403 strain, we identified 19 genes with absolute intensities lower than the cut-off value and thus declared as absence using the statistical method a (see Material and Methods). These genes were thus tagged as missing on the microarray platform and omitted in the subsequent analyses. Therefore, the 1,929 genes were analyzed in the CGH experiments. No control was necessary to identify false positive genes (genes declared as presence while they are absent) was included in the CGH experiment.

In order to analyse genomic divergences between the strains, ratios of intensities were calculated between the tested strain and the IL1403 strain as indicated in Materials and Methods (normalization method c using likely conserved genes).

These ratios, given in Appendix D, were plotted as a function of gene position on the IL1403 strain chromosome (Figure 4.2). Results in Figure 4.2 showed that only a few genes had low ratios (no more than 10% of the genes exhibited ratios lower than 0.76 and was thus declared as divergence), indicating strong similarities between the sequences of the six strains. However, one can observe that the sequence of the reference LD61 strain was very close to the sequence of the IL1403 strain, while the other five strains shared diversity in the same regions. Seven diversity regions were detected: i) regions 2, 3, 5, 6 containing phage-related genes *(i.e. pi1*, *pi2*, *pi3* and *ps3*) were visually enriched in low ratios in these five strains (S86, LD55, LL08, LL52, UCMA5713), indicating that these transposable elements could be a source of sequence divergence between *L. lactis* subsp. *lactis* strains; and ii) regions 1 and 7 containing in particular genes related to the cell envelope (*ycbBDFHIJ* genes in locus 1 and *pspB* in locus 7) and region 4 including genes involved in citrate and malate metabolism (*citRCDEF* and *mae* genes, respectively). These divergent regions could include absent or highly divergent genes but also present genes with low genomic divergence.

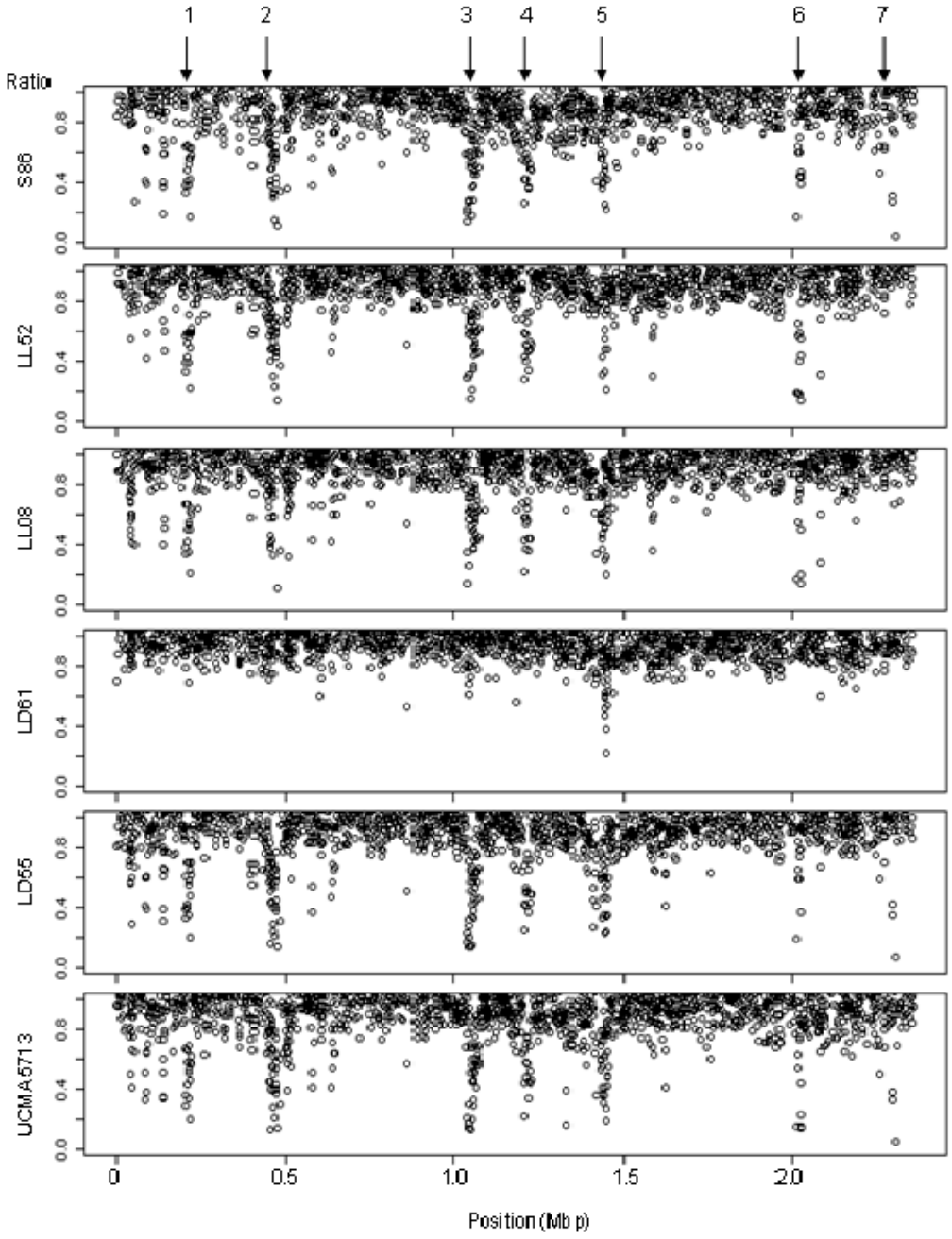

Figure 4.2 CGH results normalized using likely conserved genes and expressed as ratios of the signal intensities of the studied strains against the reference IL1403 strain (statistical method c, Table S1). Ratios lower than 1 in the six strains were plotted as a function of gene position on the IL1403 strain

chromosome. Locus 1: position ~ 0.20 Mbp, *ycbABCDKFGHIJ* and *ycaFG* genes; Locus 2: position ~ 0.44 Mbp, pi1 genes; Locus 3: position ~ 1.03 Mbp, pi2 genes; Locus 4: position ~ 1.20 Mbp, *citRCDEF*, *mae*, *ymbCDHIJK* and *ymcABCDEF* genes; Locus 5: position ~ 1.41 Mbp, pi3 genes; Locus 6: position ~ 2.01 Mbp, ps3 genes; Locus 7: position ~ 2.30 Mbp, *pspAB* and *ywjABCDEFGH* genes. Genes were declared as only divergence when their ratios were lower than 0.76.

Utilisation of these CGH ratios in order to predict gene deletions was not trivial when strains were genetically close. The low ratios could indeed correspond to the absent genes as well as the genes with the low sequence identity with IL1403 strain and the low hybridization efficiencies (Van Hijum *et al.*, 2008). Only sequencing experiments had the resolving power to give a definitive answer on the presence or the absence of the specific gene in the tested strains. In our experiment, after subtraction of the local background value, we have identified absent or highly divergent genes when the genes were declared as absence by the three different statistical methods a, b and c (see Materials and Methods). Method a was based on the absolute signal intensity with empty spot threshold constraint. The two other procedures b and c were comparative to the IL1403 strain and based on two different statistical normalisations, *i.e.* normalisation by whole spot mean intensity (method b) and by a subset of conserved genes (method c). From the 1,929 ORFs spotted on the microarray membranes, only 16 genes were identified as absence in at least one of the six strains tested in CGH experiment (Table 4.4): UCMA5713, *pspB*, *citR*, *pi109*, *pi147*, *pi202*, *pi207*, *ps313* and *ps315*, in LD55, *citR*, *pi147*, *pi202*, *pi203*, *pi204*,

pi207, *pi213*, *pi225* and *pi226*, in LL08, *citR*, *pi202* and *ps315*, in LL52, *citR*, *pi204*, *ps305*, *ps310*, *ps311* and *ps313*, and in S86 *pspB*, *citR*, *pi202*, *pi203 and pi204* were absent. In order to discriminate absent genes from highly divergent genes (false negative genes), PCR amplifications specific of these 16 genes were also performed on genomic DNA using hybridization temperatures between 42 and 55 ^{o}C. In our PCR conditions, amplified fragments were detected for gene *pi202* in strains UCMA5713, LD55 and S86, for genes *pi109* and *pi207* in strain UCMA5713, and for genes *ps311* and *ps313* in strain LL52. Following these PCR experiments, the number of genes declared absent in at least one of the six strains was therefore reduced to 14 genes.

No ORF was commonly absent in the six strains, meaning that no IL1403-specific ORF was detected. The number of absent ORFs ranged from 8 in the LD55 strain to 0 in the LD61 strain even if the divergent locus could be observed in the LD61 strain on Figure 4.2. Among the 14 genes absent ORFs, 12 ORFs were involved in the phage related functions and prophages functional category, more specifically related with prophages pi1, pi2 and ps3. Only two absent ORFs were linked to metabolic functions, *pspB* encoding a glucosyltransferase-S of the cell envelope functional category and *citR*, coding for a citrate lyase regulator. This experiment underlined a very large core genome with 1,915 genes of the IL1403 strain genome present in all of the six studied strains with a dairy phenotype.

Table 4.4 Absent genes identified by CGH analysis in at least one of the six strains UCMA5713, LD55, LL08, LL52 or S86. Successful PCR amplifications are shown by *.

Genes	Strains				
	UCMA 5713	LD55	LL08	LL52	S86
pspB	absent	-	-	-	absent
pi109	absent*	-	-	-	-
pi147	absent	absent	-	-	-
pi202	absent*	absent*	absent	-	absent*
pi203	-	absent	-	-	absent
pi204	-	absent	-	absent	absent
pi207	absent*	absent	-	-	-
pi213	-	absent	-	-	-
pi225	-	absent	-	-	-
pi226	-	absent	-	-	-
ps305	-	-	-	absent	-
ps310	-	-	-	absent	-
ps311	-	-	-	absent*	-
ps313	absent	-	-	absent*	-
ps315	absent	-	absent	-	-
citR	absent	absent	absent	absent	absent

4.3 Gene expression analysis

The differential transcriptomic analysis of the core genome of the five strains, UCMA5713, LD55, LL08, LL52 and S86, compared with the reference LD61 strain were examined with DNA arrays (*L. lactis* subp. *lactis* IL1403-specific amplicons spotted) after 24 hours of growth in UF-cheese. Total RNA was extracted from cells grown 24 hours in UF-cheese. The quality and quantity of total RNA were verified and were used to perform retrotranscription. Afterwards, the synthesis of radiolabelled cDNA, nylon arrays hybridizations and washings were performed as described in Materials and Methods. Membranes were exposed to a phosphoimager screen for

three days and scanned with a phosphofluoroimager. Signal intensities were quantified, tested statistically, and assigned to gene names with the Bioplot software. Local background was removed from all spot intensities. Signals were normalized by the mean intensity of the corresponding membrane. Expression ratios were calculated between the strain of interest and LD61 strain which was considered as the reference strain for expression studies in milk (Raynaud *et al*., 2005). The complete transcriptomic ratios were given in Appendix D. Significant ratios corresponding to the genes differentially expressed in each strain in comparison with the LD61 strain were selected with the usual statistical criterion of the FDR ≤ 7%.

In order to check if the transcriptomic ratios were under-estimated for the genes with low hybridization efficiencies (corresponding to low CGH ratios), we have calculated the correlation coefficients between all the 1,915 genes expression ratios and the CGH genes ratios using the LD61 strain as the reference strain. With Pearson, Spearman or Kendall methods, correlation coefficients were lower than 0.05, indicate that such a bias was not significantly present in our experiments.

To establish the relationships of the different strains, the two-dimensional hierarchical clustering of the entire data set (gene expression ratios of the significant and non-significant regulated-genes for the five strains UCMA5713, LD55, LL08, LL52 and S86) resolved the gene expression ratios back to their strain origin (Figure 4.3). Gene expression ratios were clustered along the vertical axis with strains along the horizontal axis. Clustering parameter was an agglomeration method of Ward. Figure 4.3 exhibited that the strain dendrogram grouped LD55 strains (dairy origin) and the UCMA5713 strain (non-dairy origin) while the LL08 strain was the most divergent compared with the other four strains.

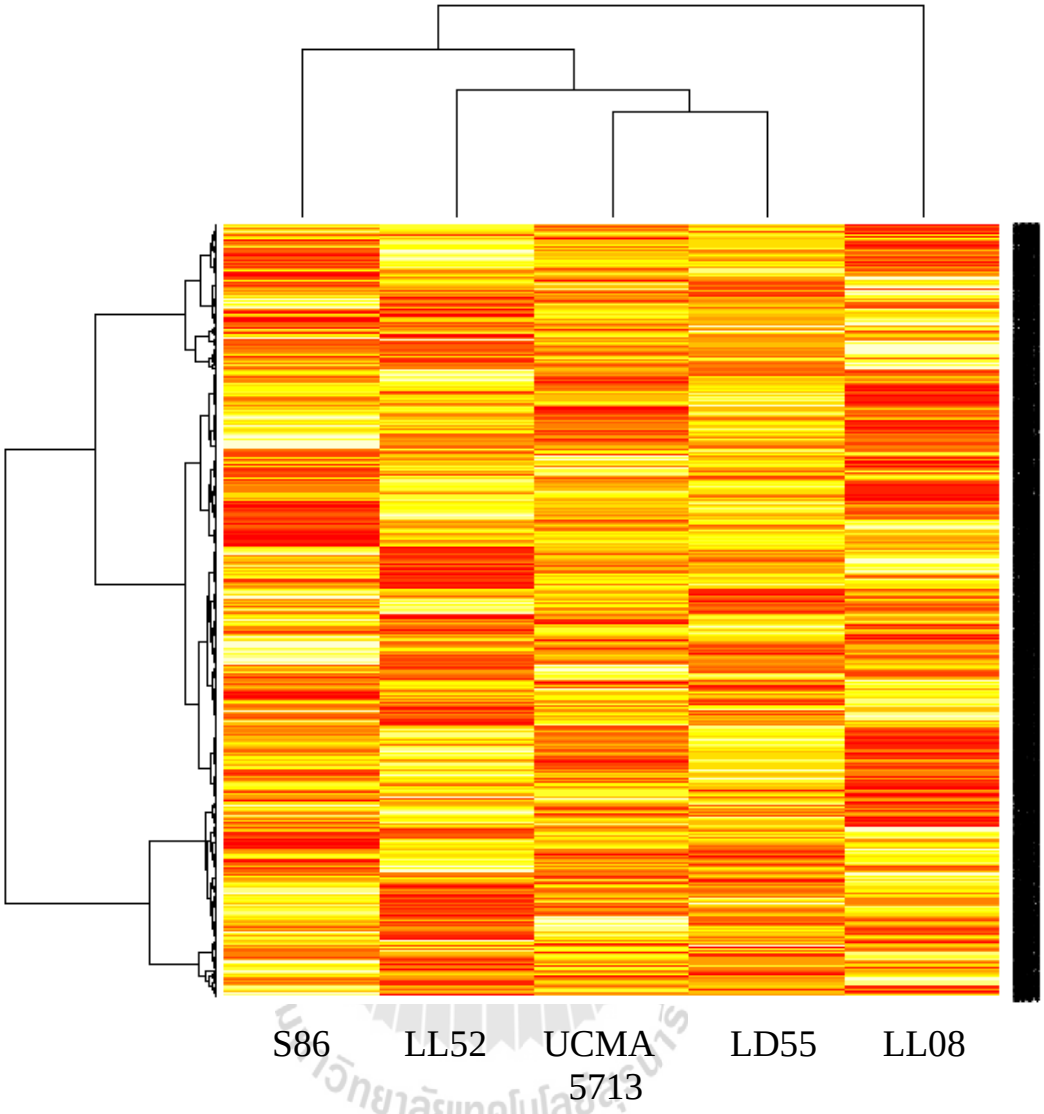

Figure 4.3 Two-dimensional hierarchical clustering resolving the gene expression ratios back to their strain origin.

To establish a link between genomic composition and gene regulation, we have measured if genes declared as divergence were statistically over-represented in the group of genes whose expression were significantly different from the LD61 strain (ratio selected with FDR ≤ 7%). By using a hypergeometrical distribution that is a discrete probability distribution that describes the probability of *k* successes in *n* draws from a finite population without replacement, probabilities higher than 12%

were observed for each of the tested strain showing no significant enrichment of divergent genes in the group of differentially regulated genes.

The number of genes differentially expressed was determined for each strain. A total of 968 different genes were differentially regulated in at least one of the five strains compared with the LD61 strain. For each strain, genes were equally partitioned between higher- and lower-expression levels compared with the LD61 strain. The strains, UCMA5713, LD55 and S86 exhibited 254, 332 and 270 differentially expressed genes, respectively, while the other two strains, LL08 and LL52, revealed even higher numbers (562 and 586 genes, respectively) (Table 4.5).

Table 4.5 Number of different genes in each strain compared with the LD61 strain (Reference strain).

Strains	The expressed genes
UCMA5713	254
LD55	332
S86	270
LL08	562
LL52	586

In order to analyse the contributions of these individual gene expression regulations into the metabolic traits of the different strains, we determined for each strain, the over- and under-expressed gene enrichments in the various functional categories by the Wilcoxon test on the whole category (p-value < 0.05 significance) as previously described by Dressaire *et al*. (2008). The significantly regulated functional (sub)categories compared with the LD61 strain were reported in Table 4.6.

Table 4.6 revealed that strong divergence of the five strains compared with strain LD61 was observed within many regulated categories distributed all over the metabolism. Even if some (sub)categories showed a similar type of regulation for the five strains, most of the functional categories exhibited different regulations between the strains underlining the large transcriptomic expression variability between the five strains.

In addition, significant differentially regulated genes were observed and shown in Table 4.7. The efficient proteolysis of *L. lactis* LD61 in UF-cheese was reported to lead to accumulation of free amino acids (in particular glutamate) and to a low expression of the nitrogen metabolism (Cretenet *et al*., 2011). In our study, after 24 hours of growth on UF-cheese model, the five strains displayed lower expression levels of the amino acid biosynthesis pathways (*e.g*. aromatic amino acids (*aro* and *trp* genes), branched chain amino acids (*ilv* and *leu* genes) and amino acids of the histidine family (*his* genes)) than the LD61 reference strain.

Table 4.6 Significantly regulated functional categories in each of the five strains (compared with the LD61 strain) according to Wilcoxon test (P-value < 0.05). Black and gray boxes were higher and lower gene expression levels than in the LD61 strain, respectively.

Functional categories or sub-categories	UCMA5713	LD55	LL08	LL52	S86
AMINO ACID BIOSYNTHESIS					
Aromatic amino acid family					
Aspartate family					
Branched chain family					
Histidine family					
CELLULAR PROCESSES					
Chaperones					
Transformation					
Protein and peptide secretion					
BIOSYNTHESIS OF COFACTORS, PROSTHETIC GROUPS, AND CARRIERS					
Menaquinone and ubiquinone					
Thioredoxin, glutaredoxin and glutathione					
FATTY ACID AND PHOSPHOLIPID METABOLISM					
CENTRAL INTERMEDIARY METABOLISM					
Other					
ENERGY METABOLISM					
Anaerobic					
ATP-PMF conversion					
Fermentation					
Sugars					
TCA cycle					
OTHER CATEGORIES					

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Table 4.6 (Continued).

Functional categories or sub-categories	UCMA5713	LD55	LL08	LL52	S86
Adaptations and atypical conditions					
Transposon related functions					
PURINES, PYRIMIDINES, NUCLEOSIDES AND NUCLEOTIDES					
Purine ribonucleotide biosynthesis					
Pyrimidine ribonucleotide biosynthesis					
Salvage of nucleosides and nucleotides					
Sugar-nucleotide biosynthesis and interconversions					
REGULATORY FUNCTIONS					
AraC-family regulators					
General					
LysR-family regulators					
MarR-family regulators					
Two-component systems					
REPLICATION					
Degradation of DNA					
TRANSLATION					
Translation factors					
Ribosomal proteins: synthesis and modification					
Amino acyl tRNA synthetases					
TRANSPORT AND BINDING PROTEINS					
Amino acids, peptides and amines					
Anions					
Carbohydrates, organic alcohols and acids					

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In UF-cheese model, it was shown that LD61 strain induced specific responses to counteract different sources of stress including acidic and oxidative stresses and carbon limitation (Cretenet *et al*., 2011). Compared with the LD61 strain, the four strains, LL08, LL52, S86 and UCMA5713, exhibited modification of expression of genes involved in acidic stress resistance. Several genes involving in the arginine deiminase pathway that produced NH_3 through the conversion of arginine into ornithine were more highly expressed in LL08 strains (*arcBC1* and the arginine/ornithine antiporter *arcD2*), LL52 (genes *arcABC1)* and S86 (*arcBC1*). In the LL08 strain, concomitantly, genes involved in glutamate transport and conversion to ornithine (*gltQS* and *argBCDEJ*) were more highly expressed than those in the LD61 strain. In this strain, the genes *citCDEF* involved in the citrate metabolism which was an alternative acid stress response, were also more highly expressed. In the LL52 strain, the genes *atpDEH* encoding the different subunits of the ATPase were expressed at lower levels compared with the LD61 strain. This enzyme catalyzes proton expulsion and was thus directly involved in resistance to acidic stress. In the plant-associated strain, UCMA5713, a net consumption of protons could be provided via the glutamate decarboxylase converting glutamate to the biogenic amine γ-aminobutyrate (GABA). The glutamate transporter (*gadC* coding for a glutamate/GABA antiporter) and the glutamate decarboxylase (*gadB*) expression was specifically increased in UCMA5713 strain compared with the other strains. Generally, it was found that the lactococcal *gadC* and *gadB* genes were maximally expressed at low pH (Cotter and Hill, 2003) which were in agreement with the lower final pH observed for this particular strain.

In response to oxidative stress, the LL52 strain exhibited lower expression of genes involved in maintaining a favorable redox balance such as *gshR* and *trxB1* which coded for glutathione reductase and thioredoxin reductase, respectively. Significant higher transcription of gene *tpx* coding for a peroxidase was observed in the S86 strain.

Concerning carbon limitation, a global over expression of genes involved in carbon metabolism and sugar transport was observed in the LD61 strain (Cretenet *et al.*, 2011). Here, all the five strains induced a higher expression of the Leloir pathway that was dedicated to lactose assimilation (genes of the *gal* and *lac* families) compared with the LD61 strain. In addition, expression of several genes involved in alternative sugar metabolism such as *scrK*, *ypdBD* and *yrcA* was specifically more highly transcribed in the LL08 strain than in the LD61 strain. Consistent with this observation, increased expression was observed for genes related to sugar transport such as the PTS system for cellobiose (*celB*, *ptcABC)* or genes coding for permease, binding proteins and transporter (*rgpC*, *ypdA*, *ypcGH* and *yngF)* involved in general carbohydrate transport system for N-galactosides, sugars, and polysaccharides in the LL08 strain. In the LL08 strain, increased expression of genes involved in carbon transport was consistent with the increased expression of the *ldh* gene coding for lactate dehydrogenase that catalysed the lactate production from pyruvate, the last step of carbon catabolism in homolactic bacteria. More surprisingly, the pathway of pyruvate conversion into acetolactic acid was also highly expressed with an increased transcription of the *als* gene in the LL08 strain compared with the LD61 strain. Inversely, genes involved in central carbon metabolism in the LL52 strain were

specifically transcribed to a lower level in this strain, (glycolytic genes, *yjhF* and *pgmB*, and those involved in the functional category fermentation).

Finally, some strains exhibited specific traits compared with the LD61 strain. The production of biogenic amines (*i.e.* putrescine) from ornithine was likely in the LL08 strain as increased expression of genes *potA* and *potD* involved in spermidine/putrescine transport was observed. Another important peculiarity in this strain was the over-expression of the functional category ribosomal proteins (*rpl* and *rps* genes) and of the *infC* gene coding for the translation initiation factor. Cell envelop modifications were suggested in both LL08 and LL52 strains since higher expressions were observed in the LL08 strain for *rgpBEF* and *ycbBDFHIJ* genes (potentially involved in biosynthesis, assembly and transport of cell wall polysaccharides and important for phage adsorption (Bolotin *et al*., 2001; Dupont *et al*., 2004; Yamashita *et al*., 1998) and in the LL52 strain for three genes *murA1BC* involved in amino sugar metabolism. Finally, the significant strain-specific expressions of regulators belonging to different families (except for strain UCMA5713) could be underlined (Table 4.7).

Table 4.7 Differentially regulated genes (Student test, FDR ≤ 7% significance) sustaining regulated functional categories in the five strains (compared with the LD61 strain as shown in Table 4.6). The expression ratios were listed in the Annex 1. Genes underlined exhibited increased expression compared with the LD61 strain, while the genes not underlined showed lower expression compared with the LD61 strain.

Functional categories or sub-categories	UCMA5713	LD55	LL08	LL52	S86
AMINO ACID BIOSYNTHESIS					
Aromatic amino acid	*trpA B*	*aroH*	*trpAB*	*aroCDH*	*aroH*
Branched chain	*ilvCD, leuC*	*ilvD, leuC*	*ilvBCD, leuBD*	*ilvBD, leuBC*	*ilvBCDN leuBC*
Histidine	*hisZ*	*hisCZ*	*hisACDGHIKZ*	*hisDHIZ*	*hisCZ*
TRANSPORT and ENERGY METABOLISM					
Sugars	*galKMT glk, lacCZ, thgA*	*galM glk, gntZ, lacCZ*	*bglA, galKMT gntZ, lacCZ scrK, thgA uxaC, ypdBD, yrcA*	*galEKMT, lacZ, thgA yidC*	*galKM, thgA uxaC*
Glutamate / GABA	*gadC, gadB*				
Glutamate / ornithine			*gltQS, argBCDEJ*		
Arginine deiminase pathway			*arcD2 arcBC1*	*arcABC1*	*arcBC1*
Biogenic amine synthesis			*potAD*		
Citrate metabolism			*citCDEF, icd*		
Glycolysis			*ldh*	*yjhF, pgmB*	
Fermentation			*als , frdC, butB*	*ackA1A2*	
Alternative sugar metabolism			*scrK, yrcA ypdBD*		
ATP-PMF conversion				*atpDEH*	
PTS and carbohydrate transport			*celB, ptcABC, rgpC, ypdA, yngF ypcGH*		
BIOSYNTHESIS OF COFACTORS, PROSTHETIC GROUPS and CARRIER					
Thioredoxin, glutaredoxin and glutathione				*gshR, trxB1*	
CELL ENVELOP					

Table 4.7 (Continued).

Functional categories or sub-categories	UCMA 5713	LD55	LL08	LL52	S86
ADAPTATIONS and ATYPICAL CONDITIONS					*tpx*
Polysaccharide			*rgpBEF*, *ycbBDFHIJ*		
Peptidoglycan				*murA1BC*	
TRANSLATION					
Ribosomal proteins and translation factors			*rplBCNT*, *rpsCDGQS* *rpmI*, *rnpA*, *infC*		
REGULATORY FUNCTIONS					
AraC-family regulators			*xylR*		
DeoR-family regulators		*lacR*			
General		*purR*	*birA2*, *rmeD*, *ydcG*, *yjaJ* *ypfD*, *yrtfA* *ysfD*	*argR* *birA1*, *copR* *tenA*, *yabA*, *ybeD*, *ydcG* *yecA*, *ykhI* *yliA*	*glnR* *rmeB* *ymiA* *ynaB*
GntR-family regulators					*rgrB*
GTP-binding proteins				*yphL*, *yyaL*	*ylqL* *yyaL*
LacI-family regulators			*rliB*	*rliC*	
LysR-family regulators		*fhuR*			
MarR-family regulators		*rmaG*	*rmaA*, *zitR*	*rmaC*, *rmaIJ*	
Two-component systems				*llrG*	

4.4 Confirmation of the significant genes by RT-PCR

In order to verify the array data, the four selected genes (*yaiA*, *feoA*, *ipd* and *pyrR*) were selected to perform RT-qPCR analysis of their expression. The specific primers for specific genes were designed with Bio-Rad Beacon Designer software. The reverse transcription was performed with mRNA template as described previously (Maligoy *et al*., 2008). The specificities of the primers for the genes of interest were controlled by using the *L. lactis* IL1403 genome with Vector NTI software. The *pbp2A* and *smc* genes were chosen as internal normalization controls. Afterwards, the Pfaffl analysis method was used to calculate the change in transcript

levels between strains UCMA5713, LD55, LL08, LL52 and S86 and the reference LD61 strain. The results of these studies were shown in Table 4.8.

The results showed the direct comparison between transcriptomic data and quantitative reverse transcription-PCR expressed as ratios in transcript concentrations between the strain of interest and the reference strain and corrected by using the *pbp2A* or *smc* normalization controls. These ratios of transcriptomic data were similar to those observed by qPCR analyses.

Table 4.8 Comparison of the gene expression ratios between the strain of interest and the reference LD61strain, in the transcriptomic and the qPCR analyses (corrected in qPCR experiments by using the *pbp2A* or *smc* normalization ratios).

Relative gene expression	Increased				Unchanged	Decreased					
Gene name	*feoA*	*feoA*	*feoA*	*yaiA*	*feoA*	*ipd*	*Ipd*	*ipd*	*ipd*	*pyrR*	*pyrR*
Strain	UCMA5713	LD55	LL52	LL52	LL08	UCMA5713	LD55	LL52	S86	LL52	S86
Transcriptomic ratio	3.5	3.3	4.9	5.6	1.0	0.1	0.2	0.1	0.1	0.2	0.3
qPCR ratio normalized with :											
pbp2A	5.2	4.7	1.3	2.8	1.0	0.2	0.1	0.1	0.1	0.5	0.1
smc	6.8	4.8	1.2	2.7	1.0	0.3	0.1	0.1	0.1	0.4	0.1

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4.5 Physiological characteristics analysis of *L. lactis* subsp. *lactis*

4.5.1 Nitrogenous bases requirements of the six *L. lactis* strains

The present study was performed to analyse the growth ability of the strains on CDM medium with different nitrogenous base composition. The experimental conditions were CDM medium without purine base or without pyrimidine base or without both bases. All cultures were grown in the test tube for 24 h at 30 ^{o}C. The resulting maximal growth rate in each growth condition was compared with the one observed in classic CDM medium (Table 4.9). The results showed that all the strains were growing slower in the CDM medium without purine or without pyrimidine. In addition, when removing both bases from CDM medium, growth was even more reduced for all strains (with the exception of the LD61 strain). In conclusion, no major phenotypic difference could be detected between the strains at the level of nitrogenous base metabolism.

Table 4.9 Maximal growth rate of *L. lactis* subsp. *lactis* strains in CDM media with different nitrogenous bases composition.

Strains	Maximal specific growth rate (h^{-1})			
	*classic CDM	without purine	without pyrimidine	without both bases
UCMA5713	1.66±0.02	1.16±0.02	1.32±0.04	0.87±0.04
LD55	1.29±0.01	1.00±0.02	1.22±0.01	0.89±0.03
LD61	1.32±0.02	0.98±0.07	1.14±0.04	1.28±0.01
LL08	1.37±0.07	1.07±0.09	1.18±0.03	0.81±0.03
LL52	1.37±0.04	1.20±0.17	1.21±0.06	1.00±0.01
S86	1.19±0.09	0.89±0.01	1.11±0.07	0.68±0.06

*Classic CDM medium: CDM medium with all bases; Without purine: CDM medium without purine; Without pyrimidine: CDM medium without pyrimidine ; without both bases: CDM medium without purine and pyrimidine.

4.5.2 Branched chain amino acid (BCAA) requirements of the six *L. lactis* strains

To determine the branched chain amino acid requirements of the six *L. lactis* strains, the strains were cultivated in CDM medium under various conditions defined as follows; without isoleucine or without leucine or without valine or without all $BCAA_s$. The control contained all branch chain amino acids (classic CDM medium). The results of these studies were shown in Table 4.10. It revealed that the two strains, LL08 and LL52, had significant growth rate in the media without Ile or Leu or Val in the medium, as well as in the absence of $BCAA_s$. In addition, they harbored only slightly reduced maximal growth rate in these conditions compared with the reference CDM medium. On the contrary, the two other strains, UCMA5713 and S86, were not able to grow in both medium without Leu or Val and in the absence of $BCAA_s$. The LD55 strain showed no growth ability in the medium without Val or without all $BCAA_s$ while the LD61 strain could not grow only in the medium without Val.

Table 4.10 Maximal growth rate of *L. lactis* subsp. *lactis* strains in CDM media with different branched chain amino acid (BCAA) composition.

Strains	Maximal specific growth rate (h^{-1})				
	*classic CDM	without Ile	without Leu	without Val	remove all $BCAA_s$
UCMA5713	1.66±0.02	0.99±0.02	-	-	-
LD55	1.29±0.01	1.11±0.01	0.97±0.02	-	-
LD61	1.32±0.02	1.16±0	1.17±0.02	-	0.95±0.14
LL08	1.37±0.07	0.99±0.02	0.92±0.01	0.77±0.04	0.83±0.01
LL52	1.37±0.04	1.04±0.09	1.03±0.04	0.83±0.04	0.97±0.03
S86	1.19±0.09	0.26±0.04	-	-	-

*Classic CDM medium: CDM medium with all BCAA;
Without Ile: CDM medium without Isoleucine;
Without Leu: CDM medium without Leucine;
Without Val: CDM medium without Valine;
Remove all $BCAA_s$: CDM medium with no $BCAA_s$ = no significant growth.

In conclusion, the behavior of the six *L. lactis* strains with regard to the BCAA requirement was strongly strain dependent. All the tested strains were able to grow only in the absence of isoleucine, while the S86 strain could slightly grow on the medium without isoleucine.

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CHAPTER V

DISCUSSION

The importance of strains belonging to the species *Lactococcus lactis* in the manufacture of fermented dairy products is well known. Generally, it occupies in a niche related to plant or animal surfaces and the animal gastrointestinal tract (Wegmann *et al*., 2007). The current literatures reveal *L. lactis* strains could be models for study on metabolism, physiology, genetics, and molecular biology of lactic acid bacteria (LAB), especially in two subspecies strain as *lactis* and *cremoris* (Bolotin *et al*., 2001) since the diversity was found within these species.

Recently, physiological properties and genetics of lactococci have considerably changed (Van Hylckama *et al*., 2006). To assess the intra-subspecies diversity of *L. lactis* subsp. *lactis*, therefore, the present study performed comparisons at the phenotypic, genomic and transcriptomic levels of the six strains of *L. lactis* subsp. *lactis*. The six strains of *L. lactis* subsp. *lactis* isolates representative of the dairy diversity of the Génoferment collection were selected. One strain, UCMA5713, was a plant-associated strain whereas the other five strains LD55, LD61, LL08, LL52 and S86, were found in dairy fermentations. The phenotypic analysis of *L. lactis* strains on UF-cheese model was firstly investigated. Their ability of strains to grow and acidify in UF-cheese was compared for all along the cheese repining by growing these strains on skimmed raw milk ultrafiltration (UF) retentate. From such result, no

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significant difference in terms of growth rate and final cell density was observed between the strains during growth in UF-cheese. However, phenotypic differences were detected when comparing final cheese pH, since a 0.3 pH unit variation can lead to different cheese quality such as cheese flavor, cheese texture and cheese safety (Pandey *et al*., 2003). In addition, De Giori *et al*. (1985) found that variations in pH had a strong influence on casein hydrolysis in streptococci more than in lactobacilli.

Strains were also tested for their ability to grow with other sugars, like glucose as carbon source on media that it is commonly used in laboratories. Since chemically defined medium (CDM) is a synthetic medium that contains 18 amino acids, 12 vitamins and 6 bases, and can support growth of the bacteria at a constant specific growth rate (Novak *et al*., 1997), therefore, CDM medium was selected for further study. From the results, strains exhibited only weak differences in the final cell population (1.21 to 1.83 x 10^9 CFU/ ml) and pH (4.12 to 4.57). It was LL52 strain that reached the low final pH value of 4.12.

From the previous experiment, the result showed that the synthetic medium (CDM) containing glucose supplemented with high amino acids, vitamins, and bases could be used as the growth media for these strains. The other types of media which were complex medium (M17) and modified synthetic medium (M13), were also studied for their ability to grow and acidify. From the results, strains could grow in M17 medium since this complex medium was formulated by including ingredients of natural origin (Zhang and Greasham, 1999) while none of the six strains were able to grow on M13 medium because of the lacking in 12 amino acids, 7 vitamins, and all nitrogenous bases. Novak *et al*. (1997) showed that the biomass was detected from

the culture grown in M17 medium was higher than the other media (MS10, MS13, and MS14, respectively) which was correlated with glucose consumption.

Since the genomes of the six strains of *L. lactis* subsp. *lactis* exhibiting a dairy phenotype were not sequenced, their similar genomic contents (core genome) were compared by using array-based comparative genome hybridization during growth on M17 medium. The genome of the sequenced strain *L. lactis* subsp. *lactis* IL1403 was used as a reference strain. DNA array technology has recently been used for the analysis of genome variability among bacterial species or closely related bacteria (Vijayendran *et al*., 2007). Array-based comparative genome hybridization is commonly used to determine the genomic content of bacterial strains. It is applied frequently to study the genomic content of closely related microorganism (Van Hijum *et al*., 2008). Detection of genomic variation between related organisms can elucidate relations between genotypic and phenotypic traits of organisms (Bayjanov *et al*., 2009). In present study, open reading frames (ORF_s) were predicted in the assembled draft genome sequences of the six strains. The genome of the sequenced strain *L. lactis* subsp. *lactis* IL1403 was used for microarray platform design. In consideration of genome content, the six strains shared a large core genome of 1,915 genes corresponding to more than 99% of the IL1403 ORFs spotted on the membranes. In addition, significantly lower core genome values were obtained for other species (Rasmussen *et al*., 2008). Due to the represent of genome content in all dairy *L. lactis* strains, here, the core genome could be named a “dairy” core genome since it was representative of the very homogeneous group of strains sharing a dairy phenotype (growth on UF-cheese model and high acidification rate). However, we could not exclude a certain genomic variability between the strains related to their accessory

genomic content notably for the LL52 strain harbouring an additional 260 kb compared with the IL1403 strain. According to CGH analysis, the accessory genome (or non-core) of the IL 1403 strain (defined as genes present in the IL1403 strain but not in all strains) was able to analyse. The results showed that fourteen genes constituting part of the accessory genome could be divided into two groups. First group was in the mobilome or mobile DNA that invaded or left the unnecessary genome and then added to the fitness of the strain such as phage-related genes. The diversity region involved in the strain specific fitness (genes involved in citrate metabolism *citR* and surface polysaccharides *pspB*) was grouped to the second one. This small accessory genome did not contain any genes unique to the IL1403 strain.

Due to an attention in gene expression of LAB, various specific techniques that fall under the rubric of omics and bioinformatics approaches were used for monitoring the global changes in phenotype of transcripts, proteins, and metabolites (Downs, 2006). The expression of the common genes to the six studied strains was compared in UF-cheese model conditions using DNA arrays corresponding to the IL1403 genome. The comparison was performed at 24 hours of growth since the dynamic study of LD61 strain under similar conditions has shown that the number of differentially expressed genes did not change thereafter (Cretenet *et al*., 2011). To establish the relationships of the different strains, the expression data set of the 1,915 core genome gene of the five strains (S86, LD55, LL08, LL52 and UCMA5713) was classified by a hierarchical clustering. From the strain dendrogram, based on gene expression ratios, showed the strains grouping between the LD55 and UCMA571 strains. In contrast to the LL08 strain, which was the most divergent compared with the other four strains. Moreover, the diversity between the six strains at the level of

gene expression of their core genome (1,915 genes) was demonstrated. Such a level of regulation was already reported in the literature between two species *E. coli*/*Shigella* (in adaptation to their environment) or two closely related *E. coli* K12 sub-strains (during growth) (Le Gall *et al*. (2005), Vijayendran *et al*. (2007)). Variation in gene expression was present between plant- and dairy-associated strains, but more interestingly, also between strains of the same dairy origin (starters and raw milk).

Classification by functional categories revealed that gene products involved in the formation of the translation complex and in the general metabolism constituted the largest groups of identified proteins (Schrtl *et al*., 2005) and it has been also used in this present study. The specific gene regulation in the plant-associated UCMA5713 strain was mainly restricted to the increased expression of genes involved in the glutamate uptake and conversion to GABA. These genes involved in the function of controlling pH by combination between amino acid decarboxylation and amino acid antiport (Sanders *et al*., 1998). Nomura *et al*. (1998) showed the relationship between the pH values and the production of gamma-aminobutyric acid during cheese ripening that gamma-aminobutyric acid increased linearly in the experimental cheeses as the pH of the cheese decreased. Siezen *et al*. (2010) did not report modification of the genomic content of the two *L. lactis* plant isolates, KF147 and KF282, related to GABA metabolism even if two polyamine transporter systems were present in these strains. Recently, a GABA-producing *Lactococcus lactis* strain grown in the medium supplemented with or without glutamate was studied using the combined transcriptome/proteome analysis (Mazzoli *et al*., 2010). It was found that most glutamate-induced responses consisted in under-expression of metabolic pathways, with the exception of glycolysis where either over- or under-expression of specific

genes was observed. In addition, the energy-producing arginine deiminase pathway, the ATPase, and also some stress proteins were down-regulated, suggesting that glutamate was not the only an alternative means to get energy, but also a protective agent against stress for the strain studied. Although being a plant associated strain, the UCMA5713 strain exhibited a dairy phenotype and it was closely related to the dairy origin strains (expression dendrogram, Figure 4.3). This could be explained by the observation that UCMA5713 strain was isolated from grassland neighbouring a dairy factory, not excluding the fact that the UCMA5713 strain was not a real grassland strain but a strain with a dairy origin found on the plant. Recently, Passerini *et al* (2010) revealed that the gene-based phylogeny was not fully consistent with the traditional classification into dairy and non-dairy strains but supported a new classification based on ecological separation between the environmental strains and the domesticated strains. Among the dairy origin strains, the LL08 strain displayed the largest transcriptomic divergence as shown by its separated branch in the dendrogram and its large amount of strain-specific transcriptomic features. Increased expression of genes involved in central metabolism, glutamate consumption, ribosomal protein synthesis and translation factors and the stronger induction of the alternative sugar metabolism and transport indicated that this strain was still able to maintain metabolic activity even after 24 hours of growth in UF-cheese. From the industrial point of view, the potential capabilities of the LL08 strain to produce diacetyl, a butter flavor compound, and polysaccharides potentially involved in cheese texture, were interesting properties even if the concomitant synthesis of the undesired biogenic amines was also possible.

We have tried to link the different observation levels, phenotype (growth on UF-cheese matrix and acidification capability), genomic content and transcriptomic profile. After 24 hours of growth on UF-cheese model, the transcriptomic profiles (common for the five strains or strain-specific) were in agreement with the genomic content of the strains and corresponded to the responses previously observed for the LD61 strain (Cretenet *et al*., 2011), *i.e.* acid and oxidative stresses and carbon but not nitrogen limitation. In the case of citrate utilization a discrepancy was however observed. Even if citrate was present in the cheese matrix, the five strains (UCMA5713, LD55, LL08, LL52 and S86) should not be able to metabolise citrate since they do not belong to the biovar *diacetylactis*. The absence of the *citR* gene in the five strains was consistent with this observation. However, more surprisingly, a *citDEF* operon was present in all these five strains and increased expression of the *cit*DEF was observed in the LL08 strain compared with the LD61 strain belonging to the biovar *diacetylactis*. In addition, we observed using PCR (results not shown) that neither the *cit*P gene (coding for the citrate permease) nor the *citM-citRCDEFGX* region was present in the UCMA5713, LD55, LL08, LL52 and S86 strains. One explanation could be the presence of cross-hybridization of other genes (plasmid-borne genes) with the microarray probes designed against the IL1403 *citDEF* genes.

Despite the strong similarity of the core genome between the strains, a large transcriptomic polymorphism was observed. We had reconfirmed that there was no significant bias in expression-change determination linked to low hybridization efficiencies. In addition, for each tested strain, we showed that genes declared as divergence was not over represented in the group of genes differentially regulated compared with the LD61 strain. The strain-specific expression of a similar core

genome could be related to the ability of the accessory genome expression to interact with the core genome expression. However the strain with the largest chromosome (LL52) and the potentially largest accessory genome was not the most divergent strain at the transcriptomic level. The discrepancy between genomic similarity and transcriptomic diversity could more probably reveal strain-dependent regulatory networks. This conclusion was supported by variable regulations within the functional category regulatory functions between the strains, related to 37 regulatory genes exhibiting strain-specific expression differences.

Nucleotides are obligatory metabolites in all organisms. They are substrates for RNA and DNA synthesis, and serve as the main energy donors for cellular processes. Some nucleotides are constituents of coenzymes in central metabolic pathways (Martinussen and Hammer, 1998), while others are used for activation of precursors in polysaccharide and lipid synthesis. In addition, nucleotides serve an important role in the regulation of numerous cellular processes from the metabolic level to the level of gene expression (Kilstrup *et al*., 2005). Most bacteria are able to produce nucleotides *de novo*, while others including some lactic acid bacteria, require addition of either purines or pyrimidines to the growth medium. Milk does not contain sufficient levels of purine compounds to support the growth of *L. lactis* even a pyrimidine source is present in milk but only some strains of the lactic acid bacteria can utilize it. Therefore, *de novo* biosynthesis is necessary (Bolotin *et al*., 2001). Recently, Bolotin *et al*. (2001) showed that *L. lactis* has sufficient and fairly active capacities for biosynthesis and also for salvage of nucleic acid compounds. Therefore, the growth ability of the strains on the modified bases CDM medium was studied. After cultivating of strains in CDMs medium containing no purine base, no

pyrimidine base or without both bases, the results showed that all the strains were not grown well in the missing of at least one of these bases in the CDM medium. Recently, additional of purines to *L. lactis* MG1363 grown in chemically defined GSA medium was demonstrated to stimulate the growth rate by approximately 15% while pyrimidine had no effect (Martinussen *et al*., 2003). Hence, the addition of exogenous purines to the growth medium led to the repression of the purine biosynthetic genes in *L. lactis* as well as *de novo* pyrimidine synthesis was also inhibited due to repression of the expression of the pyrimidine biosynthetic genes encoded by the *pyr* operon when uracil was present in the culture medium (Arsène-Ploetze *et al*., 2006). In addition, Martinussen *et al*. (1994) showed that hypoxanthine, adenine, and guanine facilitate growth of a purine-requiring mutant. It had been shown that all pyrimidine derivatives except cytosine could be metabolized, demonstrating permeability for these compounds as well as BmpA-NupABC recently was found to be an ABC transporter with the ability to actively transport all common nucleosides, whereas UriP was shown to be responsible for the uptake of only uridine and deoxyuridine (Martinussen *et al*., 2010).

Generally, LAB use amino acids not only to synthesize proteins but also use as an energy source to obtain the optimal internal pH under an acid environment. In addition, this source is used to regenerate co-substrates, and also for biosynthesis (Ardö, 2006). Besides the limited capacity for biosynthesis of amino acids, *Lactococci* require essential amino acids for growth, and the number of essential amino acids is strain-dependent (Van Kranenburg *et al*., 2002). Branch chain amino acids (BCAA) was selected to test for growth requirements of strains since these amino acids are highly relevant to substrates for cheese flavor development. Besides the essential

growth of *L. lactis* in at least the six amino acids such as glutamate, leucine, isoleucine, valine, histidine, and methionine (Wegmann *et al*., 2007), synthetic medium (CDM) was used as growth medium. Although in most cases complex and semi-defined media provided greater biomass yields than these of CDM medium. For this reason, the CDM that supports reasonable cell growth can be very useful in studies of gene regulation, protein expression, and metabolic fluxes (Zhang *et al*., 2009). Moreover, CDM medium are usually preferred in laboratory research since they permit one to determine the specific requirements for growth and product formation by systematically adding or eliminating chemical species from the formulation, with minimal complicated medium interactions and reproducible culture conditions. Defined media are thus well suited for fundamental studies of metabolism (Zhang and Greasham, 1999). Strains were cultivated in CDM medium under various kinds of BCAAs as following; without isoleucine, without leucine, without valine, and without all $BCAA_s$. The different kinds of BCAAs in the CDM medium were carried out to investigate the growth requirements for amino acids in the five strains compared with the LD61 strain which was the reference strain. The results showed that all these strains could grow at least in the modified CDMs medium. However, these results showed no correspondence between the physiological characteristics and global gene expression. Therefore, the physiological characteristics analysis and the global transcriptome led to the conclusion that the genome expression and physiological characteristics analysis should not link with these closely related sub-strains of *L. lactis* subsp. *lactis*. However, the growth requirement for specific amino acids could result from either the absence of the functional specific biosynthesis genes (Godon *et al*., 1993) or from the specific regulatory mechanisms (Chopin, 1993) or

the difference of the environment such as medium that used to cultivate the strains that may affect the growth ability.

From the study of comparative genomic hybridization and gene expression in the six *L. lactis* strains, the strains exhibited different gene expression under acidity condition, oxidative stress, and carbon limitation. These results showed that some strains could grow under the acidity condition and inhibit the pathogenesis since this strain was used for cheese manufacturing or dairy fermentation.

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CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

The six strains of *Lactococcus lactis* subsp. *lactis* isolates form the Génoferment collection were selected. They were isolated from various sources, and all of them showed a dairy phenotype. After these *L. lactis* strains were grown on skimmed raw milk ultrafiltration (UF) retentate, it was found that no significant difference in the final cell density was observed between the strains during growth in UF-cheese. However, phenotypic differences were detected when final cheese pHs were compared, since a 0.3 pH unit variation could lead to different cheese quality such as cheese flavor, cheese texture and cheese safety (Pandey *et al*., 2003). The other types of media which were synthetic medium (chemically defined medium; CDM), complex medium (M17) and modified synthetic medium (M13), were also studied for their ability to grow with glucose as a carbon source on media commonly used in laboratories. Strains also exhibited only weak differences in the final cell density and pH whereas none of the strains was able to grow on M13 medium.

According to the scanning of a whole genome for variations in DNA copy number, CGH analysis was used to determine the core genome (similar genomic content) of the six strains of *L. lactis* subsp. *lactis* exhibited a dairy phenotype. After CGH experiments and signal normalization, the intensities ratios were calculated between the tested strain and IL1403 strain in order to evaluate the genomic divergences between the strains. It was found that only a few genes had low ratios,

indicated strong similarities between the sequences of the six strains. The sequence of the reference LD61 strain was close to the sequence of IL1403 strain, while the five other strains shared diversity in the same regions. From the calculation with three statistical methods, PCR experiment and comparing of genome content, it was found that the six strains shared a large core genome of 1,915 genes, corresponding to more than 99% of the IL1403 ORFs spotted on the membranes.

The expression of the genes common to the six studied strains was compared under UF-cheese model conditions using DNA arrays corresponding to the IL1403 genome. After signal normalization, evaluative statistical significance of expression ratios using false discovery rate (FDR) calculations and a statistical threshold of 7%, the expression changed at the level of the functional (sub) categories was then further determined. The number of genes differentially expressed was observed for each strain. This investigation showed that a total of 968 different genes were differentially regulated in at least one of the five strains compared with the LD61 strain. For each strain, genes were equally partitioned between higher and lower expression levels compared with the reference strain. From the calculation of over- and under-expressed gene enrichments in the different groups using the Wilcoxon test, it was found that strong divergence of the five strains compared with the LD61 strain was observed within many regulated categories distributed throughout metabolism. Even if some (sub)categories showed a similar type of regulation for the five strains, most of the functional categories exhibited different regulation between the strains, underlining the large transcriptomic expression variability between the five strains. After a hierarchical clustering analysis of the expression data set of the 1,915 core genome genes of the five strains (S86, LD55, LL08, LL52 and UCMA5713), the strain

dendrogram based on gene expression ratios showed the strains grouping between the LD55 and the UCMA571 strains. In contrast to the LL08 strain, which was the most divergent compared with the other four strains.

To determine the physiological characteristics, therefore, the six of *L. lactis* strains were investigated for the nitrogenous bases and branched chain amino acids (BCAAs) requirements in CDM medium under various conditions as described above. Results showed that no major phenotypic difference could be detected between the strains at the level of base metabolism. The behaviour of the strains with regards to the BCAA requirement was strongly strain dependent. All the tested strains were able to grow in the absence of isoleucine, except the S86 strain could slightly grow on the medium without isoleucine.

Recommendation for further studies

The expression of the genes in the six studied strains was compared in UF-cheese model condition in order to establish the relationships of the different strains with the clustering analysis. However, it will be interesting to compare the gene expression and the clustering groups of the strains after cultivation in the CDM medium and compare them with the former results. This study will enable better understanding of the gene expression and the clustering analysis after the strain grown in the UF-cheese model compared with that in CDM medium.

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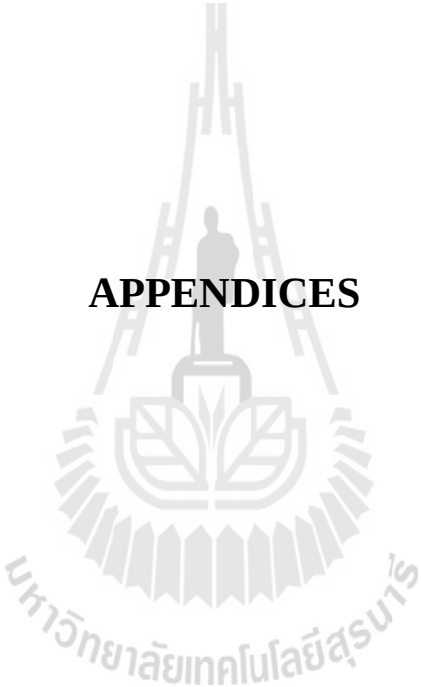
APPENDICES



APPENDIX A

CULTURE MEDIA AND REAGENTS

1. Culture media for growth of *L. lactis* subsp. *lactis* strains

1.1 Chemically defined media (CDM)

CDM was used for the cell cultivation and physiological characteristics analyse. All compounds were weighted individually and prepared with distilled water. All solutions were mixed in the order indicated (Solution 1 to Solution 8). The pH was maintained at 6.6 and the final volume was adjusted with distilled water. Afterwards, the culture media were sterilized by filtering through the membrane (0.2 µm; Sartorius). The culture media were prepared as described by Otto *et al*. (1983) and modified by Poolman & Konings (1988). It has composition as follows:

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1.1.1 Solution 1: Sugars and Salts1

Composition per liter (concentration 1X):

Glucose	10 g
Sodium acetate	1 g
Ammonium citrate	0.6 g
KH_2PO_4	9 g
K_2HPO_4	7.5 g

This solution was prepared at concentration 10X .

1.1.2 Solution 2: Salt2

Composition per liter (concentration 1X):

$MgCl_2 \cdot 6H_2O$	0.2 g
$FeSO_2 \cdot 7H_2O$	0.011 g
$CaCl_2 \cdot 2H_2O$	0.05 g
$ZnSO_4 \cdot 7H_2O$	0.005 g
$CoCl_2 \cdot 6H_2O$	0.0025 g

This solution was prepared at concentration 50X.

1.1.3 Solution 3: Amino acids

Composition per liter (concentration 1X):

Alanine	0.24 g
Arginine	0.12 g
Asparagine	0.34 g
Glutamine	0.51 g
Glycine	0.17 g
Histidine	0.11 g
Isoleucine	0.2 g
Leucine	0.47 g
Lysine	0.35 g
Methionine	0.12 g
Proline	0.68 g
Serine	0.34 g
Threonine	0.23 g
Tryptophane	0.05 g

Valine 0.33 g

This solution was prepared at concentration 20X.

Preparation of medium: To determine the growth ability of bacterial strains in the medium with and without branch chain amino acids, therefore, branch chain amino acids such as isoleucine, leucine or valine, were individually added.

1.1.4 Solution 4: Phenylalanine

Composition per liter (concentration 1X):

Phenylalanine 0.28 g

This solution was prepared at concentration 20X. A few drops of 37% HCl were added.

1.1.5 Solution 5: Tyrosine

Composition per liter (concentration 1X):

Tyrosine 0.29 g

This solution was prepared at concentration 20X. A few drops of 10N KOH were added.

1.1.6 Solution 6: Bases

Composition per liter (concentration 1X):

Adenine 0.01 g

Guanine	0.01 g
Inosine	0.005 g
Thymidine	0.005 g
Uracile	0.01 g
Xanthine	0.01 g

This solution was prepared at concentration 50X. A few drops of 10N KOH were added.

Preparation of medium: To determine the growth ability of bacterial strains in the medium with and without bases, therefore, purine bases (adenine, guanine, xanthine, and inosine) or pyrimidine (uracile and thymidine) were individually removed from the solution.

1.1.7 Solution 7: Vitamins

Composition per liter (concentration 1X):

para-aminobenzoic acid	0.01 g
Biotine	0.01 g
Cyano-cobalamine (B12)	0.001 g
Folic acid	0.001 g
Nicotinic acid	0.001 g
Orotic acid	0.005 g
Calcium-pantothenate	0.001 g
Pyridoxamine	0.005 g
Pyridoxine	0.002 g

Riboflavine (B2)	0.001 g
Thiamine	0.001 g
DL-6,8-thioctic acid	0.0025 g

This solution was prepared at concentration 20X.

1.1.8 Solution 8: Cysteine

Cysteine	0.17 g

This solution was prepared at concentration 20X.

1.2 M13 medium

M13 medium is a synthetic medium. All compounds were weighted individually and prepared with distilled water. All solutions were mixed in the order indicated (Solution 1 to Solution 3). The pH was maintained at pH 6.6 and the final volume was adjusted with distilled water. Afterwards, the culture media were sterilized by filtering through the membrane (0.2 µm; Sartorius). The culture media were prepared as described by Cocaign-Bousquet *et al.* (1995). It has composition as follows:

1.2.1 Solution 1: Sugars and Salts1

Composition per liter (concentration 1X):

Glucose	5 g
KH_2PO_4	4.5 g
K_2HPO_4	3.75 g

This solution was prepared at concentration 10X .

1.2.2 Solution 2: Salt2

Composition per liter (concentration 1X):

$MgCl_2.6H_2O$	0.1 g

This solution was prepared at concentration 50X.

1.2.3 Solution 3: Amino acids

Composition per liter (concentration 1X):

Glutamic acid	0.05 g
Isoleucine	0.1 g
Leucine	0.235 g
Methionine	0.06 g
Serine	0.17 g
Valine	0.165 g

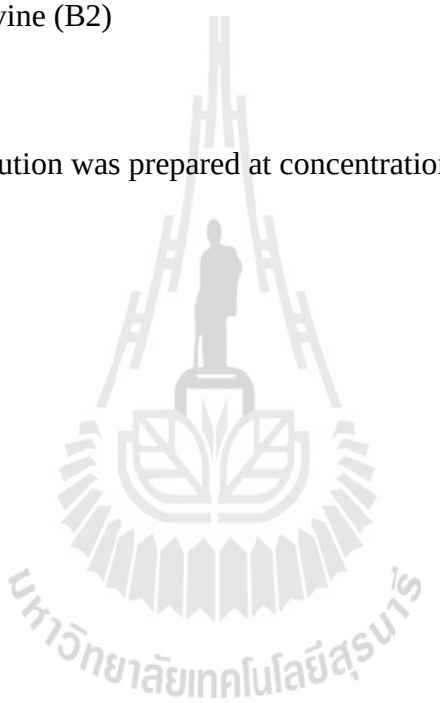
This solution was prepared at concentration 20X.

1.2.4 Solution 4: Vitamins

Composition per liter (concentration 1X):

Biotine	0.1 g
Nicotinic acid	0.01 g
Calcium-pantothenate	0.01 g
Pyridoxamine	0.05 g
Riboflavine (B2)	0.01 g

This solution was prepared at concentration 20X.



2. Reagents for gel electrophoresis

2.1 Agarose agar: 1.5% of agarose agar.

2.2 Loading buffer: 0.25% bromphenol blue, 0.25% Xylene cyanol FF and 40% (w/v) sucrose was diluted in Milli Q water.

2.3 Lysis buffer: 50 mM glucose, 25 mM Tris-HCl, and 10 mM EDTA

2.4 Phenol, TE saturated.

Dissolved phenol was transferred to 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH8

2.5 TE buffer: Ten mL of 1 M Tris-HCl, pH 8.0 was mixed with 0.2 mL of 0.5 M EDTA, pH 8.0 and made up volume to 1.0 L.

APPENDIX B

FORMULAR

The volume of inocula was determined to obtain an initial optical density at 600 nm of 0.1 as described by the follow formulas,

$$OD_i = OD_f/e^{\mu\Delta t} \quad \text{.....(1)}$$

OD_i = the initial OD_{600} unit of the culture

OD_f = the final OD_{600} unit of the culture

μ = specific growth rate of the interest culture (h^{-1})

Δt = the different of time between initial and final OD_{600} unit of the culture

$$V_iOD_i = V_fOD_f \quad \text{.....(2)}$$

V_i = the initial volume of the fresh culture

OD_i = the initial OD_{600} unit of the culture

V_f = the final volume of the needed culture

OD_f = the final OD_{600} unit of the culture

APPENDIX C

Table C1 Sequences of primer pairs for qPCR experiments table.

Gene	Sequences (5’ - 3’)	
	Forwards	Reverses
feoA	TCAGACGCCGCTTGATGGAC	AGTTCAAGAGGGTCGCCAAGTG
yaiA	CAGAAGAAGATGGGCATGGAGC	GTCGGTACACGGAATGAAGCAC
pyrR	GACCGTGGACATCGTGAATTGC	CATTGCCATCGTGTTCAGACATTTG
ipd	TGGCTGATGGCTATGCTCGTAC	CGGCGTAACTTCCTGCTAATCC
pbp2A	TGGAGCAGTCACAGGCAGATAC	GTCAGACCATACTTGCGTTCCG
smc	CGCAGTTGTCGGTCCAAATGG	ACGAAGTGCTTTAGCCGATTGC

APPENDIX D

Table D1 Expression ratios of the 1,915 genes of the core genome.

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
accB	1.53	3.56	3.52	2.43	4.14
accC	1.2	1.12	1.06	0.91	1.08
accD	1.11	1.13	1.2	0.96	1.04
ackA1	0.6	0.58	0.62	0.56	0.71
ackA2	0.71	0.82	1.11	0.7	1.17
acmA	1.28	0.94	1.18	1.56	1.21
acmB	0.84	0.84	0.75	0.63	0.88
acmC	1.28	1.02	1.16	1.37	0.99
acmD	0.84	0.74	0.48	0.92	0.67
acpA	1.17	0.93	0.79	0.74	1.14
acpD	1.47	1.06	1.08	1.03	1.15
adaA	1	0.9	0.77	0.81	0.61
add	1.18	0.95	0.7	0.77	0.85
adhA	1.45	1.18	0.45	0.83	1.15
adhE	1.48	1.2	0.47	1.24	1.27
adk	0.7	0.71	0.58	0.63	0.73
ahpC	0.93	0.71	0.65	0.84	1.09
ahpF	1.1	0.86	0.79	0.96	1.26
ahrC	1.89	1.6	1.34	1.65	1.66
alaS	1.11	1.16	0.83	0.9	0.87
aldB	1.04	1.05	1.28	0.69	1.16
aldR	1.14	1.6	1.47	1.09	1.59
als	1.1	1.26	2.35	0.91	1.18
amtB	0.94	1.1	0.55	0.68	0.51
amyL	1.13	1.04	1.1	1.11	1.51
ansB	1.05	0.92	0.89	1.12	1.31
apbE	1.16	0.83	1.23	1.02	1.08
apl	1.21	1	0.82	1.19	1.31
apt	1.15	0.87	1.01	1.06	1.04
apu	0.73	0.65	0.64	0.74	0.71
araT	0.62	0.56	0.92	0.49	1
arcA	1.12	0.58	0.89	1.77	1.18
arcB	1.49	0.93	1.28	2.51	1.32
arcC1	1.57	1.25	1.46	1.35	1.84
arcC2	1.2	1.05	1.06	1.11	1.13
arcC3	1.23	1.1	0.93	0.92	1.16
arcD1	1.12	0.91	0.94	1.19	1.29
arcD2	0.97	0.86	1.39	0.86	1.01

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
arcT	1.13	0.87	0.92	1.08	1.19
argB	1.18	1.6	3.32	1.4	1.44
argC	1.46	1.07	8.29	1.46	1.46
argD	1.43	1.44	2.65	1.24	1.45
argE	0.53	0.5	2.36	0.65	0.55
argF	1.05	0.96	1.03	1.06	1.42
argG	0.51	0.47	0.48	0.44	0.4
argH	0.42	0.57	0.47	0.45	0.33
argJ	0.99	1.65	7.18	1.51	1.09
argR	0.84	0.9	1.1	0.48	0.97
argS	0.82	0.67	0.99	0.68	0.66
aroA	1.01	1	1.15	0.82	0.87
aroB	1.2	0.85	0.89	1.06	1.19
aroC	0.95	0.83	1.16	0.61	1.14
aroD	0.82	0.86	1.02	0.6	0.91
aroE	1.33	1.07	1.14	1.15	1.16
aroH	0.48	0.43	0.71	0.43	0.48
arsC	0.69	1.29	0.96	0.96	1.29
asd	0.78	0.88	0.78	0.75	0.73
asnB	1	0.83	0.76	0.8	0.88
asnH	1.09	0.88	0.77	0.98	0.93
asnS	0.86	0.86	1.08	0.78	0.91
aspB	0.88	0.77	0.99	0.81	0.9
aspC	0.88	0.73	1.03	0.87	1.06
aspS	0.82	0.71	0.93	0.87	0.95
atpB	0.8	0.87	0.82	0.81	0.99
atpD	0.66	0.76	0.81	0.65	1.03
atpE	0.89	0.8	0.89	0.74	0.99
atpF	0.89	1.07	0.95	0.78	0.94
atpG	0.86	0.87	0.68	0.78	1
atpH	0.74	0.81	0.9	0.66	0.96
bacA	1.09	1.03	1.13	1.11	1.01
bar	1.21	1.04	0.91	0.98	1.56
bcaT	0.79	0.64	0.89	0.63	0.68
bglA	1.22	1.12	1.64	1.04	1.41
bglH	0.65	0.62	0.87	0.51	1.14
bglR	0.38	0.35	0.29	0.4	1.16
bglS	1.23	1.18	1.29	1.02	1.33
birA1	1.22	1.25	0.95	1.37	1.21
birA2	1.02	1.17	0.75	0.87	1.09
blt	1.15	1.16	0.91	0.78	0.67
bmpA	0.77	0.67	0.55	1.02	0.9
busAA	0.72	0.92	0.7	0.61	0.75
busAB	0.6	0.8	0.78	0.67	0.84
busR	0.93	1.01	0.94	0.82	0.96
butA	0.92	1.01	1.34	0.56	0.74
butB	0.43	0.53	0.64	0.32	0.65

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
cadA	0.41	0.51	1.46	0.49	1.23
carA	0.3	0.39	0.69	0.24	0.38
carB	0.74	0.49	0.59	0.47	0.52
cbr	1.28	1.2	1.53	1.59	1.26
ccpA	0.88	1.01	1.14	1.08	0.75
cdd	1.2	1.47	1.22	1.25	1.14
cdsA	1.48	1.38	0.98	1.53	0.71
celB	1.66	1.32	2.8	0.93	1.97
ceo	0.67	0.93	0.58	0.43	1
chiA	1.25	1.97	2.54	0.8	1.77
choS	0.84	0.8	1.04	0.7	1.49
citB	1.37	1.08	1.38	1.03	0.98
citC	1.2	1.14	3.8	1.17	1.19
citD	1.28	1.5	9.16	1.63	1.57
citE	0.84	1.02	8.03	1.18	1.03
citF	0.94	0.85	5.73	1.06	0.87
clpB	1.02	1.54	0.76	1.43	1.12
clpC	1.07	1.11	0.98	0.76	1.24
clpE	0.74	0.85	0.78	0.63	0.91
clpP	0.65	0.95	0.81	0.51	0.94
clpX	1	0.83	1.02	0.9	1.21
clsA	0.77	0.75	0.98	0.7	1.09
clsB	0.94	0.88	1.1	0.75	1.13
cmk	1.22	0.95	0.83	0.97	1.05
coaA	0.77	0.82	1.01	0.94	0.79
cobC	1.02	1.01	1.04	1.23	1.01
cobQ	1.04	0.9	0.97	1.06	0.93
codY	0.91	0.88	0.93	0.93	0.96
codZ	1.26	1.13	0.91	1.16	1.08
coiA	1.99	3.2	2.11	2.53	1.46
comC	1.2	1.32	1.16	1.16	1.18
comEA	0.88	0.91	0.74	0.93	0.95
comEC	0.93	0.94	0.72	0.83	1.02
comFA	1.28	1.07	0.95	1.1	1.47
comFC	0.95	1.54	1	1.16	1.97
comGA	1.17	0.87	0.97	0.97	1.42
comGB	0.92	0.93	0.85	0.97	1.1
comGC	0.89	0.83	0.76	1.03	1.07
comGD	1.03	0.77	0.71	1.2	1.26
comX	0.89	0.91	0.81	0.83	1.45
copB	5.61	1.6	2.84	0.99	2.06
copR	1.03	0.94	0.79	0.61	1.15
cpo	1.04	0.83	0.91	0.7	1.06
cpsM	1.4	0.99	1.08	1.17	1.18
crtK	1.59	1.84	2.47	1.01	3.02
cshA	0.99	0.92	1.18	0.78	0.94
cspE	1.22	1.03	0.84	0.92	1.09

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
cstA	3.24	3.74	5.37	2.86	1.45
ctrA	1.18	1.1	0.78	0.8	0.86
ctsR	0.91	1.09	0.85	0.79	1.34
cydA	0.81	0.88	0.57	0.61	0.82
cydC	1	1.22	1.01	0.82	0.96
cydD	1.25	1.45	1.24	1.03	1.02
cysD	1.74	1.46	1.09	1.78	1.12
cysE	1.1	1.02	1.02	0.96	1.06
cysK	1.89	1.49	2.12	1.69	1.55
cysM	1.12	1.01	1.78	0.95	0.71
cysS	1.01	0.86	0.89	1.09	0.94
dacA	1.3	0.93	0.86	1.32	1.27
dacB	1.03	0.97	1.23	1	0.86
dal	1.09	1.19	0.9	1.12	1.05
dapA	1.26	1.22	0.99	1.26	1.31
dapB	0.93	0.94	0.75	0.87	0.99
dcdA	1.05	0.94	0.92	1.13	0.97
ddl	1.29	1.28	1.23	1.09	1
def	1.38	1.49	0.95	1.11	1.35
deoB	0.97	0.99	1.09	0.8	1.06
deoC	1.06	0.95	1.07	0.86	0.91
deoD	1.11	1.08	1.72	0.86	1.08
dexB	1.13	0.92	0.95	1.18	1.2
dfpB	1.01	0.99	1	0.99	1.17
dfrA	0.81	0.8	0.95	0.93	0.79
dgkA	1.06	0.99	0.98	1.14	1.1
dhaK	0.96	1	1.07	0.89	0.96
dhaL	0.52	0.32	0.93	0.38	1.15
dhaM	0.67	0.58	0.76	0.63	1.03
dinF	0.93	0.96	1.25	0.97	0.86
dltC	0.32	0.35	0.5	0.27	0.46
dnaC	0.96	1.12	0.97	0.77	0.84
dnaD	1.34	1.32	1.11	1.69	0.97
dnaE	0.96	1.1	1.44	1.02	0.9
dnaH	1.38	1.11	1.07	1.33	1.34
dnaJ	1.21	0.96	0.85	1.11	0.93
dnaK	2.41	2.67	1.32	2.28	1.27
dnaN	0.74	0.68	0.82	0.72	0.82
dnaQ	1.06	0.97	0.83	1.08	0.83
dpsA	1.31	1.15	1	1.1	1.23
dukA	0.99	0.9	0.92	0.97	1.03
dukB	0.78	1	0.85	0.71	0.82
dut	1.05	1.22	1.08	0.84	1.13
dxsA	1.07	1.05	0.87	0.99	1.15
dxsB	1.44	1.59	1.58	1.11	0.9
ecsB	0.93	1.02	1.06	0.87	0.82
efp	0.92	0.81	1.02	0.96	0.81

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
enoA	1.17	0.96	1.09	1.28	1.19
enoB	1.31	1.16	1	1.4	1.15
eraL	0.93	0.77	0.85	1.27	0.78
exoA	1.21	1.1	1.13	0.66	0.89
ezrA	0.88	0.82	0.95	0.84	0.67
fabF	1.36	1.41	1.46	0.91	1.44
fabG1	1.38	1.07	1.16	0.95	1.09
fabG2	0.92	0.96	1.13	1.12	1.31
fabI	1.12	0.99	0.94	1.06	0.96
fabZ1	1.14	0.98	1.02	1.52	1.02
fabZ2	0.95	1.05	1.12	0.95	1.21
fadA	1.02	1.05	1.25	1.86	1.65
fadD	1.35	1.28	1.07	1.73	1
fbaA	1.2	0.96	0.84	1.09	1.03
fbp	0.75	0.96	0.97	0.85	0.95
femD	1.21	1.22	0.98	1.11	1.02
feoA	3.5	3.27	1.05	4.92	0.98
feoB	2.17	1.88	0.89	2.12	0.92
fer	0.65	0.71	0.65	0.94	0.75
ffh	1.17	0.8	0.99	0.91	0.97
fhs	0.65	0.7	0.8	0.73	0.82
fhuB	1.11	0.79	0.73	1.35	0.91
fhuD	1.38	1.23	1.01	1.22	1.16
fhuG	1.3	1.12	0.84	1.17	0.98
fhuR	0.85	0.71	0.81	0.84	0.82
fmt	1.46	1.5	1.18	1.62	1.46
folB	1.01	0.94	0.93	0.94	0.86
folC	1.22	0.87	0.76	1.04	1.02
folD	1.28	0.95	1.12	1.43	1.01
folE	1.19	1.17	1.03	0.83	1
folP	1.01	1.05	1.04	0.67	0.9
frdC	0.84	0.64	0.89	0.68	0.89
frr	1.15	0.88	0.88	1	0.83
ftsA	1.04	0.85	0.89	1.04	0.82
ftsE	1.06	0.88	0.85	1.14	0.8
ftsH	1.34	0.98	0.88	1.07	1
ftsW1	1	1.14	0.79	1.08	0.87
ftsW2	0.19	0.19	0.58	0.39	0.88
ftsX	1.18	1.11	0.99	1.07	0.79
ftsY	1.12	1.05	1.01	0.87	1.07
ftsZ	0.95	0.99	0.99	0.94	0.79
fur	1.02	1.02	1.14	0.85	1.03
fusA	1.06	0.93	1.01	0.97	1.06
gadB	1.49	0.86	0.7	0.89	0.91
gadC	1.48	0.71	0.41	0.73	0.95
gadR	1.18	1	1.14	1.08	1.06
galE	1.31	0.76	1.33	1.45	1.02

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
galK	2.72	1.45	3.97	3.05	2.3
galM	2.34	1.69	3.58	2.3	3.57
galT	1.63	1	2.1	1.68	1.26
gapA	1.51	1.73	1.27	1.12	1.66
gapB	1.42	1.36	1.32	1.54	1.27
gatB	1.11	1.08	1.24	0.87	0.92
gatC	0.9	0.91	1.18	0.84	0.98
gcp	1.26	1.16	0.99	1.21	0.95
gidA	0.89	1.15	1.05	0.81	0.75
gidB	2.23	1.71	1.49	2.06	1.25
gidC	0.88	0.94	0.82	0.73	0.7
glgA	0.59	0.64	0.58	0.43	0.57
glgC	0.55	0.61	0.75	0.4	0.54
glgD	0.69	0.69	0.84	0.36	0.75
glgP	0.48	0.58	0.53	0.4	0.61
glk	1.46	1.26	0.91	1.27	0.83
glmS	0.69	0.83	1.61	0.7	0.69
glmU	1.01	0.99	0.96	1.01	0.96
glnA	1.03	0.84	0.66	0.87	0.56
glnB	1.01	1.26	0.85	0.78	0.7
glnP	0.94	0.97	0.94	0.7	0.66
glnR	0.71	0.66	0.5	0.75	0.47
glpD	0.69	0.89	0.67	0.58	0.79
glpF1	0.71	0.76	1.04	0.94	0.84
glpF2	0.73	0.84	0.73	0.82	0.96
glpK	0.74	0.88	0.69	0.79	0.88
glpT	0.59	0.58	0.5	0.61	0.58
gltA	1.09	0.92	1.25	1	0.74
gltD	0.99	1.17	0.66	0.97	1.18
gltQ	0.92	0.92	1.84	0.87	0.68
gltS	0.43	0.36	2.15	0.38	0.32
glyA	1.02	1.06	1	0.87	0.83
glyS	1.39	1.32	1.02	1.28	0.69
gnd	1.46	1.38	1.28	1.41	1.07
gntK	1.07	1.15	1.31	1	1.11
gntR	0.99	1.14	1.26	0.94	1.11
gntZ	1.03	1.47	1.42	1.13	0.98
gpdA	0.92	0.87	1.03	1.06	0.87
gpo	0.86	0.73	1.08	0.77	1.38
greA	1.55	1.39	2.81	1.2	1.26
groES	1.46	1.23	0.7	1.03	1.24
grpE	2.2	2.24	1.09	1.59	1.08
gshR	0.83	0.96	1.02	0.54	0.85
guaA	1.25	1.19	1	1.23	0.74
guaB	1.04	0.93	0.82	0.93	0.54
guaC	0.57	0.55	0.48	0.96	0.65
gyrA	1.08	0.91	0.8	1.22	0.85

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
gyrB	0.91	0.91	0.8	1.18	0.72
hemH	0.88	0.78	0.93	1.03	0.66
hemK	1.06	1.21	1.09	0.74	0.88
hemN	0.99	1.06	1.27	1.03	0.83
hexA	0.84	0.88	0.99	0.97	0.92
hexB	1.03	1.05	1.04	1.13	0.8
hflX	1.4	1.41	1.42	1.43	0.94
hisA	0.88	0.81	0.58	0.77	0.64
hisB	0.77	0.68	0.66	0.89	0.93
hisC	0.6	0.56	0.3	1.04	0.56
hisD	0.86	0.79	0.65	0.64	0.92
hisG	0.85	0.9	0.64	0.67	0.83
hisH	0.87	0.99	0.81	0.72	0.93
hisI	0.73	0.76	0.67	0.63	0.89
hisK	0.65	0.78	0.59	0.7	0.76
hisS	1.02	1.18	0.99	1.13	0.73
hisZ	0.66	0.71	0.52	0.61	0.59
hly	0.77	0.88	0.95	0.82	0.66
hmcM	1.84	2.28	2.17	1.86	1.27
holB	1.24	1.06	0.9	1.27	0.84
hom	0.96	1.1	0.84	0.89	0.56
hprT	1.11	1	0.95	1.11	0.96
hpt	1.17	0.95	0.82	1.29	0.92
hrcA	1.38	1.57	0.96	1.22	1.18
hsdM	0.34	0.39	1.58	0.33	0.3
hsdR	0.97	0.8	0.94	0.75	0.72
hsdS	0.77	1.45	2.24	1.03	0.43
hslA	0.92	0.7	0.89	0.87	1.02
hslB	2.17	1.72	1.01	0.99	1.15
htrA	0.78	0.84	0.67	0.75	0.59
icaA	0.84	1.62	0.85	0.83	0.82
icaC	0.91	1.32	0.96	1.01	0.62
icd	1.43	1.28	1.6	0.93	0.93
ileS	1.41	1.35	0.81	1.35	0.85
ilvB	0.83	0.85	0.75	0.8	0.61
ilvC	0.48	0.7	0.66	0.55	0.49
ilvD	0.37	0.54	0.46	0.32	0.4
ilvN	0.73	0.83	0.77	0.79	0.66
infA	0.83	0.81	0.97	1.07	1.03
infB	1.1	1.11	1.26	1.06	0.76
infC	1.1	1.07	1.68	1.21	0.81
ipd	0.14	0.21	0.43	0.15	0.1
ispA	1.22	1.19	1.07	1.38	0.89
ispB	1.52	1.18	0.93	1.31	0.9
kdgA	0.7	0.72	0.89	0.72	1.03
kdgK	1.07	0.99	0.82	1.27	1

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
kdtB	1.11	1.1	0.92	0.82	0.95
kinA	1.03	1.31	1.07	1.02	1
kinB	0.78	0.9	1.06	0.74	0.71
kinC	1.14	1.12	0.93	1.13	1.03
kinD	1.26	1.13	1.13	1.28	0.89
kinE	0.96	0.96	0.96	1.2	0.95
kinF	2.28	2.28	1.89	1.58	0.77
ksgA	1.63	1.27	1.23	1.54	1.16
kupA	0.72	1.11	1.19	0.88	0.83
kupB	1.12	1.51	0.81	1.16	0.99
lacC	2.2	3	2.75	1.78	1.9
lacR	1.2	1.78	1.86	1.16	1.08
lacZ	1.82	1.45	2.4	2.07	1.76
lcnC	0.5	0.32	0.65	0.61	0.66
lcnD	0.44	0.57	0.83	0.47	0.85
lctO	0.77	0.78	0.78	0.86	0.92
ldh	1.16	1.43	2.15	1.3	1.11
ldhB	1.03	0.94	0.95	1.14	0.8
ldhX	1.16	1.14	1.16	1.31	1.03
lepA	0.88	0.98	0.92	0.91	0.66
leuB	0.6	0.65	0.65	0.52	0.52
leuC	0.45	0.41	0.58	0.33	0.41
leuD	0.62	0.57	0.68	0.75	0.76
leuS	1.11	1.32	1.11	1.25	0.72
lgt	1.07	1.41	1.51	1.33	1.01
ligA	0.5	0.46	0.58	0.62	0.58
llrA	1.08	1.21	1.23	0.77	1.04
llrB	0.65	1	1.1	0.82	0.73
llrC	0.76	0.84	0.97	0.92	0.71
llrD	1.44	1.31	1.12	1.49	0.99
llrE	0.77	0.86	0.91	1.07	0.78
llrF	1.62	2	1.55	1.27	1.08
llrG	1.03	1.2	0.93	0.81	0.83
llrH	0.71	0.29	0.3	0.69	0.58
lmrA	0.71	0.77	0.59	0.77	0.67
lnbA	0.99	0.77	0.8	0.75	0.85
lplL	1.66	1.44	2.96	1.89	1.18
lspA	0.84	0.89	1	0.97	0.87
lysA	0.65	0.69	0.69	0.51	0.69
lysP	0.85	0.83	0.78	0.75	0.88
lysQ	0.4	0.37	0.73	0.25	0.42
lysS	0.67	0.74	1.11	0.92	0.9
mae	1.04	0.98	2.98	1.22	1.19
malQ	1.24	1	1.12	0.88	1.08
menB	1.17	1.15	0.95	1.15	1.09
menD	1.2	1.23	1.16	0.95	1.26

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
menE	0.98	1.13	0.92	1.16	1.11
menF	1.2	1.36	1.26	0.91	1.18
menX	1.52	1.66	1.8	1.24	1.37
mesJ	1.8	1.38	0.93	1.91	1.25
metB1	1.05	0.93	1.23	0.98	1.52
metB2	1.9	1.71	2.52	1.45	1.38
metE	0.48	0.46	0.28	0.37	0.54
metF	0.42	0.52	0.37	0.48	0.59
metK	0.95	1.09	1.41	0.98	1.13
metS	0.7	0.73	0.8	0.67	0.77
mgtA	0.43	0.35	0.47	0.36	0.34
miaA	0.7	0.53	0.66	0.59	0.65
mleR	0.91	0.8	0.77	0.89	0.86
mleS	1.15	1.22	1.38	0.77	1.2
mreC	1.05	0.87	0.88	1.14	1.03
mreD	0.89	1.02	0.82	0.84	0.84
mscL	1.72	2.07	1.9	1.28	2.1
msmK	1.12	1.14	1.16	0.79	0.97
mtlD	0.91	1.29	0.97	0.8	0.84
mtlF	0.93	1.01	0.85	0.81	1.15
mtlR	0.92	0.99	1.02	0.98	1.14
mtsA	2.12	2.14	0.65	2.43	1.46
mtsB	1.7	1.6	0.64	2.35	1.32
mtsC	2.21	2.57	0.56	2.6	1.44
murA1	1.26	1.18	1.03	1.42	1.29
murA2	0.98	0.81	0.82	1.06	1.01
murB	1.35	1.22	1.05	1.4	1.14
murC	1.55	1.28	1.06	1.28	1.16
murD	1.13	1	0.93	0.92	1.04
murE	0.98	0.92	0.84	0.92	0.98
murF	1.05	1.13	1.07	1.09	1.04
murI	0.76	0.71	0.62	0.83	0.84
mutM	1.46	0.95	1.37	0.92	1.35
mutS	1.31	1	1.27	1.38	1.24
mutX	1.09	0.88	1.06	1.04	1.14
mvaA	1.33	0.89	0.75	1.28	1.02
mycA	1.2	1.71	1.1	1.48	1.06
nadE	1.29	1.23	1.31	0.8	1.01
nadR	1.1	1.1	1.13	1.04	1.32
nagA	1.54	1.34	1.06	1.32	1.17
nagB	1.14	1.27	0.89	1	1.32
nah	0.88	0.94	1.1	0.85	1.19
napB	1.07	0.97	1.26	0.82	1.09
napC	0.63	0.51	0.57	0.85	0.74
ndrH	0.7	0.88	0.75	0.39	0.8
ndrI	0.63	0.74	0.71	0.45	0.75
nifS	0.92	0.95	0.83	0.92	0.89

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
nifU	0.9	0.91	0.73	0.85	0.87
nifZ	1.28	1.4	0.9	1.55	1.13
noxA	0.9	0.78	0.8	0.72	0.62
noxB	1.25	1.06	0.87	1.2	0.77
noxC	1.74	1.74	0.92	1.58	1.38
noxD	1.8	2.14	1.15	1.71	0.92
noxE	1.25	1.03	0.33	1.62	1.38
nrdD	0.63	0.55	0.61	0.5	0.63
nrdE	0.77	0.83	0.72	0.53	0.76
nrdF	0.88	0.85	0.75	0.78	0.89
nrdG	1.07	0.92	1.07	0.86	0.7
nth	2.28	2.11	1.3	3.23	1.63
nucA	0.64	0.77	0.68	0.54	0.71
nusA	0.94	0.91	0.86	0.86	0.81
nusB	1.54	1.43	1.42	1.28	1.08
nusG	1.05	1	1.23	0.94	0.88
obgL	1.05	1.18	0.92	1.27	1.06
ogt	0.83	0.86	0.76	0.81	0.76
oppA	0.52	0.69	0.49	0.36	0.78
oppB	0.74	0.71	0.71	0.54	1.24
oppC	0.74	0.75	0.72	0.55	1.13
oppD	0.65	0.65	0.83	0.71	1.05
oppF	0.82	0.86	0.71	0.66	1.1
optA	1.46	1.09	0.61	0.88	0.93
optB	0.86	1	0.52	0.7	0.82
optC	0.53	0.78	0.42	0.5	0.74
optD	0.83	1.19	0.54	0.68	0.75
optF	0.71	1.03	0.44	0.62	0.72
optS	1.16	1.1	0.79	1.02	0.97
osmC	0.79	0.85	0.45	0.8	1.16
otcA	1.13	1.38	1.09	1.47	1.95
pabB	1	0.95	0.78	1.02	1.07
pacB	0.93	0.85	0.83	0.97	0.93
pacL	0.97	0.89	0.76	0.99	1.14
panE	0.98	0.97	0.67	0.75	1.44
papL	0.9	0.93	0.89	0.98	0.92
parA	1.79	1.84	1.02	1.47	1.02
parC	1.14	1.2	0.99	1.08	0.83
parE	1.08	0.88	1.15	0.73	0.8
pbp1B	0.92	0.97	1.11	0.97	1
pbp2A	1.1	1	1.04	0.96	1.13
pbp2B	1.3	0.93	1	1.24	1.26
pbpX	1.4	1.08	0.85	1.23	1
pbuX	0.64	0.72	0.55	0.61	0.6
pcaC	1.28	1.08	0.92	1.27	1.38
pcrA	0.84	0.83	0.85	0.9	0.69

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pdc	0.83	0.97	0.85	0.68	0.95
pdhA	0.54	0.74	0.93	0.65	0.9
pdhB	1.07	1.19	1.26	0.92	1.07
pdhC	0.7	0.92	1.1	0.75	1.01
pdhD	0.87	1	1.11	0.91	1.12
pdp	1.1	1.07	1.19	0.98	1.2
pepC	0.73	0.73	1	0.8	0.8
pepDA	1.11	0.68	0.91	0.99	0.98
pepDB	1.52	1.31	1.37	0.98	1.36
pepF	1.19	1.35	1.11	1.07	0.92
pepM	0.96	0.97	0.99	1.09	0.93
pepN	0.9	0.94	0.93	0.92	1.12
pepO	0.58	0.75	0.63	0.5	0.81
pepP	0.84	0.95	1.21	0.57	1
pepT	1.63	1.28	1.34	1.3	1.57
pepV	0.82	0.96	0.96	0.64	0.86
pepXP	2.41	2.13	2.45	1.87	1.16
pfl	0.68	0.62	0.69	0.71	0.54
pflA	1.13	0.93	0.96	1.03	0.93
pfs	1.03	1.21	0.88	1.25	1.15
pgk	1.14	1.04	1.03	0.91	1.08
pgmB	0.79	0.88	0.86	0.7	0.87
pgsA	1.16	1.1	1	0.9	0.78
pheA	1.14	1.25	0.89	0.8	0.85
pheS	1.13	1.24	1.01	0.93	1.17
pheT	1.33	1.21	1.11	1.1	1.06
phnA	3.01	2.39	1.05	2.52	1.12
phnB	0.86	0.72	0.84	0.84	0.93
phnC	0.64	0.52	0.69	0.72	0.66
phnE	0.64	0.6	0.89	0.72	0.7
phoL	0.99	1.02	1.04	0.99	0.87
phoU	1.11	1.17	1.1	0.87	1.13
pi101	1.6	1.26	2.41	1.01	2.73
pi102	1.14	0.92	2.88	1.93	1.06
pi103	1.17	1.29	1.98	1.37	0.91
pi104	1.24	0.86	0.77	1.22	1.05
pi105	1.32	1.01	0.91	0.88	3.35
pi106	0.69	0.78	1.01	0.95	0.82
pi107	1.15	1.13	2.19	1.13	1.3
pi108	1.56	1.21	1.15	1.38	1.52
pi109	1.19	0.97	1.54	1.24	1.52
pi110	1.24	1.01	0.81	1.1	1.04
pi111	1.29	1.31	1.09	1.3	1.14
pi113	1.52	0.83	0.95	1.03	1.33
pi114	1.22	1	0.89	1.22	1.1
pi115	1.16	0.91	0.93	1.25	1.22
pi116	1.12	0.89	0.75	1.21	1.42

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pi117	0.57	0.51	0.44	0.44	0.58
pi118	1.08	1.08	1.03	0.95	0.92
pi120	0.5	0.54	0.45	0.47	0.56
pi122	0.9	1.06	1.04	0.88	0.86
pi123	0.69	0.71	1	0.77	0.75
pi124	1.15	0.8	0.91	0.88	0.94
pi125	0.07	0.05	2.01	0.06	0.07
pi127	0.73	0.6	0.96	0.7	1.08
pi128	1.4	1.08	1.48	1.28	1.63
pi129	1.54	1.42	1.13	1.55	1.43
pi130	1.02	0.91	1.16	0.87	1.02
pi133	1.52	1.12	0.91	1.31	1.37
pi135	1.22	0.99	0.93	1.07	1.21
pi137	0.79	0.9	0.87	0.77	0.86
pi138	0.96	0.89	1.01	1.01	0.96
pi139	1.19	1.17	1.1	1.27	1.44
pi140	1.01	0.97	0.86	0.93	0.97
pi141	1.13	1.22	1.19	1.4	1.44
pi142	1.46	0.83	0.77	1	1.33
pi143	1.09	0.99	1.03	1.12	1.21
pi144	1.12	1.08	0.95	1.03	1.1
pi145	1.11	1.18	3.47	0.9	0.82
pi205	0.85	1.08	1.25	1.05	0.85
pi208	0.09	0.1	0.47	0.9	0.46
pi209	0.78	1.14	0.78	0.78	0.87
pi210	0.25	0.37	0.31	0.28	0.37
pi211	0.49	0.65	0.94	0.59	0.68
pi215	0.8	0.93	0.56	0.85	1.1
pi216	0.84	1.03	1	1	0.85
pi217	1.09	0.95	0.85	1.05	1.06
pi218	1.05	0.77	0.74	1.09	1.19
pi222	0.49	0.56	0.52	0.47	0.67
pi223	0.72	0.8	1.2	0.73	0.86
pi224	1.05	1.02	1.17	1.16	1.27
pi227	1.15	1.23	1.38	1.12	1.28
pi228	1.45	1.12	0.95	1.11	1.07
pi229	1.1	1.06	0.98	1.38	0.93
pi230	0.98	0.89	0.91	1.17	1.15
pi231	1.14	1.15	0.88	1.3	0.88
pi232	1.22	1.27	1.08	1.14	0.98
pi233	1.27	1.08	0.85	1.39	1.44
pi234	1.04	0.8	0.8	1.03	1.27
pi235	1.41	1.03	0.96	0.99	1.09
pi236	1.14	1.01	0.99	1.24	1.18
pi237	1.55	1.37	1.1	1.35	0.82
pi238	0.89	1.12	1.07	1	0.95
pi239	1.3	1.22	1.09	1.14	1.27

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pi240	1.01	0.98	0.94	0.97	1.07
pi241	1.29	1.05	0.85	1	1.13
pi242	1.06	1.11	1.22	1.04	1.11
pi243	1.46	1.18	1.15	1.52	1.46
pi244	1.12	1.11	1.22	1.47	1.45
pi245	1.33	1.13	0.9	1.56	1.3
pi246	1.12	1.08	0.9	1.34	0.97
pi247	0.98	0.75	0.76	1	0.88
pi248	1.09	1.2	1.07	1.03	1.05
pi249	1.05	0.95	1.1	1.15	1.46
pi251	1.03	1.07	1.24	0.81	1.3
pi301	0.54	0.62	0.59	0.63	0.6
pi302	0.82	0.87	0.86	0.74	0.97
pi303	1	0.66	0.86	1.07	1.47
pi307	1.26	1.27	1.02	1.1	0.79
pi308	1.07	1.05	1.09	0.84	1.32
pi316	0.81	0.78	0.72	0.78	0.83
pi317	1.08	0.93	1	1.22	1.2
pi318	1.18	0.97	0.88	1.34	1.21
pi319	1.18	1	1.01	1.46	1.65
pi320	1.16	1.21	1	1.43	1.39
pi321	1.05	1.18	1.04	1.02	1.35
pi322	1.18	1.31	1.11	1.3	1.64
pi323	1.19	1.08	0.99	1.14	1.31
pi324	1.2	1.21	1.11	1.32	1.22
pi325	1.02	0.97	0.8	0.93	0.85
pi326	1.13	1.16	0.91	1.04	0.75
pi327	1.13	1.07	1.19	1.01	1.25
pi328	1.1	1.18	0.97	1.02	1.18
pi329	1.11	1.16	1.21	1.38	1.38
pi330	1.17	0.95	0.9	1.12	1.11
pi331	1.22	1.02	0.98	1.37	1.13
pi333	0.7	0.94	0.76	0.86	1.06
pi334	1.04	0.86	0.8	1.03	1
pi336	0.75	1.19	0.84	0.72	0.93
pi337	0.6	0.57	0.55	0.59	0.64
pi338	1.15	1.07	0.83	1.35	1.18
pi339	0.95	1	0.83	0.98	1.27
pi341	0.8	0.94	1.03	0.88	1.05
pi343	1.31	0.94	0.82	0.99	0.96
pi345	0.99	1.08	0.88	1.13	1.07
pi347	1.03	1.16	1.03	0.95	1.14
pi348	0.52	0.55	0.56	0.59	0.6
pi349	0.35	0.35	0.29	0.33	0.39
pi350	0.94	1.09	0.88	1.09	0.95
pi353	0.4	0.38	0.39	0.41	0.53
pi354	0.91	1.01	0.85	1.11	1.47

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pi355	1.21	1.73	1.19	1.6	1.01
pi356	0.96	0.65	0.65	1.14	1.12
pi357	1	0.87	0.81	1.29	1.06
pi358	1.93	1.33	1.82	3.27	1.37
pi359	0.14	0.16	0.17	0.15	0.16
pi360	0.84	1	0.98	3.79	2.02
pip	1.04	1.17	1.4	1.03	0.72
pknB	1.2	1.06	0.88	1.23	0.82
plpA	1.26	1.14	1.51	1.12	0.73
plpB	1.49	1.49	1.78	1.16	1
plpC	1.43	1.46	1.49	1.22	0.97
plpD	1.19	1.07	1.24	1.21	0.96
plsX	1.08	0.99	0.99	0.89	1.15
pmg	0.95	0.78	0.79	0.84	0.73
pmpA	0.71	0.79	0.86	0.81	0.55
pmrA	1.09	1.25	0.79	0.91	1.08
pmsR	0.85	0.89	1.22	0.71	0.94
pmsX	1.34	1.37	1.19	1.06	1.53
pnpA	1.37	0.95	1.11	1.26	0.74
pnuC2	1.25	1.25	0.92	1.27	0.95
polA	1.26	1.19	1.06	1.14	1.4
polC	0.91	1.01	1.14	1.01	1
ponA	1.25	1.01	1.08	0.92	1.02
potA	1.24	1.11	1.33	1.08	0.88
potB	1.12	0.98	1.3	0.98	0.9
potC	0.8	0.89	1.08	0.67	0.62
potD	1.19	1.14	1.7	0.82	0.93
poxL	0.58	0.51	0.47	0.52	0.96
ppiA	0.82	0.78	0.91	0.79	0.56
ppiB	1.57	1.14	0.78	1.57	1.77
preA	1.15	1.17	0.84	0.68	1.25
prfA	1.43	1.33	1.11	1.43	1.08
prfB	1.01	0.98	1.26	1.06	1.11
prfC	0.99	1.05	1.16	0.97	0.73
prmA	1	0.9	0.98	0.86	0.98
proA	0.91	0.89	0.99	0.72	0.7
proB	0.84	0.72	0.85	0.76	0.63
proC	0.94	1.09	0.93	0.65	0.82
proS	0.94	0.98	1.04	0.97	0.76
prsA	1.11	1.13	0.87	1.34	0.92
prsB	1.13	0.85	0.89	1.22	0.88
ps101	1.37	1.17	1.13	1.78	1.39
ps102	0.98	1.35	1.36	1.43	0.75
ps104	1.13	1.3	1.05	1.14	1.22
ps105	1.25	1.15	0.81	1.14	0.99
ps106	1.18	1.81	0.85	1.26	1.18
ps107	1.16	1.9	1.11	1.13	1.06

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ps108	0.89	0.62	0.56	0.92	0.94
ps109	1.05	0.93	0.86	0.98	1.2
ps110	1.1	0.99	0.9	1.02	1.31
ps111	0.92	1.07	0.92	1.14	1.33
ps112	1.34	1.39	1.22	1.44	1.22
ps113	1.09	1.09	1.02	1.21	1.14
ps114	1.33	1.07	1.03	1.22	1.06
ps115	1.03	1.15	1.13	1.52	1.22
ps116	1.5	1.38	1.27	1.79	1.62
ps117	0.72	0.84	2.17	1.64	1.09
ps118	5.76	2.46	1.86	3.79	3.68
ps119	1.32	1.15	1.51	1.55	1.52
ps120	0.92	1.03	1.76	1.33	1.44
ps121	2.05	2.08	1.61	1.55	1.46
ps122	1.21	1.23	1.17	1.42	0.84
ps123	1.23	1.13	0.96	1.07	0.57
ps201	1.76	1.38	1.62	1.92	2.2
ps202	2.04	1.09	1.98	1.16	1.08
ps203	0.72	0.3	0.7	0.2	0.91
ps205	1.51	1.25	1.6	1.39	1.28
ps206	1.1	0.87	1.01	0.95	1.13
ps207	1.46	0.98	0.88	1.23	1.51
ps209	1.06	1.72	0.85	0.95	1.04
ps211	0.99	1.16	0.85	1	1.15
ps212	0.99	0.88	0.9	1.01	1.29
ps213	1.51	1.29	1.43	1.08	1.21
ps214	1.22	1.05	0.96	1.14	0.93
ps216	1.2	1.15	0.99	1.25	1.11
ps218	1.26	1.79	1.01	1.57	0.99
ps219	1.24	1.06	0.88	1.43	1.17
ps220	1.61	1.21	1	1.93	1.34
ps301	0.89	0.92	6.19	0.89	0.74
ps302	1.04	0.89	9.14	0.89	1.08
ps303	0.57	0.28	0.69	0.78	1.02
ps304	1.1	0.99	0.98	1.04	1.16
ps306	1.13	1.11	0.99	0.96	1.54
ps307	0.88	1.32	0.9	1	1.81
ps308	0.88	0.95	0.86	0.76	1.17
ps309	0.97	1.11	1	1.08	0.96
ps311	0.94	1.25	0.92	0.73	0.75
ps312	0.97	1.1	1.17	0.97	1.01
ps314	0.86	1.01	0.92	1.13	1.09
ps316	0.25	0.24	1.56	0.41	0.37
pstA	1.01	0.9	1.02	0.87	0.82
pstB	0.99	0.96	0.97	0.81	0.8
pstC	0.96	1.04	1.27	0.84	0.8
pstE	2.22	1.13	1.99	0.77	1.22

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pstF	2.61	0.96	2.7	0.48	1.13
pta	0.77	0.78	0.89	0.89	0.77
ptbA	0.32	0.31	0.3	0.19	0.77
ptcA	1.31	0.92	3.06	0.99	1.12
ptcB	1.11	0.72	1.72	0.9	0.66
ptcC	1.41	1.16	1.88	0.94	0.93
ptk	0.74	0.96	0.97	0.65	1.07
ptnAB	0.58	0.64	0.82	0.64	0.74
ptnC	1.24	1.31	1.61	1.33	0.68
ptnD	1.14	1.25	1.39	1.16	0.8
ptpL	0.59	0.81	0.63	1.06	0.63
ptsH	0.96	0.83	0.87	1	0.93
ptsI	0.99	1.21	0.93	1.17	0.74
ptsK	0.73	0.87	0.91	0.77	0.63
purB	0.8	0.8	0.9	0.89	0.6
purC	0.74	0.7	0.87	1.02	0.49
purD	0.81	0.74	0.92	1.13	0.58
purE	0.95	0.97	1.03	1.23	0.58
purF	0.74	0.66	0.62	0.57	0.64
purH	0.82	0.74	0.7	1.09	0.55
purK	0.82	0.79	0.92	1.01	0.47
purL	0.48	0.66	0.72	0.62	0.48
purM	0.73	0.7	0.83	0.95	0.72
purN	0.92	0.84	1.09	1.02	0.77
purR	0.64	0.57	0.93	0.82	0.69
pycA	0.9	0.99	0.75	0.82	0.7
pydA	0.63	0.62	0.5	0.55	0.6
pydB	0.61	0.47	0.62	0.31	0.3
pyk	1.21	1.22	1.08	1.08	0.82
pyrB	0.36	0.43	0.63	0.19	0.34
pyrC	0.56	0.42	0.66	0.39	0.32
pyrE	0.81	0.43	0.5	0.47	0.32
pyrF	0.54	0.7	0.77	0.42	0.39
pyrG	1	0.92	0.89	0.77	0.79
pyrH	1.02	0.57	0.84	1.07	0.82
pyrR	0.45	0.47	0.66	0.26	0.31
pyrZ	0.49	0.22	0.4	0.22	0.21
qor	1.25	1.51	1.26	2.03	1.1
queA	1.22	1.26	1.07	1.3	0.78
racD	1.31	1.29	1.04	1.21	0.8
radA	1.15	1.29	1.11	1.08	1.02
radC	1.09	1.08	1.26	1.19	1.14
rarA	0.82	0.59	0.71	0.81	1.2
rbfA	1.17	1.07	1.19	1.11	0.88
rbsA	1.7	1.47	1.35	1.58	1.08
rbsB	1.25	1.45	1.64	1.66	1.29
rbsC	1.46	1.61	1.32	1.55	0.96

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
rbsD	0.86	1.07	1.01	1.15	0.79
rbsK	1.98	1.87	1.2	1.39	1.37
rbsR	1.01	0.95	0.78	1.71	0.86
rcfA	1.03	1.12	0.88	1.21	0.9
rcfB	1.15	0.97	0.85	1.21	1.1
rdrA	1.09	1.05	0.91	1.12	0.81
rdrB	1.11	1.33	0.66	1.01	0.88
recA	0.94	0.93	0.86	1.11	0.81
recD	0.96	1.58	0.82	0.85	0.91
recJ	1.83	1.79	1.8	1.68	1.7
recM	1.31	0.99	0.89	1.17	1.17
recN	1.76	1.4	1.17	1.63	1.71
recQ	1.28	1.09	0.86	1.17	1.03
relA	0.76	0.73	0.91	0.85	0.82
rexA	1.09	1	0.89	1.01	1.09
rexB	0.95	0.95	0.83	0.97	1.24
rgpA	0.96	0.78	0.92	0.84	0.81
rgpB	0.77	0.62	1.49	0.62	0.67
rgpC	1.04	0.89	5.35	0.84	1
rgpE	1.08	0.87	1.52	1.15	1.37
rgpF	1.06	1.13	5.46	0.9	1.04
rgrA	1.1	1.02	0.99	0.99	1.24
rgrB	0.94	0.84	0.79	0.73	0.66
rheA	1.5	1.06	0.64	1.47	1.59
rheB	1.06	0.88	0.87	1.11	0.88
ribA	1.18	0.87	0.8	1.1	1
ribC	1.24	0.97	0.89	1.19	1.21
ribH	0.93	0.97	0.95	1.13	1.52
rimM	1.02	0.89	1.1	0.99	0.85
rliA	1.09	0.72	0.76	1.09	1.1
rliB	1.01	0.95	1.24	1.1	0.9
rliC	1.29	1.39	0.96	1.76	1.51
rliDB	1.08	1.02	0.71	0.93	1.27
rlrA	0.8	0.83	0.85	0.96	0.7
rlrB	0.84	0.84	0.98	1.36	1.3
rlrC	0.57	0.54	0.48	0.56	0.48
rlrD	0.4	0.55	0.7	0.75	1.14
rlrG	0.92	0.96	1.31	0.89	1.29
rluA	1.18	0.9	1	0.95	1.34
rluB	1.42	1	0.98	1.31	1.44
rluC	1.61	1.63	1.85	1.76	1.24
rluD	1.05	0.93	1.02	1.03	1.02
rmaA	1.05	0.92	2.37	1.11	1.26
rmaB	0.71	0.56	0.32	0.6	0.53
rmaC	1	1.23	0.73	0.64	1.1
rmaD	1.07	1.08	0.98	1.07	1.11
rmaE	1.16	0.94	0.86	1.09	1.15

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
rmaF	0.56	0.49	0.49	0.56	0.56
rmaG	1.05	0.72	0.88	0.94	1.03
rmaH	1.02	0.85	0.81	1.07	1.1
rmaI	1.33	1.03	0.97	1.59	1.06
rmaJ	1.02	0.52	0.77	1.75	0.91
rmeA	1.12	0.86	0.78	1.27	1.36
rmeB	0.95	0.97	0.85	0.92	1.58
rmeC	1.05	0.74	0.67	1.15	1.32
rmeD	1.42	1.34	1.66	1.25	1.19
rmlA	1.25	1.29	1.5	1.19	1.2
rmlB	1.35	1.17	1.31	1.07	0.94
rmlC	1.16	0.88	1.19	1.14	0.91
rnc	1.02	0.85	0.92	1.04	1.08
rnhA	0.99	0.98	1.53	1.11	1.03
rnhB	1.15	0.95	1.52	1.23	1
rnpA	1.22	0.83	1.36	1.37	1.18
rpe	1.08	1.08	1.24	1.21	1.23
rpiA	1	0.77	0.72	0.94	0.91
rplA	1.13	0.96	1.03	1.13	0.85
rplB	1.51	1.54	1.99	1.43	1.31
rplI	1.09	1	0.99	0.7	0.98
rplM	1.17	0.99	1.01	0.97	0.85
rplN	1.06	1.35	1.65	1.3	1.39
rplO	1.05	0.86	0.82	0.91	0.87
rplQ	0.8	0.75	0.78	1.02	0.99
rplR	1.24	1.38	1.43	1.29	1.02
rplS	1.17	1.2	1.23	1.11	1.1
rplT	1.67	1.23	2.47	1.48	1.03
rplU	0.99	0.88	1.03	0.8	0.8
rplV	1.11	1.2	1.52	1.14	1.08
rplX	0.88	0.77	1.23	0.87	1.15
rpmA	1.15	0.87	1.18	0.92	0.99
rpmB	0.99	0.89	0.95	0.79	1.05
rpmD	1.19	0.85	0.9	1.26	1.18
rpmE	1	0.79	0.98	1.09	1.04
rpmF	0.76	0.69	0.81	0.97	0.72
rpmGA	0.96	1.19	1.15	0.9	1.11
rpmGB	0.76	0.64	0.89	0.85	0.9
rpmGC	0.78	0.96	0.72	0.95	0.78
rpmH	1.2	1.39	0.91	1.29	1.13
rpmI	1.55	1.25	2.09	1.55	1.07
rpmJ	1.01	0.91	0.97	1.05	1.02
rpoA	1	0.88	1.06	1.42	1.07
rpoB	0.99	0.95	1	0.95	0.81
rpoC	0.85	0.87	0.99	0.76	0.83
rpoD	0.99	0.87	0.91	0.93	1.11
rpoE	1.11	0.92	0.96	1.23	0.86

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
rpsA	1.24	1.22	1.08	1.2	1.08
rpsB	0.94	0.77	0.8	1.03	0.83
rpsC	0.99	1.23	1.62	0.99	1.02
rpsD	1.14	0.96	1.3	0.99	0.85
rpsE	0.96	0.83	1.17	1.04	1.08
rpsF	0.86	0.81	1.15	0.79	0.95
rpsG	1.16	0.88	1.25	0.96	1.03
rpsH	0.89	1.01	1.2	1.23	0.92
rpsI	1.23	1.08	1.07	1.05	0.83
rpsJ	0.96	1.25	1.44	1.2	0.87
rpsK	0.81	0.91	1.01	1.09	0.86
rpsL	0.86	0.87	1.08	0.91	0.72
rpsM	0.92	1.23	1.18	1.11	0.79
rpsN	1.14	0.87	1.26	0.93	1.13
rpsN2	1.01	0.82	1.02	0.6	0.91
rpsO	0.84	0.78	1	0.78	0.84
rpsP	0.66	0.55	0.59	0.65	1.09
rpsQ	1.23	1.15	1.65	1.46	1.22
rpsR	1.04	0.92	0.91	1.2	0.99
rpsS	1.2	1.14	1.46	1.41	0.77
rpsT	0.97	0.99	0.79	0.9	0.88
rpsU	1.25	0.97	0.94	1.09	1.04
rsuA	1.91	1.34	0.87	1.76	1.18
ruvA	0.95	1.02	0.95	1.01	1.19
ruvB	0.88	0.79	0.95	1.06	1.09
sbcC	1.14	1.18	1.19	1.19	2.54
sbcD	0.86	0.96	1.04	0.79	2.53
scrK	1.24	1.04	2.02	0.88	1.39
sdaA	1.42	1.07	1.24	1.06	1.37
sdaB	0.86	0.77	0.76	0.93	0.72
secA	0.75	0.84	0.89	1.04	0.79
secE	1.21	0.95	0.93	1	1.33
secG	0.91	0.82	0.65	0.69	1.02
secY	0.73	0.65	0.7	0.73	0.69
serA	1.06	0.97	0.86	0.83	0.71
serB	1.17	1.22	1.03	1.03	0.74
serC	1.08	0.74	0.84	0.9	0.78
serS	1.01	1.02	0.84	1.02	1.03
sigX	0.56	0.59	0.71	0.49	0.81
sipL	0.95	0.94	1.1	0.78	0.92
smc	1.06	0.99	1.01	0.92	1.04
smpB	0.98	0.84	0.83	0.88	0.97
snf	1.13	1.26	1.08	0.97	0.96
sodA	0.88	1.12	0.98	0.77	0.91
ssbA	0.95	1.02	0.69	1.86	1.83
ssbB	0.57	0.61	0.86	0.69	0.82
sugE	1	1.13	1.02	1.12	1.12

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
sunL	0.88	0.74	0.87	0.96	0.64
tag	1.09	1.01	0.94	0.82	0.95
tagB	0.45	0.44	0.44	0.31	1.12
tagD1	0.92	0.93	5.4	1.02	0.99
tagD2	1.7	1.22	1.11	1.26	1.49
tagF	1.42	0.98	1.01	0.98	2.64
tagH	1	0.97	0.89	0.91	1.38
tagR	0.94	0.99	0.92	1.04	0.93
tagX	0.93	0.91	0.76	1.03	1.15
tagY	0.81	1.01	0.58	0.68	0.9
tagZ	0.63	0.99	0.81	0.61	1.08
tenA	1.17	1.18	0.86	1.36	1.08
thdF	0.98	0.93	0.84	0.68	1.02
thgA	1.86	1.18	2.02	1.88	2.03
thiD1	0.78	0.73	0.96	0.74	0.98
thiD2	1.19	1	1.33	1.11	1.05
thiE	1.18	0.94	0.95	0.98	1.33
thiL	0.99	0.89	0.67	1.23	0.83
thiM	0.67	0.75	0.7	0.79	0.75
thrA	0.66	0.77	0.79	0.8	0.66
thrB	1.24	1.05	0.92	1.04	1.2
thrC	0.9	0.83	0.8	0.97	0.85
thrS	0.95	1.04	0.88	0.91	0.85
thyA	0.99	0.85	0.93	1.13	0.79
tig	1.5	1.05	0.82	1.19	0.85
tkt	0.97	0.94	1.21	0.96	1.04
topA	0.67	0.71	0.86	0.61	0.66
tpiA	1.03	1	0.92	0.97	1.11
tpx	1.11	1.19	1.15	0.95	1.42
tra1077B	1.81	1.38	5.12	2.69	1.76
tra904A	1.81	1.47	2.14	0.3	1.06
tra905	1.19	0.93	0.76	1.26	1.48
tra981C	1.13	1.3	1.14	1.16	0.77
tra983L	1.38	1.22	2.37	1.6	1.12
trmD	1.09	1.13	0.92	0.96	0.79
trmU	1.3	1.08	0.94	1.35	0.97
trpA	0.58	0.73	0.6	0.94	0.69
trpB	0.5	0.7	0.59	0.81	0.75
trpC	0.63	1.22	0.92	0.82	0.72
trpD	0.74	1.23	0.88	0.92	0.83
trpE	0.72	0.95	1.17	1.06	0.56
trpF	1.07	1.29	0.88	1.05	1.2
trpG	0.47	1.07	1.13	0.7	0.37
trpS	1.19	1.7	2.24	1.92	0.8
truA	0.94	0.91	1.35	1.01	0.99
truB	0.77	0.89	0.92	0.8	0.77
trxA	0.87	1	0.91	0.8	1.06

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
trxB1	0.83	1.08	1.05	0.6	1.03
trxB2	1.25	1.41	0.84	0.94	1.38
trxH	0.77	0.91	0.98	0.73	0.76
tsf	1.03	0.96	1.05	1.14	0.92
tuf	0.88	0.93	0.82	1.14	0.89
typA	1.11	1.06	0.96	1.14	0.94
tyrA	1.06	1.21	0.84	0.75	0.86
tyrS	0.69	0.8	0.7	0.77	0.56
udk	1.14	1.23	1.05	0.93	1.32
udp	0.68	0.84	0.86	0.63	0.9
umuC	0.57	0.6	0.51	0.5	0.75
ung	1.55	1.4	1.38	1.56	1.3
upp	1.03	0.77	0.77	0.88	0.81
usp45	0.87	0.69	0.78	1	0.7
uvrA	1.39	1.34	1.7	1.43	1.25
uvrB	1.36	1.36	1.16	1.37	1.23
uvrC	1.02	1.2	1.11	1.04	1.12
uxaC	1.28	1.2	1.28	1.08	1.68
uxuA	0.97	1.04	1.06	0.98	0.95
uxuB	1.12	1.07	0.93	1.08	0.84
uxuT	1.59	1.53	1.52	1.02	1.17
vacB1	1.15	1.07	0.99	1.05	1.36
vacB2	0.9	0.88	1.22	0.93	0.8
valS	1.41	1.33	1.27	1.35	1.06
xerD	1.29	1.13	1.17	1.4	1.43
xpt	0.68	0.68	0.66	0.83	0.66
xseA	1.36	1.14	1.3	1.4	1.13
xylA	0.65	0.69	1.26	0.75	1.03
xylB	0.98	0.98	0.91	0.98	1.02
xylH	0.86	0.82	0.89	0.76	1.06
xylM	1.41	0.87	0.8	1.29	1.27
xylR	0.88	0.8	0.57	0.91	0.91
xylT	1.25	1.08	0.98	1.35	1.08
xylX	1.13	1.08	1.01	1.11	1.18
xynB	1.21	1.33	1.5	1.34	0.93
xynD	0.89	0.76	1.02	0.76	0.7
xynT	1.08	0.94	0.98	0.86	0.94
yabA	1.01	1.19	1.06	1.74	1.3
yabB	1.27	1.13	1.09	1.29	1.2
yabC	0.29	0.33	0.35	0.27	0.4
yabD	0.37	0.42	0.67	0.46	0.57
yabE	1.33	1.02	0.84	1.4	1.37
yabF	1.01	0.94	0.88	1.1	0.98
yacB	0.81	0.82	0.85	1.14	0.73
yacC	1.02	0.96	1.09	1.35	0.81
yacG	1.07	1.05	0.79	1.23	0.89
yacI	0.47	0.47	0.48	0.44	0.5

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yafB	0.38	0.39	0.26	0.39	0.32
yafC	0.96	0.85	0.8	0.93	0.97
yafD	0.37	0.35	0.89	0.3	0.85
yafE	1.8	1.35	0.67	2.53	1.72
yafF	2.11	1.23	0.89	2.21	2.57
yafJ	0.67	0.62	0.64	0.56	0.71
yagA	1.49	2.21	3.76	1.68	1.2
yagB	1.13	1.74	2.52	1.96	0.84
yagE	0.5	0.66	0.52	0.72	1.02
yahA	1.51	1.2	1.35	1.44	1.07
yahB	0.89	0.92	0.64	0.83	1.03
yahC	0.56	0.48	0.53	0.51	1.28
yahD	4.18	3.6	2.15	4.04	3.14
yahG	0.86	0.76	0.81	0.9	0.78
yahI	1.5	1.56	1.17	1.43	1.06
yaiA	6.58	5.39	3.48	5.63	4.86
yaiB	4.39	3.7	2.98	3.71	4.57
yaiE	1.09	1.11	1.29	0.9	0.78
yaiF	0.9	0.79	1.38	0.88	0.97
yaiG	1.09	1.05	1.04	1.14	1.37
yaiI	1.19	1.14	0.95	1.04	0.97
yajB	0.67	0.85	1.49	0.79	1.42
yajE	1.47	1.78	1.66	1.77	1.1
yajH	1.44	1.08	0.97	1.71	1.49
ybaA	1.04	1.03	0.97	1.28	1.4
ybaB	1.07	0.85	1.01	0.89	1.01
ybaC	0.71	0.76	1.01	0.92	1.05
ybaD	0.96	1.03	1.46	0.95	1.11
ybaF	1.32	1.43	1.3	1.18	0.9
ybaG	1.21	1.15	1.11	1.07	0.86
ybaH	0.93	0.97	0.86	1.05	0.75
ybaI	0.82	0.78	0.81	1.14	0.9
ybbA	1.14	1.19	1.02	1.12	1.17
ybbB	0.9	1.12	0.91	0.76	1.08
ybbC	1.34	1.11	1.45	1.18	0.78
ybbE	1.22	0.95	1.06	1.3	1.01
ybcC	1.4	1.42	0.9	1.5	1.41
ybcG	1.19	1.13	0.92	1.27	1.08
ybcH	1.05	1.06	1.16	0.67	0.61
ybdA	1.03	0.91	0.68	1.37	0.9
ybdC	1.15	1	0.95	1.22	0.89
ybdD	1.38	1.19	1.04	1.28	0.9
ybdG	0.28	0.28	0.37	0.29	0.99
ybdH	0.35	0.26	0.4	0.41	0.59
ybdI	0.62	0.68	0.62	0.63	0.95
ybdJ	0.77	0.87	0.82	0.66	1.01
ybaH	0.93	0.97	0.86	1.05	0.75

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ybaI	0.82	0.78	0.81	1.14	0.9
ybbA	1.14	1.19	1.02	1.12	1.17
ybbB	0.9	1.12	0.91	0.76	1.08
ybbC	1.34	1.11	1.45	1.18	0.78
ybbE	1.22	0.95	1.06	1.3	1.01
ybcC	1.4	1.42	0.9	1.5	1.41
ybcG	1.19	1.13	0.92	1.27	1.08
ybcH	1.05	1.06	1.16	0.67	0.61
ybdA	1.03	0.91	0.68	1.37	0.9
ybdC	1.15	1	0.95	1.22	0.89
ybdD	1.38	1.19	1.04	1.28	0.9
ybdG	0.28	0.28	0.37	0.29	0.99
ybdH	0.35	0.26	0.4	0.41	0.59
ybdI	0.62	0.68	0.62	0.63	0.95
ybdJ	0.77	0.87	0.82	0.66	1.01
ybdK	0.49	0.74	0.98	0.32	0.81
ybdL	1.05	1.31	2.93	1.42	1.23
ybeA	0.92	0.99	1.02	0.94	0.93
ybeB	1.1	0.97	0.95	1.14	0.99
ybeC	1.09	1.1	0.94	0.96	0.97
ybeD	1.14	1.28	0.96	1.84	1.04
ybeF	0.88	1.88	0.65	5.88	0.8
ybeH	1.03	1.1	0.65	1.19	1.13
ybeI	1.45	1.23	1.31	1.67	1.71
ybeM	1.05	1.02	0.93	1.03	1.07
ybfA	1.31	1.4	1.75	0.86	1.21
ybfB	0.94	1.02	0.9	1.11	0.94
ybfC	3.68	2.07	2.22	1.66	2.05
ybfD	1.6	1.64	0.88	1.45	1.52
ybfE	1.18	1.19	1.29	0.9	1
ybgA	5.05	2.9	2.21	3.22	2.87
ybgD	0.86	0.89	1.01	0.81	1.26
ybgE	1.13	1.14	0.97	0.87	1.07
ybhA	1.09	1.03	0.93	0.85	0.86
ybhB	0.96	0.84	0.72	0.94	0.81
ybhC	1.11	0.98	0.88	1.36	1
ybhD	0.86	0.92	1.23	1	0.92
ybhE	1.42	1.35	1.86	1.44	1.49
ybiB	1	1.02	0.93	1.35	1.18
ybiC	1.43	1.26	1.01	1.37	1.12
ybiD	1.12	1.05	1	1.15	0.82
ybiE	0.75	0.73	1.04	0.77	0.73
ybiG	1.16	1.29	1.08	0.79	1.09
ybiH	0.59	0.67	0.5	0.61	0.4
ybiI	0.59	0.59	0.55	0.55	0.55
ybiJ	0.54	0.45	0.58	0.52	0.49
ybiK	1.81	1.19	1.01	1.69	1.55

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ybjA	1.43	1.72	1.5	1.21	1.09
ybjB	1.58	1.7	1.23	1.48	1.52
ybjD	1.22	0.89	1.13	1.02	0.82
ybjJ	1.09	1.11	0.89	1.01	0.89
ybjK	1.09	1.04	0.84	1.19	1
ycaF	1.14	0.93	2.19	1.25	1.17
ycaG	0.84	1.09	5.91	1.09	1.05
ycbA	1.05	1.05	4.99	1.04	0.81
ycbB	0.87	0.96	4.9	0.79	0.93
ycbC	1.1	1.02	3.16	1.06	1.15
ycbD	1.31	1.09	1.64	1.14	1.08
ycbF	0.99	1.13	2.07	0.99	0.85
ycbH	0.88	1.53	4.77	1.13	0.97
ycbI	1.13	1.47	3.26	1.14	0.93
ycbJ	1.03	1.17	4.46	1.02	1.07
yccB	0.85	0.92	1.84	0.77	0.67
yccE	1.35	1.52	1.63	1.67	1.38
yccF	1.27	1.11	1.18	1.25	0.84
yccG	1.4	1.36	1.63	1.37	1.16
yccH	1.39	1.3	1.67	1.28	1.18
yccI	1.5	1.49	1.63	1.4	1.32
yccJ	1.11	1.16	1.29	1.06	1.06
yccK	1.2	1.39	0.88	1.4	1.36
yccL	1.12	1.1	0.93	1.02	1.1
ycdA	0.49	0.6	0.94	0.56	0.5
ycdB	1.02	0.89	0.84	1.01	0.79
ycdC	1.05	1.12	0.94	1.23	0.7
ycdE	0.95	1.09	0.94	1.09	1.07
ycdF	1.08	1.03	0.92	1.12	1.25
ycdG	0.86	0.89	0.98	0.85	0.92
ycdH	1.16	1.17	1.03	1.17	1.2
yceA	1.23	0.98	0.88	1.3	0.87
yceD	1.79	1.67	1.41	1.77	1.23
yceE	1.13	1.23	1.13	1.43	1.23
yceG	1.28	1.92	1.9	1.67	1.14
yceJ	0.56	0.52	0.87	0.52	1.06
ycfA	0.95	1.02	1.08	1.02	0.81
ycfB	0.84	0.71	0.88	0.71	0.71
ycfD	0.76	0.73	1	0.83	0.67
ycfF	0.95	1.05	1.19	0.95	0.99
ycfG	1.07	1.24	1.12	1.34	1.2
ycfH	0.96	0.96	0.88	0.91	1.11
ycfI	0.63	1.24	1.03	1.36	0.88
ycgA	0.95	1.55	1.22	1.17	1.03
ycgB	0.83	1.31	1.25	1.2	1.29
ycgC	0.67	0.86	1.23	0.54	0.92
ycgD	1.26	1.22	1.12	1.23	1.06

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ycgE	0.91	0.96	0.88	0.91	0.81
ycgF	0.93	1.21	1	0.82	1.01
ycgG	1.07	1.15	0.98	0.91	0.93
ycgH	0.95	0.94	0.81	0.87	0.82
ycgI	0.71	0.72	0.92	0.73	0.76
ycgJ	0.93	0.83	0.79	0.86	0.95
ychC	1.46	1.33	1.23	1.56	0.97
ychD	1.07	1.01	0.89	1.33	0.81
ychE	1.3	1.1	1.1	1.47	1.04
ychG	0.98	1.04	1.03	0.87	0.75
yciA	0.54	0.57	0.98	0.69	0.55
yciC	1.1	1.26	1.16	1.41	0.98
yciD	1.23	1.29	1.12	1.34	0.98
yciF	0.7	0.72	0.63	0.73	0.71
yciG	0.54	0.8	0.56	0.47	0.57
ycjA	0.86	1.01	0.81	0.89	0.91
ycjB	1.01	1.53	1.15	1.27	0.76
ycjC	1.25	1.01	0.96	1.18	0.87
ycjD	1.11	1.16	0.95	1.18	0.98
ycjG	0.63	0.74	0.84	0.82	0.89
ycjH	0.8	0.79	0.8	0.85	0.78
ycjI	0.65	0.45	0.41	0.98	0.43
ydaE	1.18	1.8	1.64	1.35	1.18
ydaF	0.64	0.88	0.88	0.54	0.77
ydaG	0.78	1.04	1.14	0.66	0.49
ydbA	0.85	1.08	0.98	0.69	0.64
ydbC	1.93	1.21	1.42	1.56	1.53
ydbD	0.67	0.88	0.87	0.85	0.6
ydbE	1.11	1.09	1.12	0.82	0.98
ydbF	0.8	1.16	1.13	0.99	0.93
ydcB	1.34	1.05	1.2	0.89	1.08
ydcD	0.98	1.14	1.05	1.03	0.9
ydcE	1.03	1.06	1.09	0.96	0.95
ydcF	0.6	0.97	0.93	0.6	0.78
ydcG	1.16	0.81	0.59	1.28	0.97
yddA	0.89	1.21	1.06	1.21	1.31
yddB	0.89	1.13	0.85	0.94	0.9
yddC	0.89	1.02	1.14	0.87	0.92
yddD	0.82	1.02	1.06	0.93	0.78
ydgB	0.55	0.94	0.63	0.69	0.67
ydgC	0.46	0.65	0.49	0.75	0.48
ydgD	0.64	0.68	0.78	0.72	0.71
ydgE	0.76	0.9	0.84	0.88	0.86
ydgF	0.69	0.81	0.75	1.14	0.83
ydgG	0.72	0.82	0.81	0.76	0.83
ydgH	0.81	0.85	0.77	1.25	1.34
ydgI	0.75	0.83	0.99	0.66	0.59

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ydhB	1.02	1.17	1.02	1.1	0.81
ydhF	1.69	1.35	1.31	1.39	0.97
ydiA	0.42	0.42	1.36	0.65	0.52
ydiB	1.18	1.13	1.17	1.21	0.66
ydiC	0.9	1.17	1.07	1.2	1.1
ydiD	0.9	1.12	0.85	1.07	0.77
ydiE	0.73	0.82	0.71	0.88	1.29
ydiF	0.86	1.06	0.59	0.95	0.99
ydiG	0.91	1.41	0.88	0.93	1.09
ydjB	0.86	0.73	1.01	0.73	1.04
ydjD	0.73	0.86	0.76	0.7	0.83
yeaA	0.36	0.39	0.8	0.3	0.31
yeaC	1.2	1.29	1.33	1.1	1.27
yeaD	0.78	0.64	1.07	0.67	0.58
yeaF	1.34	1.74	1.38	1.51	1.84
yeaG	1.08	0.92	0.92	1.06	0.96
yeaH	1.56	1.1	1.35	1.39	1.19
yebB	0.69	0.6	0.4	0.2	0.28
yebE	1.01	0.73	0.75	0.94	0.92
yebF	1.13	0.95	1.06	1.31	1.22
yecA	0.99	0.9	1.06	0.77	1.29
yecD	0.18	0.17	0.23	0.2	0.48
yecE	1.04	1.08	1.17	0.88	1.14
yedA	1.11	1.01	0.83	0.99	1.16
yedE	0.9	0.66	0.8	0.74	0.75
yedF	1.22	0.91	0.92	1.02	1.01
yeeC	1.28	1.26	1.04	1.11	1.2
yeeD	1.25	1.28	1.17	1.14	1.31
yeeF	1.24	0.93	0.86	1.2	1.16
yeiD	0.52	0.61	0.25	1.12	0.6
yeiE	1.26	1.24	1.09	0.77	1.25
yeiF	0.79	0.72	0.66	0.62	0.93
yeiG	1.35	1.14	1.14	1.03	0.76
yejC	0.82	0.83	0.82	0.71	0.62
yejD	1.27	1.14	0.94	1.24	1.08
yejE	2.11	0.72	1.67	1.12	1.17
yejI	0.95	0.46	0.72	1.06	1.02
yfaA	1.06	0.85	1.36	0.93	1.05
yfbB	0.84	1.45	0.84	1.05	1.03
yfbG	0.37	0.45	0.42	0.61	0.92
yfbI	1.02	0.96	1.13	0.85	1.12
yfbJ	1.29	1.15	1.16	1.21	1.5
yfbK	1.12	1.22	1.58	1.17	1.9
yfbM	1.98	2.02	2.15	1.6	3.09
yfcB	2.16	2.71	2.56	1.46	4.05
yfcF	1.25	0.99	1.03	1.1	1.32
yfcI	0.82	0.68	0.87	0.95	0.93

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yfdA	0.84	0.65	0.95	0.95	0.82
yfdB	1.71	0.8	0.97	1.22	0.91
yfdD	0.93	0.95	0.94	1.28	1.22
yfdE	0.58	0.42	0.44	0.66	0.73
yfeA	1.44	1.26	1.31	1.34	1.5
yffA	1.24	0.97	0.82	1.06	1.05
yffB	0.76	0.85	0.73	0.57	0.93
yffD	0.91	1.03	0.67	0.75	0.75
yfgC	1.08	1.03	0.77	0.99	0.84
yfgG	1.04	0.78	0.84	1.05	0.93
yfgH	0.6	0.64	0.89	0.79	0.86
yfgL	1.19	0.98	0.9	1.4	1.37
yfhA	1.42	1.7	0.9	2.22	1.37
yfhF	0.82	1.17	0.8	1.07	1.08
yfhH	1.27	1.67	1.26	1.35	1.46
yfhI	0.97	0.95	7.36	1.17	9.81
yfhJ	1.16	0.79	6.42	0.95	7.46
yfhK	1.37	1.52	0.93	1.11	1.36
yfhL	1.23	1.2	1.25	1.24	1.42
yfiC	1.06	0.74	0.93	0.96	1.03
yfiE	1.79	1.71	1.76	1.62	2.66
yfiG	0.57	0.6	0.63	0.72	0.79
yfiH	0.77	0.63	0.64	0.72	0.76
yfiJ	0.84	1.01	1.05	0.82	0.9
yfiL	1.43	1.52	1.23	1.33	1.52
yfjA	1.09	1.3	1.59	0.99	1.07
yfjC	1.1	1.01	1.07	0.99	1.04
yfjD	1.18	1	1.47	0.9	1.58
yfjF	1.87	1.16	1.75	1.71	1.53
ygaB	0.47	0.36	1.1	0.46	0.4
ygaC	0.47	0.4	0.76	0.53	0.42
ygaD	0.96	0.93	1.05	1.16	0.96
ygaE	1.35	1.2	1.03	1.13	1.2
ygaF	0.5	0.67	0.76	1.02	0.73
ygaI	1.24	1.19	1.19	0.99	1.22
ygaJ	1.79	1.09	3.68	1.03	1.22
ygbB	1.21	0.99	1.23	1.26	0.95
ygbD	1.36	1.05	1.28	1.21	1.04
ygbE	1.26	1.19	1.12	1.03	1.3
ygbF	1.99	1.84	2.18	2.37	2.31
ygcA	0.97	0.83	1.14	0.92	1.26
ygcC	1.29	0.97	1	1.13	1.32
ygdC	1.41	1.2	1.2	1.77	1.51
ygdD	0.87	0.82	0.95	0.81	1.03
ygdE	1.2	1.21	1.2	1.52	0.99
ygdF	1.6	1.75	1.69	1.6	1.67
ygeB	0.27	0.23	0.39	0.24	0.28

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ygeC	0.7	0.67	0.74	0.62	0.74
ygeD	1.02	0.98	0.71	1.39	0.99
ygfA	1.16	1.16	0.95	1.22	1.04
ygfB	2.77	3.16	2.2	2.71	2.01
ygfC	1.66	2.31	1.64	1.65	1.84
ygfE	0.96	0.88	1.3	0.77	1.2
yggA	0.91	1.38	0.95	1.02	1.05
yghB	1.15	1.27	1.56	0.96	1.25
yghC	0.81	0.79	0.79	0.83	0.73
yghD	1.51	1.3	1.37	1	1.37
yghE	1.32	1.28	1.33	1.17	1.24
yghG	0.94	1.06	0.74	0.99	0.94
ygiC	0.9	1.28	0.95	1.1	1.09
ygiG	0.49	0.46	0.45	0.46	1.38
ygiI	1.04	0.98	0.82	1.13	0.99
ygiJ	1.56	1.38	1.37	1.31	1.21
ygiK	1.6	1.25	1.09	1.18	1.07
ygjB	1.03	0.85	1.01	0.87	0.87
ygjD	0.63	0.9	1.49	0.52	0.8
yhbE	0.58	0.45	0.35	0.49	0.54
yhbH	0.95	0.86	0.74	1.04	1.03
yhcA	2.62	1.53	1.57	1.62	1.35
yhcB	3.04	2.69	1.71	5.76	2.66
yhcC	1.15	1.03	1.04	1.26	1.34
yhcE	1.18	1.05	2.2	1.31	1.31
yhcH	0.84	0.96	0.95	0.9	1.24
yhcI	0.94	1.16	1.01	0.88	1.09
yhcK	1.06	1.05	0.68	1.52	1.18
yhdA	1.12	1.71	1.46	1.5	1.18
yhdB	1.09	1.31	1.12	1.22	1.03
yhdC	0.67	0.81	1.04	1.09	0.86
yheA	1.08	0.9	1.41	1.02	1.2
yheB	1.17	1.01	1.27	1.05	1.01
yhfB	0.67	0.78	0.81	0.76	0.82
yhfC	0.93	1.08	0.88	0.95	0.8
yhfD	1.15	1.17	1.02	1.09	1.03
yhfE	1.27	1.05	0.94	1.13	1.05
yhfF	1.42	1.25	1.03	1.25	1.11
yhgA	1.25	1.18	0.8	1.45	0.99
yhgB	1.67	1.61	1.48	1.88	1.81
yhgC	0.69	0.67	0.7	0.72	0.82
yhgD	1.27	1.06	0.91	1.19	1.36
yhgE	1.1	0.8	0.79	1.02	1
yhhA	1.14	1.06	0.94	0.97	0.97
yhhB	1.37	1.18	1.01	1.14	1.17
yhhD	0.94	0.99	1.07	0.93	1.03
yhhE	1.08	0.86	0.87	0.85	0.99

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yhhG	1.17	1.15	1.31	0.95	0.98
yhjA	1.53	1.59	1.14	1.05	1.75
yhjB	1.75	1.51	1.05	1.97	1.68
yhjC	1.92	1.51	1.09	1.72	1.68
yhjE	1.31	1.11	1.03	1.11	0.93
yhjF	1.84	1.3	1.11	1.81	1.28
yhjG	1.07	1.14	1.38	0.98	1.15
yiaA	0.74	0.77	0.94	0.7	0.79
yiaB	0.82	0.84	0.91	0.81	0.88
yiaC	0.93	0.86	0.86	1	0.78
yiaD	1.03	1.61	2.08	0.93	1.74
yibB	1.23	1.44	1.17	1.18	1.23
yibC	1.22	1.13	1.13	1.23	1.14
yibD	1.43	1.83	1.36	1.13	0.77
yibE	1.52	1.67	1.1	1.43	1.45
yibF	1.17	1.06	0.92	1.01	0.78
yibG	3.37	3.06	1.18	0.99	1.29
yicA	1.25	1.58	1.4	1.57	1.29
yicB	0.93	1.13	1.29	1.01	0.94
yicC	1.1	1.21	1.22	1.21	1.11
yicE	1.17	0.99	0.83	1.92	0.81
yidA	1.68	1.53	1.59	1.64	1.35
yidB	1.11	0.92	0.99	1.22	1.23
yidC	1.36	1.31	1.28	1.29	1.63
yidE	1.09	0.97	0.91	0.99	1.03
yieF	0.93	0.94	1.07	0.81	1.54
yieH	1.86	2.01	0.91	2.52	1.24
yifA	1.81	1.73	0.73	1.77	1.07
yigC	1.95	1.8	3.04	1.58	1.89
yihA	1.33	1.21	1.02	1.33	2.1
yihB	1.12	1.06	0.94	1.19	2.02
yihD	1.19	1.19	1.09	1.2	1.74
yihF	1.22	1.11	1.34	1.18	1.37
yiiB	1.45	1.38	1.17	1.54	1.26
yiiD	1.07	0.92	0.78	1.1	0.89
yiiE	0.71	0.82	0.76	1.04	0.81
yiiF	0.84	0.89	0.64	0.89	0.78
yiiG	1.04	0.98	0.69	1.13	1.01
yiiH	0.93	0.79	0.71	0.99	0.73
yiiI	0.89	0.73	0.58	0.77	0.74
yijB	1.36	1	1.08	1.07	1.29
yijC	1.58	1.09	1.01	1.09	0.96
yijD	1.63	1.29	1.12	1.27	1.32
yijE	1.01	1.02	1.17	0.86	0.97
yijF	1	1.12	0.98	1.08	1
yijG	0.96	0.84	0.73	0.93	0.97
yijH	3.32	3.52	3.59	3.01	2.06

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yjaB	1.8	1.33	1.03	0.91	1.31
yjaD	0.92	0.83	1.02	0.98	0.75
yjaE	1.06	0.9	0.85	0.78	1.05
yjaF	0.87	0.8	0.61	1.02	0.84
yjaH	0.5	0.5	0.65	0.56	0.75
yjaI	0.89	0.82	0.78	0.91	1.06
yjaJ	0.79	0.45	0.48	0.82	0.84
yjbB	1.62	1.37	1.58	1.48	1.01
yjbC	1.81	1.16	1.21	1.55	1.34
yjbE	1.73	1.3	1.14	1.52	1.57
yjbF	1.45	1.43	1	1.5	1.42
yjcA	1.62	2.03	1.55	1.07	1.08
yjcD	0.76	0.99	1.01	0.99	0.93
yjcE	0.64	0.59	0.82	0.94	0.7
yjcF	1.09	0.89	0.84	1.31	0.85
yjdA	1.5	2.19	1.24	1.5	1.52
yjdB	2.54	2.89	2.25	3.02	1.89
yjdE	0.72	0.95	0.84	0.5	0.56
yjdI	0.79	0.96	0.93	0.86	1.06
yjdJ	0.91	0.9	0.87	1.1	0.95
yjeA	1.43	1.22	1	1.26	1.14
yjeD	0.73	0.83	0.67	0.42	0.84
yjeF	1.16	0.98	1.09	1.07	1
yjeG	0.91	1.17	0.97	0.91	1.85
yjfB	1.12	1.24	1.24	1.22	1.41
yjfG	0.86	1.02	1.27	0.77	1.13
yjfI	0.79	0.73	0.79	0.76	1.41
yjfJ	0.68	0.64	0.69	0.49	1.07
yjgB	0.71	0.6	0.42	0.94	0.96
yjgC	1.49	1.41	2.17	1.18	0.96
yjgD	1.17	1.18	1.37	0.97	0.78
yjgF	1.23	1.23	1.29	1.26	1.26
yjhA	1.25	1.11	1.24	1.15	1.04
yjhB	1.02	1.1	0.9	0.99	1.01
yjhC	0.98	0.94	1.25	0.88	0.86
yjhD	0.87	0.91	1.79	0.76	0.56
yjhF	0.75	0.85	1.57	0.75	0.65
yjiB	2	2.16	1.43	2.58	1.27
yjiE	1.13	1.11	0.99	0.94	0.91
yjjA	1.2	1.12	1.14	0.96	1.02
yjjB	1.06	0.92	0.89	1.14	0.98
yjjC	1.07	1.25	1.91	1.1	1.46
yjjD	1.4	1.19	1.95	1.02	1.79
yjjE	0.91	1.05	0.92	0.88	0.98
yjjF	0.84	0.91	0.85	0.81	0.85
yjjH	0.83	0.81	1.18	0.92	1.12
ykaE	0.87	0.96	1.02	1.2	0.94

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ykaF	1.14	0.81	0.89	1.26	0.86
ykbA	1.18	0.9	0.89	1.19	1.06
ykbC	0.4	0.33	0.43	0.4	0.26
ykbE	0.68	0.56	0.57	0.68	0.68
ykbF	0.58	0.59	0.47	0.59	0.59
ykcA	0.51	0.45	0.48	0.67	0.52
ykcB	0.43	0.61	0.62	0.71	0.62
ykcC	0.8	0.77	0.7	0.89	0.94
ykcF	0.54	0.71	0.84	0.64	0.66
ykcG	1.27	1.03	0.99	1.59	1.31
ykdA	1.28	1.06	1.16	1.47	1.05
ykdB	1.28	1.08	1.05	1.46	1.11
ykhE	1.23	1.26	2.02	1.49	0.7
ykhF	0.99	0.88	0.96	1.13	0.95
ykhG	0.98	1.33	1.32	1.21	0.8
ykhI	1.22	1.3	1.03	2.07	1.12
ykhJ	0.81	1.07	1.07	0.96	1.17
ykhK	1.33	1.42	1.19	2.26	1.12
ykiC	1.88	1.75	1.14	2.41	1.44
ykiD	1.8	2	1.29	2.89	1.54
ykiF	0.95	0.95	0.95	0.93	0.81
ykiG	0.86	0.87	0.93	0.81	0.94
ykiH	0.85	0.94	1.2	0.79	0.8
ykjA	1.3	1.37	1.2	1.2	0.85
ykjB	0.81	1.4	0.89	0.73	3.87
ykjC	1.1	1.09	0.67	0.94	2.27
ykjE	1.1	0.98	1.08	0.96	1.01
ykjF	1.05	0.96	1.09	0.89	0.93
ykjH	1.19	1.49	1.21	1.21	1
ykjJ	1.35	1.4	1.35	1.53	1.25
ylaC	0.91	1.03	1.09	0.95	0.98
ylaE	1.83	1.47	1.16	1.71	1.05
ylbA	1.1	1.23	1.46	1.16	1.19
ylbB	1.22	1.26	1.58	1.09	1.44
ylbD	1.02	0.91	1.05	0.96	0.83
ylbE	1.08	1.01	1.16	0.77	0.96
ylcA	1.37	1.35	1.22	0.95	1.17
ylcC	0.34	0.39	0.32	0.27	0.61
ylcD	0.51	0.53	0.35	0.51	0.51
ylcE	0.81	0.75	0.59	0.79	0.75
ylcF	0.59	0.59	0.38	0.68	0.59
yldA	1.13	1.04	0.96	1.16	1.11
yldC	0.96	1.21	1.35	0.78	1.27
yldE	1.28	1.72	1.79	1.29	1.51
yleB	1.27	1.19	1.64	3.44	1.07
yleE	1.08	1.25	1.07	1.15	1.03
yleF	1.14	1.21	1.28	1.28	1.15

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ylfA	1.15	1.43	1.24	1.46	0.99
ylfB	0.82	0.79	0.56	0.79	0.64
ylfC	0.68	0.67	0.59	0.78	0.56
ylfD	0.68	0.67	0.55	0.86	0.55
ylfF	1.21	0.95	1	1.01	0.62
ylfH	1.41	1.09	1.08	0.78	0.82
ylfI	1.16	1.5	1.58	1.16	1.36
ylgB	1.31	1.47	1.15	1.68	1.38
ylgC	0.85	0.93	0.65	1.13	0.92
ylgG	1.12	1.08	1.09	0.72	0.9
ylhB	1.54	2.08	1.96	1.18	2.08
yliA	0.91	0.82	0.89	0.71	1.04
yliC	1.28	1.15	0.84	1.29	0.97
yliD	1.15	1.11	1.03	1.28	0.82
yliE	0.95	1.04	0.97	1.16	1.04
yliF	0.88	0.97	0.95	0.79	1.23
yljB	1.18	1.07	1.1	1.05	1.12
yljC	1.1	0.98	1.18	0.94	1.11
yljD	0.97	0.9	0.83	0.81	0.91
yljE	0.71	0.89	0.9	0.97	0.72
yljF	1.08	1.17	1.06	1.04	0.78
yljG	1.15	1.25	1.34	1.13	0.77
yljH	0.86	1.14	0.9	0.87	0.95
yljI	0.91	1.23	0.82	0.95	0.77
yljJ	1.01	1.26	1.04	1.17	0.73
ylqL	1.05	0.86	0.91	1.02	0.71
ylxQ	1.19	1.03	1.06	1.08	0.88
ymaB	0.93	0.96	1.01	0.84	1.03
ymbC	1.11	1.03	0.96	1.26	0.94
ymbD	1.36	1.17	1.01	1.2	1.35
ymbG	0.93	1.03	1.04	0.79	0.96
ymbJ	0.98	1.06	1.14	1.21	1.03
ymbK	1.63	1.61	1.17	1.98	0.91
ymcA	1.02	1.25	1	1.15	0.96
ymcB	0.8	1.36	1.02	0.9	0.67
ymcC	1.08	1.35	0.98	1.16	0.97
ymcF	1.27	1.18	0.99	1.24	1.21
ymdC	0.47	0.57	0.47	0.41	0.43
ymeB	0.44	0.58	0.87	0.34	0.39
ymfD	0.84	1.06	0.69	1.3	0.84
ymfE	1.16	0.95	0.93	1.15	0.94
ymgB	1.13	1.16	1.1	1.2	1.81
ymgC	1.49	1.53	1.62	1.52	1.49
ymgF	1.72	1.97	1.54	1.37	2.86
ymgG	0.6	0.75	0.46	0.95	0.69
ymgH	0.62	0.56	0.43	0.86	1.04
ymgI	0.64	0.67	0.45	0.97	0.77

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ymgJ	0.51	0.84	0.62	0.86	0.81
ymgK	0.4	0.52	0.37	0.4	0.93
ymhC	1.44	1.03	1.01	1.33	2.35
ymhG	0.47	0.76	0.77	0.6	0.59
ymiA	0.73	0.94	0.85	0.82	0.58
ymjE	1.24	1.78	1.17	2.32	1.04
ymjF	1.13	1.73	1.19	2.18	0.85
ynaA	1.1	0.91	0.84	1.18	1.17
ynaB	0.85	0.89	0.76	0.92	0.5
ynaC	0.83	0.98	1.13	1.06	0.64
ynaD	0.9	0.78	0.69	1.16	0.71
ynaE	0.92	0.93	0.65	0.97	0.86
ynaG	1.43	1.48	1.17	1.2	0.97
ynbA	1.22	1.23	1.49	1.24	0.95
ynbB	1	1.06	1.1	1.1	0.96
ynbC	0.96	1.06	0.97	1.03	0.7
ynbD	0.89	0.86	0.75	1.03	0.66
ynbE	0.78	1.01	0.88	1.02	0.86
yncA	0.7	0.91	1.06	0.68	0.66
yncB	0.87	0.83	0.66	0.89	0.61
yndA	0.83	0.95	0.7	0.94	0.59
yndB	0.97	1.08	1.23	0.87	0.86
yndC	1.15	1.26	1.2	1.12	1.17
yndD	1.05	1.08	0.88	1.13	1.12
yndE	0.94	1.02	0.96	0.9	1.15
yndF	1.09	1.25	1	1.36	0.99
yndG	0.77	2.03	0.85	1.01	1.21
yneB	1.22	0.65	1.26	1.34	1.31
yneC	1.11	1.2	1.45	1.18	1.44
yneE	0.73	0.97	0.85	0.83	0.76
yneF	0.71	0.69	0.6	0.84	0.45
yneG	4.06	3.37	2.18	4.46	1.3
yneH	3.65	3.88	2.64	4.3	1.11
ynfC	0.9	1.15	1.22	0.8	0.85
ynfD	1.43	1.35	1.29	1.52	2.57
ynfG	0.22	0.34	0.34	0.22	0.3
ynfH	1.54	1.14	0.99	1.65	1.02
yngA	0.79	0.9	0.83	0.72	0.82
yngB	0.71	0.89	0.85	0.75	0.85
yngE	1.29	1.58	2.15	1.59	1.09
yngF	0.96	1.21	1.86	1.14	0.94
ynhA	1.27	1.38	1.28	1.01	1.19
ynhC	2.58	2.03	1.7	2.82	1.16
ynhD	1.95	1.78	1.65	2.18	1.15
ynhH	0.84	1.11	1.05	1.47	0.93
ynhI	0.75	1	0.83	1.05	0.66
yniC	0.73	0.87	0.92	0.68	0.74

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yniG	1.03	1.27	0.91	1.05	0.91
yniH	1.41	1.18	1.24	1.4	1
yniI	1.02	0.49	0.52	0.76	0.56
yniJ	1.02	1.05	0.85	1.11	1.19
ynjC	1.64	6.21	1.58	11.21	2.56
ynjD	1.9	2.36	0.93	3.71	1.12
ynjE	1.86	1.52	0.92	2.54	1.06
ynjF	1.41	1.49	0.93	1.8	1.09
ynjG	1.52	1.06	0.73	2.1	1.47
ynjH	2.53	2.51	4.58	1.75	1.5
ynjI	1.6	2.17	2.05	2.33	1.92
ynjJ	1.9	1.92	1.83	2.22	1.73
yoaD	1.18	0.82	0.81	1.27	1.03
yoaG	1.03	1.17	0.99	1.25	0.8
yoaH	1.03	1.29	0.88	1.2	0.87
yoaI	0.22	0.65	0.28	0.64	0.72
yobA	0.09	0.86	0.68	0.75	0.67
yobC	1.14	0.78	0.75	1.08	1.54
yofM	0.73	0.56	0.52	0.8	0.83
yogE	1.22	0.93	1.07	1.1	1.06
yogG	0.89	0.75	0.84	1	0.92
yogI	1.17	0.94	1.03	0.9	1.46
yogJ	1.14	0.77	1.16	0.79	1.42
yogL	1.03	0.77	0.7	1.13	1.11
yogM	1.16	0.77	0.9	0.93	1.2
yohC	0.59	0.63	0.48	0.35	0.84
yohD	0.95	0.8	0.75	0.8	1.04
yohH	1.03	1.11	0.81	0.81	1.33
yohJ	1.09	1.28	1.01	0.83	1
yoiB	1.18	1.16	0.91	1.25	1.07
yoiC	0.86	1.09	0.9	1.11	1.19
yojB	0.69	0.8	0.66	0.82	0.89
yojC	0.8	1.03	0.93	0.92	1.22
ypaA	1.11	1.12	0.76	0.63	1.12
ypaC	0.85	0.78	1	0.68	0.99
ypaG	0.33	0.27	0.25	0.26	0.47
ypaH	0.74	0.73	0.83	0.84	0.84
ypaI	1.13	1.08	1.18	1.06	1.1
ypbB	0.89	1.02	0.97	0.86	1.19
ypbC	1.47	2.07	1.49	1.72	0.55
ypbD	1.49	1.79	1.39	1.3	0.23
ypcA	1.2	1.02	1.36	0.67	1.06
ypcB	1	0.83	0.95	0.73	1.07
ypcC	1.1	0.99	0.88	0.96	1.1
ypcD	0.76	0.61	0.62	0.85	1.18
ypcG	1.44	1.21	2.21	0.92	1.27
ypcH	1.13	1.33	2.83	0.79	1.04

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ypdA	1.1	1.44	3.7	1.03	1.04
ypdB	1.14	1.35	3.41	1.03	1.34
ypdC	1.15	1	1.92	1.09	1
ypdD	1.27	1.3	1.84	1.2	0.81
ypfD	0.74	0.83	1.38	0.91	0.8
ypfE	1.77	2.74	2.29	1.75	1.08
ypfF	1.08	0.9	1.01	0.96	1.21
ypgB	0.94	0.79	0.88	0.79	1.02
ypgC	1.29	0.82	0.88	1.17	1.35
ypgD	1.13	0.73	1.16	1.06	1.09
yphA	1.3	1.22	1.25	1.16	1.46
yphC	0.81	0.84	0.84	0.83	0.54
yphH	1.09	0.82	0.84	1.16	1.09
yphI	0.96	1.02	1.27	0.97	0.92
yphJ	1.26	1.21	1.52	1.22	0.95
yphK	1.08	1.28	1.56	1.21	0.98
yphL	1.38	1.33	1.01	1.3	1.19
ypiA	1.34	1.08	1.06	1.3	1.6
ypiB	1.33	1.27	0.97	1.23	1.25
ypiE	2.1	1.27	1.21	1.77	1.48
ypiH	2.04	1.48	1.49	1.93	1.14
ypiJ	1.65	1.18	1.14	1.44	1.36
ypiK	1.08	1.13	0.95	1.02	0.95
ypiL	1.09	1.22	0.74	1.61	0.84
ypjA	1	0.96	0.89	1.03	1.1
ypjB	0.96	0.93	0.92	0.96	1.08
ypjC	1.08	0.78	0.87	1.24	1.1
ypjF	1	0.96	0.83	1.12	1.22
ypjH	0.93	0.92	0.95	0.9	0.84
ypjI	1.06	1.04	0.86	1.03	1.05
yqaB	0.72	0.6	0.59	0.57	1.02
yqaC	0.88	0.92	0.69	0.77	0.97
yqaD	1.05	0.8	0.81	0.77	0.89
yqaG	0.48	0.38	0.55	0.6	0.65
yqbA	1.17	0.96	0.8	0.91	1.08
yqbF	1.03	1.15	1.04	1.21	1.46
yqbH	0.16	0.18	0.18	0.17	0.2
yqbI	0.18	0.15	0.16	0.14	0.16
yqbJ	0.63	0.57	0.63	0.72	0.83
yqcB	0.2	0.22	0.23	0.23	0.29
yqcC	0.15	0.17	0.18	0.18	0.2
yqcD	0.19	0.18	0.22	0.19	0.25
yqcE	0.73	0.7	0.54	0.74	0.6
yqcF	1.16	1.2	0.89	0.9	1.02
yqcG	1.15	0.91	1.18	1.23	1.14
yqdA	0.92	0.81	0.91	0.66	1.05
yqeA	0.9	0.81	1.04	0.73	1.28

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yqeB	0.72	1.08	0.87	0.81	0.89
yqeD	0.92	0.88	1.02	0.99	0.77
yqeH	1	0.76	0.87	1.03	1.01
yqeL	0.95	0.96	0.96	0.99	0.92
yqfA	0.96	1.01	0.83	1.33	1.07
yqfC	0.76	0.85	0.79	0.73	0.82
yqfE	0.97	0.23	1.57	0.66	1.4
yqfF	0.86	0.72	0.89	0.73	1.4
yqfG	1.34	1.4	1.03	1.95	1.82
yqgA	1.22	1.16	1.01	1.12	1.08
yqgC	1.05	0.99	0.92	0.93	0.88
yqgE	0.65	0.75	0.66	0.65	1.33
yqgF	1.55	1.35	1.1	1.73	1.73
yqgG	1.84	1.62	1.33	2.08	1.92
yqhA	1.05	1.1	1.23	0.9	1.4
yqiA	1.07	1.3	1.1	1.42	1.07
yqjA	0.77	0.81	0.83	0.93	0.9
yqjB	1.05	0.81	0.9	1.35	0.8
yqjD	1.29	0.94	1.04	1.21	1.2
yqjE	0.88	0.91	0.86	1.11	0.63
yraA	0.91	0.85	1.13	0.97	0.96
yraB	0.76	0.75	0.88	0.82	0.85
yraC	1.32	1.05	1.39	1.21	1.35
yraD	1.25	1.06	1.19	1.11	1.18
yraE	1.09	1.05	1.02	0.95	0.92
yraF	0.8	1.06	0.79	1.08	0.96
yrbA	1.01	0.98	0.99	0.88	0.97
yrbB	0.97	0.97	0.68	1.76	0.76
yrbC	0.76	0.69	0.44	1	1.26
yrbD	1.31	1.16	0.97	1.52	1.57
yrbE	1.06	1.02	0.82	1.12	0.93
yrbF	1.31	0.88	0.79	1.48	1.57
yrbH	1	1.21	0.99	1.15	1.49
yrbI	1.05	0.91	0.95	0.94	0.98
yrcA	1.19	1.2	2.08	1.06	1.2
yrcB	1.19	0.99	0.8	1.14	1.05
yreB	1.13	0.87	0.83	0.94	1.09
yreD	1.13	0.97	0.81	1.02	1.14
yreE	1.1	0.95	0.91	1.06	1.1
yrfA	0.8	0.75	0.74	0.79	0.88
yrfB	1.44	2.13	1.93	1.23	1.86
yrfC	1.18	0.91	0.85	0.99	1.3
yrfD	1.44	1.35	1.2	1.26	1.11
yrfE	0.84	0.81	0.94	1.35	0.97
yrgA	0.85	0.85	1.02	0.9	1.07
yrgE	0.94	1.11	0.88	1.08	0.97
yrgF	0.64	0.74	0.85	0.47	0.83

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yrgG	1.21	1.11	1.02	1.18	1.77
yrgH	0.91	1.18	1.13	0.74	0.63
yrgI	0.83	1.06	0.91	0.5	0.59
yrhH	1.12	0.88	0.83	1.11	1.11
yriB	1	0.87	1.04	0.87	0.96
yriC	0.85	0.96	1.12	0.73	0.8
yrjA	0.68	0.6	0.8	0.55	0.54
yrjB	0.47	0.59	1.17	0.72	0.79
yrjC	1.18	1.42	1.15	2.07	1.21
yrjD	1.48	1.57	1.07	2.66	1.37
yrjE	2.31	2.15	1.58	2.11	2.01
yrjF	1.9	1.87	2.35	1.73	1.77
yrjG	0.76	0.71	0.8	1.06	0.69
yrjI	0.93	1.19	1.02	0.88	0.98
ysaA	0.86	1.04	0.94	0.78	1.02
ysaB	0.3	0.33	0.3	0.23	0.44
ysaC	0.45	0.38	0.31	0.41	0.64
ysaD	0.35	0.25	0.2	0.34	0.47
ysbA	0.79	0.83	1.24	0.6	0.95
ysbB	0.86	1.1	1.11	0.73	0.94
ysbC	0.75	0.9	1.03	0.79	1.02
ysbD	1.25	0.88	0.83	1.14	0.91
yscA	0.25	0.2	0.7	0.87	0.73
yscB	0.96	1.23	0.79	1.08	1.08
yscD	0.84	0.82	0.83	0.8	0.87
yscE	0.72	0.87	1.03	0.91	1.45
ysdA	0.91	0.96	1.13	0.71	1.16
ysdB	1.04	0.86	0.98	0.98	1.23
ysdC	1.36	1.31	0.99	1.3	1.44
ysdE	2.92	2.64	3.52	2.09	2.27
yseA	0.91	0.93	0.95	1.22	1.02
yseC	1.02	1.11	1.21	1.5	1.36
yseD	0.81	0.85	0.94	0.96	1.17
yseE	0.76	1.06	1.41	2.12	1.52
yseF	0.94	0.9	0.77	1.05	0.82
yseH	0.91	1.07	0.78	0.95	0.9
ysfB	0.95	0.99	0.58	0.79	0.73
ysiA	0.8	0.8	0.73	1.17	0.86
ysiB	0.51	0.52	0.44	0.66	0.44
ysiC	1.16	1.16	1.06	0.85	1.07
ysiD	1.45	1.4	1.37	1.39	1.19
ysiE	0.98	0.89	1.01	0.88	0.88
ysiG	1.22	1.2	1.33	1.14	1.24
ysjA	0.57	0.65	0.7	0.56	0.44
ysjC	1.43	1.04	1.03	0.91	1.39
ysjD	1.01	0.86	0.91	0.93	1.22
ysjE	0.85	0.9	1.1	0.78	1.27

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ysjF	0.35	0.4	0.46	0.5	0.43
ysjG	1.09	0.97	1.05	1.22	1
ysjH	1.13	0.86	0.75	1.2	0.95
ysxL	1.19	1	0.87	1.3	1.32
ytaA	1.01	1.34	1.22	1.41	1.22
ytaB	1.14	0.98	0.78	1.22	1.17
ytaD	0.58	0.58	0.51	0.66	0.55
ytbA	0.92	0.91	0.92	0.91	0.86
ytbB	1.25	1.21	1.34	1.16	1.2
ytbC	1.13	1.16	0.73	0.85	1.53
ytbD	0.85	0.86	0.95	0.77	1.19
ytbE	1.21	1.05	1.04	1.37	1.2
ytcA	1.05	1.03	0.98	1.13	1.06
ytcB	1.05	0.96	1.12	1.21	0.88
ytcC	1.19	0.97	0.16	1.32	1.09
ytcD	1.43	1.48	1.37	1.42	1.6
ytcE	1.27	0.9	0.78	1.09	1.11
ytdA	1.16	0.99	0.98	1.09	0.91
ytdB	1.11	0.93	0.72	1.13	0.96
ytdC	0.68	0.71	0.66	0.8	0.99
ytdF	1.1	1.03	0.99	0.98	0.83
yteA	1.01	0.86	0.87	0.83	0.87
yteB	1.6	1.94	1.79	1.34	1.79
yteC	1.16	1.03	1.14	1.03	1.11
yteD	0.66	0.73	1.11	0.65	1.47
yteE	1.19	1.09	0.93	1.74	1.65
yteG	1.35	1.08	1.07	1.44	1.39
ytfA	1.63	1.25	0.91	1.54	1.53
ytfB	1.16	0.9	0.83	1.1	1.07
ytgA	2.34	2.6	2.43	2.67	3.01
ytgB	2.35	2.43	3.13	2.75	2.97
ytgC	1.22	0.94	0.91	1.26	1.18
ytgD	1.24	1.3	1.15	1.23	1.17
ytgE	1.32	1.17	1.45	1.01	1.27
ytgF	1.12	0.98	1.17	1.09	0.99
ytgG	1.53	1.46	1.9	0.93	1.19
ytgH	2.71	2.28	2.65	2.52	3.03
ythA	2.47	1.34	0.91	1.93	1.98
ythB	2.43	1.52	1.06	2.12	2.15
ythC	2.27	1.64	1.05	1.96	1.78
ytiA	1.83	2.06	2.09	1.63	2.05
ytjA	1.3	1.23	1.07	1.1	1.06
ytjD	0.95	0.92	0.61	0.62	1.6
ytjE	0.91	1.14	1.2	0.88	0.94
ytjF	1.19	1.15	0.94	0.87	1.25
ytjG	0.92	0.83	0.93	0.85	1.01
ytjH	1.19	1.28	1.2	1.37	1.1

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yuaA	1.1	1.09	1.3	0.94	1.08
yuaB	1.13	1.12	1.27	0.92	1.15
yuaC	1.19	1.01	1.04	1.25	1.19
yuaD	1.03	0.87	1.17	0.93	1.01
yuaE	1.12	0.75	0.69	1.06	0.81
yucF	2.28	1.94	2.38	1.88	1.79
yucG	1.69	1.96	2.11	1.3	1.41
yudA	1.2	0.98	0.99	1.11	1.06
yudB	0.98	0.81	0.81	0.95	0.59
yudD	1.27	0.97	0.92	1.14	0.79
yudE	0.87	0.66	0.7	1.2	0.7
yudF	0.79	0.77	0.73	1.07	0.69
yudG	1.35	1.21	1.08	0.98	1.51
yudI	1	0.92	1.03	0.84	1.13
yudJ	1.18	0.79	0.79	0.96	0.91
yudK	0.79	0.97	0.77	0.61	0.75
yudL	0.83	0.91	0.97	0.9	0.81
yueA	1.12	0.9	0.91	1.15	0.99
yueB	0.36	0.34	0.3	0.26	0.32
yueC	0.88	0.61	0.62	0.84	0.79
yueD	0.9	0.86	0.74	0.88	0.68
yueE	1.31	1.12	0.9	1.3	1.35
yueF	1.47	1.24	1.12	1.17	1.34
yufA	0.93	0.96	1.06	0.99	0.9
yufC	1.01	0.86	0.64	1.04	0.64
yugA	1.17	1.05	1.27	1.38	1.29
yugB	1.62	1.48	1.75	1.63	1.57
yugC	1.12	1.08	1.43	0.96	1.13
yugD	0.72	0.8	1.14	0.79	0.94
yuhA	1.17	1.17	0.93	1.11	1.15
yuhB	0.94	0.84	0.86	1.08	0.86
yuhC	0.95	0.87	0.71	1.05	1
yuhD	0.96	0.89	0.59	0.93	0.75
yuhE	1.32	1.06	1.01	1.19	0.83
yuhH	0.84	0.82	0.85	0.89	0.61
yuhI	0.68	0.95	0.8	0.81	0.92
yuhJ	0.97	1.1	0.92	1.08	1.31
yuiA	0.99	0.99	0.84	0.95	1.11
yuiB	0.88	0.97	2.34	0.53	1.1
yuiC	0.98	0.94	0.86	1.11	0.96
yuiD	1.1	1.28	1.02	1.24	0.87
yuiE	1.57	1.43	1.06	1.15	1.29
yujA	0.88	1.03	1.29	0.87	1.02
yujB	1.27	1.38	1.53	1.36	1.34
yujD	0.97	0.91	0.84	0.95	0.91
yujE	0.86	0.88	0.89	0.85	0.89
yujF	1.23	1	0.9	1.19	1.19

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yujG	1.19	1.08	0.94	1.29	1.31
yvaA	0.7	0.8	0.87	0.86	0.88
yvaB	1.16	1.08	0.84	1.25	0.78
yvaD	1.05	0.83	0.91	1.18	1
yvcA	1	0.79	0.85	1.15	0.92
yvcC	1.59	1.29	1.13	1.38	1.23
yvdB	1.44	1.59	1.01	1.7	1.43
yvdC	0.74	1.06	1.08	0.67	0.68
yvdD	0.72	0.7	0.54	0.69	0.65
yvdE	1.77	2.23	2.07	1.46	0.78
yvdF	1.03	1.02	1.1	0.58	0.69
yvdG	0.68	0.67	0.44	0.44	0.47
yveB	1.07	0.87	1.02	0.81	0.97
yveC	1.44	0.87	1	1.48	1.39
yveD	1.88	1.46	1.23	2.36	1.43
yveE	1.42	1.26	1.09	1.59	1.07
yveF	1.91	1.6	1.45	2.29	1.37
yveG	0.98	0.98	0.91	0.78	0.87
yveH	1.36	1.12	1.02	1.36	1.05
yveI	0.85	1.12	1.43	0.79	1.08
yvfA	1.22	1.08	0.86	0.84	0.81
yvfB	0.96	1.16	0.95	0.71	0.82
yvhA	1.03	1.14	0.73	1.28	1.13
yvhB	1.49	1.65	1.25	1.4	1.01
yviA	2.13	2.29	1.58	1.93	2.03
yviC	1.8	2.19	1.69	2.28	2.11
yviH	1.15	1.02	0.93	1.1	1.04
yviI	1.56	1.28	0.96	1.57	1.4
yviJ	1.19	0.91	0.93	0.94	0.8
yvjA	1.21	1.38	0.89	1.17	0.75
yvjB	0.91	1.06	0.95	1	0.76
ywaB	0.83	1	0.81	1.02	0.84
ywaC	0.93	0.95	0.81	0.7	0.94
ywaD	1.1	1.22	1.37	1.25	1.09
ywaE	0.77	0.78	0.87	0.81	0.52
ywaF	0.98	0.95	0.95	1.24	0.84
ywaG	1.16	1.08	0.95	1.37	0.93
ywaH	3.22	2.33	0.87	3.99	2.25
ywaI	1.8	1.53	1.15	1.8	0.99
ywbA	1.12	1.04	0.7	1.1	0.81
ywbB	1.07	0.78	0.85	0.74	1.32
ywcC	0.86	0.83	1.25	0.76	1.1
ywdA	1.38	0.92	0.68	1.39	1.07
ywdB	1.48	1.21	1.01	1.26	1.33
ywdC	1.34	1.73	0.97	1.48	1.1
ywdD	1.29	1.44	1.11	1.45	1.28
ywdE	0.95	0.97	0.98	0.94	0.93

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ywdF	1.08	1.16	1.09	0.94	0.91
yweA	1.04	1.01	0.78	0.92	0.81
yweB	0.8	1.09	1.17	0.8	0.74
yweC	0.65	0.78	1.48	0.59	0.96
yweD	1.78	1.37	1.48	1.44	1.11
yweE	1.1	1.03	0.72	1.32	1.38
yweF	1.08	0.97	1.03	1.35	0.94
ywfA	1.12	1.37	1.42	1.65	1.17
ywfB	1.02	1.23	0.91	1	1.02
ywfC	0.99	0.94	1	1.42	0.89
ywfD	0.92	1.1	1.09	0.97	0.72
ywfE	0.8	1.01	1.12	0.97	0.75
ywfF	0.76	0.8	0.94	1.05	0.63
ywfH	0.63	1.58	1.33	3.2	0.75
ywgA	1.17	1.31	0.86	1.68	0.87
ywhA	1.03	1.1	1.24	1.13	0.85
ywiA	1.23	1.01	0.83	1.21	0.85
ywiB	1.48	1.31	1.07	1.67	1.09
ywiC	1.25	1.14	1.12	1.13	1.21
ywiD	1.01	1.23	1.11	1.85	1.4
ywiE	1.32	0.86	0.94	1.24	1.28
ywiH	1.38	1.75	0.98	1.93	1.5
ywjA	1.33	1.33	1.01	1.13	0.91
ywjB	0.7	0.9	0.94	1.04	0.74
ywjC	1.17	1.06	0.89	1.1	0.98
ywjD	0.26	0.35	0.79	0.29	0.33
ywjE	0.35	0.46	1	0.37	3.29
ywjG	0.84	0.96	0.8	1.07	0.98
yxaB	1	1.22	0.84	1.73	1.24
yxaC	1.07	1.07	1.05	1.11	0.71
yxaF	1.26	1.13	0.85	1.28	1
yxbA	0.99	1.04	0.9	1.08	0.84
yxbC	0.87	1.4	1.32	0.79	0.51
yxbD	0.86	1.1	0.86	0.95	1.12
yxbE	0.68	0.57	0.74	0.98	0.95
yxbF	1.04	1.12	0.99	0.91	1.02
yxcA	0.68	0.87	1.29	0.84	0.87
yxcB	0.45	0.61	0.68	0.58	0.53
yxcD	1.12	1.31	0.89	1.61	0.78
yxdB	0.53	0.5	0.58	0.82	0.55
yxdC	0.59	0.95	0.79	0.44	0.42
yxdD	2.63	1.57	1.4	1.44	1.2
yxdE	1.89	1.31	0.92	1.09	1.2
yxdF	1.07	1.12	0.83	1.14	1.11
yxdG	0.91	0.83	1.28	0.79	1.06
yxeA	2.94	2.73	2.59	2.33	2.27
yxeB	2.86	3.01	1.89	2.81	1.39

Table D1 (Continued).

Expression ratios between the strains of interest and the reference LD 61 strain					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yxfA	0.47	0.83	0.21	0.51	0.5
yxfB	0.83	0.9	0.75	0.85	0.75
yxfC	0.97	1.12	1.1	1.27	1
yyaL	0.92	0.97	0.96	1.29	0.71
zitP	1.11	0.97	0.89	1.01	0.76
zitQ	1.03	1.09	1.04	0.92	0.97
zitR	0.94	0.88	0.7	0.74	0.84
zitS	0.56	0.54	0.48	0.57	0.5
zwf	0.59	0.84	0.94	0.53	0.88

Table D2 CGH ratios of the 1,915 genes of the core genome.

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
accB	1.09	0.83	0.94	0.88	0.96
accC	1.06	0.92	0.99	0.95	0.89
accD	1.16	0.98	1.12	1.07	0.99
ackA1	1.24	1.18	1.21	1.14	1.19
ackA2	1.31	1.27	1.27	1.17	1.12
acmA	1.21	1.04	1.04	1.08	1.06
acmB	1.47	1.3	1.11	1.06	1.05
acmC	0.91	0.85	0.98	0.79	0.81
acmD	1.12	1.12	1	1.04	1.01
acpA	0.98	0.79	0.84	0.89	0.85
acpD	0.9	0.82	0.94	0.78	0.81
adaA	1.27	1.06	1.13	1.15	1.22
add	1.27	1.02	1.09	0.96	0.96
adhA	1.23	1.15	1.11	1.19	0.95
adhE	1.24	1.09	1.23	1.06	1.13
adk	1.01	0.93	1.04	0.93	0.94
ahpC	1.39	1.24	1.26	1.08	1.13
ahpF	1.4	1.32	1.23	1.13	1.1
ahrC	0.85	0.82	0.81	0.77	0.75
alaS	1.03	1.11	1.06	1.04	1
aldB	1	0.88	1.02	0.83	0.94
aldR	0.97	0.71	1	0.96	0.95
als	0.96	0.85	0.89	0.8	0.92
amtB	1.12	0.89	0.95	0.94	0.89
amyL	1.09	0.85	1.04	0.95	0.98
ansB	1.24	1.14	1.19	1.12	1.19
apbE	1.01	0.95	0.98	0.89	0.89
apl	1.12	1.08	1.06	0.97	1.03
apt	1.21	1.15	1.13	1.05	1.1
apu	0.98	1.02	1.05	0.88	0.99
araT	1.04	0.98	1.12	0.9	1.01
arcA	1.04	1.08	1.1	0.97	1.03
arcB	1.2	1.02	1.08	0.99	1.21
arcC1	1.05	0.92	0.9	0.88	0.98
arcC2	1.2	1.1	1.16	1.1	1.07
arcC3	1.11	1.03	1.07	1	0.98
arcD1	1.36	1.3	1.26	1.2	1.2
arcD2	1.14	1.13	1.07	1.03	1.12
arcT	1.12	1.1	1.1	0.99	0.99
argB	1.36	1.37	1.29	1.18	1.22
argC	1.02	1.03	1.06	0.91	1.01
argD	0.91	0.95	0.97	0.86	0.92
argE	1.17	1.16	1.28	1.06	1.18
argF	1.12	1.11	1.05	1.07	1.12
argG	1.21	1.02	1.16	1.12	1.2
argH	1.09	1.07	1.04	1.09	1.12

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
argJ	1.19	1.07	1.11	1.06	1.06
argR	1.16	1.07	1.09	1.05	1.09
argS	1.11	1.14	1.14	1.08	1.04
aroA	1.11	1.05	1.02	1.03	0.95
aroB	1	0.98	0.98	0.95	0.96
aroC	1.05	1.09	0.96	1.01	0.92
aroD	1.43	1.37	1.29	1.33	1.41
aroE	1.09	1.08	1	0.94	0.98
aroH	0.78	0.76	0.99	0.8	0.83
arsC	0.89	0.45	0.87	0.89	0.88
asd	1.04	0.92	0.99	1.01	0.96
asnB	1.08	1.09	1.09	1.05	1.02
asnH	1.34	1.23	1.27	1.25	1.25
asnS	1.41	1.35	1.28	1.36	1.3
aspB	1.08	1.07	0.98	1.03	0.97
aspC	1.06	1.06	1	1.02	0.93
aspS	1.3	1.32	1.2	1.16	1.18
atpB	0.81	0.93	0.91	0.82	0.82
atpD	0.98	1.14	1.09	1.02	1.15
atpE	0.86	0.88	0.86	0.84	0.9
atpF	1	0.93	1.07	1	0.96
atpG	1.03	1.01	1.02	1	0.96
atpH	1.11	1.03	1.11	1.04	1
bacA	1.03	0.99	0.98	0.94	0.93
bar	1.08	1.11	1.01	1.11	1.06
bcaT	0.96	1.09	1.01	1.04	0.99
bglA	1.08	1.05	0.96	0.96	1
bglH	1.31	1.24	1.15	1.14	1.09
bglR	0.79	1.06	1.03	1	0.97
bglS	1.07	1.07	1.15	1.03	1.04
birA1	1.18	1.09	1.25	1.12	1.09
birA2	1.05	1.08	1.11	1.03	1.01
blt	1.24	1.09	1.22	1.1	1.13
bmpA	1.07	0.97	0.98	0.9	0.95
busAA	0.98	0.94	0.99	0.99	0.92
busAB	1	0.95	0.91	1.03	0.87
busR	1.14	1.16	1.05	1.2	1.07
butA	1.11	1.02	1.06	1.06	0.98
butB	1.09	1.04	1.08	1.03	1.01
cadA	1.26	1.22	1.17	1.04	1.12
carA	1.11	1.19	1.19	1.13	1.06
carB	0.86	0.88	0.96	0.87	0.82
cbr	1.1	1.06	1.23	1.05	1.05
ccpA	1.15	1.19	1.15	1.17	1.26
cdd	1.07	0.93	1.19	0.94	1.11
cdsA	1.3	1.13	1.26	1.08	1.03

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
celB	1.24	1.19	1.13	1.17	1.22
ceo	1.64	1.14	1.13	1.53	1.47
chiA	1.16	1.17	1.16	1.19	1.09
choS	1.04	0.98	1	0.88	0.92
citB	1.15	1.09	1.16	1.03	0.98
citC	0.66	0.68	0.99	0.58	0.59
citD	0.7	0.5	0.98	0.58	0.51
citE	0.48	0.41	1.05	0.37	0.4
citF	0.58	0.53	1.15	0.54	0.58
clpB	1.05	0.95	1.02	0.94	0.98
clpC	0.92	0.9	0.96	0.94	0.91
clpE	1.38	1.37	1.23	1.29	1.21
clpP	1.17	1.17	1.13	1.25	1.09
clpX	1.14	1.09	1.06	1.01	0.98
clsA	1.16	1.16	1.14	1.11	1.11
clsB	1.04	0.98	1.08	0.9	0.88
cmk	0.75	0.89	0.86	0.72	0.73
coaA	0.98	0.95	1.02	0.89	0.86
cobC	1.06	1.06	1.09	1.01	1.03
cobQ	1.01	0.95	1.09	1	1
codY	0.98	1.02	0.97	0.99	1.02
codZ	0.94	0.79	0.79	0.71	0.92
coiA	1.34	1.22	1.18	1.17	1.07
comC	1.17	1.15	1.06	1.15	1.12
comEA	1.02	1.04	0.96	1.04	0.93
comEC	0.89	0.97	0.91	0.95	0.9
comFA	1.07	1.08	1.05	0.97	1
comFC	1.06	1.07	1.15	0.89	0.94
comGA	1.09	1.05	1.07	0.86	0.96
comGB	0.9	0.93	0.96	0.82	0.87
comGC	0.81	0.79	0.83	0.76	0.82
comGD	0.74	0.74	0.65	0.56	0.74
comX	0.7	0.8	0.83	0.8	0.78
copB	1.2	2.61	1.81	1.09	1.2
copR	0.87	0.88	0.88	0.96	0.9
cpo	0.97	0.93	0.95	0.99	0.9
cpsM	0.86	0.85	1.04	0.96	0.96
crtK	1.04	1.05	1.02	0.94	0.94
cshA	1.39	1.38	1.51	1.17	1.29
cspE	0.91	0.76	0.83	0.83	0.75
cstA	1.16	1.09	1.12	0.9	0.95
ctrA	1.09	0.99	1.07	0.9	0.96
ctsR	1.08	1.05	1.19	0.96	1.05
cydA	0.87	0.86	0.93	0.83	0.89
cydC	1.05	1.05	1.02	1.17	1.2
cydD	1.28	1.18	1.26	1.15	1.16

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
cysD	1.07	1.1	1	1.07	1.06
cysE	1.13	1.09	0.9	1.08	1.06
cysK	1.08	1.07	0.94	1.02	1.05
cysM	0.86	0.97	0.92	0.99	0.88
cysS	1.02	1.07	0.92	0.99	1.01
dacA	0.84	0.86	0.89	0.85	0.84
dacB	1.04	0.96	0.9	0.84	0.86
dal	1	0.84	0.95	0.9	0.89
dapA	0.96	0.88	0.97	0.98	0.96
dapB	1.17	1.07	1.15	1.04	0.96
dcdA	0.96	1.02	1.05	0.97	0.95
ddl	1.14	1.2	1.06	1.07	1.12
def	1.25	1.3	1.12	1.24	1.18
deoB	0.96	1.01	0.93	0.99	0.95
deoC	0.99	0.93	0.82	0.96	0.91
deoD	0.98	1.08	1.02	0.99	1
dexB	0.78	0.83	0.86	0.84	0.73
dfpB	1.02	1.04	1.12	0.97	0.99
dfrA	0.93	0.89	1.13	0.87	0.99
dgkA	0.71	0.76	0.72	0.82	0.84
dhaK	1.09	1.01	1.1	1.01	1.12
dhaL	1.09	1.16	0.93	1.1	1.11
dhaM	1.24	1.24	1.24	1.28	1.24
dinF	1.12	1.22	1.08	1.17	1.13
dltC	1.11	0.83	1.14	1.07	1.04
dnaC	1.22	1.1	1.16	1.13	1.12
dnaD	1.01	1.04	1.02	1	1
dnaE	1.09	1.2	1.15	1.14	1.12
dnaH	1.07	1.13	1.06	1.11	1.12
dnaJ	0.9	1.12	1.05	1.05	1.03
dnaK	1.04	1.03	1.08	1.13	1.01
dnaN	1.08	1.13	1.08	1.12	0.99
dnaQ	0.92	0.92	0.95	0.83	0.85
dpsA	0.96	0.93	0.9	0.94	0.86
dukA	1.12	1.09	1.07	1.04	1.04
dukB	0.93	0.94	0.9	0.9	0.99
dut	1.08	0.98	1.08	1.05	1.1
dxsA	1	1.01	0.89	1.01	0.97
dxsB	1.15	1.18	1.14	1.23	1.16
ecsB	0.87	1.07	1	1	0.94
efp	1.17	1.25	1.35	1.27	1.26
enoA	1.1	1.17	1.22	1.11	1.05
enoB	1.13	1.09	1.13	1.09	1
eraL	1.23	1.22	1.3	1.15	1.12
exoA	1.04	1.02	1.05	1.05	1.02
ezrA	1.03	1.03	1.11	1.06	1.07

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
fabF	1.07	1.01	1.04	1.03	1.09
fabG1	1.06	1.05	1.06	1.1	1.04
fabG2	1.06	1.13	1.1	1.08	1.07
fabI	0.99	1.05	1.08	1.03	0.96
fabZ1	0.93	1.09	1.18	1.04	0.99
fabZ2	0.96	0.98	1.02	0.94	0.93
fadA	1.24	1.31	1.3	1.23	1.2
fadD	1.27	1.24	1.35	1.24	1.15
fbaA	1.28	1.2	1.15	1.16	1.1
fbp	1.24	1.13	1.27	1.23	1.24
femD	1.15	1.09	1.13	1.13	1.11
feoA	0.93	0.95	0.89	0.78	0.85
feoB	1.24	1.22	1.19	1.09	1.19
fer	0.91	0.83	0.95	0.94	0.88
ffh	1.05	1.1	1.02	1.14	1.03
fhs	1.09	1.13	1.19	1.23	1.14
fhuB	0.95	1.04	0.95	0.88	0.94
fhuD	1.01	1.11	1.1	1.02	1.05
fhuG	1.14	1.22	1.3	1.05	1.11
fhuR	1.25	1.27	1.35	1.08	1.14
fmt	0.89	0.95	0.95	0.91	0.86
folB	1.02	0.96	1.08	1.05	0.96
folC	0.89	0.91	1.08	0.94	0.9
folD	1.06	1	1.14	1.05	1.04
folE	1.03	0.99	1.03	1.04	0.91
folP	1.04	1.01	1.02	1.04	0.99
frdC	1.01	1.07	1	1.04	1.02
frr	0.86	0.99	0.86	0.92	0.91
ftsA	1.13	1.09	1.13	0.99	1.03
ftsE	1.06	0.96	1.06	0.89	0.94
ftsH	1.15	1.17	1.16	0.96	1.13
ftsW1	1.03	1.05	1.12	1.07	1.01
ftsW2	0.98	0.92	0.9	0.91	0.97
ftsX	1	0.92	1.07	1.05	0.99
ftsY	1.03	1	0.99	1.01	0.93
ftsZ	1.05	1.19	1.12	1.16	1.07
fur	0.94	1.17	1.16	1	1.12
fusA	0.93	1.1	1.09	1.06	1.09
gadB	0.86	0.95	0.94	0.9	0.89
gadC	0.92	0.97	0.95	0.93	0.9
gadR	0.9	1.02	1.04	0.96	0.95
galE	1.18	1.17	1.75	1.16	1.13
galK	1.04	1.14	1.13	1.15	1.07
galM	0.92	0.97	1.03	0.95	0.99
galT	0.94	1.03	1.02	0.98	0.97
gapA	1.04	1.01	1.03	1.11	0.98

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
gapB	1.09	1.21	1.05	1.14	1.05
gatB	0.94	1.13	1.14	1.06	1.17
gatC	0.81	0.97	0.98	0.88	0.93
gcp	1.02	1.1	1.04	1.02	1.13
gidA	1.06	1.14	1.03	1.04	1.07
gidB	1.04	1.06	1.08	1.06	1.03
gidC	0.97	1.01	1.14	0.98	1
glgA	1.1	1.09	1.14	1.14	0.99
glgC	0.89	0.97	0.98	0.93	0.9
glgD	0.97	1.01	1.05	0.95	1.01
glgP	1.16	1.1	1.09	1.06	1.24
glk	1.16	1.09	1.08	1.07	1.12
glmS	0.91	0.94	1	0.92	0.93
glmU	1.11	1.18	1.07	1.11	1.11
glnA	1.2	1.34	1.18	1.05	1.31
glnB	0.92	0.91	0.92	0.96	0.99
glnP	1.08	1.19	1.02	1.1	1.05
glnR	1	1.1	0.96	0.98	1.01
glpD	0.82	0.9	1	0.96	0.9
glpF1	0.91	1.02	1	1.06	1.02
glpF2	0.82	0.87	0.89	0.81	0.89
glpK	0.8	0.84	0.91	0.89	0.9
glpT	1.03	1.03	1.03	1.07	0.99
gltA	1.05	1.15	1.15	1.15	1.16
gltD	0.76	0.81	0.91	0.81	0.78
gltQ	0.93	1.02	0.98	0.89	0.93
gltS	0.96	1.07	1.02	1	1.04
glyA	1.01	1.1	1.07	1.03	1.08
glyS	0.81	0.89	0.99	0.87	0.9
gnd	0.84	0.87	0.92	0.92	0.86
gntK	0.95	1.07	1.12	0.97	1.04
gntR	1.12	1.16	1.18	1.06	1.25
gntZ	1.25	1.13	1.19	1.25	1.21
gpdA	0.69	0.73	0.8	0.79	0.75
gpo	0.83	0.9	0.95	0.88	0.91
greA	0.94	1.07	1.02	1.05	1.05
groES	1.05	0.62	1	0.94	0.91
grpE	0.95	1	0.99	0.93	0.95
gshR	0.95	1.02	0.97	0.97	0.99
guaA	0.99	0.99	0.94	1.03	1.01
guaB	1.11	1.26	1.25	1.18	1.11
guaC	0.89	0.88	0.94	1.02	0.93
gyrA	0.78	0.78	0.72	0.76	0.79
gyrB	0.83	0.9	0.94	0.86	0.88
hemH	0.84	0.95	0.87	0.91	0.95

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
hemK	0.92	1.13	1.07	1.03	1.02
hemN	0.92	0.98	1.01	0.93	0.99
hexA	1.16	1.29	1.19	1.08	1.25
hexB	0.97	1.09	1.06	0.99	1.08
hflX	1.18	1.13	0.95	0.98	1.05
hisA	0.95	0.97	1.01	0.92	0.96
hisB	0.87	0.89	0.84	0.87	0.89
hisC	0.99	0.93	0.95	1.02	0.94
hisD	0.89	0.9	0.88	0.94	0.93
hisG	0.86	0.87	1.03	0.87	0.96
hisH	0.96	0.97	1.13	0.95	0.97
hisI	0.83	0.93	0.93	0.94	0.92
hisK	0.87	1.01	0.98	0.93	0.98
hisS	0.82	0.97	0.94	0.88	0.94
hisZ	0.79	0.85	0.88	0.85	0.85
hly	1.13	1.16	1.04	1.06	1.11
hmcM	0.91	0.93	0.86	0.9	0.85
holB	0.83	0.94	0.91	0.89	0.86
hom	0.76	0.84	0.85	0.89	0.83
hprT	0.98	1	1.03	0.98	0.89
hpt	0.81	0.84	0.91	0.94	0.83
hrcA	0.99	1.04	1.04	1.04	1.02
hsdM	0.64	0.65	1.06	0.91	0.67
hsdR	0.83	0.94	1.13	0.99	0.85
hsdS	0.64	0.67	1.19	0.71	0.75
hslA	0.96	0.89	0.96	0.89	0.93
hslB	0.75	0.86	0.91	0.85	0.87
htrA	1.12	1.2	1.13	1.09	1.14
icaA	1.04	1.02	0.93	0.96	1.01
icaC	0.95	1.02	0.96	0.99	0.99
icd	1	1.01	0.94	1.03	1.01
ileS	0.93	1.04	0.95	0.95	0.94
ilvB	0.89	0.94	0.95	0.85	0.88
ilvC	0.95	1.02	1.04	1.06	0.98
ilvD	0.79	0.88	0.96	1	0.92
ilvN	0.72	0.8	0.88	0.8	0.77
infA	1.03	0.97	0.96	0.96	0.93
infB	0.9	0.99	0.98	1.02	1.02
infC	0.88	1.03	1.04	1	0.99
ipd	0.87	0.98	0.95	0.94	0.97
ispA	0.95	0.93	1.02	1.01	0.97
ispB	0.84	0.92	0.93	0.92	0.94
kdgA	0.9	0.91	0.72	0.94	0.89
kdgK	0.81	0.87	0.78	0.84	0.87
kdtB	1.1	1.22	1.11	1.08	1.1

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
kinA	0.94	1.01	1	0.97	0.94
kinB	0.8	0.9	0.94	0.9	0.85
kinC	0.98	1.08	1.04	1.07	1.06
kinD	0.74	0.83	0.9	0.83	0.84
kinE	0.92	0.94	1.01	0.96	0.97
kinF	0.94	0.96	0.94	0.76	0.78
ksgA	0.97	1.01	0.99	1.07	1.01
kupA	0.89	0.97	0.91	0.98	0.87
kupB	0.92	0.94	0.93	0.9	0.93
lacC	0.94	0.99	0.93	0.89	1.07
lacR	0.95	1.06	1.01	0.77	0.99
lacZ	1.01	1.12	1.09	0.96	1.04
lcnC	0.51	0.6	1.05	0.94	1.05
lcnD	0.33	0.41	1.16	1.03	1.17
lctO	0.86	0.94	1	0.91	0.93
ldh	0.84	0.97	1.02	0.98	1
ldhB	0.88	0.87	0.99	0.95	0.97
ldhX	0.82	0.93	1.05	0.88	0.98
lepA	0.74	0.87	0.87	0.83	0.78
leuB	0.81	0.87	0.88	0.85	0.81
leuC	0.86	0.92	0.97	0.97	0.99
leuD	0.87	0.96	0.97	0.89	0.93
leuS	1.06	1.14	1.09	0.96	1.1
lgt	0.79	0.94	1.01	0.95	0.97
ligA	0.91	1.05	1.17	1.06	1.01
llrA	0.91	1.04	1.07	1.03	1.08
llrB	0.86	0.99	1.12	0.98	0.93
llrC	1.19	1.28	1.27	1.24	1.29
llrD	0.79	0.87	0.94	0.84	0.87
llrE	0.88	0.96	1.07	0.9	0.96
llrF	0.73	0.95	0.92	0.88	0.85
llrG	0.88	0.95	0.92	0.93	0.85
llrH	0.6	0.63	0.75	0.92	0.8
lmrA	1	1.17	1.06	1.03	1.21
lnbA	1.42	1.1	1.3	1.39	1.04
lplL	1.24	1.13	1.12	1.16	0.78
lspA	0.99	0.8	1.04	0.99	0.86
lysA	1.05	0.92	1.13	1.09	1
lysP	1.31	1.01	1.18	1.14	1.13
lysQ	1.15	1.03	1.1	1.04	1
lysS	1.37	1.31	1.22	1.16	1.18
mae	0.44	0.42	0.9	0.43	0.43
malQ	1.14	0.95	1.09	1.13	0.85
menB	1.2	1.02	1.19	1.18	1.08
menD	1.15	0.99	1.16	1.12	1.1
menE	1.14	0.86	1.16	1.11	1.04

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
menF	0.99	0.91	0.94	0.88	0.87
menX	1.07	0.97	0.99	0.93	0.85
mesJ	1.16	1.07	1.04	1.02	1.04
metB1	0.99	0.95	1.13	1.03	0.92
metB2	1.22	1.08	0.86	1.08	1.01
metE	1	0.8	0.96	0.98	0.98
metF	1.12	0.96	0.96	0.93	0.98
metK	1.21	1.05	1.05	1.1	1.13
metS	1.26	1	1.17	1.1	1.17
mgtA	1.15	1.02	1.13	1.08	1.09
miaA	1.22	1.09	1.12	0.99	1.09
mleR	0.99	0.82	1.01	0.85	0.96
mleS	0.9	0.93	0.97	0.8	0.91
mreC	1.24	1.16	1.12	0.98	1.11
mreD	1.29	1.04	1.04	1.04	1.21
mscL	1.14	0.93	0.98	1	1.05
msmK	1.11	0.94	1.05	1.09	1.02
mtlD	1.46	1.21	1.25	1.23	1.32
mtlF	1.11	1	1.2	1.23	1.25
mtlR	1.21	1.13	1.13	1.03	1.22
mtsA	1.12	1	1.11	0.98	1.02
mtsB	1.2	1.05	1.11	0.99	1.08
mtsC	0.92	0.92	0.9	0.76	0.84
murA1	1.03	1	1.09	0.85	1
murA2	1.06	0.99	1	0.85	1.02
murB	0.7	0.68	0.85	0.76	0.75
murC	1.34	1.13	1.28	1.11	1.25
murD	1.04	0.92	0.96	1.02	1
murE	1.04	0.96	1	1.02	0.99
murF	1.09	1.03	1.09	1.11	1.08
murI	1.17	1.03	1.18	1.06	1.11
mutM	1.05	0.98	0.94	0.96	0.97
mutS	1.09	1.08	1.06	1.03	1.07
mutX	0.96	0.96	0.91	0.82	0.91
mvaA	0.82	0.81	0.9	0.81	0.89
mycA	1	0.93	1.05	0.91	0.99
nadE	0.9	0.76	1.02	0.95	0.95
nadR	1.16	1.14	1.12	1.19	1.1
nagA	1.05	0.96	0.98	1	1
nagB	1.27	1.27	1.25	1.33	1.21
nah	1.2	1.18	1.15	1.22	1.2
napB	1.02	1.02	0.98	1.01	0.97
napC	1.22	1.15	1.08	1.03	1
ndrH	0.86	0.77	0.78	0.79	0.8
ndrI	0.95	0.9	0.9	0.88	0.9
nifS	0.96	1.05	1	0.87	0.97

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
nifU	0.84	0.87	0.93	0.81	0.88
nifZ	0.83	0.94	0.9	0.83	0.81
noxA	1.11	0.98	1.11	1.04	1.04
noxB	1.25	1.11	1.13	1.26	1.13
noxC	1.02	0.95	1.02	1.01	0.98
noxD	1.03	0.97	1.05	1.13	1.05
noxE	1.32	1.32	1.18	1.35	1.24
nrdD	1.06	1.05	0.99	1.05	0.98
nrdE	0.95	0.92	0.96	0.93	0.93
nrdF	0.96	0.99	1.01	0.95	0.92
nrdG	1.03	0.99	0.93	0.9	0.9
nth	1.03	1.04	1.08	1.01	1.01
nucA	0.89	0.8	0.98	0.82	0.83
nusA	0.87	0.92	0.96	0.85	0.85
nusB	1.33	1.06	1.34	1.35	1.31
nusG	1.2	1.11	1.15	1.08	1.14
obgL	1.06	0.94	1.01	1.08	1.05
ogt	1.31	1.14	1.19	1.25	1.19
oppA	1.62	1.66	1.44	1.55	1.08
oppB	2.12	2.07	1.74	1.62	1.92
oppC	2.18	2.21	1.89	1.86	1.92
oppD	1.77	1.78	1.7	1.58	1.81
oppF	2.19	2.28	2.13	1.91	2.16
optA	1.21	1.22	1.32	1.12	1.14
optB	1.03	0.97	1.13	0.96	1.02
optC	1.24	1.27	1.15	1.07	1.25
optD	1.02	0.89	1.01	0.97	0.97
optF	1.23	1.02	1.22	1.13	1.02
optS	1.13	1.1	1.16	1.07	1.07
osmC	1.04	1.04	1.03	1.06	0.99
otcA	1.14	1.14	1.08	1.13	1.02
pabB	0.77	0.84	0.96	0.83	0.75
pacB	0.87	0.83	0.84	0.79	0.77
pacL	1.03	0.97	1	0.98	1.01
panE	0.96	0.87	1.05	0.97	0.87
papL	0.99	0.95	0.99	0.97	0.92
parA	1.29	1.13	1.21	1.14	1.17
parC	0.88	0.85	0.88	0.91	0.87
parE	1.14	1.05	1.01	1.08	1.06
pbp1B	1.15	1.09	1.08	1.18	1.07
pbp2A	0.91	0.95	0.91	0.95	0.89
pbp2B	1.08	0.98	1.02	0.95	0.94
pbpX	1.13	1.02	1.11	0.96	0.99
pbuX	1.11	1.18	1.12	0.96	1.01
pcaC	0.93	0.96	0.96	0.92	0.9
pcrA	1.16	1.04	1.17	1.09	1.06

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pdc	1.14	1.08	1.25	1.13	1.11
pdhA	0.98	1.13	1.04	1.09	1.19
pdhB	1.12	1.01	1.13	0.95	1.09
pdhC	0.92	0.89	0.93	0.98	0.84
pdhD	1.12	1.08	1.12	1.09	1.04
pdp	0.97	0.97	0.87	1.01	0.88
pepC	1.01	1.04	1.01	1.01	0.98
pepDA	0.96	0.93	1.03	0.86	0.89
pepDB	1.02	0.98	0.98	0.75	0.85
pepF	1.41	1.38	1.34	1.07	1.26
pepM	1.24	1.1	1.2	0.99	1.12
pepN	1.03	0.96	1.03	0.93	0.92
pepO	0.86	0.9	0.99	0.87	0.96
pepP	1.14	1.01	1.1	1.07	1.08
pepT	1.34	1.34	1.09	1.23	1.19
pepV	1.07	1.06	1.06	1.14	1.02
pepXP	1.06	1.13	1.04	0.98	0.88
pfl	1.06	0.99	1.13	0.88	0.94
pflA	1.07	1.05	1.06	0.83	0.89
pfs	1.15	1.07	1.12	0.99	1.03
pgk	1.1	1.08	1.26	0.97	1.05
pgmB	0.76	0.81	0.86	0.79	0.84
pgsA	1.1	1.05	1.1	0.98	1.06
pheA	1.16	1.17	1.07	1.08	1.07
pheS	1.34	1.42	1.17	1.31	1.33
pheT	1.22	1.23	1.04	1.11	1.07
phnA	1.01	1.03	0.93	0.96	1.01
phnB	0.95	1.05	0.93	0.93	0.94
phnC	1.08	1.09	1.07	0.93	0.95
phnE	1.21	1.1	1.17	1.03	0.98
phoL	1.02	0.87	1.11	0.92	0.97
phoU	1.06	0.96	1.02	0.91	0.99
pi101	0.65	0.66	1.19	0.65	0.65
pi102	0.45	0.57	0.93	0.58	0.57
pi103	0.81	0.77	0.97	0.76	0.76
pi104	0.7	0.7	0.71	0.69	0.7
pi105	0.81	0.74	1.08	0.76	0.83
pi106	0.77	0.73	0.87	0.76	0.68
pi107	0.78	0.87	0.99	0.83	0.81
pi108	0.45	0.59	1.11	0.38	0.6
pi109	0.13	0.16	0.92	0.75	0.89
pi110	0.79	0.61	0.75	0.77	0.75
pi111	0.4	0.43	0.92	0.41	0.4
pi113	0.99	0.83	0.98	0.83	0.8
pi114	0.88	0.51	0.87	0.46	0.48
pi115	0.83	0.56	1.13	0.9	0.72

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pi116	0.67	0.7	0.95	0.78	1.08
pi117	0.86	0.73	0.92	0.94	0.85
pi118	1.12	0.69	1.05	0.82	0.69
pi120	0.8	0.6	1.11	0.74	0.91
pi122	0.55	0.44	1.01	0.43	0.49
pi123	0.4	0.24	0.98	0.33	0.3
pi124	0.97	0.64	1.02	0.85	0.82
pi125	0.62	0.53	0.84	0.76	0.53
pi127	0.66	0.55	0.84	0.72	0.66
pi128	0.5	0.59	0.89	0.58	0.57
pi129	0.55	0.59	1.07	0.59	0.61
pi130	0.39	0.4	1.2	0.33	0.48
pi133	0.28	0.29	1.06	1.16	0.48
pi135	0.79	0.66	0.89	0.97	0.81
pi137	0.21	0.21	1.02	1.06	0.23
pi138	0.54	0.58	1.03	0.97	0.58
pi139	0.58	0.61	1	0.94	0.6
pi140	0.74	0.77	1	1	0.69
pi141	0.63	0.65	0.87	0.83	0.63
pi142	0.37	0.4	1.03	0.96	0.43
pi143	0.43	0.45	1.07	1.09	0.46
pi144	0.4	0.38	0.89	0.93	0.49
pi145	0.14	0.14	1.18	0.11	0.14
pi205	0.89	0.71	0.79	0.72	0.63
pi208	0.17	0.2	0.68	0.62	0.58
pi209	1.42	0.32	0.61	0.71	0.9
pi210	0.94	1.12	1.67	1	0.9
pi211	0.3	0.28	1.14	0.26	0.31
pi215	1.06	1.07	1.67	1.08	1.44
pi216	0.7	0.65	0.84	0.63	0.67
pi217	0.68	0.51	0.73	0.52	0.6
pi218	0.51	0.57	0.8	0.65	0.7
pi222	0.81	0.6	1.02	0.89	1
pi223	0.45	0.42	1.34	0.49	0.57
pi224	0.68	0.74	0.97	0.75	0.72
pi227	0.6	0.67	0.9	0.72	0.66
pi228	0.56	0.63	0.95	0.6	0.71
pi229	0.29	0.33	1.08	0.37	0.35
pi230	0.46	0.5	0.99	0.43	0.49
pi231	0.52	0.57	1	0.5	0.5
pi232	0.45	0.46	1.04	0.43	0.44
pi233	0.33	0.38	0.94	0.38	0.37
pi234	0.57	0.6	0.81	0.57	0.63
pi235	0.51	0.43	1.07	0.47	0.51
pi236	0.75	0.77	1.15	0.77	0.75
pi237	0.69	0.64	0.81	0.58	0.71

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pi238	0.84	0.86	1.12	0.8	0.86
pi239	0.6	0.63	0.98	0.62	0.6
pi240	0.51	0.53	0.97	0.55	0.53
pi241	0.86	0.74	1	0.73	0.78
pi242	0.41	0.45	1.04	0.44	0.45
pi243	0.89	0.92	1.09	0.89	0.9
pi244	0.56	0.64	0.97	0.66	0.59
pi245	0.63	0.64	0.97	0.69	0.73
pi246	0.67	0.75	0.92	0.62	0.7
pi247	0.6	0.57	0.78	0.43	0.6
pi248	0.58	0.66	1	0.63	0.62
pi249	0.56	0.46	0.94	0.45	0.46
pi251	0.69	0.88	1.17	1.04	0.98
pi301	1.06	0.97	1.08	1.06	1.13
pi302	1.05	0.93	0.96	0.88	0.92
pi303	0.64	0.63	0.68	0.61	0.71
pi307	0.71	0.72	0.75	0.79	0.75
pi308	0.36	0.41	0.72	0.34	0.81
pi316	0.69	0.75	0.83	0.74	0.83
pi317	0.6	0.62	1.01	0.6	0.94
pi318	0.79	0.83	0.9	0.72	0.83
pi319	0.72	0.77	0.93	0.77	0.74
pi320	0.59	0.62	0.76	0.58	0.79
pi321	0.53	0.59	0.82	0.56	0.8
pi322	0.6	0.59	0.98	0.57	0.94
pi323	0.55	0.6	0.91	0.61	0.86
pi324	0.36	0.46	1.03	0.47	1.07
pi325	0.41	0.46	0.86	0.44	0.43
pi326	0.37	0.33	0.9	0.37	0.31
pi327	0.31	0.35	0.92	0.76	0.84
pi328	0.79	0.86	0.89	0.94	1.03
pi329	0.76	0.68	0.82	0.75	1.03
pi330	0.92	0.99	1.13	0.96	0.9
pi331	1.06	0.99	1.09	0.91	0.97
pi333	1.03	0.84	0.91	0.93	0.91
pi334	0.72	0.73	0.7	0.73	0.94
pi336	0.77	0.53	0.52	0.51	0.48
pi337	0.61	0.53	0.62	0.74	0.55
pi338	0.63	0.6	0.59	0.52	0.61
pi339	0.41	0.47	0.47	0.78	0.71
pi341	0.36	0.23	0.6	0.3	0.33
pi343	0.76	0.65	0.8	0.62	0.87
pi345	0.77	0.84	0.86	0.83	0.79
pi347	0.19	0.24	0.22	0.2	0.21
pi348	0.85	0.84	0.83	0.9	0.93
pi349	0.83	0.93	0.79	0.86	0.97

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
pi350	0.27	0.34	0.38	0.32	0.88
pi353	0.48	0.35	0.79	1.06	1.03
pi354	0.65	0.55	0.67	0.64	0.92
pi355	0.81	0.75	0.89	0.71	0.75
pi356	0.76	0.75	0.81	0.89	0.85
pi357	0.79	0.84	0.71	0.77	0.85
pi358	0.49	0.7	0.75	0.6	0.74
pi359	0.42	0.46	0.54	0.67	0.48
pi360	0.55	0.6	0.63	0.55	0.86
pip	1.05	1.1	1.15	1.1	0.99
pknB	0.99	1.03	1.08	1.03	1
plpA	1.02	1.02	1.12	1.03	1
plpB	1.06	1.03	1.03	1.11	0.98
plpC	1.01	1.11	1.04	1.1	1.06
plpD	0.74	0.92	0.85	0.9	0.87
plsX	0.97	1.15	1.15	1.07	1.14
pmg	0.93	1.02	1.01	0.98	0.97
pmpA	0.93	0.94	1.01	0.93	0.89
pmrA	0.88	0.93	0.99	0.89	0.87
pmsR	0.91	0.94	1	0.98	0.89
pmsX	0.92	0.96	1.01	0.93	0.89
pnpA	0.97	0.97	0.88	1.04	1.03
pnuC2	1	0.92	1	0.95	1.01
polA	1.21	1.25	1.18	1.14	1.2
polC	0.93	1.03	1.01	0.95	1.01
ponA	0.93	1.14	1.02	1	1.07
potA	0.76	0.87	0.92	0.89	0.88
potB	0.9	0.93	0.96	0.87	0.96
potC	0.91	0.93	1.02	1	0.92
potD	0.95	1.12	1.1	1.01	0.98
poxL	0.74	0.88	0.88	0.85	0.84
ppiA	1.02	1.04	1.11	1.13	1.04
ppiB	0.88	0.98	1.04	0.95	0.97
preA	1.07	1.12	1.17	1.04	1.21
prfA	0.95	0.88	0.94	0.99	0.9
prfB	0.98	0.98	1.02	1.01	1
prfC	0.97	1.07	1.04	0.99	0.99
prmA	0.81	0.94	0.95	0.94	0.98
proA	0.83	0.88	0.83	0.89	0.88
proB	0.86	0.95	0.94	1.01	0.92
proC	0.68	0.81	0.83	0.78	0.78
proS	0.98	1.03	1.14	1.11	1.06
prsA	1.03	1.07	1.16	1.11	1.07
prsB	1.07	1.15	1.19	1.11	1.09
ps101	0.75	0.78	0.78	0.72	0.72
ps102	0.89	0.71	0.89	0.87	0.76

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ps104	0.8	0.68	0.94	0.73	0.81
ps105	1.13	1.2	1.14	1.04	1.13
ps106	1.01	1.03	0.96	0.83	1.17
ps107	0.96	0.94	0.88	0.81	0.99
ps108	1.08	1.1	0.99	0.6	0.97
ps109	0.91	0.82	0.84	0.46	0.9
ps110	0.92	0.93	0.87	0.77	0.97
ps111	1.09	0.56	1.02	0.5	1.15
ps112	0.81	0.79	0.81	0.68	0.9
ps113	0.9	0.81	1.03	0.69	0.98
ps114	0.5	0.57	0.99	0.56	0.55
ps115	0.66	0.6	0.97	0.58	0.79
ps116	0.78	0.77	0.92	0.76	0.79
ps117	0.88	0.87	1.05	0.85	0.86
ps118	1	1.2	0.87	0.6	1.08
ps119	0.96	0.73	1	0.8	1.05
ps120	0.41	0.29	1.03	0.41	1.18
ps121	1.24	0.97	1.09	0.6	1.17
ps122	0.75	0.78	0.8	0.75	0.77
ps123	0.99	1.01	0.97	0.96	0.98
ps201	0.79	0.96	1	0.79	0.97
ps202	0.64	0.84	0.84	0.75	0.75
ps203	0.39	1.11	1.08	1.08	1.14
ps205	0.68	0.78	0.85	0.77	0.7
ps206	0.57	0.9	0.92	0.77	0.71
ps207	0.73	1.06	0.94	0.59	0.94
ps209	0.71	0.97	0.93	0.71	0.65
ps211	0.65	1.04	0.81	0.64	0.94
ps212	0.85	1.24	1.02	0.82	1.13
ps213	0.76	0.95	0.93	0.76	0.74
ps214	0.99	1.06	1.06	0.94	1.07
ps216	0.6	0.99	0.91	0.63	1.08
ps218	0.83	1.12	0.94	0.74	0.91
ps219	0.8	0.89	0.84	0.8	0.84
ps220	0.72	0.87	0.79	0.68	0.79
ps301	0.15	0.19	1.18	0.17	0.19
ps302	0.63	0.65	0.87	0.69	0.65
ps303	0.9	0.84	1.03	1.01	1.29
ps304	0.54	0.59	0.87	0.55	0.57
ps306	0.99	1.08	1.09	1.08	1.1
ps307	0.75	1.05	1.09	0.94	1.04
ps308	0.87	0.93	0.9	0.77	0.98
ps309	0.94	0.74	0.89	0.84	0.59
ps311	0.98	1.23	1.24	1.11	0.18
ps312	0.73	0.78	0.94	0.87	0.4
ps314	0.44	0.79	1.05	0.5	0.44

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ps316	0.23	0.37	1.05	0.94	0.55
pstA	1.02	1.1	0.97	1.05	0.98
pstB	1	1.02	1.02	1.06	0.99
pstC	1.17	1.2	1.18	1.1	1.09
pstE	1.04	1.29	1	1.12	1.13
pstF	1.04	1.25	1.03	1.01	1
pta	0.99	1.09	1.02	1.07	1.08
ptbA	1.03	1.09	1.13	1.1	1.09
ptcA	1.21	1.41	1.16	1.22	1.29
ptcB	0.97	1.03	1.03	1.05	1
ptcC	1.24	1.28	1.19	1.18	1.24
ptk	1	1.02	0.86	1	0.95
ptnAB	0.96	0.97	0.97	1.09	0.94
ptnC	0.99	0.96	0.96	1	1.01
ptnD	1.1	1.1	1.12	1.04	1.05
ptpL	1.07	1.1	1.02	1	1.08
ptsH	0.93	0.93	0.92	1.01	0.91
ptsI	0.94	1.08	0.9	1.03	1.01
ptsK	1.02	1.05	1.08	1.04	1.06
purB	0.85	0.96	0.96	0.95	0.91
purC	0.92	1.05	0.97	0.93	0.96
purD	0.91	0.96	0.97	0.92	0.91
purE	0.74	0.93	0.91	0.87	0.83
purF	0.74	0.84	0.72	0.84	0.77
purH	0.96	1.01	0.98	1.01	0.95
purK	0.81	0.84	0.88	0.87	0.81
purL	1.14	1.17	1.05	1.02	1.04
purM	0.95	0.96	0.88	0.91	0.89
purN	0.98	1.06	0.99	0.96	0.91
purR	0.96	1.11	1.16	1.09	1.09
pycA	0.83	0.97	1.05	0.99	0.89
pydA	1	0.99	0.95	0.9	0.96
pydB	0.93	1.08	1	0.95	0.96
pyk	0.91	1.04	0.95	1.07	0.99
pyrB	1	1.05	0.96	1.06	1.05
pyrC	0.81	0.83	0.88	0.88	0.83
pyrE	1.01	0.88	0.96	0.92	1.02
pyrF	0.95	0.96	1.03	0.88	0.98
pyrG	1.07	1.22	1.16	1.11	1.11
pyrH	0.92	1.09	1.02	0.98	1.02
pyrR	0.81	0.86	0.93	0.92	0.93
pyrZ	0.93	1.06	1.11	0.98	1.07
qor	1.05	1.12	1.15	1.04	1.15
queA	0.99	1.1	1.09	1.05	1.09
racD	1.07	1.15	0.96	1.02	1.06
radA	0.85	0.93	0.83	0.88	0.88

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
radC	0.77	0.79	0.85	0.83	0.86
rarA	0.82	0.74	0.71	0.7	0.83
rbfA	1.12	1.12	1.13	1.01	1.03
rbsA	1.1	1.23	1.11	1.11	1.09
rbsB	0.98	1.2	1.14	1.09	1.1
rbsC	0.94	1.04	1.04	1.04	1.13
rbsD	0.81	0.96	0.97	0.85	0.95
rbsK	0.91	1.04	1.13	1.05	1.06
rbsR	0.93	1.05	1.1	0.95	1.04
rcfA	1.01	1.16	1.06	1	1.13
rcfB	0.94	1.12	0.92	1.02	1
rdrA	0.79	0.85	0.83	0.87	0.81
rdrB	0.68	0.85	0.86	0.9	0.87
recA	0.86	0.96	0.88	0.79	1.04
recD	1.16	0.92	1.01	1.15	0.98
recJ	1.4	1.12	1.24	1.24	1.1
recM	1.15	0.99	1.07	1.02	0.86
recN	1.11	0.89	1.09	1.08	0.99
recQ	1.12	0.91	1.07	1	0.99
relA	0.98	0.8	0.97	0.91	0.84
rexA	1.02	0.82	0.98	0.89	0.91
rexB	0.95	0.94	0.88	0.89	0.92
rgpA	1.04	0.93	0.87	0.92	0.83
rgpB	1	0.94	0.79	0.87	0.95
rgpC	0.67	0.61	0.87	0.67	0.57
rgpE	0.66	0.7	0.91	0.67	0.59
rgpF	0.58	0.42	0.92	0.5	0.52
rgrA	0.88	0.86	0.82	0.94	0.71
rgrB	0.98	0.8	0.95	1.05	0.81
rheA	1.02	0.91	1	1	0.96
rheB	0.99	0.96	1	0.99	0.87
ribA	0.99	0.86	0.92	0.92	0.88
ribC	0.94	0.78	0.93	0.82	0.84
ribH	0.85	0.83	0.78	0.81	0.8
rimM	0.83	0.76	0.72	0.78	0.76
rliA	0.95	0.87	0.96	0.91	0.9
rliB	0.9	0.91	0.79	0.87	0.67
rliC	1.16	0.94	1.01	0.96	1.04
rliDB	0.87	0.82	0.89	0.84	0.82
rlrA	1.03	0.84	0.98	0.96	0.9
rlrB	1.14	0.94	1.01	1.06	1.09
rlrC	0.99	0.93	0.95	1.04	0.91
rlrD	0.98	0.91	1.01	0.96	0.95
rlrG	0.94	0.85	0.87	0.83	0.86
rluA	1.14	1.1	1.05	0.97	1.12
rluB	0.83	0.82	0.82	0.77	0.81

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
rluC	0.85	0.85	0.88	0.72	0.84
rluD	0.98	0.8	0.97	0.8	0.91
rmaA	0.81	0.79	0.79	0.67	0.76
rmaB	1.2	1	1.04	1.07	1.17
rmaC	0.93	0.76	0.87	0.9	0.85
rmaD	1.11	0.95	1.07	1.09	1.02
rmaE	0.89	0.79	0.91	0.9	0.87
rmaF	0.89	0.88	0.83	0.9	0.89
rmaG	0.83	0.81	0.81	0.79	0.82
rmaH	0.94	0.91	0.93	0.85	0.87
rmaI	0.9	0.78	0.8	0.71	0.84
rmaJ	0.71	0.83	0.82	0.8	0.83
rmeA	0.86	0.87	0.9	0.75	0.81
rmeB	1.07	1.01	1.02	0.94	1.11
rmeC	0.97	1.07	0.93	0.89	0.82
rmeD	1.02	0.91	1.01	0.9	1
rmlA	0.97	0.9	0.89	0.98	0.89
rmlB	0.95	0.88	0.97	0.9	0.9
rmlC	0.98	0.92	0.94	0.99	0.92
rnc	0.88	0.88	0.86	0.89	0.79
rnhA	0.96	0.94	0.91	0.91	0.9
rnhB	0.89	0.87	0.87	0.88	0.81
rnpA	0.87	0.87	0.92	0.89	0.84
rpe	0.85	0.9	0.94	0.87	0.9
rpiA	1.07	0.95	1.04	0.9	1.03
rplA	0.87	0.92	0.85	0.82	0.83
rplB	1.1	0.9	1.03	0.99	1.08
rplI	0.95	0.82	0.92	0.94	0.89
rplM	0.88	0.9	0.84	0.81	0.78
rplN	0.71	0.75	0.87	0.75	0.77
rplO	1.1	1.17	1.21	1.05	1.17
rplQ	0.78	0.88	0.78	0.76	0.7
rplR	1.11	0.97	1.04	1.03	1.06
rplS	1.17	1	1.04	1.14	1.1
rplT	1.08	1.04	1.03	1.03	0.96
rplU	0.82	0.8	0.89	0.87	0.77
rplV	1.1	1.08	1.04	1.07	1.02
rplX	1.2	1.21	1.12	1.2	1.12
rpmA	1.07	0.94	0.97	1.13	0.99
rpmB	1.03	0.95	0.95	0.98	1.03
rpmD	1.15	1.14	1.04	1.12	1.05
rpmE	0.97	0.93	0.93	0.96	0.9
rpmF	0.88	0.91	0.87	0.84	0.86
rpmGA	0.8	0.81	0.89	0.72	0.82
rpmGB	0.91	0.88	0.92	0.88	0.92
rpmGC	0.62	0.79	0.74	0.7	0.69

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
rpmH	0.92	0.8	0.84	0.79	0.9
rpmI	0.86	0.81	0.8	0.87	0.82
rpmJ	1.12	0.97	1.03	1.06	0.98
rpoA	1.09	1.09	1.04	0.99	0.99
rpoB	0.93	0.92	0.92	0.95	0.88
rpoC	1.09	0.97	1.01	0.98	0.99
rpoD	0.94	0.98	0.99	0.93	0.98
rpoE	1.18	0.97	1	1.08	1.03
rpsA	0.99	0.96	1.03	0.97	0.97
rpsB	1.26	1.2	1.21	1.21	1.13
rpsC	0.98	0.98	0.99	0.97	0.92
rpsD	1.11	1.12	1.03	1.1	0.99
rpsE	0.99	0.96	0.88	0.93	0.93
rpsF	0.93	0.87	0.88	0.93	0.84
rpsG	1.02	1	1.01	0.98	0.95
rpsH	0.89	0.88	0.8	0.88	0.88
rpsI	1.18	1.21	1.13	1.18	1.17
rpsJ	0.91	0.89	0.98	0.89	0.88
rpsK	0.65	0.79	0.85	0.75	0.84
rpsL	1.22	1.2	1.05	1.04	1.15
rpsM	1.04	0.89	0.95	0.97	0.97
rpsN	1.22	1.22	1.19	1.27	1.23
rpsN2	1.02	0.94	0.91	1.01	0.94
rpsO	0.9	0.9	0.9	0.91	0.84
rpsP	1.14	1.17	1.1	1.1	1.02
rpsQ	0.94	0.93	0.85	0.9	0.87
rpsR	0.8	0.87	0.81	0.78	0.82
rpsS	0.96	0.94	1.06	0.98	0.96
rpsT	0.81	0.89	0.95	0.86	0.88
rpsU	0.92	0.81	0.88	0.92	0.89
rsuA	1.19	1.1	1.18	1.11	1.09
ruvA	1.18	1.02	1.01	1.06	1.1
ruvB	1.26	1.26	1.1	1.21	1.14
sbcC	1.16	1.07	1.03	1.08	0.95
sbcD	1.08	1.11	1.05	1	1
scrK	1.1	1.06	1.12	0.99	1.03
sdaA	1.04	0.97	0.96	0.87	0.92
sdaB	1.06	1.04	1.05	0.89	0.97
secA	1.11	1.12	1.21	1.08	1.14
secE	1.43	1.22	1.36	1.24	1.45
secG	1.13	0.96	1.06	1.01	0.98
secY	1.19	1.05	1.12	1.08	1.05
serA	0.89	0.96	0.85	0.87	0.91
serB	1.03	0.88	1.09	0.96	0.93
serC	1.31	1.23	1.26	1.34	1.2
serS	1.22	1.17	1.11	1.22	1.16

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
sigX	0.97	0.97	0.98	0.93	0.87
sipL	1.12	1.13	1.14	1.06	1.05
smc	1.01	0.96	1	0.97	0.92
smpB	0.83	0.87	0.9	0.79	0.76
snf	1.15	1.17	1.11	0.96	1.03
sodA	1.15	1.14	1.17	1.09	1.07
ssbA	1.03	0.96	1.03	0.94	0.93
ssbB	0.89	0.88	0.94	0.84	0.91
sugE	0.73	0.82	0.77	0.83	0.87
sunL	1.21	1.07	1.23	1.06	1.13
tag	1.01	0.99	1.04	1.09	0.98
tagB	1.15	1.08	1.07	1.08	1.07
tagD1	0.62	0.62	0.97	0.6	0.59
tagD2	0.86	0.81	0.85	0.81	0.78
tagF	1.13	1.11	1.11	1.06	1.07
tagH	1.04	1.04	1.1	0.89	0.94
tagR	0.94	0.89	0.95	0.77	0.82
tagX	0.92	0.86	0.93	0.82	0.9
tagY	0.8	0.76	0.87	0.81	0.78
tagZ	1.01	0.95	1	0.97	1.05
tenA	1.16	1.05	1.08	1.07	1.11
thdF	1.31	1.39	1.19	1.26	1.28
thgA	1.11	1.04	0.98	1.12	1.06
thiD1	1.01	1.03	1.02	1	0.96
thiD2	1.07	1.14	1.08	1	1
thiE	1.05	1.01	1.11	0.87	0.98
thiL	0.95	0.96	1.02	0.79	0.8
thiM	1	0.95	1.05	0.87	0.93
thrA	1.19	1.05	1.19	1.07	1.12
thrB	0.83	0.86	0.95	0.88	0.85
thrC	0.98	0.94	0.85	0.96	1.03
thrS	1.09	1.08	1.06	0.99	1.06
thyA	1.14	1.12	1.03	1.16	1.14
tig	1.28	1.33	1.12	1.31	1.3
tkt	1.06	1.09	0.95	1.04	1.02
topA	0.91	1.01	0.95	0.98	0.97
tpiA	1.01	1.06	1.07	1.01	0.97
tpx	1.07	1.06	1.15	1.05	0.98
tra1077B	0.77	0.65	1.9	0.51	0.47
tra904A	0.65	0.67	1.23	0.4	0.73
tra905	0.46	0.49	1.08	1.11	0.51
tra981C	1.32	1.07	1.36	0.6	0.9
tra983L	0.92	0.96	0.97	0.28	0.31
trmD	1.17	1.04	1.02	1.18	1.08
trmU	1.08	1.14	1.02	1.19	1.07
trpA	0.98	1.01	0.84	0.98	0.91

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
trpB	0.86	0.96	0.92	0.91	0.89
trpC	1.08	1.14	1.18	1.08	1.02
trpD	1.04	1.07	1.18	0.99	0.92
trpE	0.81	0.8	0.85	0.74	0.76
trpF	0.91	0.87	0.99	0.89	0.9
trpG	1.06	1.01	1.13	1.05	0.93
trpS	1.2	1.16	1.13	1.06	1.12
truA	0.95	0.95	0.88	1.02	1
truB	1.01	0.91	1.08	1.08	1.05
trxA	1.19	1.23	1.05	1.27	1.16
trxB1	1.26	1.24	1.21	1.29	1.22
trxB2	1.05	1.15	1.04	1.18	1.1
trxH	1.18	1.35	1.26	1.31	1.24
tsf	1.07	1.16	1.26	1.21	1.2
tuf	1.27	1.36	1.38	1.36	1.14
typA	1.24	1.35	1.33	1.19	1.15
tyrA	1.37	1.35	1.27	1.26	1.15
tyrS	1.23	1.13	1.12	1.2	1.17
udk	1.04	0.94	0.97	1.08	0.94
udp	1.05	0.96	1.03	0.99	0.99
umuC	1.28	1.27	1.13	1.28	1.3
ung	1.02	1.07	0.99	1.07	1.07
upp	1.26	1.36	1.12	1.33	1.26
usp45	0.95	1	1.02	1	0.97
uvrA	0.99	1.21	1.09	1.16	1.08
uvrB	1.11	1.3	1.29	1.31	1.14
uvrC	0.98	1	1.09	1.07	0.93
uxaC	1.01	1.04	1.07	1	0.9
uxuA	1.01	1.02	1.01	1.09	0.98
uxuB	0.95	0.9	0.89	0.99	0.89
uxuT	0.93	0.81	0.9	0.93	0.91
vacB1	0.83	0.81	0.82	0.82	0.85
vacB2	1.04	0.95	1.02	1.02	1
valS	0.93	0.98	0.93	0.98	0.97
xerD	0.41	0.47	1.22	0.42	0.46
xpt	1.07	1.16	1.18	1.16	1.16
xseA	0.93	1.07	1.08	1.09	1.01
xylA	1	1.09	1.15	1.09	1.02
xylB	1.11	1.12	1.14	1.05	0.99
xylH	0.94	0.84	0.84	1.01	0.98
xylM	1.03	1.06	1.11	1.06	0.99
xylR	0.99	0.93	0.95	0.94	0.89
xylT	0.91	0.91	0.93	0.94	0.88
xylX	0.92	0.9	0.91	1.03	0.88
xynB	0.87	0.9	0.92	0.96	0.75
xynD	1.19	1.17	0.94	1.11	1.04

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
xynT	1.03	1.1	1.03	1.09	0.74
yabA	1.03	1.14	1.21	1.18	1.09
yabB	0.87	1.01	1.15	1.14	1.17
yabC	0.96	1.16	1.23	1.15	1.11
yabD	1	1.12	1.23	1.18	1.04
yabE	1.02	0.99	1.14	1.01	0.92
yabF	0.89	0.9	0.94	0.83	0.9
yacB	0.98	0.93	0.92	0.94	0.87
yacC	1.09	1.04	1.12	1.1	1.07
yacG	1.19	1.12	1.05	1.23	1.1
yacI	0.79	0.8	0.78	0.85	0.87
yafB	1.28	1.13	1.11	1.09	1.17
yafC	1.07	1.15	1.14	1.12	1.11
yafD	1	1.06	1.05	1.1	1
yafE	1.06	1.16	0.97	1.04	1.09
yafF	0.88	0.86	0.82	0.92	0.84
yafJ	0.97	0.9	0.92	0.93	0.94
yagA	1.03	1.14	1.18	1.06	1.14
yagB	1.37	1.33	1.32	1.13	1.37
yagE	1.17	1.17	1.24	1.01	1.08
yahA	1.02	1.03	1.05	0.96	0.96
yahB	0.92	0.93	0.98	0.92	0.9
yahC	1.01	0.96	1	1.03	1.01
yahD	1	0.98	0.96	0.94	0.99
yahG	0.8	0.8	0.91	0.93	0.9
yahI	1.03	0.99	0.97	1.02	1.05
yaiA	1.09	1.15	1.08	1.1	1.09
yaiB	0.97	1.13	1.09	1.07	1.06
yaiE	0.79	0.92	1.33	1.18	0.86
yaiF	0.38	0.39	1.45	1.2	0.42
yaiG	0.64	0.6	0.89	0.91	0.75
yaiI	0.65	0.61	0.93	0.93	0.59
yajB	0.7	0.75	0.9	0.87	0.9
yajE	1.17	1.05	1.24	0.85	0.92
yajH	0.89	0.88	0.91	0.94	0.86
ybaA	0.97	0.95	0.95	0.96	0.97
ybaB	0.94	0.91	0.94	0.89	0.85
ybaC	0.77	0.78	0.82	0.76	0.77
ybaD	0.92	0.93	0.89	0.93	1.04
ybaF	1.28	1.3	1.2	1.23	1.13
ybaG	0.95	1.06	0.98	1.04	1.04
ybaH	1.12	1.32	1.23	1.19	1.23
ybaI	1.08	1.33	1.29	1.08	1.2
ybbA	0.84	0.95	0.94	0.91	1
ybbB	1.09	1.15	1.16	1.07	1.12
ybbC	1.21	1.25	1.21	1.15	1.17

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ybbE	0.91	0.99	1.01	0.94	0.93
ybcC	0.95	1.02	1.01	0.99	1
ybcG	0.86	0.87	0.92	0.9	0.8
ybcH	1.07	1.16	1.2	1.15	1.2
ybdA	0.93	0.91	0.93	0.92	0.92
ybdC	1.11	1.1	1.08	1.12	1.09
ybdD	1.1	1.24	1.13	1.16	1.14
ybdG	0.34	0.39	1	0.98	1.1
ybdH	0.35	0.31	1.11	0.94	1.04
ybdI	0.73	0.77	1.13	0.99	1.13
ybdJ	0.51	0.61	0.95	0.89	0.94
ybdK	0.6	0.66	1.06	0.4	0.67
ybdL	0.79	0.78	1.86	0.57	0.6
ybeA	1.21	1.03	1.16	1.06	1.06
ybeB	1.05	1.06	1.02	0.97	1.05
ybeC	0.92	0.96	0.93	0.91	0.91
ybeD	1.01	1	1.04	1.06	0.94
ybeF	1.19	1.23	1.3	1.26	1.23
ybeH	1.08	1.05	1.07	1	1.1
ybeI	1.1	0.94	1.03	1.03	0.96
ybeM	0.96	0.77	0.88	0.94	1.05
ybfA	1.23	1.05	1.18	1.13	1.19
ybfB	1.22	1.33	1.24	1.15	1.23
ybfC	0.98	0.99	0.94	1.02	0.98
ybfD	1.1	1.23	1.13	1.03	1.14
ybfE	0.94	1.09	1.16	0.99	1.07
ybgA	1.19	1.1	1.04	1.24	1.09
ybgD	0.65	0.75	1.03	0.95	0.98
ybgE	1.08	1.16	1.14	1.06	1.08
ybhA	0.89	0.92	0.88	0.96	0.95
ybhB	0.98	1.03	1.01	1.05	0.99
ybhC	0.84	0.86	1.08	1.02	0.95
ybhD	1	1.15	1.13	1.16	1.14
ybhE	0.82	0.92	0.99	0.94	0.97
ybiB	1.1	1.12	1.09	1.08	1.09
ybiC	0.97	1.02	1.03	0.99	1
ybiD	1.12	1.23	1.2	1.13	1.24
ybiE	1.23	1.46	1.41	1.25	1.45
ybiG	1.14	1.22	1.26	1.12	1.2
ybiH	0.97	1.12	1.17	1.1	1.1
ybiI	1.28	1.31	1.21	1.34	1.29
ybiJ	1.1	1.02	0.96	1.02	1.02
ybiK	1.14	1.07	0.98	1.08	1.01
ybjA	1.18	1.17	1.12	1.19	1.19
ybjB	0.92	1.1	1	1.09	1.01
ybjD	1.18	1.16	1.09	0.86	0.97

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ybjJ	1.01	1.05	1.08	1.19	1.13
ybjK	0.84	0.94	0.97	0.95	0.9
ycaF	0.36	0.39	1.04	0.38	0.39
ycaG	0.29	0.33	0.9	0.34	0.33
ycbA	0.43	0.4	1	0.42	0.43
ycbB	0.34	0.35	1.17	0.35	0.39
ycbC	0.51	0.58	1.16	0.53	0.59
ycbD	0.53	0.55	1.13	0.53	0.56
ycbF	0.69	0.7	0.69	0.55	0.71
ycbH	0.2	0.2	1.3	0.21	0.22
ycbI	0.46	0.48	0.96	0.5	0.49
ycbJ	0.58	0.65	0.96	0.62	0.59
yccB	0.57	0.7	0.91	0.64	0.62
yccE	0.86	0.92	0.91	0.91	0.78
yccF	0.94	0.95	0.95	0.98	0.98
yccG	0.99	1.06	1.01	1.06	1.06
yccH	1.2	1.16	1.12	1.13	1.18
yccI	1.06	1.22	1.11	1.09	1.14
yccJ	1.01	1.22	1.13	1.01	1.05
yccK	1.03	1.12	1.1	1.06	1.13
yccL	0.99	1.1	1.05	0.99	0.98
ycdA	0.9	1.02	1.02	1.02	0.94
ycdB	1.09	1.14	1.07	1.13	1.12
ycdC	1.04	1.04	1.03	1.05	0.97
ycdE	0.86	0.87	0.92	0.94	0.85
ycdF	0.97	1.03	0.91	1.07	1.05
ycdG	1	0.91	0.83	0.76	0.89
ycdH	0.96	1.02	0.95	0.93	1.01
yceA	0.8	0.82	0.84	0.78	0.78
yceD	1.13	1.13	1.11	0.64	0.67
yceE	0.9	0.96	0.95	0.87	0.94
yceG	0.76	0.94	0.91	0.82	0.82
yceJ	1.04	1.15	1.15	1.06	1.15
ycfA	1.03	1.12	1.08	1.06	1.07
ycfB	0.85	0.94	0.8	0.77	0.87
ycfD	0.95	1.02	0.95	1.08	0.99
ycfF	0.96	1.03	0.99	1.03	1.03
ycfG	0.92	0.95	0.89	1.04	0.96
ycfH	0.63	0.73	0.77	0.76	0.71
ycfI	0.93	0.93	1.03	1.09	1.04
ycgA	1.2	1.25	1.25	1.22	1.26
ycgB	1.01	1.09	1	1.03	1.07
ycgC	0.83	0.99	0.98	0.96	0.97
ycgD	0.99	1.19	1.12	1.04	1.19
ycgE	0.82	0.96	0.9	0.87	0.84
ycgF	1.03	1.1	1.07	1.06	1.11

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ycgG	0.95	1	0.99	1.1	1.11
ycgH	1.02	1.06	0.97	1.01	1.04
ycgI	0.8	0.9	0.86	0.96	0.88
ycgJ	1.03	0.99	1.04	1	1
ychC	1.03	1.04	0.96	1.02	1.02
ychD	0.98	1.06	0.97	1.02	1.03
ychE	0.9	0.9	0.94	0.84	1
ychG	1	1.03	1.07	0.93	1.02
yciA	1.13	1.3	1.19	1.21	1.18
yciC	1.02	1.18	1.12	1.12	1.19
yciD	0.84	0.97	1.02	0.93	0.94
yciF	0.78	0.81	0.82	0.82	0.85
yciG	1.27	1.37	1.36	1.26	1.21
ycjA	0.92	0.85	0.95	0.98	1.04
ycjB	1.07	1.09	1.07	1.11	1.08
ycjC	1.02	1.12	1.04	1.11	1.03
ycjD	0.9	0.92	0.78	0.89	0.83
ycjG	0.99	0.92	0.88	0.93	0.99
ycjH	0.99	1.04	1	1.06	1.11
ycjI	1.03	0.89	0.96	0.97	0.99
ydaE	1	1.1	0.95	1.02	1
ydaF	1.15	1.34	1.16	1.18	1.15
ydaG	1.08	1.31	1.17	1.13	1.2
ydbA	0.91	1.05	1.04	0.97	0.9
ydbC	1.43	1.26	1.21	1.37	1.33
ydbD	0.89	1.04	1.07	1.08	0.98
ydbE	0.81	0.99	0.93	0.95	0.92
ydbF	0.79	0.89	0.92	0.95	0.83
ydcB	1.09	1.09	1.02	1.05	1.05
ydcD	0.8	0.82	0.89	0.88	0.84
ydcE	0.89	0.85	0.84	0.84	1.02
ydcF	0.89	1.07	1.19	0.79	1.04
ydcG	1.12	1.08	1.02	1.1	1.1
yddA	1.1	1.31	1.18	1.16	1.13
yddB	1.01	1.23	1.1	1.1	1.12
yddC	0.91	1.17	1.1	1.07	1.05
yddD	0.89	0.95	0.92	0.8	0.89
ydgB	1.2	1.41	1.43	1.3	1.47
ydgC	1.06	1.09	1.12	1.12	1.13
ydgD	0.68	0.79	0.82	0.84	0.74
ydgE	0.75	0.88	0.83	0.89	0.77
ydgF	0.81	0.87	0.81	0.87	0.86
ydgG	0.98	1.06	0.95	0.99	1.08
ydgH	0.77	0.89	0.84	0.85	0.93
ydgI	1.12	1.24	1.11	1.02	1.14
ydhB	1.15	1.44	1.44	1.14	1.22

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ydhF	1.01	1.14	1.17	1.1	0.91
ydiA	1.15	1.27	1.83	1.12	1.26
ydiB	1.07	1.18	1.23	1.16	1.2
ydiC	0.97	1.02	1.07	0.93	1.05
ydiD	0.95	1.04	0.97	1.02	1.06
ydiE	0.99	1.04	0.85	1	1.01
ydiF	0.9	0.91	0.91	0.98	0.96
ydiG	0.87	0.93	0.97	1	0.96
ydjB	1.04	0.97	1.04	1.14	0.99
ydjD	0.73	0.69	0.75	0.58	0.82
yeaA	0.66	0.55	0.86	0.9	0.58
yeaC	0.78	0.65	0.92	0.74	0.61
yeaD	0.86	0.69	0.96	0.93	0.76
yeaF	0.87	0.88	0.84	0.89	0.9
yeaG	1.08	0.94	0.94	0.95	0.92
yeaH	0.99	0.94	0.95	0.87	0.91
yebB	1.24	1.14	1.16	1.05	1.21
yebE	0.93	0.92	0.89	0.85	0.89
yebF	1.01	0.91	0.97	0.88	0.88
yecA	0.98	0.99	0.99	0.88	0.85
yecD	0.91	0.86	0.78	0.87	0.85
yecE	0.84	0.65	0.75	0.81	0.77
yedA	1.04	0.88	0.89	0.96	0.84
yedE	1.05	0.83	0.95	0.99	0.89
yedF	0.92	0.76	0.86	0.89	0.85
yeeC	1.07	0.96	0.98	0.94	0.94
yeeD	0.79	0.79	0.87	0.8	0.78
yeeF	1.05	0.94	0.95	0.88	0.96
yeiD	0.6	1.08	1	0.99	0.67
yeiE	1.19	1.09	1.2	1.03	1.1
yeiF	0.98	1.02	1.03	0.89	0.86
yeiG	1.26	0.96	1.04	1.01	1.13
yejC	0.95	0.82	0.86	0.83	0.86
yejD	0.9	0.77	0.84	0.88	0.82
yejE	0.64	0.83	0.95	0.93	0.91
yejI	0.68	0.96	0.88	0.85	0.92
yfaA	0.91	0.88	1.14	0.84	0.82
yfbB	0.92	1.23	0.99	0.32	0.91
yfbG	0.87	0.9	1.06	1.27	1.24
yfbI	1.1	1	1	0.89	0.99
yfbJ	0.91	0.81	0.85	0.78	0.85
yfbK	0.55	0.59	1.02	0.66	0.98
yfbM	1.2	1.05	1.04	0.95	1.1
yfcB	0.94	0.75	0.81	0.93	0.88
yfcF	0.91	0.83	0.9	0.89	0.83
yfcI	0.96	0.87	0.89	0.87	0.94

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yfdA	1.13	1.03	1.04	0.99	0.99
yfdB	1.23	1.14	1.11	1.04	1.2
yfdD	0.8	0.89	0.78	0.85	0.87
yfdE	1.03	0.98	1.06	0.85	0.92
yfeA	0.95	0.95	1.06	0.92	0.98
yffA	0.93	1.07	1.05	0.97	0.81
yffB	1.22	0.91	1.08	1.01	1.08
yffD	1.09	0.98	0.97	1.08	1.08
yfgC	1.17	1.07	1.03	1.06	1.05
yfgG	0.93	0.96	0.94	0.99	0.91
yfgH	1.01	0.94	0.92	0.97	0.93
yfgL	1	0.96	0.95	0.96	0.92
yfhA	1.27	1.2	1.08	1.12	1.22
yfhF	0.81	0.9	0.94	0.82	0.83
yfhH	0.91	0.86	0.85	0.93	0.85
yfhI	0.41	0.37	1.12	0.43	1.16
yfhJ	0.51	0.54	0.95	0.66	0.95
yfhK	0.96	0.87	0.98	0.92	0.97
yfhL	0.96	0.88	0.9	0.97	0.88
yfiC	1.11	1.06	0.95	0.98	0.98
yfiE	0.94	0.96	0.94	0.92	0.91
yfiG	0.92	0.99	0.98	0.84	0.87
yfiH	1.02	0.97	1.1	0.95	1.03
yfiJ	0.98	1.04	0.99	0.96	0.96
yfiL	1.09	0.9	0.93	0.92	0.97
yfjA	1.13	1.04	0.98	1.01	1.07
yfjC	1.18	1.07	1.11	1.17	1.11
yfjD	1.02	0.95	0.92	1.04	0.94
yfjF	0.95	0.96	0.87	0.97	0.88
ygaB	1.3	1.3	1.17	1.15	1.18
ygaC	0.73	0.75	0.72	0.66	0.74
ygaD	0.89	0.82	0.97	0.94	0.84
ygaE	1.31	1.14	1.15	1.32	1.22
ygaF	0.88	0.84	0.79	0.93	0.84
ygaI	1.02	1.1	1.1	0.96	1.04
ygaJ	0.96	0.83	0.92	0.96	0.9
ygbB	0.92	0.93	0.93	0.95	0.89
ygbD	1.02	0.96	0.97	1	0.96
ygbE	1.06	1.11	1	1.16	1.05
ygbF	0.94	0.94	0.95	0.98	0.9
ygcA	1.11	1.04	0.94	1.02	0.98
ygcC	1.09	1.04	0.99	0.99	0.97
ygdC	0.8	0.86	0.81	0.81	0.82
ygdD	0.71	0.69	0.77	0.7	0.73
ygdE	0.98	0.94	1.06	0.98	0.94
ygdF	0.54	0.57	1.15	0.6	0.56

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ygeB	0.8	0.79	0.91	0.8	0.69
ygeC	1.03	0.93	0.97	0.88	0.9
ygeD	0.9	1.01	0.98	0.9	0.92
ygfA	0.97	0.85	0.99	0.87	0.93
ygfB	1.09	0.94	1.08	1.15	1.01
ygfC	1.29	1.12	1.07	1.26	1.16
ygfE	1.22	1.21	1.09	1.31	1.12
yggA	1.18	1.12	1.04	1.16	1.03
yghB	0.98	0.99	0.96	1.01	0.97
yghC	1.05	1.05	1.07	0.98	0.96
yghD	0.83	0.82	0.86	0.8	0.75
yghE	1.02	1.07	1.17	0.89	1.11
yghG	0.91	0.89	0.9	0.81	0.87
ygiC	0.79	0.82	0.8	0.79	0.81
ygiG	0.87	0.88	0.94	0.83	0.87
ygiI	0.94	0.96	1.02	0.97	0.95
ygiJ	1.19	1.07	1.16	1.04	1.07
ygiK	1.17	1.05	1.11	1.17	1.09
ygjB	1.01	1	0.98	1.06	0.98
ygjD	1.05	0.99	1.02	1.12	1.1
yhbE	0.95	1.11	1.04	1.13	1
yhbH	1.07	1.08	1.06	0.92	0.94
yhcA	0.74	0.94	0.91	0.81	0.84
yhcB	0.99	1.01	1.03	0.96	0.94
yhcC	0.98	0.95	1.04	0.98	0.96
yhcE	1.18	1.06	1.22	1	1.09
yhcH	1.07	1.08	1.1	1.04	1.13
yhcI	1.03	0.92	1.03	0.98	0.99
yhcK	1.15	1.02	1.02	1.08	1.08
yhdA	1.12	0.93	1.01	1.08	1.07
yhdB	1.13	1.16	0.96	1.22	1.07
yhdC	1.15	1.18	1.1	1.14	1.13
yheA	1.02	0.97	0.96	0.98	1.01
yheB	1.03	1.05	1.05	0.96	0.95
yhfB	0.99	1.04	1.12	0.98	1.01
yhfC	1.2	1.1	1.13	1.23	1.07
yhfD	1.07	0.95	1.01	1.05	1
yhfE	0.86	0.87	0.87	0.96	1.01
yhfF	1	1.01	0.92	1.06	1.01
yhgA	1.11	1.17	1.06	1.13	1.02
yhgB	1.01	1.1	0.95	1	1.04
yhgC	1.04	1.07	1.03	0.94	0.98
yhgD	1.06	0.94	1.05	0.91	0.91
yhgE	0.91	0.89	0.91	0.8	0.81
yhhA	0.88	0.86	0.91	0.87	0.91
yhhB	1.08	0.98	1.16	0.98	1.03

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yhhD	1	0.8	0.95	1.01	1.02
yhhE	1	1	0.99	0.96	1.02
yhhG	1.11	1.1	1.11	1.19	1.1
yhjA	0.95	0.91	0.91	0.97	0.96
yhjB	1.16	1.16	1.1	1.26	1.2
yhjC	1.06	1.1	0.84	1.06	0.97
yhjE	1.22	1.04	0.93	1.07	1.13
yhjF	1.06	1.11	1.02	1.09	1.11
yhjG	1.08	1.06	0.94	1.14	1.06
yiaA	0.89	0.87	0.95	0.95	0.9
yiaB	0.94	1	1.04	1.05	0.88
yiaC	0.94	0.87	0.97	0.92	0.83
yiaD	0.99	0.99	1.06	0.98	0.99
yibB	0.95	0.88	0.94	0.88	0.94
yibC	1.05	1	1.13	1.05	1.02
yibD	0.85	0.92	0.96	0.86	0.95
yibE	1	0.97	1.04	1.1	1
yibF	1.08	1.09	1.01	1.11	1.06
yibG	0.99	1	0.91	1.03	0.92
yicA	1.08	1.12	0.92	1.1	1.06
yicB	1.14	1.21	1.09	1.17	1.13
yicC	0.97	0.97	1	1	0.96
yicE	1.08	1.04	1.06	1.1	0.93
yidA	1.11	1.02	1.04	0.98	0.95
yidB	0.98	0.99	1.08	1.02	0.99
yidC	1.02	0.93	1.07	0.99	0.92
yidE	0.88	0.89	0.9	0.82	0.87
yieF	0.99	0.9	0.9	0.88	0.85
yieH	0.86	0.91	0.91	0.88	0.87
yifA	1.06	0.96	1.02	1.08	1.08
yigC	1.07	1.05	1.03	1.12	1
yihA	1.13	1.21	1.03	1.18	1.16
yihB	1.08	1.2	1.1	1.22	1.17
yihD	0.99	1.29	1.13	1.19	1.17
yihF	0.97	1.08	1.02	1.11	1.02
yiiB	0.95	1.03	1.05	1.04	0.92
yiiD	0.91	0.89	0.91	0.92	0.91
yiiE	0.79	0.89	0.89	0.83	0.77
yiiF	1.06	0.99	1.01	0.92	0.89
yiiG	1.14	1.08	1.07	1	1.04
yiiH	0.74	0.78	0.86	0.8	0.75
yiiI	0.84	0.87	0.93	0.86	0.8
yijB	1.04	0.94	0.98	0.83	0.91
yijC	1.06	1.06	0.98	1.05	1.02
yijD	1.03	1.05	1.09	1.13	1.13
yijE	1.04	1.1	1.05	1.18	1.07

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yijF	0.9	1.03	0.99	1.04	1.01
yijG	1.1	1.21	1.26	1.37	1.22
yijH	1	1	1.01	1.18	0.96
yjaB	1.05	1.02	1.14	1.15	0.96
yjaD	1.13	1.1	1.13	1.11	1.02
yjaE	1.01	1	1.03	1.11	0.91
yjaF	0.82	0.81	0.92	0.88	0.85
yjaH	0.87	0.78	1.01	0.9	0.88
yjaI	0.82	0.87	0.92	0.79	0.75
yjaJ	1	0.94	0.84	0.84	0.95
yjbB	1.09	0.88	1	1.02	0.99
yjbC	0.92	0.93	0.9	1	0.9
yjbE	1	1.06	1.07	1.12	1.02
yjbF	1.07	1.11	1.07	1.2	1.15
yjcA	0.88	1.05	1.05	1.03	1.06
yjcD	1.19	1.09	1.28	1.32	1.31
yjcE	1.07	1.14	1.2	1.06	1.04
yjcF	0.94	0.93	1.02	0.96	0.88
yjdA	1.03	1.04	1.1	1.03	0.93
yjdB	1.1	1.01	1.04	1.03	1
yjdE	0.86	0.85	0.87	0.96	0.88
yjdI	1.04	0.97	1.07	1.01	0.99
yjdJ	1.12	1.1	1.1	1.12	1.07
yjeA	1.12	1.02	0.97	1.07	1.08
yjeD	1.08	1.13	1.08	1.17	1.13
yjeF	0.97	1.01	1.03	1.11	1.05
yjeG	1	1.09	1.07	1.09	1.08
yjfB	0.89	0.96	1.14	1.14	1.12
yjfG	0.83	0.82	0.93	0.77	0.85
yjfI	1	0.98	1.03	0.93	0.91
yjfJ	0.94	0.94	0.98	0.87	0.9
yjgB	0.86	0.9	0.88	0.89	0.84
yjgC	1.04	0.95	1.1	1.06	0.97
yjgD	1.01	0.95	1.04	1	0.94
yjgF	1.03	0.99	0.95	0.98	0.96
yjhA	0.95	0.96	0.93	0.98	0.92
yjhB	0.94	0.98	0.97	1.07	1
yjhC	1.01	1.01	1.16	1.05	1.01
yjhD	0.96	1.12	1.03	1.1	1.12
yjhF	1.11	1.05	1.18	1.01	1.05
yjiB	0.96	0.92	0.99	0.98	0.89
yjiE	0.83	0.86	0.9	0.83	0.79
yjjA	0.96	1.03	1.15	1.11	1.04
yjjB	0.83	0.83	0.92	0.83	0.82
yjjC	1	0.9	0.95	0.97	0.93
yjjD	0.98	1.01	0.97	1	1.01

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yjjE	0.88	0.83	0.86	0.95	0.89
yjjF	0.9	1.05	1.02	1.05	1.09
yjjH	0.96	0.99	1.12	0.94	0.97
ykaE	0.89	0.81	0.87	0.83	0.89
ykaF	0.83	0.89	0.89	0.89	0.85
ykbA	0.91	0.73	0.84	0.85	0.88
ykbC	0.91	0.84	1.05	0.88	0.88
ykbE	1.05	0.93	0.96	1.04	1.08
ykbF	0.91	0.91	0.96	0.95	0.88
ykcA	0.98	1	1	1.02	0.98
ykcB	0.84	0.98	0.96	0.96	0.94
ykcC	0.95	1.03	0.99	0.88	1.03
ykcF	0.96	1	1.02	1.01	0.98
ykcG	0.89	0.99	0.95	0.98	0.94
ykdA	0.68	0.78	0.8	0.77	0.74
ykdB	0.85	0.86	0.95	0.92	0.88
ykhE	0.87	1	1.09	0.99	0.98
ykhF	1.06	1.02	1.05	0.99	1.04
ykhG	0.94	0.86	0.94	1.03	0.92
ykhI	0.92	1.01	0.95	0.95	0.99
ykhJ	0.89	1.03	1.12	0.97	1.03
ykhK	0.85	0.87	0.93	0.88	0.97
ykiC	1	0.94	1	1.05	1.05
ykiD	0.93	0.82	1.14	0.97	0.91
ykiF	0.86	0.89	1.15	0.94	0.9
ykiG	0.97	0.97	1.06	1.06	1
ykiH	0.92	0.97	1.04	0.97	0.91
ykjA	0.95	0.84	0.96	0.94	0.91
ykjB	0.94	0.94	1.1	1	1.01
ykjC	0.9	0.87	0.89	0.88	0.86
ykjE	0.87	0.9	1	0.91	0.89
ykjF	1	1.03	1.06	0.96	1.04
ykjH	0.88	0.93	0.99	0.94	1.03
ykjJ	0.93	1.02	1.03	1.01	0.99
ylaC	1.2	1.11	1.12	1.2	1.18
ylaE	0.88	0.83	0.96	0.98	0.88
ylbA	0.9	0.83	0.89	0.99	0.91
ylbB	0.94	0.97	0.93	1.07	1.06
ylbD	0.95	0.98	1.04	0.96	1.02
ylbE	0.9	1.03	1.1	1.03	1.1
ylcA	0.99	1	1	0.94	1.01
ylcC	0.99	0.98	0.98	0.91	1
ylcD	0.88	0.94	0.96	0.96	0.97
ylcE	0.9	0.97	0.87	0.99	0.94
ylcF	0.9	0.94	1	1.04	1
yldA	0.9	0.89	0.96	1	0.94

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yldC	0.87	0.92	0.9	0.87	0.9
yldE	0.94	0.92	1.01	1	0.98
yleB	0.99	1.02	1.11	1.02	1.15
yleE	1.05	1.04	1.12	1.07	1.17
yleF	0.93	1.02	1.1	1.09	1.08
ylfA	0.91	0.91	0.87	0.94	0.9
ylfB	1	0.97	0.99	1.05	0.93
ylfC	0.9	0.92	0.96	0.94	0.9
ylfD	0.96	1	0.94	1.05	0.9
ylfF	0.84	0.86	0.84	0.86	0.81
ylfH	0.97	0.91	0.96	0.92	0.95
ylfI	0.93	0.87	1.02	1.03	0.99
ylgB	0.95	1.03	1.03	1.11	1.12
ylgC	0.91	1.04	1.08	1.02	1
ylgG	0.88	0.96	0.98	0.98	1.12
ylhB	0.89	0.93	1.01	0.97	0.96
yliA	0.91	0.91	0.9	0.91	0.85
yliC	0.78	0.85	0.91	0.94	0.81
yliD	0.78	0.76	0.77	0.75	0.78
yliE	0.65	0.65	0.56	0.63	0.68
yliF	0.83	0.78	0.96	0.93	0.99
yljB	1	0.95	0.94	0.94	1.05
yljC	0.87	0.86	0.94	0.88	0.88
yljD	0.89	0.89	0.82	0.88	0.88
yljE	0.94	1.09	1.02	1.01	1.07
yljF	1.02	1.08	1.13	0.98	1.05
yljG	0.78	0.87	0.96	0.85	0.88
yljH	0.95	0.93	0.98	0.95	1.01
yljI	0.97	1.02	1.05	1	1.04
yljJ	0.9	0.91	0.91	0.91	0.88
ylqL	0.88	0.9	0.85	0.97	0.88
ylxQ	0.88	0.94	0.89	0.94	0.92
ymaB	0.64	0.64	0.81	0.68	0.68
ymbC	0.75	0.72	0.8	0.64	0.66
ymbD	0.57	0.51	0.87	0.56	0.5
ymbG	0.74	0.76	0.82	0.69	0.72
ymbJ	0.34	0.37	0.99	0.36	0.34
ymbK	0.45	0.65	1.16	0.44	0.47
ymcA	0.69	0.72	0.91	0.76	0.73
ymcB	0.44	0.43	0.98	0.44	0.48
ymcC	0.52	0.49	0.93	0.44	0.53
ymcF	0.86	1.01	1.01	0.96	0.99
ymdC	0.95	0.96	1	0.96	0.95
ymeB	0.89	0.99	0.97	1	0.93
ymfD	0.88	0.92	0.89	0.92	0.87
ymfE	0.87	0.82	0.84	0.77	0.82

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ymgB	0.79	0.82	0.82	0.85	0.87
ymgC	0.89	0.93	0.96	0.94	0.95
ymgF	0.85	0.81	0.95	0.9	0.95
ymgG	1.47	1.21	1.29	1.5	1.5
ymgH	1.93	1.64	1.68	1.88	2.18
ymgI	1.57	1.34	1.34	1.47	1.69
ymgJ	1.61	1.33	1.24	1.52	1.51
ymgK	1.01	1	1.01	0.95	1
ymhC	0.91	1.04	1	1	1
ymhG	0.9	1.03	1	1.01	0.98
ymiA	0.9	0.98	1	1.01	1.04
ymjE	0.94	0.99	1.07	1.07	1.01
ymjF	0.85	0.88	0.86	0.87	0.83
ynaA	1.18	1.17	1.1	1.24	1.12
ynaB	0.91	0.97	0.91	0.96	0.91
ynaC	0.91	0.95	0.94	0.97	0.93
ynaD	0.84	0.89	0.79	0.91	0.85
ynaE	0.87	0.89	0.94	0.94	0.94
ynaG	0.81	0.79	0.81	0.83	0.85
ynbA	0.8	0.93	0.95	0.92	0.86
ynbB	0.84	0.95	0.98	0.97	0.91
ynbC	0.87	0.96	0.9	1.01	1
ynbD	0.91	1	1.02	0.99	0.99
ynbE	0.95	0.93	0.83	0.95	0.93
yncA	0.92	0.95	0.99	0.94	1
yncB	0.85	0.8	0.87	0.84	0.83
yndA	0.83	0.86	0.91	1	0.88
yndB	0.91	0.94	0.81	0.89	0.89
yndC	0.7	0.79	0.7	0.79	0.71
yndD	0.78	0.82	0.88	0.9	0.88
yndE	0.16	0.8	0.85	0.86	1.06
yndF	0.39	1	1.02	0.63	0.95
yndG	0.82	0.89	0.88	0.83	0.85
yneB	0.75	0.91	0.9	0.85	0.87
yneC	1.11	1.08	1.04	1.19	1.28
yneE	0.89	1.02	1.03	0.94	0.97
yneF	0.93	1.08	1.28	1.15	1.26
yneG	0.69	0.78	0.91	0.8	0.83
yneH	0.7	0.91	0.96	0.91	0.94
ynfC	0.76	0.86	0.86	0.93	0.87
ynfD	1.13	1.08	1.02	1.14	1.1
ynfG	0.91	0.91	0.9	1.04	0.93
ynfH	0.67	0.73	0.8	0.83	0.81
yngA	0.81	0.89	0.8	0.81	0.87
yngB	0.99	1.02	1.02	0.85	0.93
yngE	0.89	1.05	1.01	0.93	1.01

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yngF	0.92	1.17	1.11	1.02	1.05
ynhA	0.98	0.98	1.01	1.05	1.14
ynhC	0.9	1.05	1.11	0.99	1.03
ynhD	0.95	1.02	1.1	1.07	1.08
ynhH	0.66	0.78	0.84	0.79	0.77
ynhI	0.72	0.92	0.86	0.92	0.93
yniC	0.82	0.79	0.93	0.81	0.75
yniG	0.85	0.87	0.87	0.88	0.86
yniH	0.83	0.9	0.87	0.93	0.92
yniI	0.56	0.66	0.72	0.84	0.8
yniJ	0.83	0.81	0.85	0.76	0.91
ynjC	0.99	0.75	0.85	0.88	0.85
ynjD	0.98	0.84	0.91	0.86	0.83
ynjE	1.22	0.89	0.98	0.92	0.97
ynjF	0.83	0.73	0.85	0.78	0.76
ynjG	0.68	0.63	0.93	0.88	0.87
ynjH	0.95	0.93	0.95	0.72	0.71
ynjI	0.91	0.85	0.88	0.84	0.83
ynjJ	0.99	0.83	0.91	0.86	0.85
yoaD	0.94	0.82	0.92	0.82	0.78
yoaG	0.96	0.75	0.76	0.87	0.81
yoaH	1.1	0.82	0.89	0.88	0.79
yoaI	1.15	0.27	0.99	1.08	0.95
yobA	0.76	0.53	0.78	0.72	0.75
yobC	0.94	0.79	0.9	0.83	0.88
yofM	0.99	0.88	0.87	0.88	0.83
yogE	0.93	0.89	0.85	0.86	0.86
yogG	1.11	0.9	1.04	0.99	0.95
yogI	0.92	0.79	0.87	0.83	0.8
yogJ	1	0.86	0.99	0.87	0.94
yogL	0.77	0.71	0.62	0.64	0.7
yogM	0.87	0.84	0.82	0.84	0.86
yohC	0.93	0.83	0.93	0.82	0.89
yohD	0.75	0.83	0.87	0.85	0.86
yohH	0.85	0.8	0.8	0.74	0.64
yohJ	0.89	0.72	0.81	0.8	0.8
yoiB	1.03	0.82	0.87	0.93	0.94
yoiC	0.86	0.74	0.82	0.81	0.82
yojB	0.84	0.76	0.79	0.83	0.79
yojC	0.98	0.94	0.99	0.97	0.97
ypaA	0.91	0.84	0.87	0.85	0.85
ypaC	0.8	0.8	0.81	0.79	0.79
ypaG	0.89	0.85	0.77	0.8	0.81
ypaH	0.87	0.87	0.88	0.8	0.84
ypaI	0.95	0.87	0.93	0.84	0.87
ypbB	0.86	0.87	0.85	0.8	0.83

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ypbC	1.04	0.89	0.88	0.91	0.95
ypbD	1.09	0.9	0.91	1	0.9
ypcA	1.02	0.91	0.98	1.02	0.99
ypcB	1	0.92	0.86	0.91	0.9
ypcC	0.93	0.9	0.9	0.88	0.88
ypcD	0.91	0.88	0.85	0.87	0.86
ypcG	0.95	0.88	0.87	0.84	0.91
ypcH	1.03	0.98	0.99	0.91	0.96
ypdA	0.86	0.86	0.92	0.78	0.89
ypdB	1.01	0.91	0.99	0.92	1
ypdC	0.82	0.88	0.82	0.84	0.69
ypdD	0.98	0.81	0.94	0.91	0.93
ypfD	1.11	0.93	0.97	1.07	1.02
ypfE	1.05	0.91	0.99	0.99	0.98
ypfF	1.08	0.93	1.03	1.02	0.98
ypgB	0.88	0.86	0.86	0.86	0.79
ypgC	1.11	0.99	0.98	1.2	1.09
ypgD	0.88	0.87	0.86	0.88	0.83
yphA	1.01	0.97	0.96	1.02	1.02
yphC	0.84	0.88	0.83	0.79	0.81
yphH	0.86	0.75	0.78	0.67	0.83
yphI	0.96	1.01	1.04	1.04	1.03
yphJ	0.7	0.8	0.85	0.72	0.76
yphK	0.9	0.75	0.8	0.87	0.87
yphL	1.14	1.07	1.02	1.15	1.11
ypiA	1.01	0.98	0.95	1.02	0.94
ypiB	0.95	0.9	0.95	1.05	0.95
ypiE	0.85	0.79	0.74	0.84	0.84
ypiH	0.96	0.9	0.9	0.56	0.58
ypiJ	0.68	0.72	0.74	0.58	0.56
ypiK	1.16	1.06	1.03	0.36	0.3
ypiL	1.01	0.99	0.97	0.77	0.79
ypjA	0.63	0.65	0.71	0.6	0.63
ypjB	0.85	0.79	0.81	0.81	0.91
ypjC	0.97	0.89	0.94	0.91	0.91
ypjF	0.84	0.86	0.8	0.81	0.78
ypjH	0.93	0.9	0.87	0.87	0.92
ypjI	0.94	0.91	0.88	0.93	0.86
yqaB	0.92	0.92	0.91	0.95	0.84
yqaC	1.11	1.14	1	1.16	1.09
yqaD	0.8	0.82	0.8	0.86	0.78
yqaG	0.93	0.92	0.81	0.89	0.85
yqbA	0.99	0.97	0.88	0.98	0.84
yqbF	1	0.98	0.94	0.84	0.78
yqbH	0.73	0.82	0.8	0.81	0.71
yqbI	0.96	0.95	1.03	0.9	0.89

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yqbJ	0.95	0.75	0.88	0.86	0.91
yqcB	0.66	0.62	0.9	0.93	0.9
yqcC	0.66	0.63	0.97	1.03	0.94
yqcD	0.41	0.41	0.94	1.03	0.91
yqcE	1.03	0.84	0.83	0.92	1.02
yqcF	0.81	0.82	0.74	0.91	0.77
yqcG	1	1.09	0.9	1.12	1.04
yqdA	0.9	0.86	0.85	0.9	0.85
yqeA	0.91	0.9	0.83	0.89	0.85
yqeB	1.05	0.95	0.88	0.86	0.98
yqeD	0.98	0.92	0.98	0.92	0.89
yqeH	0.89	0.87	1	0.92	0.93
yqeL	1	0.85	1.12	0.92	0.88
yqfA	1.09	0.96	1	1.05	0.97
yqfC	1.12	0.99	0.93	1.13	1.05
yqfE	1.12	1.09	1.04	1.24	1.05
yqfF	0.96	0.98	0.91	0.97	0.9
yqfG	0.92	0.9	0.93	0.91	0.88
yqgA	0.89	0.92	0.91	0.84	0.75
yqgC	0.91	0.9	0.88	0.8	0.82
yqgE	1.02	0.98	1.06	0.95	1.03
yqgF	0.85	0.99	0.93	0.96	0.97
yqgG	0.9	0.88	0.93	0.89	0.85
yqhA	0.91	0.98	0.97	1.02	0.99
yqiA	1.21	1.1	1.1	1.03	1.08
yqjA	1.05	1.12	1.07	1.14	1.08
yqjB	1	0.95	0.97	1.11	0.98
yqjD	1.06	0.98	1	1.05	0.98
yqjE	1.05	1.06	1.05	1.16	1
yraA	0.93	0.96	0.91	0.95	0.87
yraB	1.02	0.98	0.98	1.06	1.06
yraC	1.02	1.04	0.94	0.99	0.95
yraD	0.97	0.93	0.96	0.87	0.85
yraE	0.86	0.91	0.84	0.77	0.77
yraF	0.92	0.97	0.94	1	1.01
yrbA	0.99	0.92	0.96	0.95	0.95
yrbB	0.93	0.89	0.98	0.99	0.98
yrbC	0.8	0.81	0.79	0.78	0.79
yrbD	1.1	0.98	1.07	0.97	1
yrbE	1.08	1.03	1.05	1	0.83
yrbF	0.95	0.91	0.84	0.82	0.9
yrbH	1.11	1.07	1	1.14	1.06
yrbI	1.07	1.09	0.86	1.13	0.95
yrcA	1.06	1.14	1	0.99	1
yrcB	0.79	0.83	0.79	0.8	0.8
yreB	1.04	0.99	0.91	0.89	0.9

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yreD	1	0.96	1	0.92	1.05
yreE	0.94	0.98	0.97	0.9	0.96
yrfA	0.83	0.89	1.09	0.86	0.84
yrfB	1.13	1.03	1.08	1.01	0.95
yrfC	0.99	1.01	0.96	1.03	0.99
yrfD	1.06	1.07	0.9	1.12	0.98
yrfE	1.02	1.13	0.97	1.08	1
yrgA	1	1.07	0.93	0.96	0.94
yrgE	0.81	0.83	0.86	0.81	0.82
yrgF	0.93	0.93	0.95	0.78	0.82
yrgG	0.93	0.92	0.94	0.79	0.77
yrgH	0.84	0.83	0.86	0.81	0.81
yrgI	0.95	0.98	0.99	0.87	0.93
yrhH	0.94	0.99	1.03	1	0.99
yriB	1.07	1.09	0.99	1.02	1.03
yriC	1.2	1.23	1.09	1.24	1.08
yrjA	1.05	1.13	0.93	1.08	1.06
yrjB	1.06	1.16	0.99	1.13	1.13
yrjC	1.05	1.13	1	1.08	1.02
yrjD	1.07	1.12	0.99	1.07	0.94
yrjE	0.93	0.92	0.93	0.91	0.82
yrjF	0.83	0.82	0.86	0.82	0.83
yrjG	0.84	0.83	0.85	0.88	0.85
yrjI	0.9	0.87	0.86	0.85	0.84
ysaA	0.9	0.99	0.96	1	1
ysaB	1.08	1.06	0.99	1.14	1.09
ysaC	1.19	1.21	1.06	1.23	1.19
ysaD	1.27	1.45	1.03	1.33	1.22
ysbA	1.09	1.13	0.9	1.15	1.1
ysbB	1.02	1.1	1.01	1.1	1.03
ysbC	0.99	1.1	1.04	1.08	0.98
ysbD	0.99	1	0.99	1.01	0.82
yscA	0.99	0.96	0.91	0.92	0.85
yscB	0.82	0.84	0.85	0.8	0.83
yscD	0.86	0.84	0.76	0.77	0.81
yscE	0.97	1	0.98	1	0.94
ysdA	1.06	1.02	1.04	1.04	1.09
ysdB	0.83	0.81	0.82	0.86	0.89
ysdC	0.83	0.84	0.82	0.83	0.87
ysdE	1.03	1.08	0.92	1.09	0.97
yseA	0.98	1.01	0.91	1.02	0.94
yseC	0.91	1.08	0.97	1.03	1.05
yseD	0.82	0.91	0.88	0.82	0.81
yseE	0.96	1.04	1.02	1.05	1
yseF	1.02	1.05	0.98	1.03	0.91
yseH	0.9	0.96	0.97	0.94	0.84

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ysfB	1.11	1.05	1.01	1.11	1.06
ysfC	1	0.94	0.92	1	0.9
ysfD	0.89	0.87	0.84	0.86	0.83
ysfG	1.13	1.18	0.95	1.11	1.1
ysgA	1.02	1.13	1	1.16	1.02
ysgB	1.04	1.07	1.02	1.13	1
yshA	1.01	1.2	1.02	1.21	1.06
yshB	0.88	1.05	1	1.11	0.92
ysiA	0.97	1.04	1.03	1.07	0.92
ysiB	1.2	1.22	1.16	1.08	1
ysiC	0.79	0.88	0.85	0.9	0.78
ysiD	0.95	0.95	0.96	0.97	0.89
ysiE	0.9	0.87	0.87	0.97	0.9
ysiG	1.04	1.06	0.96	0.97	0.96
ysjA	1.06	0.96	0.92	1	0.99
ysjC	0.92	0.99	0.87	0.96	0.96
ysjD	0.91	1.05	0.92	1.08	0.93
ysjE	0.82	1.01	1.01	1.04	0.96
ysjF	0.82	0.93	0.93	0.91	0.93
ysjG	0.94	1.01	0.89	1	0.86
ysjH	0.72	0.83	0.84	0.77	0.76
ysxL	0.77	0.81	0.82	0.81	0.77
ytaA	1.05	1.02	1.02	1.08	0.97
ytaB	0.92	0.92	0.83	1.03	0.91
ytaD	0.94	0.9	0.83	0.94	0.86
ytbA	1.17	1.19	1	1.17	1.19
ytbB	1.1	1.13	0.98	1.22	1.03
ytbC	0.84	0.98	0.92	1.03	0.9
ytbD	0.92	1.07	0.97	1.01	1.01
ytbE	0.71	0.83	0.83	0.87	0.8
ytcA	0.71	0.85	0.79	0.79	0.76
ytcB	0.95	0.94	0.97	1.01	0.9
ytcC	0.98	1.06	0.99	0.94	0.92
ytcD	0.87	0.93	0.85	0.94	0.85
ytcE	0.71	0.76	0.74	0.81	0.73
ytdA	0.98	0.93	0.77	0.88	1
ytdB	0.95	0.84	0.81	0.98	0.88
ytdC	0.76	0.76	0.71	0.81	0.8
ytdF	1	1.03	0.93	1.09	0.96
yteA	1.07	1.12	1	1.1	1.08
yteB	0.96	1.1	0.87	1.03	1.02
yteC	0.91	1	0.98	0.98	0.99
yteD	1.1	1.16	1.13	1.03	1.04
yteE	0.99	0.99	0.95	0.93	0.91
yteG	0.8	0.88	0.87	0.85	0.8
ytfA	1.02	1.05	0.94	1.05	1.02

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ytfB	0.74	0.8	0.8	0.75	0.74
ytgA	0.71	0.71	0.78	0.77	0.69
ytgB	0.9	0.96	0.83	1.01	0.89
ytgC	0.76	0.82	0.78	0.8	0.77
ytgD	0.71	0.74	0.73	0.82	0.73
ytgE	1.22	1.22	1.04	1.28	1.17
ytgF	1	1.07	0.9	1.11	0.95
ytgG	1	1.16	0.97	1.06	1.09
ytgH	1.01	1.19	1	1.07	1.18
ythA	0.92	0.98	0.9	0.92	0.96
ythB	1.11	1.03	0.87	0.95	1.05
ythC	0.96	1	0.98	0.93	0.95
ytiA	0.98	0.98	1	0.94	0.91
ytjA	0.83	0.9	0.84	0.87	0.85
ytjD	0.95	0.97	0.99	1.03	0.89
ytjE	1.04	1.04	1.01	1.05	1
ytjF	1	1	0.98	0.93	0.97
ytjG	0.95	0.9	0.85	0.95	1.03
ytjH	1.11	1.07	1.04	1.02	1.04
yuaA	1.03	1.07	1	1.07	1.02
yuaB	1.08	1.24	1.1	1.19	1.03
yuaC	0.98	1.13	1.07	1.05	1.06
yuaD	0.97	1.14	1	1.02	1.07
yuaE	0.77	0.94	0.88	0.93	0.96
yucF	0.84	0.94	0.86	0.83	0.79
yucG	0.86	1.03	0.94	0.93	0.98
yudA	0.83	0.92	0.91	0.94	0.93
yudB	0.82	0.9	0.88	0.88	0.87
yudD	0.85	0.93	0.99	0.94	0.9
yudE	0.81	0.91	0.84	0.94	0.83
yudF	0.9	0.97	0.9	0.93	0.9
yudG	0.92	0.92	0.81	0.9	0.9
yudI	0.86	1.06	0.88	0.9	0.99
yudJ	0.87	1.1	0.96	0.94	1.08
yudK	0.86	1	0.9	0.95	0.95
yudL	0.8	0.99	0.86	0.92	0.87
yueA	1.02	1.07	1.01	1.09	0.88
yueB	1.03	1.07	1.04	1.07	0.99
yueC	0.77	0.85	0.83	0.85	0.81
yueD	1.18	1.12	1.15	1.21	1.06
yueE	0.93	0.98	1.01	1.02	0.98
yueF	0.75	0.83	0.9	0.87	0.82
yufA	0.84	0.83	0.82	0.84	0.83
yufC	0.98	0.92	0.95	0.96	0.99
yugA	0.9	0.97	0.92	0.92	0.97
yugB	1.06	1.09	1.05	0.98	1.11

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yugC	0.85	1.02	0.94	0.94	0.97
yugD	0.9	0.99	0.93	0.94	1.01
yuhA	1.1	0.99	0.93	0.91	0.93
yuhB	0.94	0.99	0.9	0.92	0.94
yuhC	1.04	1.08	0.91	0.96	0.98
yuhD	0.83	0.88	0.83	0.83	0.85
yuhE	0.85	0.93	0.83	0.9	0.89
yuhH	0.84	0.88	0.87	0.9	0.83
yuhI	0.78	0.83	0.82	0.89	0.79
yuhJ	1.29	1.23	1.25	1.26	1.34
yuiA	0.78	0.86	0.86	0.88	0.88
yuiB	0.81	0.84	0.86	0.79	0.89
yuiC	0.65	0.67	0.6	0.6	0.68
yuiD	0.99	1.02	0.93	1.12	1.04
yuiE	0.89	0.96	0.89	0.92	0.95
yujA	0.99	1.06	1.02	1.02	1.11
yujB	0.88	1.02	1.06	1.02	1.04
yujD	0.82	1	0.84	0.96	0.92
yujE	0.88	1.01	0.85	0.97	0.98
yujF	0.69	0.83	0.82	0.84	0.83
yujG	0.86	0.91	0.88	0.95	0.84
yvaA	1.1	0.97	0.99	1.04	0.92
yvaB	0.7	0.78	0.8	0.83	0.76
yvaD	0.72	0.8	0.86	0.81	0.8
yvcA	0.78	0.82	0.78	0.78	0.82
yvcC	0.96	0.98	0.98	1	1.03
yvdB	0.86	1.01	1.01	0.92	0.93
yvdC	0.95	1.11	1.03	1.03	1.03
yvdD	0.84	0.92	0.87	0.88	0.91
yvdE	0.82	0.94	0.87	0.88	0.87
yvdF	0.82	0.93	0.87	0.88	0.86
yvdG	1.05	1.1	1.04	1.15	1.09
yveB	0.91	0.89	0.83	1.01	0.94
yveC	0.77	0.77	0.72	0.8	0.78
yveD	0.95	1.02	0.91	0.94	1.02
yveE	1.06	1.03	0.98	1.05	1.01
yveF	0.96	1.04	0.89	0.95	1.03
yveG	0.96	1.19	1.03	1.01	1.09
yveH	0.93	1.05	0.99	1	1.09
yveI	1.17	1.26	1.08	1.11	1.19
yvfA	0.91	0.75	0.69	0.73	0.84
yvfB	1.5	1.45	1.24	1.33	1.43
yvhA	0.94	1.02	0.88	1.01	1.02
yvhB	0.94	1	0.95	1.01	0.99
yviA	0.96	0.99	0.96	1.04	0.94
yviC	0.86	1.05	0.95	0.97	0.99

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
yviH	0.96	0.89	0.87	0.93	0.88
yviI	0.87	0.91	0.8	0.79	0.88
yviJ	1.03	0.91	0.93	1.01	0.94
yvjA	0.9	0.86	0.96	0.92	1
yvjB	1.02	1.07	1.02	1.04	1.16
ywaB	0.93	1.05	0.96	0.96	0.94
ywaC	1.02	1.18	1.05	1.01	1.06
ywaD	0.98	0.94	1	1.04	0.96
ywaE	1.01	1.09	1.13	1.09	1.12
ywaF	1.08	1.14	1.13	1.13	1.18
ywaG	0.86	0.93	0.85	0.89	0.91
ywaH	0.8	0.84	0.78	0.87	0.76
ywaI	0.9	0.98	0.92	0.96	0.93
ywbA	0.77	0.89	0.81	0.84	0.8
ywbB	1.02	1.06	0.91	1.15	1.08
ywcC	0.86	0.96	0.99	0.98	0.99
ywdA	1.1	1.04	0.92	1.05	1.02
ywdB	0.88	0.82	0.89	0.92	0.88
ywdC	1.12	1.15	1.1	1.12	1.16
ywdD	1.01	1.18	1.02	1.08	1.14
ywdE	0.86	1.04	0.93	0.91	0.93
ywdF	0.99	1.14	1.04	1	1.09
yweA	0.95	1.02	0.98	1.01	1.06
yweB	0.77	0.83	0.9	0.98	0.98
yweC	1.01	1.1	0.95	1	1
yweD	0.92	0.99	0.89	1.02	0.99
yweE	0.88	0.86	0.89	1.02	0.93
yweF	0.69	0.75	0.76	0.78	0.76
ywfA	0.96	1.01	0.93	0.97	1
ywfB	1.22	1.1	1.04	1.1	1.01
ywfC	0.94	0.9	0.94	0.89	0.94
ywfD	1.31	1.28	1.19	1.08	1.22
ywfE	1.23	1.34	1.21	1.17	1.17
ywfF	0.73	0.92	0.94	0.9	0.84
ywfH	0.5	0.59	0.97	0.99	0.98
ywgA	0.69	0.92	0.93	0.93	0.91
ywhA	0.85	0.95	0.9	0.85	0.91
ywiA	0.97	1	0.94	1.04	1.02
ywiB	0.94	1	0.88	0.96	0.96
ywiC	0.91	0.98	0.96	1.05	0.95
ywiD	0.82	0.9	0.89	0.9	0.92
ywiE	1.22	1.26	1.11	1.29	1.12
ywiH	1.16	1.24	1.16	1.15	1.2
ywjA	0.84	0.94	0.9	0.91	0.89
ywjB	0.85	0.96	0.97	0.98	0.98

Table D2 (Continued).

CGH ratios between the strains of interest and the IL1403 strain (normalization by the intensities of a subset of conserved genes, method C)					
Gene id	**UCMA5713**	**LD55**	**LL08**	**LL52**	**S86**
ywjC	1.12	1.18	1.01	1.13	1.12
ywjD	0.33	0.35	0.95	0.95	0.98
ywjE	0.38	0.42	1.07	1.08	1.07
ywjG	0.9	0.99	0.87	0.94	0.96
yxaB	0.98	1.01	0.96	1.09	1.02
yxaC	0.96	1.01	0.91	0.99	0.95
yxaF	0.69	0.84	0.9	0.92	0.93
yxbA	0.93	0.91	0.88	0.8	1.04
yxbC	0.97	1.15	1.08	0.69	1.11
yxbD	1.08	1.14	1.11	1.04	1.1
yxbE	0.98	1.21	1.1	1.08	1.12
yxbF	0.81	0.85	0.91	1.02	0.91
yxcA	0.97	1.23	1.15	1.12	1.15
yxcB	0.89	1.07	1.04	0.94	0.99
yxcD	1.06	1.17	1.17	1.11	1.22
yxdB	0.85	0.99	0.93	0.9	0.96
yxdC	0.89	1.01	0.91	0.92	0.96
yxdD	0.94	0.92	0.85	0.95	0.96
yxdE	0.9	0.98	0.97	1.01	1.05
yxdF	0.81	0.91	0.8	0.87	0.97
yxdG	1.03	1.06	1.1	0.97	1.16
yxeA	1.03	1.12	1.09	1.04	1.04
yxeB	1.01	1.18	1.2	1.09	1.02
yxfA	1.71	1.92	1.2	1.78	1.5
yxfB	0.95	1.06	1.07	1.03	1.07
yxfC	1.08	1.19	1.2	1.16	1.18
yyaL	0.87	0.99	1.02	0.9	1.05
zitP	0.82	1	0.92	0.96	0.95
zitQ	0.78	0.88	0.82	0.94	0.92
zitR	0.92	0.96	0.89	0.93	0.99
zitS	0.92	0.98	0.95	1.08	1.1
zwf	0.89	0.91	0.93	0.67	1.09

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