Stress responses of *Listeria monocytogenes* to antimicrobial substances from lactic acid bacteria: an explorative study

Stressresponser hos *Listeria monocytogenes* ovenfor antimikrobielle substanser fra melkesyrebakterier: et eksplorativt studium

Philosophiae Doctor (PhD) Thesis

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Faith is to believe what you do not see;
the reward of this faith is to see what you believe.
St. Augustine (354-430)

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Ås, October 2010

Girum Tadesse Tessema

ABSTRACT

Listeria monocytogenes is a gram-positive bacterium that causes severe and life threatening disease both in humans and animals. Due to the severity of the disease and the fact that the bacterium is responsible for considerable economical loss, *L. monocytogenes* is of a great concern particularly to the food industry. *L. monocytogenes* is a common food contaminant and nearly all human *L. monocytogenes* infections are due to ingestion of contaminated food. Antimicrobial components from lactic acid bacteria may serve as safe and natural food preservatives targeting unwanted microorganisms, including *L. monocytogenes*. Elucidations of how *L. monocytogenes* respond to the antimicrobial products are crucial steps to devise a knowledge-based strategy to control this deadly bacterium in food and food-related environments using food grade bacteria.

In the present study the responses of *L. monocytogenes* to the class IIa bacteriocin sakacin P and to acids (acetic, lactic and hydrochloric acid) were explored. Exposure to sakacin P gave spontaneous mutant strains with reduced susceptibility to the bacteriocin. Analysis of large number of the mutant strains using different approaches revealed substantial difference among the strains. The mutant strains displayed difference in (i) the level of resistance to sakacin P, (ii) the stability of the sakacin P resistance phenotype, (iii) growth fitness in various conditions, (iv) biofilm formation ability, (v) virulence potential, (vi) Fourier transform infrared spectroscopy profile, (vii) regulation of the bacteriocin receptor gene, and (viii) global transcriptome profile. Overall, this indicates that the incidence(s) giving rise to the sakacin P resistance involves a complex regulatory gene network possibly mediated by the bacteriocin receptor and have pleiotropic effects on the physiology of the resistant strains.

The growth of *L. monocytogenes* was reduced but not completely inhibited at pH 5 when the growth medium was acidified with hydrochloric acid (HCl), 10 mM acetic acid or 20 mM lactic acid. Acetic acid had the highest antilisterial activity followed by lactic acid and HCl. Stress due to the presence of the acids induced a large number of genes associated with acid defense, virulence, and cross-protection to other types of stresses. Acidulant type dependent responses were also observed.

The explorative transcriptome studies confirmed numerous results of previous studies on the response of *L. monocytogenes* to the class IIa bacteriocins and acid stresses. In addition, it identified a number of putative genes with possible role in the responses under investigation. Altogether, the results presented in this thesis revealed insights contributing to understand the responses of *L. monocytogenes* to the antimicrobial substances that may be encountered by this bacterium in fermented food and thereby opening new avenues for further studies.

SAMMENDRAG (NORWEGIAN ABSTRACT)

Listeria monocytogenes er en gram-positiv bakterie som kan forårsake alvorlig og livstruende sykdom blant både dyr og mennesker. Alvorligshetsgraden av sykdommen og det faktum at bakterien er årsak til stort økonomisk tap, gjør at *L. monocytogenes* forårsaker stor bekymring, spesielt hos næringsmiddelindustrien. *L. monocytogenes* forekommer i mange typer mat, og nesten alle humane infeksjoner med *L. monocytogenes* skyldes inntak av forurenset mat. Antimikrobielle forbindelser fra melkesyrebakterier, som bakteriocin og syrer, kan være trygge og naturlige konserveringsmidler mot uønskete mikroorganismer, inkludert *L. monocytogenes*. Å avdekke hvordan *L. monocytogenes* responderer på disse antimikrobielle forbindelsene er viktig for å utvikle en kunnskapsbasert strategi for kontroll av denne bakterien i mat og i matrelaterte omgivelser ved bruk av "food grade" bakterier.

I denne studien ble responsen til *L. monocytogenes* mot klasse IIa bakteriocinet sakacin P, mot lav pH og syrer (eddik- og melkesyre) undersøkt. Eksponering for sakacin P ga spontane mutanter med redusert følsomhet for bakteriocinet. Bred analyse av et stort antall mutanter viste at det var vesentlig forskjell mellom stammene. De muterte stammene hadde forskjeller i (i) resistensnivå mot sakacin P, (ii) stabilitet av resistens fenotype (iii) vekst ved ulike forhold, (iv) evne til biofilmdannelse, (v) virulenspotensial, (vi) Fourier transform infrarød spektroskopiprofil, (vii) regulering av bakteriocinreseptorgenet og (viii) global transkripsjonsprofil. Til sammen indikerer dette at hendelser som medfører økt sakacin P resistens involverer et komplekst genreguleringsnettverk, muligens styrt via bacteriocin reseptoren, som har en mangfoldig påvirkning på fysiologien til de resistente stammene.

Vekst av *L. monocytogenes* ble redusert, men ikke fullstendig hemmet ved pH 5, når vekstmediet var surgjort med saltsyre (HCl), 10 mM eddiksyre eller 20 mM melkesyre. Eddiksyre hadde den største antilisteriaeffekten, etterfulgt av melkesyre og HCl. Stress som følge av nærvær av syrer, induserte et stort antall gener assosiert med syreforsvar, virulens og kryssbeskyttelse mot andre typer stress. Det ble også observert spesifikke responser for de ulike syrene.

Transkripsjonsstudier bekreftet flere resultater fra tidligere studier på respons av *L. monocytogenes* til klasse IIa bakteriociner og syrestress. I tillegg ble det identifisert gener som muligens er involvert i responsene som ble studert. Til sammen gir resultatene presentert i denne avhandlingen innsikt som bidrar ytterligere til å forstå responser til *L. monocytogenes* mot antimikrobielle forbindelser som denne bakterien kan utsettes for, for eksempel i fermentert mat, noe som kan være interessant å undersøke videre i fremtidige studier.

LIST OF PAPERS

Paper I

Tessema, G. T., T. Møretrø, A. Kohler, L. Axelsson, and K. Naterstad. (2009). Complex phenotypic and genotypic responses of *Listeria monocytogenes* strains exposed to the class IIa bacteriocin sakacin P. Appl. Environ. Microbiol. 75:6973-6980.

Paper II

Tessema, G. T., T. Møretrø, L. Snipen, L. Axelsson, and K. Naterstad. (2010). Global transcriptional analysis of spontaneous sakacin P-resistant mutant strains of *Listeria monocytogenes* during growth on different sugars. Submitted.

Paper III

Tessema, G. T., L. Axelsson, T. Møretrø, L. Snipen, E. Heir, A. Holck and K. Naterstad. (2010). Global transcriptional responses of *Listeria monocytogenes* to hydrochloric acid, acetic acid and lactic acid stress. Submitted.

1. INTRODUCTION

Outbreaks during the early 1980s involving *Listeria monocytogenes* established this bacterium as a foodborne pathogen (1, 2). Listeriosis caused by ingestion food contaminated with *L. monocytogenes* still remains a great threat (3-6). Listeriosis occurs infrequently but despite early antibiotic treatment the fatality rate is up to 30% or higher, which is one of the highest figures for foodborne diseases (4, 7, 8). Product recalls due to contamination by *L. monocytogenes* and expenditures associated with listeriosis create a significant economical burden to the society in general and to the food industry in particular (9, 10).

Preservation of food using fermentation has been a common practice in the history of mankind, long before the invention of refrigeration and synthetic additives (11). Lactic Acid Bacteria (LAB) are one of the most common group of microorganisms involved in food fermentations (11, 12). The preservative quality of LAB relies on their ability to produce an array of antimicrobial substances such as organic acids (e.g. lactic acid and acetic acid) and bacteriocins (12, 13).

Controlling the growth of foodborne pathogens such as *L. monocytogenes* using LAB and their antimicrobial products alone, or as a part of hurdle technology, has raised an exciting possibility to use the traditional way of food preservation in a contemporary manner [(14-26) and more other works]. *L. monocytogenes*, however, shows a remarkable capacity to counteract adverse conditions including stresses due to low pH and bacteriocins (27-33). Their intrinsic properties as well as the ability of *L. monocytogenes* to acquire new mechanisms to withstand the antimicrobial activity of LAB, has created concerns regarding the application of LAB and their products as biopreservatives.

A deeper understanding of how *L. monocytogenes* respond to the antimicrobial products of LAB is a crucial step to devise a knowledge-based strategy to control this and other closely related foodborne pathogens in food and food-related environments. In addition, knowledge about stress survival strategies in pathogens may be applicable for patho-biotechnology purpose (34, 35). The present work aim to explore the responses of *L. monocytogenes* to stresses from antimicrobial products of LAB with emphasis on bacteriocin and organic acids.

This thesis is organized in chapters, and a brief description of important thematic areas is addressed in Chapter 2. The aim of this study is described in Chapter 3. Chapter 4 and Chapter 5 report the main results of the present work and the future perspectives, respectively. The papers described in this thesis (Paper I - Paper III) are presented as appendix.

2. BACKGROUND

2.1 *Listeria monocytogenes*

Listeria monocytogenes belongs to the genus *Listeria,* a genus of gram-positive bacteria closely related to *Bacillus, Clostridium*, *Enterococcus, Streptococcus* and *Staphylococcus*. The genus comprises facultative anaerobe, non spore-forming, and rod shaped bacteria 0.5 µm in width and 1-1.5 µm in length with a low GC content (36). Currently, the genus includes six species: *L. monocytogenes*, *L. ivanovii*, *L.* seeligeri, *L. innocua*, *L. welshimeri* and *L. grayi*. Of these, *L. monocytogenes* and *L. ivanovii* are pathogenic. While the former infects both human and animals the latter principally causes disease in animals. However, some of the regarded non pathogenic *Listeria* species are reported to be implicated in human infections (8, 36, 37).

The species of *L. monocytogene*s consists of 13 different serotypes which are grouped into three genetic lineages: linage I (consists of serotypes 1/2a, 1/2c, 3a and 3c), linage II (1/2b, 3b, 4b, 4d, 4e and 7) and III (4a and 4c) (38). Serotype 4ab is a variant of serotype 4b (39) and no linage designation is given to serotype 4ab. Although 13 serotypes are recognized, serotypes 1/2a, 1/2b, 1/2c, and 4b represent approximately 98% of the isolates collected from food and infected patients (40). Generally, serotype 4b causes the majority of foodborne outbreaks and human listeriosis, while serotypes belonging to 1/2 are most frequently isolated from food (41-44).

2.2 Significance of *L. monocytogenes* and human listeriosis

The bacterium *L. monocytogenes* (originally named *Bacterium monocytogenes*) was first described in 1926 as the cause of a disease of rabbits, characterized by a large mononuclear leucocytosis (45). A retrospective study has confirmed listeriosis in human even before it was described in the rodents (46). The transmission of human listeriosis was shown to be foodborne in 1980s (1, 2) and since *L. monocytogenes* is considered one of the major foodborne pathogens (4-6). Recent resurgent trend in listeriosis has been reported in several European countries (3, 5, 47, 48). In United States of America, a modest increase in the incidence of *Listeria* infection has been reported for the year 2009, compared with the last three years of average incidence (49). *L. monocytogenes* has been isolated from different food

products in developing countries such as in Africa (50-53), however, except few reports (54, 55), the human epidemiologic aspects of listeriosis is largely unknown.

Listeriosis in humans occurs infrequently, but despite early antibiotic treatment the fatality rate is up to 30% or higher, which is one of the highest figures for foodborne diseases (4, 7, 8). Immunocompromised individuals such as neonates, pregnant women, elderly persons, and patients suffering from transplantation events are at higher risk of acquiring listeriosis (8, 56). However, accumulating evidences indicate that *L. monocytogenes* also may cause febrile gastroenteritis and to the lesser extent systemic infection among otherwise healthy individuals (56-61). Recall of products contaminated with *L. monocytogenes* and cost associated with human listeriosis has created a high economic burden to the food industry and to the public health services (9, 10).

2.2.1 Pathogenicity and virulence determinants

Normally *L. monocytogenes* is a soil-dwelling bacterium and live as a saprophyte. The bacterium can also survive and multiply in diversity of environments, including in the cytoplasm of mammalian hosts (8, 62, 63). As already mentioned, ingestion of food heavily contaminated with *L. monocytogenes* appears to be the major source of infection (4). In the majority of cases, ingestion of *L. monocytogenes* does not cause any symptoms and goes unnoticed (6). The incubation period among individuals with symptoms varies and is estimated to be 6-20 hours for febrile gastroenteritis and 3-70 days for systemic infections (6, 8, 61). Febrile gastroenteritis due to *L. monocytogenes* is often acute, self-limited and cease within two days (61). In contrast, invasive listeriosis may cause severe and often fatal illness, involving otherwise sterile body organs (8).

Crossing of the intestinal mucosa by specific (legend-receptor interaction) and non-specific mechanisms [M (microfold) cells of the Peyer's patches], is the first step in systemic listeriosis (8). The bacterium multiplies in the lamina propria of the gut and translocates into the primary target organs; liver and spleen, by lymphohematogenous means. Most of the bacterial loads are cleared by the immune system. Surviving bacteria continue to proliferate in the liver parenchyma and may result in release of the bacteria into the blood circulation. *L. monocytogenes* then disseminates to the secondary target organs. Due to the tissue tropism nature of *L. monocytogenes,* it most often infects the central nervous system, the gravid uterus

and the fetus. *L. monocytogenes* has an ability to penetrate various non-phagocytic cells (e.g. epithelial cells, and hepatocytes), to multiply both in phagocytic and non-phagocytic cells and to spread to neighboring cells directly (8). Detailed description of the pathophysiology is extensively described in the review by Vazquez-Boland et al. (8).

The transition of *L. monocytogenes* from a saprophytic to a pathogenic life style involves the regulation of a number of genes associated with virulence (58, 64, 65). This includes genes encoding proteins important for survival in the gastrointestinal tract such as *gad*, *bsh, bilE opuC,* and *gbu* (58). The above mentioned genes are mostly up-regulated in the intestine and are regulated by the general stress regulator σ^B (64, 66). The proteins encoded by genes involved in intracellular parasitism (e.g. *plcA, plcB, mpl, hyl,* and *actA*) and their master positive regulatory factor A (*prfA*) are reported to be induced in blood, liver and spleen (64, 65). Genes associated with attachment and invasions (e.g. *inlA,* and *inlB*) are common for both gastrointestinal stage and intracellular parasitism, and are generally induced in the host (64, 65). Lists of confirmed and putative genes associated with virulence are described elsewhere (8, 64, 65, 67).

2.3 Food safety and biopreservatives

Listeriosis occurs almost exclusively after ingestion of contaminated food (4). Food products such as dairy, poultry, meat, fish, vegetables, fruits, and sea foods contaminated with *L. monocytogenes* have been linked to outbreaks and sporadic cases (67, 68). The above mentioned food products can be pre-processed in a ready-to-eat (RTE) form and consumed without further treatment. RTE food products are usually kept at low-temperatures and several of them are capable of supporting the growth of *L. monocytogenes* (69).

Preservation of food to control the growth of unwanted microorganism can be achieved by physical, chemical and biological means (70). Application of different preservatives together at low concentration (level) as means of hurdles can control the microbial growth, improve the sensory quality of foods as well as their nutritional properties (71). Hurdles of biological preservatives have the potential to satisfy consumer's preference to "natural "and "healthier" preservatives. Studies on the applications of LAB and their antimicrobial products, as well as of listeria specific bacteriophages, have shown promising results for better control of this bacterium in food and food related environments (14-25, 72-74). *L. monocytogenes*, however,

shows a remarkable ability to resist the killing effect of antimicrobial substances from LAB (27-32).

2.3.1 Lactic acid bacteria and their antimicrobial products

Lactic acid bacteria are a heterogeneous group of bacteria and comprise acid tolerant, non-sporulating, non-respiring, low GC content gram-positive cocci or rods. LAB are known for production of lactic acid as a major end product of carbohydrate fermentation (75). LAB are naturally found in various food products, are one of the most common groups of microorganisms involved in food fermentations, and are members of the normal flora of mammals (11, 12, 75). LAB produce an array of antimicrobial substances such as organic acids (e.g. lactic acid, acetic acid and propionic acid), hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins (13). These antimicrobial substances from LAB serve as an agent in biological warfare against closely related bacteria, in the struggle for niches and nutrients (76). LAB as a group is related to *L. monocytogenes* and LAB and *L. monocytogenes* generally share common niches and utilize similar nutrients (36, 75). It makes sense the antimicrobial substances from LAB also kills the foodborne pathogen *L. monocytogenes.* The present work mainly focuses on the responses of *L. monocytogenes* to the bacteriocin sakacin P and to acid (acetic, lactic and hydrochloric acid) stresses. An overview of the mode of antimicrobial action of these compounds, and their application in food are presented below.

Bacteriocins

Ribosomally synthesized antimicrobial peptides are widely distributed in nature. Because of their food grade quality and potential use in the food industry, bacteriocins produced by LAB have received a great interest (77, 78). According to the current release of the bacteriocin database BACTIBASE, LAB make up the predominant group of bacteriocin producers accounting for more than 63% of the total bacteriocins (79). The LAB bacteriocins have been grouped into classes and subclasses based on criteria such as producer organism, molecular size, physical property, chemical structure, mode of action and so on (78). Despite some differences in classification schemes, class I and class II bacteriocins are common to all classification systems (78, 80, 81), including according to the latest “universal” bacteriocin classification scheme (82). Class I bacteriocins, also known as lantibiotics are subjected to extensive post translational modification and contain unusual amino acid side chain residuals

such as lanthionine and methyllanthionine (78). So far, nisin (class I) is the only bacteriocin approved for use as a food preservative (E234) and is in use in more than 50 countries (83). In contrast to class I bacteriocins, class II bacteriocins are not subjected to extensive post translational modifications. Class II bacteriocins may be divided into three to five subclasses, class IIa, IIb, IIc, IId and IIe (77, 78, 80, 82). Subclass IIa bacteriocins (also called pediocin-like) are considered the most important class II bacteriocins, having strong antilisterial activity (77). These bacteriocins are described in more details in the following section.

The class-IIa (pediocin-like) bacteriocins

The class IIa group of bacteriocin constitutes the most important and well-studied group of class II bacteriocins. Common for bacteriocins in this group is the high antilisterial activity (77). Pediocin PA-1 has already been exploited for commercial use and are covered by several US and European patents. If more bacteriocins are to be approved for various industrial applications, bacteriocins of class IIa are believed to be the next in line (80, 84, 85). The overall sequence similarity among members of the class IIa bacteriocins reported to be 26.8 to 78% (86). The pediocin-like bacteriocins have between 37-48 amino acid residues and are characterized by the conserved Y-G-N-G-V/L "pediocin box" motif signature at the N-terminal ß-sheet domain region. In addition to the “pediocin box”, class IIa bacteriocins possess one or two disulphide bridges and their antimicrobial activity towards indicator strains appears to be correlated with the number of the disulphide bridges (80, 86, 87). The C-terminal domains are less conserved and are suggested to participate in target recognition (88). To date more than 20 class IIa bacteriocins have been identified (77). Based on their C-terminal domain, these class IIa bacteriocins are further classified into three to four subgroups (77). The subgroup 1 comprises the majority of the class IIa bacteriocins including pediocin PA-1 (from which the term pediocin-like bacteriocins derived) and sakacin P (77).

Sakacin P

Sakacin P is a class IIa bacteriocin produced by different strains of *Lactobacillus sakei,* all peptides of 43 amino acids with one disulfide bridge (81, 86, 87, 89-91). Comparative study on class IIa bacteriocins revealed that sakacin P is one of the class IIa bacteriocins with very low sequence similarity (26.8%) and with narrow spectrum of activity (86). Sakacin P is active against *L. monocytogenes* (18, 19, 92), and unlike many other class IIa bacteriocins, it

has modest activity also against LAB (87). Hence, sakacin P is suggested to be the most promising bacteriocin for use in LAB fermentations that are prone to *Listeria* contaminations (87). Sakacin P, as most other class IIa bacteriocins, consists of N-terminal antiparallel β-sheet region, followed by an α-helix and an extended C-terminal amino acid sequence tail that folds back onto the helical region. A hinge region between the β-sheet N- terminal region and the α-helix C-terminal region allows the two domains to move relative to each other (93) (Fig. 1). Interestingly, it has been shown that the potency, the target specificity, and the temperature activity of sakacin P can be further increased by the introduction of an additional disulfide bridge at the C-terminal region (94).

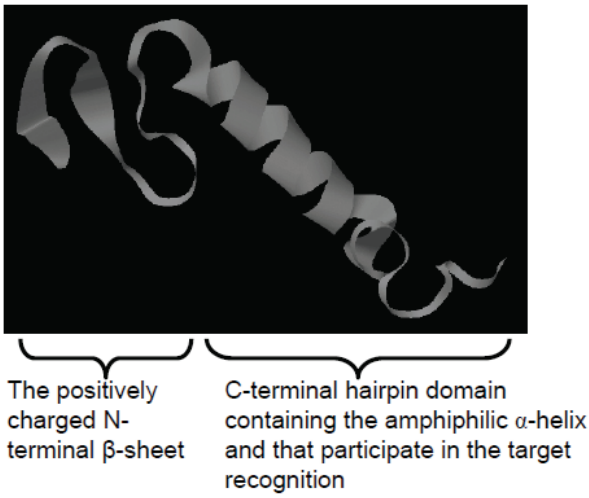


Fig. 1. A cartoon depiction of sakacin P. Image from the BACTIBASE (79).

Mode of action of class IIa bacteriocins

Initial electrostatic interaction between the positively charged bacteriocin (the N-terminal) and the negatively charged cell-envelope of target cells mediates the binding of the bacteriocin to the target (77) (Fig. 2A). It has been shown that the membrane component IIC of a phylogenetically defined subgroup of mannose phosphotransferase systems (PTS) act as receptors for class II bacteriocins (95-98). Evidences from studies involving modified class IIa bacteriocins revealed that the C-terminal hairpin-like domain is responsible for penetrating the hydrophobic core of the target membrane (77). The class II bacteriocins kill the target

cells by permeabilizing the cell membrane, resulting in disruption of the cell's proton motive force, depletion of intercellular ATP pool and leakage of electrolytes and amino acids (77, 99, 100) (Fig. 2B). In addition, the class IIa bacteriocin mesentericin Y105 has been shown not only to induce leakage of amino acids, but also inhibiting their uptake in *L. monocytogenes* (101). A more detailed response of *L. monocytogenes* to class IIa bacteriocins will be presented in the stress response section (see below).

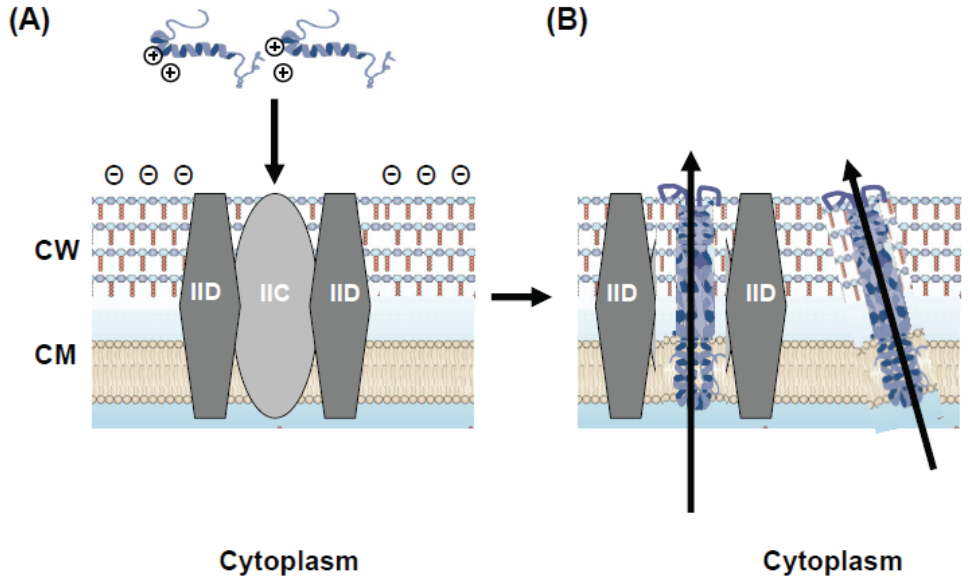


Fig. 2. Putative model for mode of action of class II bacteriocin on target cells. (A) The positively charged class IIa bacteriocins (e.g. sakacin P) interact with the negatively charged cell-envelope. The membrane component IIC of the mannose phosphotransferase systems (PTS) acts as a docking molecule to the bacteriocin. (B) A pore is formed by the bacteriocin, which induces leakage of molecules such as electrolytes and amino acids from the cell. The exact orientation of the pore relative to the docking molecule is not fully known. The model is based on several studies on the interaction between bacteriocins and the target cells (77, 80, 84, 95, 97, 100, 102). Abbreviations: CW, cell wall; CM, cell membrane; IIC and IID refer domains of the mannose-PTS.

Low pH and organic acids

Lactic acid bacteria have the ability to lower the local pH by the production of organic acids, such as lactic-, acetic- and propionic acids (103). Organic acids are also commonly used for acidification of food environments and are used as food additives to extend shelf life and to control the growth of undesirable microorganisms, including *L. monocytogenes* (104, 105). It has been documented that the inhibitory effect of acetic acid (Fig. 3A) on *L. monocytogenes* is higher than the effect of lactic acid (Fig. 3B and 3C) at equal pH and equimolar total acids (23). The difference in antilisterial activity may be partly due to the higher pKa value for acetic acid (pKa = 4.76) compared to that of lactic acid (pKa = 3.86), that gives a higher undissociated: dissociated ratio for acetic acid than lactic acid at a given pH. Factors other than the pKa affecting the antimicrobial activity of organic acids include the bacterial strain, the characteristics of the acid (e.g. isomer type), as well as growth conditions (e.g. pH, media, temperature, growth phase) (33, 104, 106-108).

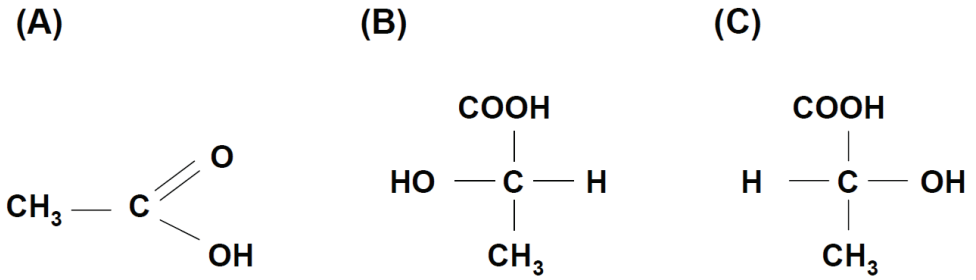


Fig. 3. Structure of (A) acetic acid (B) L-lactic acid and (C) D-lactic acid. Sources (104, 109).

Antimicrobial action of acids

Strong acids as hydrochloric acid (HCl), exert their antimicrobial effect by denaturing enzymes present on the cell surface and by lowering the cytoplasmic pH due to increased permeations of protons caused by a large pH gradient (110, 111). Despite the widespread use of organic acids, the exact mode of their antimicrobial action still has not been fully elucidated (108, 112). It is accepted that the undissociated form of organic acids can freely diffuse across the cell envelope, and once inside, the acids dissociate to give protons and

anions (105) (Fig. 4). Generally the undissociated form of the organic acid, the proton and the anion, are suggested to have effect on the cell by perturbation of the cell envelope, acidification of the cytoplasm, by osmotic stress, and by inhibition syntheses of macromolecules (105, 108, 113).

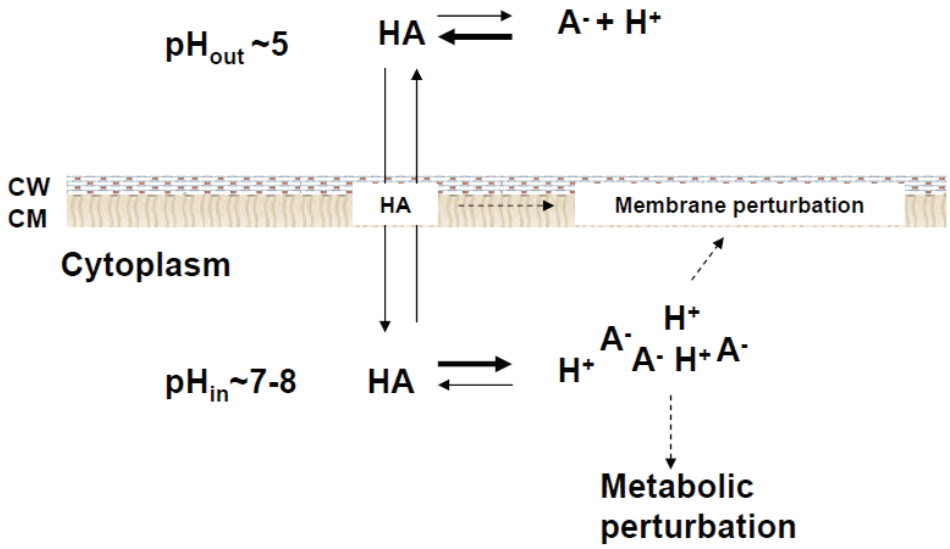


Fig. 4. Mode of action of organic acids on a target cell. The undissociated form of organic acid (HA) can freely pass the cell membrane. HA equilibrates across the membrane until the external and internal concentrations are equal. The acidic environment (pH_{out}~5) favors a higher proportion of the HA (shown by thick arrows), however, the neutral cytoplasmic (pH_{in}~7-8) (33) favors dissociations of HA to a proton (H^+) and an anion (A^-). The effects of organic acids on the cell are shown by dashed arrows. Abbreviations: CW, cell wall; CM, cell membrane; HA, undissociated form of organic acid; H^+, proton; and A^-, an anion. Modification from (112).

2.4 Stress responses of *L. monocytogenes*

In general *L. monocytogenes* optimally grows between 30°C and 37°C, in a pH range of 6 to 7, at salt concentration up to 0.5% (wt/vol), and with water activity level ≥ 0.97, in the absence of other growth-limiting factors (33, 67, 114). *L. monocytogenes* has a remarkable ability to survive, adapt and grow under non-optimal environmental conditions. For example, the bacterium has shown to survive/grow at a wide range of temperatures (<-0.1°C and 47°C), at extreme pHs (pH 3 and pH 9.5), at high concentrations of salt (up to 14%) and at water activity as low as 0.90 (67, 114-119). The ability to withstand external stress conditions facilitates the ubiquitous distribution of the bacterium (62, 120-123) and creates concern among health authorities and food industries.

For *L. monocytogenes*, as any other bacteria, changes in environmental conditions away from the optimum growth conditions inflict stress responses (110). Depending on the extent of the shift, the bacterium may respond in various ways: (i) no apparent phenotypic change, (ii) change in cellular components (e.g. expression of stress related genes), (iii) reduced growth (increased lag time, decrease the growth rate as well as the maximum yield), (iv) growth arrest, (v) and in the worst case it may be killed (124). In order to survive stressful conditions such as exposure to antimicrobial products from LAB, *L. monocytogenes* has to sense and respond rapidly and specifically to the condition. Different modes of physiological and biochemical responses, and the molecular mechanism behind the responses due to the presence of class IIa bacteriocins, low pH and organic acids, are described below.

2.4.1 Response to the class IIa bacteriocin stress

The foodborne pathogen *L. monocytogenes* encounter bacteriocins from LAB in fermented food, in food treated with protective culture or when purified or semi-purified bacteriocins are added directly to the food (18, 19, 125). It has been shown that some isolates of *L. monocytogenes* exhibit modest levels of natural tolerance to class IIa bacteriocins (92, 126, 127). *L. monocytogenes* isolates that are naturally susceptible to class IIa bacteriocins can develop acquired resistance through habituation, at frequency of 10^{-3} to 10^{-6} (28-30, 92, 126, 128-131). Previous studies grouped such spontaneous mutant of class IIa bacteriocins resistant strains into strains with high levels of resistance (500 to 10^6 times more resistant than the wild-type strain) and strains with intermediate levels of resistance (2 to 8 times more

resistant than the wild-type strain) (29, 30, 128, 132-134). Cross-resistance between class IIa bacteriocins are frequently reported (92, 129, 135). The mutant strains display differences in the stability of the acquired resistance during growth in the absence of contact with bacteriocins (28, 128-130, 135, 136). The development of bacteriocin resistance is often associated with lower growth fitness (28, 129, 136, 137) and a shift in metabolic profile toward more mixed acid fermentation (137, 138). The influence of the acquired bacteriocin resistance on antibiotic susceptibility profile of *L. monocytogenes* appears to be variable (128, 136).

Earlier studies sought to unravel the molecular mechanism behind the physiological and biochemical responses of class IIa bacteriocin-resistant strains. Several seminal studies on *L. monocytogenes* and other related gram-positive bacteria indicated abolition of the mannose-PTS (encoded by *mpt* operon and the receptor for class IIa bacteriocins) to be the most common resistance mechanism to class IIa bacteriocins [(28, 29, 96, 97, 132, 134, 135, 138, 139); the PTS is discussed in more detail in the next section]. Interestingly, increase in transcription and translation of the bacteriocin receptor gene has been reported in one of the class IIa bacteriocin-resistant *L. monocytogenes* strains studied so far (29).

Proteins known to modulate the *mpt* expression and conferring resistance to a class IIa bacteriocin includes the σ^{54} (one of the alternative sigma factors) (140-142), ManR (transcriptional activator for σ^{54}) (139), ResD (two-component response regulator) (143), Lmo0095 (the mannose-PTS activator) (144), or PrfA (29). A more recent study also showed the auto-regulatory role of the *mpt* in class IIa bacteriocin-resistant strains of *Enterococcus faecalis* (138). Recently, genes *glpQ* (encoding glycerophosphoryl diester phosphodiesterase) and *pde* (encoding phosphodiesterase) have been identified as new molecular targets conferring resistance to the class IIa bacteriocins in *E. faecalis* (145). Subsequent transcriptional analysis study on *glpQ*, *pde* and *mpt* genes in *E. faecalis* in the presence of divercin RV41, however, gave contradictory results (146). The putative orthologs of *glpQ* and *pde* in *L. monocytogenes* are reported to be *lmo0052* and *lmo1292* respectively, and their inactivation indicate that *lmo0052* but not *lmo1292* confer resistance to class IIa bacteriocins (147). In addition to the common molecular mechanism described, alterations in cell envelope fatty acid composition (133, 136, 148) and changes in cell surface charge (132) are reported to contribute to class IIa bacteriocin resistance in *L. monocytogenes*.

The PTS: an overview on sugar uptake and regulatory roles in low GC gram-positive bacteria

The phosphoenolpyruvate (PEP): carbohydrate specific PTS (simply PTS) is the most efficient and commonly preferred sugar uptake system in many bacteria (149, 150). The primary function of this system is to couple the transportation of carbohydrate across the cytoplasmic membrane with a simultaneous phosphorylation of the carbohydrates (151) (Fig. 5). The PTS consists of two general cytoplasmic components EI (enzyme I) and HPr (histidine-containing phosphocarrier protein), and carbohydrate specific enzymes called EIIs (enzyme II). The EIIs consist of two cytoplasmic functional domains (IIA and IIB), and generally one or two membrane bound domains (IIC and sometimes IID). During active transport and phosphorylation of sugars, the EI is phosphorylated by PEP generated from the end product of glycolysis. The phosphate from P~EI is then transferred to the His-15 residue in HPr (P~His-HPr) and sequentially to EIIA and EIIB. The sugar substrate is then channelled through the integral membrane component EIIC (and EIID) and subsequently accepts the phosphate from the P~EIIB (149, 150, 152). In addition to their role in transportation and phosphorylation of sugars, the P~His-HPr and several P~EIIBs are involved in regulation of different cellular functions (Fig. 5) (see below).

The HPr is the central processing unit in the regulatory function of PTS (153) (Fig. 5). Carbon catabolite control (CCC) system mediated by the HPr is complex and is dependent on factors such as the site of phosphorylation in HPr (His-15 and/or Ser-46), the presence of other regulatory proteins as catabolite control protein A (CcpA), glycolytic intermediates [fructose-1, 6-biphosphate (FBP) and glucose-6-phosphate], HPr kinase/phosphatase (HprK/P), and a specific conserved DNA sequences called catabolite responsive elements (*cre*) (153). During active transportation of a preferred sugar certain glycolytic intermediates as FBP and glucose-6-phosphate, stimulate the phosphorylation of HPr at a conserved Ser-46 residue by the bifunctional enzyme HprK/P (Fig. 5). The phosphorylated HPr (P~Ser-HPr) form a complex with the CcpA. The CcpA/ P~Ser-HPr complex may bind to the *cre* sequence (WWTGNAARCGNWWWCAWW; W is A or T; R is G or A, and N is any nucleotide). Binding of the CcpA/ P~Ser-HPr complex to the *cre* site mostly represses target genes that are under carbon catabolite repression (CCR). Depending on the location of the *cre,* however, the binding of the CcpA/P~Ser-HPr complex to the *cre* can mediate carbon catabolite activation (CCA) of target genes (154).

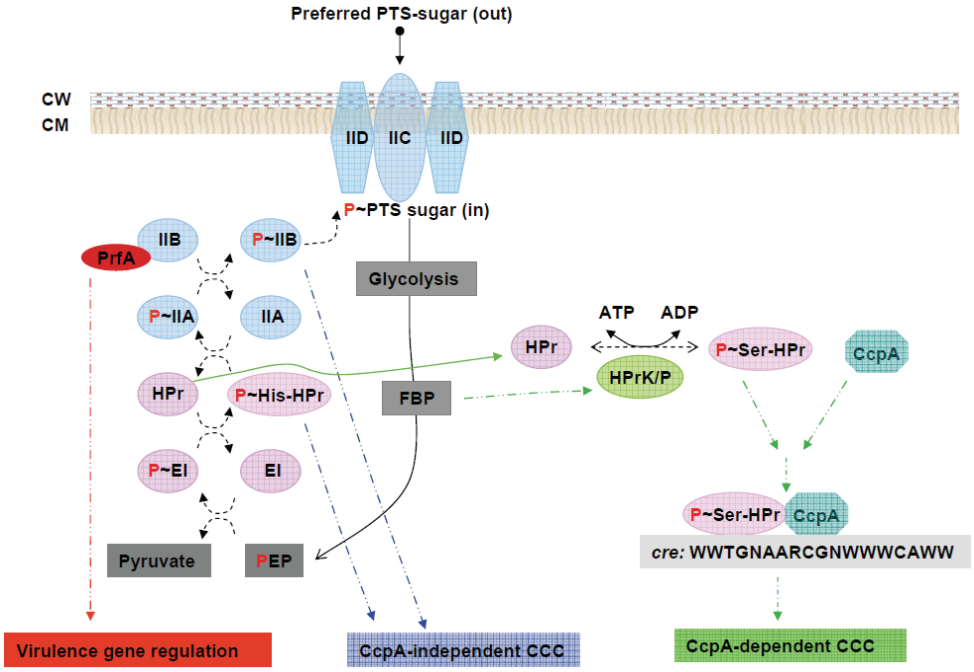


Fig. 5. Schematic presentation of the PTS components during uptake of a preferred PTS sugar and its roles in CCC and virulence regulations (see text). The presentation is based on studies on the role of PTS in Firmicutes (e.g. *B. subtilis* and *L. monocytogenes*) (144, 153-156).

The P~His-HPr and P~EIIBs are also involved in regulation of proteins in CcpA-independent manner (153). This may be achieved (i) by direct binding of P~His-HPr and/or P~EIIB to the target gene; (ii) by phosphorylation of proteins containing PTS Regulation Domain (PRD) and (iii) by phosphorylation of proteins in the glycerol metabolism (153). The overall goal of these CCC systems is to ensure that only enzymes necessary for utilizing the preferred substrate are synthesized as long as the preferred source of carbon and energy are present (154). The EIIs are also believed to be involved in regulation of virulence genes (Fig. 5). During active transportation of a preferred PTS sugar, the EIIs components of the PTS are in a non-phosphorylated state. It is speculated that the non-phosphorylated form of EIIA sequesters the PrfA and inhibits its activity (144, 156). In contrast, non-PTS sugars (e.g. glycerol) reported to induce the expression of *prfA* and *prfA*-dependent virulence genes (156-160).

Growing of *L. monocytogenes* in the presence of limiting glucose and each of other sugars (e.g. mannose or cellobiose) has indicated glucose is the preferred carbohydrate and the functionality of CCC system in this bacterium (161). Transportation of glucose is mainly mediated by the high affinity PTS uptake system (162, 163) and the class IIa bacteriocin pediocin JD appears to inhibit the uptake (164). The genome of *L. monocytogenes* EGDe contains a total of 84 *pts* genes, that can be grouped into seven PTS families depending on their sugar specificity (151, 163, 165). Four loci encoding a complete mannose PTS has been identified, and the mannose-PTS encoded by the *mpt* is reported to be the major permease for glucose and mannose (139, 163, 165) and as well as the receptor for the class II bacteriocins (see above). In most low GC gram-positive bacteria, the PTS in general and the mannose PTS in particular have central regulatory roles in the cell's physiology, including in CCC (153), in virulence [(144) also see below], in biofilm formation (166) and possibly also in global gene regulation (138, 166). In *Escherichia coli*, the mannose-PTS is reported to have also role in resistance to the bacteriophage lambda (167).

2.4.2 Response to acid stress

The capacity to survive acid stress is an important property of *L. monocytogenes* as it determines its ability to survive in the environment (acidic soil and food) and in the host (in human and animal). Constitutive and inducible strategies that consume protons (H^+) and subsequently neutralize the cytoplasm (e.g. glutamate decarboxylase and arginine deiminase systems) as well as efflux of protons from the cell using proton efflux systems (e.g. F_0F_1-ATPase) are primary acid defense mechanisms of *L. monocytogenes* (Fig. 6). The significance of the different acid defense mechanisms may vary depending on the strain, growth phase and the growth conditions (111, 168-171). Recently, Ryan et al. (32) have presented a comprehensive review on the acid stress response of *L. monocytogenes.* The main acid resistance mechanisms are summarized below (Fig. 6).

The Glutamate Decarboxylase (GAD) system

The Glutamate Decarboxylase (GAD) system comprises the glutamate decarboxylase enzymes (encoded by *gadD1*, *gadD2* or *gadD3*) and the membrane associated glutamate/γ-aminobutyrate (GABA) antiporter (encoded by *gadT1* or *gadT2*) (172). The role of the GAD system, as well as the contribution of the three GAD homologues, varies depending on the

strain, growth phase and growth conditions (32, 169, 171, 173). Generally, the GAD system reduces acidification of the cell cytoplasm by consuming protons during decarboxylation of acidic glutamate to the neutral GABA, facilitated by the glutamic acid decarboxylase. The GABA may be then exchanged for another extracellular glutamate by the antiporter (174, 175) (Fig. 6). It is also proposed that the gene *lmo0913* encoding a putative succinate semialdehyde dehydrogenase enzyme, may further metabolize the GABA to succinate (172). It has been shown that in *Lactobacillus,* the decarboxylation reaction generates ATP and this may further contribute to pH homeostasis by the F_0F_1-ATPase system (176). In general, the GAD system is found to be the major mechanism in acid defense (32, 174, 177, 178), is a major component of the acid tolerance response (ATR) (174), and also enhances the tolerance of *L. monocytogenes* to the bacteriocin nisin (179).

The Arginine Deiminase (ADI) system

Recently a study by Ryan et al. (168) characterized the Arginine Deiminase (ADI) system of *L. monocytogenes,* and showed its role in acid defense. The enzymes arginine deiminase (encoded by *arcA*), catabolic ornithine carbamoyltransferase (*arcB*) and carbamate kinase (*arcC*) mediate the conversion of arginine to ornithine, carbon dioxide and ammonia. A membrane-bound antiporter (*arcD*) transports the produced ornithine out of the cell in exchange for a molecule of arginine in an energy-independent manner. The ammonia produced as a result of the system combines with intracellular protons to yield ammonium ions (NH_4^+) and thereby neutralize the low cytoplasmic pH (168) (Fig. 6). In addition, the ADI system generates ATP, which may further contribute to pH homeostasis by the F_0F_1-ATPase (176). The induction of the *arc* genes by acidic pH and by anaerobicity suggests the role of ADI system particularly under these conditions (168).

The F_0F_1-ATPase: an active transporter of protons

The F_0F_1-ATPase is a multisubunit enzyme complex consisting of the cytoplasmic catalytic portion (F_1) and the membrane channel for proton translocation (F_0) (Fig. 6). The role of the catalytic portion is to synthesize ATP when protons move into the cell, or to hydrolyze ATP when protons expel from the cell through the membrane bound channel (170). The F_0F_1-ATPase plays an important role in the regulation of cytoplasmic pH homeostasis in a number

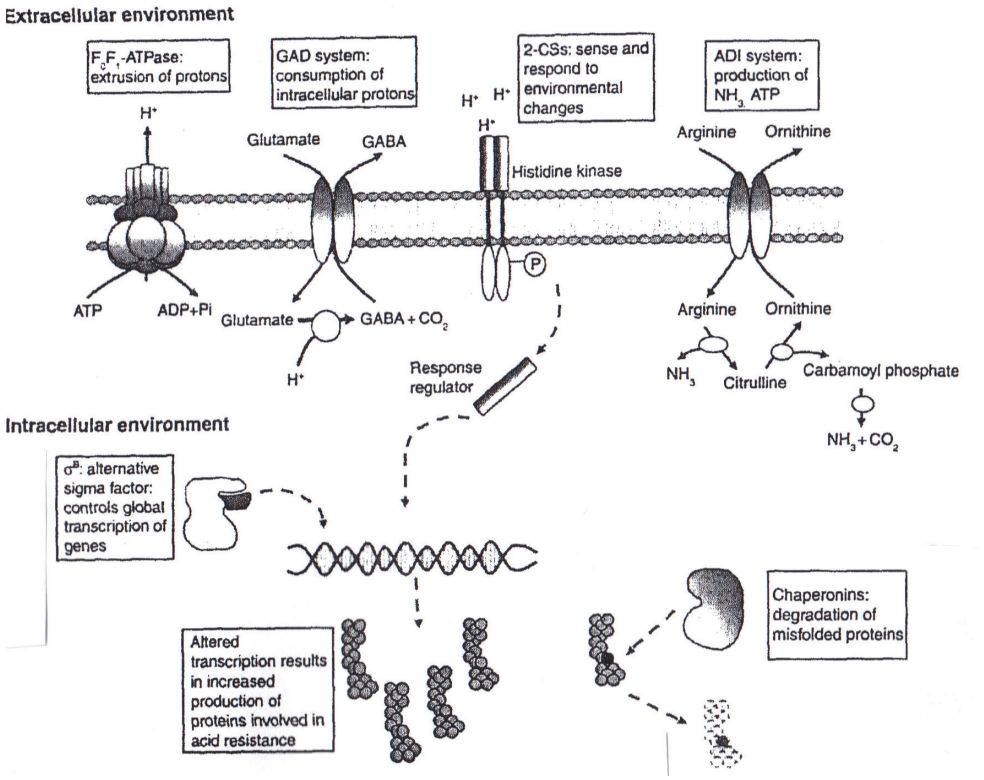


Fig. 6. Some of the known responses of *L. monocytogenes* to low pH stress (see text). The figure is reproduced from Ryan et al. (32) with permission from Elsevier.

of bacteria (180, 181). It has been shown that when *L. monocytogenes* encounter low pH induces rapid net protons influx and consequently decrease in cytoplasmic pH. Depending on the pH and availability of glucose, the bacterium may recover by switching the net protons flow from influx to efflux (111). Attempts to create mutants by deletion of genes encoding the F_0F_1-ATPase in *L. monocytogenes* have not been successful (32, 170), limiting the understanding of the exact role of the F_0F_1-ATPase, particularly in this bacterium. However, the reported induction of the components of the F_0F_1-ATPase as consequences of exposure to acidic pH (169, 182), together with studies reporting increased in sensitivity of cells to acid after treatment with ATPase inhibitor (170), strongly suggest the F_0F_1-ATPase is involved in acid defense. In accordance, a study by Shabala et al. also demonstrated that treatment with ATPase inhibitor resulted in decrease in net extrusion of protons in *L. monocytogenes* (111).

In addition to the F_0F_1-ATPase, other ATPase transporters, such as K^+-ATPase, are known to contribute to pH homeostasis in a number of gram-positive bacteria (180).

Other responses to combat acid stress

Exposure to acid stress may change the cell membrane composition, induce repair and protection systems for macromolecules and may change the track of the common metabolic pathways of target cells (32, 169). It has been shown that *L. monocytogenes* modulate the fatty acid profile of the membrane in response to low pH stress (183, 184), and this may provide additional protection against acidic pH. Several studies have shown that low cytoplasmic pH seems to induce systems aiming to repair acid-induced damage to DNA (185), chaperonins that assist folding of malformed proteins (186) and proteases and peptidases to degrade aberrant proteins (169, 187, 188) (Fig. 6). A recent transcriptome study showed that increase in the transcription of genes encoding the acetoin synthesis enzymes from pyruvate under acidic conditions, suggesting *L. monocytogenes* to alter its metabolic pathway to alleviate acid stress (169). This genome-wide transcriptional study also revealed a number of strain and acid stress dependent responses of *L. monocytogenes* exposed to HCl and the organic acid salt sodium diacetate (169).

Acid tolerance response (ATR) and cross-protection to other stress conditions

Exposure of *L. monocytogenes* to extremely low pH has been shown to select spontaneous mutants that are constitutively resistant to this condition (31, 33). A transient acid tolerance response (ATR) upon brief exposure of *L. monocytogenes* to mild low pH has also been reported (33, 189, 190). ATR is reported to protect the bacterium against extremely low pH, and can in addition induce cross-protection to other food related environmental stresses including bacteriocins (27, 33, 184, 189-193). Importantly, ATR is shown to influence the virulence capacity of *L. monocytogenes*, although there is some disagreement regarding its contribution [(194-197), see below]. The ATR is mainly meditated by *de novo* expression of a number of proteins (33) and the contribution of GAD system (174), F_0F_1-ATPase system (111, 170) and the general stress regulator σ^B (198) in ATR, has been documented.

Gene regulation under stress conditions: emphasis on acid stress

The *L. monocytogenes* genome possesses one of the highest number (>7%) of predicted transcriptional regulators reported (165). In addition, the bacterium also uses noncoding RNAs as a regulatory system (64, 199, 200). These regulatory components are crucial to sense and respond to a rapidly changing environment through modulation of gene transcription. Four alternative sigma factors (σ^B, σ^{54}, σ^C and σ^H) associated with stress response have been identified in *L. monocytogenes* [reviewed in (201)]. A more recent and comprehensive study (202) has compared the regulons of seven key regulatory proteins of *L. monocytogenes,* including the four alternative sigma factors. The study showed cross-connection between the transcriptional regulators and overlapping in regulons, suggesting certain level of compensatory and synergistic roles among the transcription regulators (202).

In addition to mediate resistance to class IIa bacteriocins (140-142), the σ^{54} has been linked to osmotic and low temperature stresses (203-205). The induction of the *sigL* by lactic acid stress, as well as, the reported impaired growth of *sigL* null mutant during growth in the presence of this acid, particular at low temperature has suggested a possible role of σ^{54} in acid stress (205). The *sigH* (encoding the σ^H) was reported to be induced by acidic pH (182) and it has been shown that the growth of a *sigH* deletion mutant was impaired in minimal media as well as under alkaline stress (206). A more recent study, however, has reported contrasting result on possible role of σ^{54} and σ^H in acid stress (202). According the study, the survival of both *sigL* and *sigH* null mutants under acidic stress appears to be higher than that of the parental strain (202). The σ^C (encoded by *sigC*) is reported to be important transcriptional regulator during heat stress and cold adaptation (207, 208).

The σ^B (encoded by *sigB*) has a central part in the general stress regulation network [portrayed in (209) and (202)] and is by far the most investigated sigma factor in *L. monocytogenes* in connection with a variety stress responses, including low pH (195, 210-218). According to a recent whole-genome transcriptional study, more than 400 genes are reported to be under the regulation of σ^B (215). The knowledge about the σ^B in acid stress response is mostly derived from the reduced survival of a *sigB* null mutant compared to the parent wild-type under acidic stress growth conditions (202, 212, 215, 216, 219, 220). A study by Heavin et al. (171), however, showed no significant difference in the growth rate between *sigB* null mutant and its isogenic wild-type in the presence of weak acids. The σ^B dependent expression of genes

encoding the main defense systems against low pH (the GAD and ADI systems) has been observed (168, 212, 216) (Fig. 6). Because the σ^B activity is regulated primarily at post-transcriptional level (218), the transcript level of *sigB* may not reflect its actual activity. In summary, a fully functional σ^B is important in activating a number of genes important for survival during stress, but is dependent on strain, stress type and growth condition.

In addition to the sigma factors, *L. monocytogenes* utilizes two-component signal transduction systems (2-CSs) to modulate gene expression in response to environmental stimulus (Fig. 6). Of the 15 histidine kinases and 16 response regulators identified in *L. monocytogenes* (221), LisRK has shown to play a major role in numerous stress conditions (208, 222-224). Mutants lacking the *lisRK* genes displayed higher resistance to low pH than the wild-type after long time exposure to low pH at stationary phase (222). However, the *lisRK* null mutant strains were more sensitive to low pH at logarithmic phase than the parental strain (222).

2.4.3 Effect of class IIa bacteriocins and low pH stresses on virulence

It has been suggested that stress of different types are factor that may influence virulence of *L. monocytogenes* (58). Relatively little is known about the effect of stress imposed by class IIa bacteriocins on the virulence potential of *L. monocytogenes* (29, 225). In one study, a targeted microarray was used to investigate the expression of 13 selected virulence genes in two class IIa bacteriocin-resistant strains (29). According to the study, five of the virulence genes were significantly down-regulated in one of the resistant strains. For the other resistant strain, the authors reported a non-significant induction of the tested virulence genes (29). In another study, exposure to class IIa bacteriocin did not appear to affect the haemolytic activity of *L. monocytogenes* (225). As outlined above, the mannose-PTS (encoded by the *mpt*) plays a central role in resistance to class IIa bacteriocins, and also regulates the expression of virulence genes. Deletion of the *mpt* induces the *prfA* controlled virulence genes but did not affect the replication of the mutant strains within the mammalian host cells (144, 163). In general, the regulation of virulence genes by the PTS is complex and largely dependent on the energy source and the phosphorylation status of the PTS components [(156, 157, 159) also see above].

Low pH is a known signal to foodborne pathogens to induce the expression of genes important for survival and adaptation in the mammalian host (32). Several studies have shown

that pre-exposure of *L. monocytogenes* to low pH may induce the expression of virulence genes (169, 195-197). Evidences from *in vivo* and *in vitro* studies also indicate that exposure to acid may aggravate the virulence potential of *L. monocytogenes* (31, 33, 194, 226). However, it is useful to note that exposure to such stress not always lead to induction in virulence potential (169, 196, 197, 227). The conflicting observations might be attributed to strain and methodological differences.

2.5 Methodological aspects of the present stress response study

Selection of *L. monocytogenes* strains for the present work was based on information from previous studies (92, 228). It has been shown that wild-type strains of *L. monocytogenes* form two distinct groups according to their natural susceptibility to sakacin P. This grouping is consistent with grouping based on other biochemical and genetic profiles of the strains (92, 228). Simultaneous analysis of all the data together using multiblock principal component analysis has allowed more distinct separation of the wild-type strains than when analysing of each profile individually (229). However, the exact biochemical and genetic basis behind this grouping of *L. monocytogenes* is not yet fully understood. In the present work, two wild-type strains with high natural susceptibility to sakacin P and two strains with low natural susceptibility to sakacin P, as well as the laboratory reference strain EGDe, were included.

Assessment of the physiological and morphological status of bacterial cells before and after being subjected to stress is common approaches in the study of stress responses. Application of both general microbiological methods (e.g. growth study) and the modern techniques (e.g. transcriptome study) offer more holistic approach to understand how bacteria respond to stresses. In the present study, the responses of *L. monocytogenes* to the class IIa bacteriocin sakacin P and to low pH stresses were investigated using different microbiological methods. Fig. 7 depicts the experimental design used. The general microbiological methods applied in the present work have been described thoroughly elsewhere, so in this introduction, only brief description of Fourier transform infrared spectroscopy, transcript quantification and the basics of principal components analysis are presented.

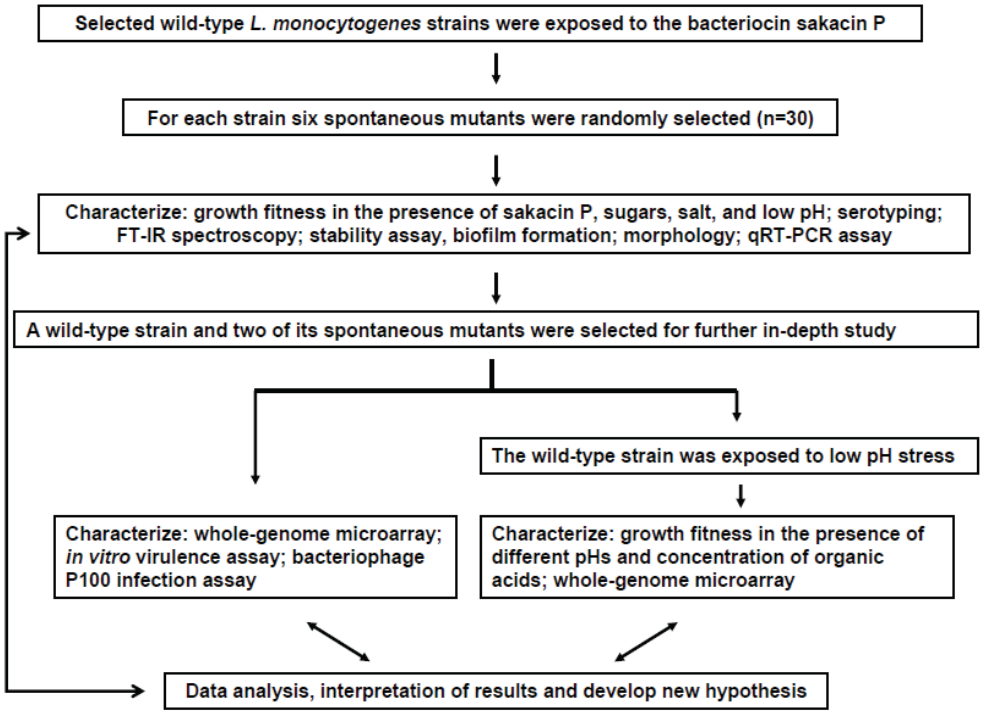


Fig. 7. Schematic presentation of the experimental design used to study the response of *L. monocytogenes* to sakacin P and low pH.

2.5.1 Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy has been widely used in microbiology for a variety of applications. This includes studying the effect of stress on bacterial cells (230-233) and for identification and differentiation of microorganisms (228, 234-236). FT-IR spectroscopy records infrared spectra originating from molecular vibrations of all functional groups found in the cell at the atomic level. The spectrum as a whole represents a fingerprint of absorption peaks which related to both phenotypic as well as genotypic information about the cell. Infrared spectra of bacteria can be divided into five spectral regions, which roughly characterize the fatty acid, protein and polysaccharide content of intact cells (Fig. 8) (237). In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present in the cell. Normalization of the data is essential to eliminate systematic variations (238) and the data can be analyzed and presented using different approaches such as by

principal components analysis (see below). The speed and the high-throughput quality of FT-IR spectroscopy make this method particularly suitable for taxonomic purposes.

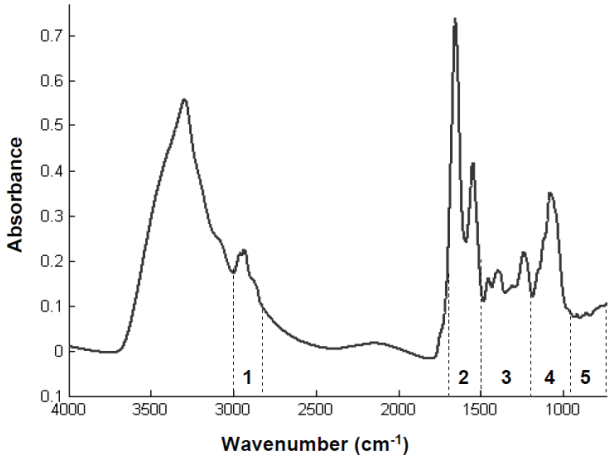


Fig. 8. A typical bacterial FT-IR spectroscopy spectrum. The various spectral regions marked with numbers are (1) 3,100-2,800 cm^{-1}, fatty acid region; (2) 1,800-1,500 cm^{-1}, protein region; (3) 1,500-1,200 cm^{-1}, mixed region of fatty acid and protein; (4) 1,200-900 cm^{-1}, polysaccharide region; and (5) 900-700 cm^{-1}, fingerprint region. Assignment of bands are according to Naumann (237).

2.5.2 Transcriptional analysis

Several methods are available to study bacterial transcript levels. DNA microarray and quantitative real-time reverse transcriptase PCR have been used widely to study transcripts of bacteria under different conditions, including in stress response studies (169, 196).

Quantitative real-time reverse transcriptase PCR

In a standard quantitative real-time reverse transcriptase PCR (qRT-PCR) technique, the mRNA is copied to cDNA (complementary DNA) by the enzyme reverse transcriptase. The copied cDNA serve as a template for quantitative real–time PCR. Detection of the cDNA can be achieved using a set of specific primers and a dual-labelled reporter (e.g. TaqMan probe). The TaqMan probe consists of a fluorophore attached to the 5'-end and a quencher at the 3'-

end. The probes are designed to anneal within the target cDNA region amplified by the specific set of primers. The purpose of the quencher is to inhibit fluorescence signals while the fluorophore and the quencher are in proximity. However, as the amplification of the target cDNA proceed degradation of the probe releases the fluorophore. The amount of fluorescence detected reflects the quantity of starting cDNA present (239). The quantification is based on threshold cycle (C_T), which is the number of PCR cycles at which the fluorescence signal of the reporter dye cross a determined threshold value. The lower C_T value reflects the higher amount of target cDNA. The C_T value is normalized using a housekeeping gene and the amount of mRNA can be compared among the samples of interest (240).

The qRT-PCR is sensitive, (semi)-quantitative, and is relatively (semi-) high throughput technique for transcript quantification (239, 240). In addition, qRT-PCR is extensively used to validate microarray transcript quantification results (241), as in the present work.

DNA microarray

Transcriptomics and genetic variation analysis are the two major applications of DNA microarrays. In standard DNA microarray transcriptomics, reporters (e.g. PCR products corresponding to genes in the whole-genome) are attached to a solid surface (e.g. glass slides) in a high density fashion (242). In "two-color" technology, the mRNA samples are copied to cDNA by the enzyme reverse transcriptase and labelled with fluorophores usually with Cy3 and Cy5 (e.g. mutant versus wild-type). The fluorophore labelled samples are mixed and co-hybridized to the microarray slide. Unbound and non-specifically bound fluorophores are removed by washing and the hybridized microarray is excited by a laser and scanned at suitable wavelengths (e.g. for the detection of the red and green channels). The amount of fluorescence emitted upon laser excitation corresponds to the amount of nucleic acid bound to each spot. The composite images from scanned arrays have different colors (e.g. red, green, yellow or black color) depending on the relative amount of each sample at a given spot. The images must be analyzed to identify the spot, and to quantify the amount of fluorescence intensity in each channel. Normalization of the data is essential for proper comparison of samples, and the data has to be filtered and analyzed (242, 243).

Generally, the DNA microarray transcriptomics approach has been a powerful tool to understand the transcriptional response of bacteria at the whole genome scale (244). An

advanced application of this technology recently uncovered the complete operon map of *L. monocytogenes* (64). The increasing knowledge regarding the function of genes and pathways, as well as development of better tools to analyze the huge amount of data are making the microarray technology more robust than before (64, 245, 246). It is important to acknowledge, however, that the technology is primarily a screening tool, and do not measure posttranscriptional events. Nevertheless, a high correlation between the gene transactional profile and proteome and enzyme levels are often reported (244).

2.5.3 Data analysis

In the present work different statistical analyses and presentations of data were used. Since Principal Components Analysis (PCA) was applied in all three papers (Paper I-III), a brief account on PCA is presented below. Analysis of multivariate data that are highly correlated to each other and again consisting of a limited number of observations require good systems for presenting them in simple visual ways. PCA is an appropriate method to analyze such complex data. PCA has been used to analyze data from FT-IR spectroscopy and DNA microarray transcriptome studies (243, 247, 248). It represents an unsupervised data analysis to summarize multidimensional data sets into new and few set of variables called Principal Components (PCs). The first PC (PC1) explains the main variation in the data set followed by PC2 and so on. The PCs are uncorrelated to each other and are presented as score plot to illustrate how the samples are grouped. Loading plots are parts of PCA presentation and shows how the variables are related to the score plot (249).

3. OBJECTIVES OF THE STUDY

The main goal of this study was to explore the responses of *L. monocytogenes* to antimicrobial products from lactic acid bacteria, particularly to the bacteriocin sakacin P and acids. The specific tasks were:

- develop spontaneous sakacin P-resistant mutant strains and perform mutant characterization using different approaches.

- further in-depth study on representative spontaneous mutant strains to elucidate the sakacin P resistance mechanisms and the global transcriptional responses of *L. monocytogenes* exposed to sakacin P.

- explore the growth and global transcriptional responses of *L. monocytogenes* to hydrochloric acid, acetic acid and lactic acid stress.

- compare the global transcriptional profile of sakacin P-resistant strains and acid stress response to identify common genes important under these conditions.

4. MAIN RESULTS AND DISCUSSION

The foodborne pathogen *L. monocytogenes* is responsible for severe and fatal illness and for significant economical losses (4, 8-10, 67). Ingestion of food contaminated with *L. monocytogenes* is the common mode of infection (3). LAB and their antimicrobial products have the potential to minimize contamination, to control the growth and eventually eliminate this bacterium from food and food related environment (17-23). *L. monocytogenes*, however, has an extraordinary ability to counteract adverse conditions including stresses due to bacteriocins and low pHs (27-33). Elucidation of how *L. monocytogenes* respond to antimicrobial products from LAB is a crucial step to devise a knowledge-based strategy to control this bacterium in food and food-related environments.

The results and discussion presented in this chapter is based on the list of papers stated by their Roman numerals (Paper I-Paper III). The work presented in Paper I and Paper II is a continuation of previous studies (92, 228). In Paper I, analysis of several spontaneous mutant strains of *L. monocytogenes* originated after exposure to sakacin P revealed clues regarding different phenotypic responses of the sakacin P-resistant mutant strains. In order to obtain deep insight into the responses of the sakacin P-resistant strains, further in-depth analysis on representative wild-type and mutant strains were conducted (Paper I and Paper II). Paper III explored the response of *L. monocytogenes* to low pH and acidulants commonly encountered by this bacterium with emphasis on lactic and acetic acid stress, using a representative wild-type strain studied in Paper II. The transcriptome results (Paper II and Paper III) were compared to identify common responses.

The class IIa group bacteriocins have potential to be used as antilisterial agents, however, development of resistance to these bacteriocins is a great concern (28-30, 77, 85, 92, 126, 128-131). To elucidate the event(s) leading to resistance to the class IIa bacteriocins, 30 spontaneous mutant strains of *L. monocytogenes* that acquired resistance after exposure to sakacin P, were characterized using phenotypic and transcriptomic approaches (Paper I and Paper II). For the transcriptome analysis, representative sakacin P-resistant strains were compared with the parent wild-type during growth on mannose or cellobiose (Paper II).

Based on the IC_{50} (50% inhibitory concentration) of sakacin P, the 30 spontaneous resistant strains could be grouped into strains with high levels of resistance (IC_{50}, $\geq$10 μg ml^{-1}) and

strains with low levels of resistance (IC_{50}, <10 µg ml^{-1}). Resistant strains belonging to the same IC_{50} group were shown to have a certain level of similar physiological and transcriptional characteristics (Paper I and Paper II). Nevertheless, the results also indicated diversity among strains belonging to the same groups. The results from the in-depth study on representative resistant strains derived from *L. monocytogenes* L40 and *L. monocytogenes* L502 are summarized in Table 1. In the study presented in Paper I, FT-IR spectroscopy analysis was applied to characterize the *L. monocytogenes* strains. The findings from FT-IR spectroscopy revealed differences between wild-type and resistant strains, albeit in a strain type dependent manner (Paper I). Despite the differences identified in Table 1, the resistant strains had indistinguishable morphology under scanning electron microscopy (Paper I and Fig. 9). The absence of morphological differences using scanning electron microscopy between sensitive and class IIa bacteriocin-resistant cells was in accordance with the results of Duffes et al. (128). However, it has been reported that, under transmission electron microscopy, the class IIa bacteriocin divergicin M35-resistant strain showed rounder and rougher outer layers than the parental wild-type strain (250).

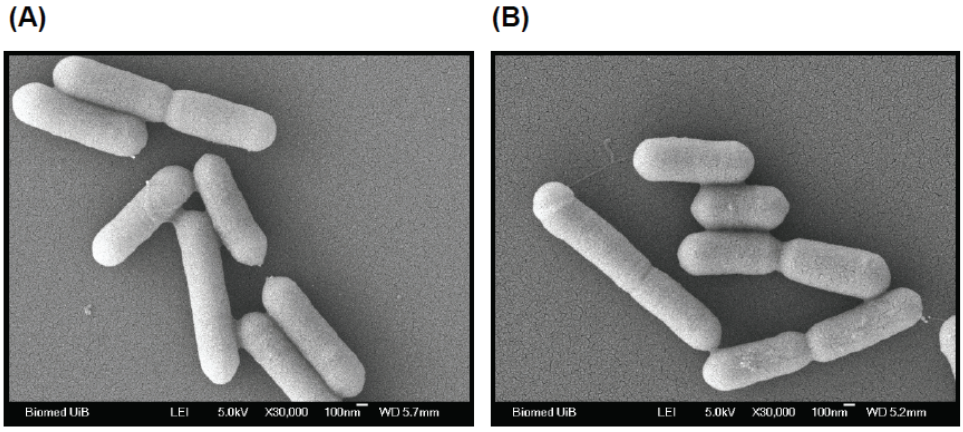


Fig. 9. Scanning electron microscopy pictures of (A) L502-1 and (B) L502-6 at exponential growth phase. The images were taken at the Molecular Imaging Center of the University of Bergen, Norway.

Interestingly, the stability of the acquired resistance during growth in the absence of bacteriocin was found to be variable (Paper I and Paper II). Previous studies also reported differences in the stability of the acquired resistance (28, 128-130, 135, 136). In the present

Table 1. The phenotypic and transcriptional profiles of representative high level sakacin P-resistant strains (L502-1 and L40-1) and low level sakacin P-resistant strains (L502-6 and L40-6) relative to their respective wild-type strains (L502 and L40) (Paper I and Paper II).

Parameters[a]	L502-1	L502-6	L40-1	L40-6
IC_{50} for sakacin P	high ($>10^6$)	low (10^3)	high ($>10^5$)	low (10^3)
Stability of the acquired resistance to sakacin P	unstable	stable	stable	unstable
Growth rate on mannose	82%	44%	73%	50%
Growth rate on glucose	94%	70%	87%	56%
Growth rate on lactose	112%	46%	42%	39%
Growth rate on cellobiose	107%	105%	108%	93%
Biofilm forming ability	L502>L502-1>L502-6		L40>L40-1=L40-6	
Food-related stress tolerance (salt, low temperature & low pH)	L502>L502-1>L502-6		L40>L40-1>L40-6	
Virulence potential	L502≥L502-1>L502-6		ND	
Growth in the presence of activated charcoal	L502=L502-1>L502-6		ND	
Growth in the presence of phage P100 at low MOI	L502=L502-1<L502-6		ND	
FT-IR spectroscopy profile	similar to L502	different from L502 and L502-1	different from L40 at fatty acid and polysaccharides regions	different from L40 and other L40 derivatives at fatty acid and polysaccharides regions
The bacteriocin receptor (*mpt* operon)	repressed	induced	repressed	induced
Total number of genes affected on mannose	87 up and 37 down	152 up and 42 down	ND	ND
Total number of genes affected on cellobiose	20 up and 19 down	30 up and 6 down	ND	ND
Serotype	no change	no change	no change	no change
Scanning electron microscopy	no change	no change	no change	no change

[a] comparisons are relative to the wild-type strains (mutant/wild-type); ND, not determined

work, for the resistant strains derived from *L. monocytogenes* L40 and EGDe, the high level resistant strains showed a more stable phenotype than the low level resistant strains (Paper I). However, the stability in the high level sakacin P-resistant strain derived from *L. monocytogenes* L502 was lower than that of the low level resistant strain (Paper II). Together, the results suggest that in addition to the level of resistance to sakacin P, other factors may influence the stability of the acquired resistance.

In regard to elucidation of the phenotypic resistance response, the expression of the bacteriocin receptor *mpt*A representing the *mpt* operon was monitored using qRT-PCR assay (Paper I and Paper II). As shown in Fig. 10, for the wild-type strains (L502 and L40), the expression of the *mpt* was induced by the presence of glucose and mannose but not by cellobiose, confirming previous reports (102, 139, 163). The *mpt* in the high level resistant strains (L502-1 and L40-1) were down-regulated, strongly suggesting that abolition of the bacteriocin receptor could be the main mechanism behind resistance in these strains (Fig. 10). This in accordance with the well established association between the presence/absence of the mannose-PTS and susceptibility/resistance to class IIa bacteriocins (28, 29, 95-97, 132, 134, 135, 138, 139, 142).

Intriguingly, the level of the bacteriocin receptor transcript (*mpt*) was significantly induced in the low level resistant strains (L502-6 and L40-6) except for strain L502-6 grown on cellobiose (Paper I and Paper II, see Fig. 10). A previous study also reported an induction of the bacteriocin receptor in a carnobacteriocin B2-resistant strain both at gene and protein levels (29). The PTS encoded by *mpt* is necessary for growth on mannose (163) and acts as a receptor for sakacin P (96, 97). Despite the induction of the *mpt*, the two low level resistant strains studied in the present work showed 1,000 times higher resistance than their respective wild-type strains. In addition, the growth of these resistant strains was reduced particularly on mannose (Table 1). Hence, this may indicate that the mannose-PTS may not be fully functional in the low level resistant strains. As outlined earlier, class II bacteriocins act on the major glucose uptake systems of target cells and inhibit the uptake of nutrients like glucose and amino acids (101, 162-164). Interestingly, this may symbolize systematic fighting of LAB against nutrient competing bacteria, including *L. monocytogenes*.

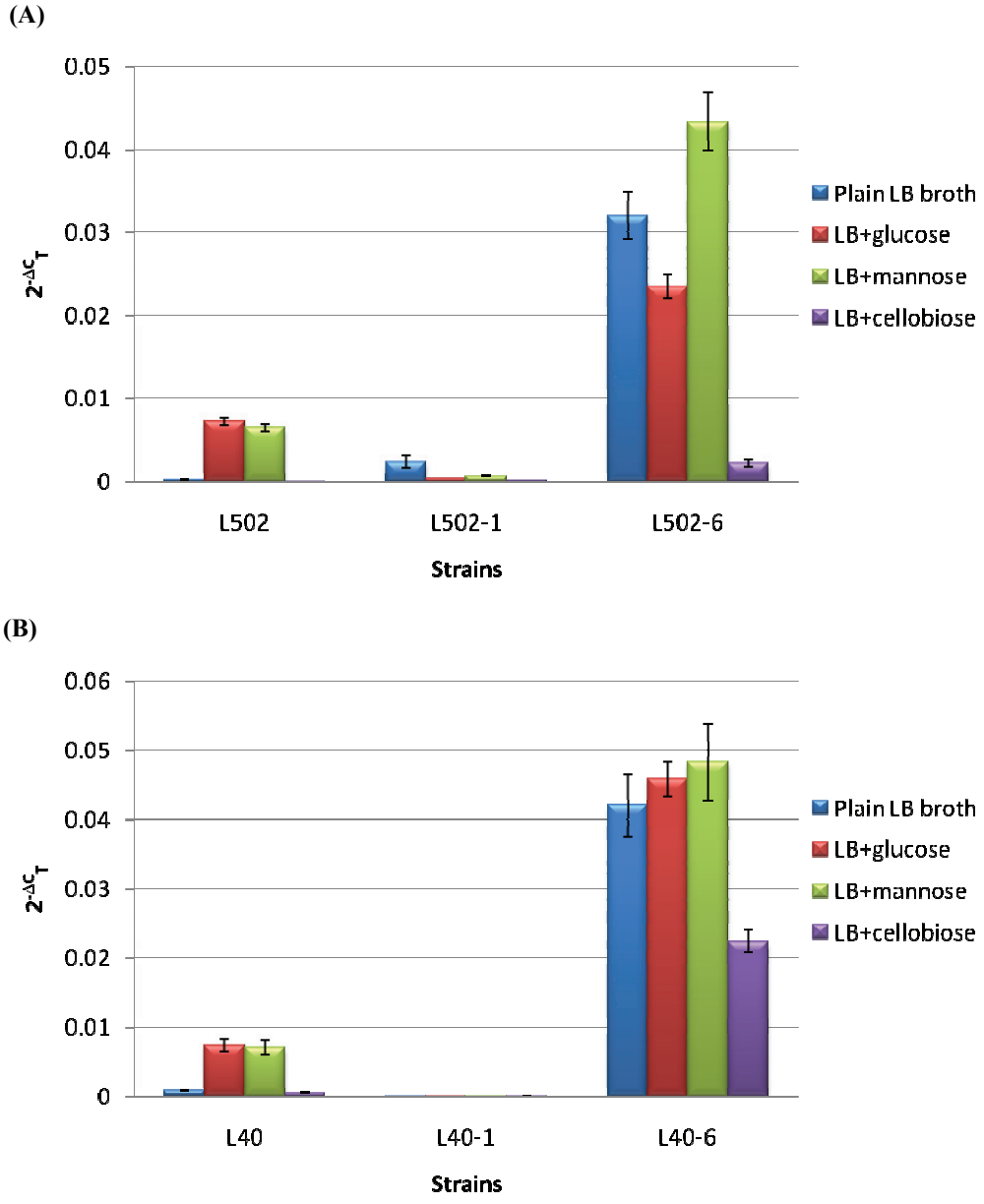


Fig. 10. The transcript level of the *mptA* gene representing the *mptACD* operon in (A) *L. monocytogenes* L502 and its sakacin P-resistant strains L502-1 and L502-6 and (B) *L. monocytogenes* L40 and its sakacin P-resistant strains L40-1 and L40-6. The strains were grown in plain Luria-Bertani broth or supplemented with glucose, mannose or cellobiose. Error bars represent standard error of the mean. The qRT-PCR data analysis was done as described by Schmittgen and Livak (240).

Our transcriptomic studies confirmed independently the results on *mpt* regulation obtained by the qRT-PCR assay (Paper II). In addition to the mannose-PTS, genes encoding proteins involved in cell envelope modifications and genes in the Twin-Arginine Translocase (TAT) system were induced particularly in L502-6 (Paper II). The link between modification of the cell envelope and resistance to class IIa bacteriocins has been shown before [Paper I and (132, 133, 148)]. The importance of the TAT system in antimicrobial resistance has been demonstrated in *Campylobacter jejuni* and *Mycobacterium smegmatis* strains (251, 252). Thus, the induced genes encoding proteins involved in cell envelope modifications and genes in the TAT system are of particular interest in connection with their possible role in resistance to class II bacteriocins.

Conceivably, most of the transcriptome changes in the sakacin P-resistant strains could be related to regulatory role of the mannose-PTS. This is largely supported by transcriptome evidences from other gram-positive bacterial strains lacking the mannose-PTS (138, 166). These studies clearly indicate the central role of mannose-PTS in regulating its own expression and the expression of other genes involved in a variety of cellular functions at global level [Paper II and references (138, 166)].

Taking into account all the results (Paper I and Paper II), the different resistant strains derived from a single strain after a single time exposure to the bacteriocin, shows substantial phenotypic and transcriptomic differences. This highlight care has to be taken before generalizations are made based on studies including a few strains and mutants.

Foodborne human pathogens such as *L. monocytogenes* frequently encounter organic acids produced from LAB in fermented food and during acidification of food products and food environment by addition of organic acids. Spontaneous mutant strains that are constitutively resistant to low pH and strains with ATR, are a challenge for the food industry (31, 33, 189, 190). The knowledge about the response of gram-positive foodborne pathogens to acetic and lactic acid stress has largely been based on studies in other bacteria than *L. monocytogenes* (253, 254). In Paper III *L. monocytogenes* L502 (the strain studied in Paper I and II) was exposed to HCl, acetic acid and lactic acid stress. The growth and transcriptome of the acid stressed cells were compared with that of cells without any acid treatment. Prior to the main experiment, a series of growth studies were performed to characterize growth conditions in the presence of the three acids. The growth of *L. monocytogenes* L502 was reduced, but not

completely inhibited at pH 5 when acidification was done by addition of HCl, 10 mM acetic acid or 20 mM lactic acid. Acetic acid had the highest antilisterial activity followed by lactic acid and HCl (Paper III), confirming previous report (23).

The transcriptomics were performed after the cells were allowed to adapt to the acidic environment (OD_{600}~1.2). This was done to ensure that the cells were in similar physiological state under the growth conditions (Paper III). Common as well as unique genes were differentially regulated in the presence of the three acidulants. Many more genes were affected by the organic acids than by HCl, indicating possible additional toxicity of the organic acids other than low pH effect (Fig. 4). Studies on other related gram-positive foodborne pathogens show similar overall acidulant dependent transcriptional responses (253, 254).

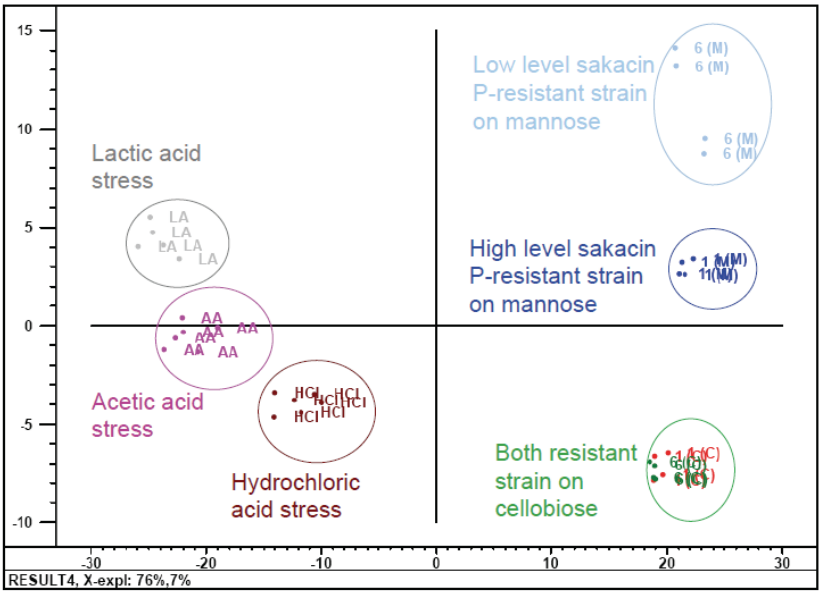
The strain responded to the acid stress by inducing confirmed and putative genes important for low pH defense (Paper III). Genes in the GAD system showed the highest induction, indicating that this is the main system in alleviating the low pH stress under the growth condition. In *L. monocytogenes,* the importance of the GAD system in acid defense has been shown using models (174, 177, 178). In addition to the GAD system, a number of genes associated with modifications of the cell envelope, with protection and repair of macromolecules, with intracellular accumulation of histidine and osmolytes as well as with genes encoding general stress proteins were induced in the acid stressed cells (Paper III). The knowledge about the importance of the above mentioned responses in acid defense and adaptation was largely based on studies on other bacterial species and their role in *L. monocytogenes* need to be confirmed (Paper III).

The acid stress also induced genes encoding proteins involved in other type of stress conditions, such as heavy metal toxicity, osmotic stress, oxidative stress and UV light stress. This is in accordance with the ATR behavior of *L. monocytogenes* in inducing protection to other types of stresses upon exposure to mild acidic condition (33, 184, 189-191, 193). The acid stress also increased the transcription of genes associated with virulence (Paper III).

Although the methodological differences influence direct comparisons, the bacteriocin resistance transcriptome (Paper II) and the acid stress response transcriptome (Paper III) were analyzed together. This was done to investigate the presences of universal stress response

important for the bacteriocin and the acids stress. As seen from the PCA score plot (Fig. 11 A), the global acid stress transcriptome is apparently different from the transcriptome of the bacteriocin-resistant strains.

(A)



(B)

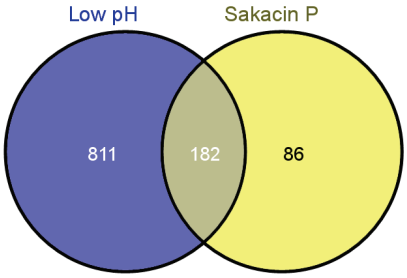


Fig. 11. Genes differentially expressed in any of the acid stressed and the sakacin P-resistant strains **(A)** PCA score plot and all the dye-swap and technical replicates from the two biological cultures were included in the PCA. The explained variances for PC1 and PC2 are 76% and 7% respectively. **(B)** Venn diagram showing the total number of genes differentially regulated in any of the transcriptomics (Paper II and Paper III).

A total of 268 genes in any of the bacteriocin-resistant transcriptomes (four conditions) and 993 genes in any of the acid stress response transcriptomes (three conditions) were differentially regulated. The Venn diagram shows 182 overlapping genes between the two transcriptomes (Fig. 11B). None of the overlapping genes were either up- or down-regulated in all growth conditions tested. Comparison of the organic acid stress response with the transcriptional response of the sakacin P-resistant mutant strains during growth on mannose showed a total of 49 core genes differentially regulated under these four conditions (not shown). Of these, an operon encoding a putative amino acid ATP-binding cassette (ABC) transporter (*lmo2250*-*lmo2252*) and a gene encoding unknown protein (*lmo0573*) were down-regulated in all the cases. In contrast, no single gene was induced under all the four conditions. The different experimental conditions employed in the two studies, as well as the disparate responses of the strain to the stresses may attribute to the disparity.

One of the key findings of the present work is the different responses of *L. monocytogenes* L502 to stresses from the sakacin P and the acids (Paper I-Paper III). One example of such difference is illustrated by regulation of genes important for acid stress defence and genes involved in virulence. While the sakacin P-resistant mutant strains showed repression of genes important for acid defence (e.g. GAD system) and other virulence related genes (Paper II), the acid stress appeared to induce these genes (Paper III). Overall, the results presented in this thesis showed strain-, growth condition-, and stress type-dependent responses of *L. monocytogenes* to the bacteriocin sakacin P and to low pH stresses.

5. MAIN CONTRIBUTION AND FUTURE WORK

The present work has provided new insight into how the foodborne pathogen *L. monocytogenes* respond and adapt to sakacin P and to low pH stresses. We have shown substantial diversity in phenotypic and transcriptomic characteristics of spontaneous mutant strains of *L. monocytogenes* obtained after a single exposure to sakacin P. In this work we have also demonstrated FT-IR spectroscopy as potential tool to study sakacin P-resistant strains and the macromolecular changes associated with this resistance. The transcriptome analyses have rendered clues on how the bacterium respond to sakacin P and low pH stresses at genome-wide level. The transcriptome results also identified putative genes that might have a role in the examined stress responses.

The present work has disclosed several new avenues of investigation for future studies. For example, some of the sakacin P-resistant strains showed induction of the bacteriocin receptor genes (*mpt*). Further characterization of the resistant strains would provide more knowledge about the resistance mechanism to class IIa bacteriocins. In order to take full advantage of the FT-IR spectroscopy technique, bands with unknown specificity should be designated. Furthermore, the discriminatory power of the absorption bands should be improved to provide information about single specific compounds. Complementing the transcriptome data by gene functional studies (e.g. knock-out/knock-down), proteomics, metabolomics and DNA sequencing (especially for the sakacin P-resistant mutant strains) could provide a more precise picture of the response of *L. monocytogenes* to the stress conditions. It would also be interesting to study the virulence capacity of the bacteriocin-resistant and the acid adapted *L. monocytogenes* strains using animal models.

An intriguing and more practically relevant study would be to investigate the interaction between *L. monocytogenes* and LAB in co-culture studies. Initially this can be done in simple membrane separated co-culture device and eventually in more complex food system containing other computing normal flora bacteria. Comparison of more representative *L. monocytogenes* strains and characterizing them under optimal growth conditions will be useful to be able to make more generalized conclusions.

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Paper I

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Complex Phenotypic and Genotypic Responses of *Listeria monocytogenes* Strains Exposed to the Class IIa Bacteriocin Sakacin P▽†

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Sakacin P is a class IIa bacteriocin that is active against the food-borne pathogen *Listeria monocytogenes*, and use of this compound as a biopreservative in foods has been suggested. In the present study, we characterized 30 spontaneous sakacin P-resistant mutants of *L. monocytogenes* obtained after single exposure to sakacin P. The frequency of development of sakacin P resistance for all strains was in the range from 10^{-8} to 10^{-9}. Using the 50% inhibitory concentration (IC_{50}) of sakacin P, the strains could be grouped into strains with high levels of resistance (IC_{50}, $\geq 10^4$ ng ml^{-1}) and strains with low levels of resistance (IC_{50}, $< 10^4$ ng ml^{-1}). Resistant strains belonging to the same IC_{50} group also had similar physiological and genetic characteristics. Generally, the resistant strains showed substantial variations in many parameters, such as differences in the stability of the acquired resistance to sakacin P, growth fitness, food-related stress tolerance, and biofilm-forming ability. Fourier transform infrared spectroscopy revealed differences between wild-type and resistant strains in polysaccharide, fatty acid, and, protein regions. A mannose-specific phosphotransferase (PTS) operon has been described for class IIa bacteriocin resistance, and the sakacin P-resistant strains displayed both up- and downregulation of the expression of the *mptA* gene encoding the PTS system. This is the first comprehensive study of the diversity of a large number of spontaneous resistant mutants obtained after one exposure to a class IIa bacteriocin, particularly to sakacin P. The great diversity among the resistant strains exposed to the same stress conditions suggests that there are different resistance mechanisms.

Listeria monocytogenes is a gram-positive human and animal pathogen, and nearly all human *L. monocytogenes* infections are due to ingestion of contaminated food (29, 30, 47). Listeriosis is a severe disease and has high hospitalization and fatality rates (30). *L. monocytogenes* is ubiquitous in nature (49), and it is relatively resistant to harsh environments, as well as to a broad range of food processing and storage conditions (16).

Bacteriocins produced by lactic acid bacteria are antimicrobial peptides and are usually active against gram-positive species with low G+C contents (5). The bacteriocins can be grouped into different classes (12, 26), and the class IIa bacteriocins are characterized by conserved sequence motifs and high specific activity against *L. monocytogenes* (9, 13). So far, nisin (class Ia) is the only bacteriocin approved for use as a food preservative (E234). If more bacteriocins are to be approved for various industrial applications, class IIa bacteriocins are considered the next in line (15). Sakacin P is a class IIa bacteriocin produced by several strains of *Lactobacillus sakei* (22, 26, 42, 43). Sakacin P is active against *L. monocytogenes* (23–25), and unlike other class IIa bacteriocins, it has modest activity against lactic acid bacteria (13).

The presence of isolates that are resistant to bacteriocins is of great concern for use of bacteriocins as biopreservatives (48). *L. monocytogenes* isolates that are naturally resistant to class IIa bacteriocins have been reported (14, 37). Susceptible strains can acquire resistance to class IIa bacteriocins through exposure to bacteriocins (19, 25). Depending on the conditions, the frequency of development of resistance to class IIa bacteriocin ranges from 10^{-3} to 10^{-6} (11, 19, 25, 38). The reported stability of the acquired resistance varies considerably (10, 11, 19, 36, 38). Class IIa bacteriocin-resistant mutants of *L. monocytogenes* have been grouped into mutants with high levels of resistance and mutants with intermediate levels of resistance (2, 10, 20, 21, 25, 44, 45).

It has been reported that in *L. monocytogenes* downregulation of either the *mpt* (mannose permease two) operon, (7, 20, 21, 36), the *mpo* (mannose permease one) operon (2), the *rpoN* gene (encoding the σ^{54} factor), or the *manR* (transcriptional activator for σ^{54}) (2, 6, 7, 39) led to resistance to class IIa bacteriocins. This is in contrast to results reported by Gravesen et al., who showed that there was upregulation of expression of the *mpt* and *manR* genes in class IIa bacteriocin-resistant mutants (20). Alterations in cell envelope fatty acid composition (33, 45) and changes in the cell surface charge (44) have also been reported to be involved in mechanisms of resistance to class IIa bacteriocins.

In numerous studies of mutants of *L. monocytogenes* strains resistant to class IIa bacteriocins only one or a few wild-type or mutant strains were included, or the mutants with acquired resistance were derived using different experimental conditions; either the strains were exposed to different types and

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TABLE 1. Wild-type strains used in this study

Strain	Description	Reference(s)
L. monocytogenes L40	Chicken isolate, serotype 3b	25, 35; Liv M. Rørvik, personal communication
L. monocytogenes L688	Sheep brain isolate, serotype 4b	25, 35; Liv M. Rørvik, personal communication
L. monocytogenes L502	Cheese isolate, serotype 1/2a	25, 35; Liv M. Rørvik, personal communication
L. monocytogenes L1037	Seawater isolate, serotype 1/2a	25, 35; Liv M. Rørvik, personal communication
L. monocytogenes EGD-e	Laboratory reference strain, serotype 1/2a	18
Lactobacillus sakei Lb790(pMLS114)	Strain producing sakacin P from plasmid pMLS114	22, 32

concentrations of bacteriocins, or they were exposed successively many times (10, 11, 19, 20, 33, 38, 46).

The purpose of this study was to characterize a large number of spontaneous mutant strains of *L. monocytogenes* that acquired resistance after a single exposure to sakacin P. To our knowledge, this is the first comprehensive study of the diversity of a large number of spontaneous resistant mutants obtained after a single exposure to class IIa bacteriocins, particularly to sakacin P.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *L. monocytogenes* wild-type strains were selected based on their natural susceptibility to sakacin P (25). *L. monocytogenes* L502 and L1037, for which the 50% inhibitory concentrations (IC_{50}) of sakacin P are high, and *L. monocytogenes* L40 and L688, for which the IC_{50} of sakacin P are low (25), as well as laboratory reference strain EGDe, were used (18). *L. sakei* Lb790(pMLS114) was used to produce cell-free supernatant (CFS) containing sakacin P (32) (Table 1).

Depending on the experiment, the *L. monocytogenes* strains were grown in tryptone soy broth (TSB) (Oxoid Ltd., Basingstoke, England), on tryptone soy agar (TSA) (Oxoid Ltd., Basingstoke, England), in brain heart infusion (BHI) broth (Oxoid Ltd., Basingstoke, England), on BHI agar (Oxoid Ltd., Basingstoke, England), in *Listeria* enrichment broth (Oxoid, Ltd., Basingstoke, England) with 0.1% Tween 80 (Sigma), or in Luria-Bertani broth (LB) (1% tryptone [Oxoid], 0.5% yeast extract [Oxoid], and 0.5% NaCl [Merck]). For *L. sakei* Lb790, deMan-Rogosa-Sharpe medium (Oxoid Ltd., Basingstoke, England) was used. Unless otherwise stated, cultivation in broth media was performed without shaking, and the sakacin P-resistant mutants were characterized in the absence of sakacin P. The *L. monocytogenes* and the *L. sakei* Lb790 stock cultures were stored frozen (−80°C) in BHI broth and deMan-Rogosa-Sharpe medium, respectively, in the presence of 15% glycerol.

Production of CFS containing sakacin P. CFS containing sakacin P was produced by heterologous expression in *L. sakei* Lb790 as previously described, with a slight modification (25). *L. sakei* Lb790 was grown overnight at 20°C (32) with shaking at 200 rpm, and the CFS was subjected to heat treatment (85°C for 20 min). Aliquots of the CFS and pure sakacin P (stock concentration, 490 mg $liter^{-1}$; kindly donated by Inga M. Aasen, SINTEF, Norway) were stored at −20°C.

Development of spontaneous mutants with resistance to sakacin P. Spontaneous mutants of *L. monocytogenes* that were resistant to sakacin P were selected after a single exposure to sakacin P as previously described (19, 25). For each strain, a total of six spontaneous mutants resistant to sakacin P (five mutants obtained after exposure to the CFS and one mutant obtained after exposure to pure sakacin P at a final concentration of 4.9 mg $liter^{-1}$) were selected. The sakacin P-resistant strains were designated by using the suffixes 1 to 6 following the wild-type strain designations (see Table 3).

Serotyping of *L. monocytogenes* strains. The serotypes of the wild-type and selected sakacin P-resistant strains were determined using a *Listeria* antiserum kit (Denka Seiken, Japan) by following the manufacturer's instructions.

Sakacin P susceptibility test. The susceptibility of the wild-type strains and the spontaneous mutants to sakacin P was determined using a pure sakacin P solution and a slight modification of a previously described method (25). The inoculum for the IC_{50} test was prepared by diluting a 24-h culture (0.001%, vol/vol) in *Listeria* enrichment broth. The IC_{50} determination was done twice on different days. Spontaneous mutants for which the IC_{50} was higher than the IC_{50} for the wild-type strain were considered resistant to sakacin P.

Stability of sakacin P resistance. The stability of the acquired sakacin P resistance of the spontaneous mutants of *L. monocytogenes* was assessed by serial cultivation in TSB in the absence of sakacin P. Every 12 h for 10 days, the cultures were transferred (1%, vol/vol) to fresh prewarmed TSB at 30°C. The stability was checked after the first 2 h of incubation (day 0) and then every other day. The frequency of retention of resistance was computed by dividing the number of CFU that grew on TSA with 30% CFS by the number of CFU that grew on TSA without CFS. The wild-type strains were included as controls.

Growth fitness in the presence of different sugars. LB broth supplemented with different sugars (0.5%, wt/vol; glucose, mannose, rhamnose, cellobiose, and lactose) was prepared and sterilized using 0.2-μm polyethersulfone membrane filters (Nalge Nunc International, United States). Growth was monitored using a Bioscreen instrument (Oy Growth Curves Ab Ltd., Helsinki, Finland) at 30°C for 18 h. The growth study experiment was done at least three times on different days.

The growth rate was computed from the slope of a linear regression line of the growth curve using the logarithmic region and was expressed in h^{-1} (31). The maximum cell density was estimated from the highest optical density at 600 nm obtained after 18 h of incubation (46). The relative fitness of a resistant strain was calculated by comparison with the wild type (resistant strain/wild-type strain). Analysis of variance in conjunction with Tukey's multiple-comparison tests was performed using Statistix 8.1 (Analytical Software Tallahassee, United States), and *P* values less than 0.05 were considered significant.

Growth fitness in the presence of food-related stresses. The protocol described above was used, with slight modifications. BHI broth media were prepared by adding hydrochloric acid (pH 4.0 to 5.5), lactic acid (pH 5; total concentration, 5 mM to 50 mM), acetic acid (pH 5; total concentration, 2 mM to 50 mM), or sodium chloride (total concentration, 0.5% to 10% [wt/vol]). Plain BHI broth was used as a control, and the growth was monitored at different temperatures (10°C, 20°C, and 30°C).

Biofilm formation. The wild-type and resistant strains of *L. monocytogenes* were screened for biofilm formation essentially as described by Borucki et al. (4). The experiment was done twice on different days.

Electron microscopy. The wild-type and resistant strains of *L. monocytogenes* were grown in BHI broth at 30°C, and for a study of flagellum formation the strains were grown at 20°C. The cells were harvested at mid-exponential and late stationary phases and were fixed as described by Giotis et al. (17). Two independent biological samples were collected, and the cells were examined by scanning electron microscopy using the standard protocol at the Molecular Imaging Center of the University of Bergen, Bergen, Norway.

FT-IR spectroscopy. Preparation of samples for Fourier transform infrared (FT-IR) spectroscopy was performed as described by Oust et al. (35). A bacterial suspension was uniformly dispensed in duplicate into 96 wells of a transmittance Si microplate (Bruker, Optics, Germany), and FT-IR measurement was performed using an HTS-XT spectrometer (Bruker Optics, Germany). For each strain, three independent biological samples were measured, and this gave a total of six spectra.

The spectra were preprocessed by taking the second derivative and applying extended multiplicative signal correction (27). The preprocessed spectra were analyzed by principal component analysis (PCA) using The Unscrambler software (The Unscrambler v9.6; CAMO AS, Norway). The PCA was performed using different biochemical fingerprint regions of the FT-IR spectra (34). The three biological samples were included in the PCA. Inspections of score plots for the first four principal components (usually PC1 and PC2) were used to determine if the wild-type strains were different from the resistant strains. Loading and line plots were used to identify chemical bands that were important for the separation of the wild-type strains from the resistant strains.

qRT-PCR. A quantitative real-time reverse transcriptase PCR (qRT-PCR) experiment was performed to quantify the level of transcription of the *mptA* gene, and spontaneous mutants of *L. monocytogenes* representing organisms with high and low levels of resistance to sakacin P were included. The strains were grown at 30°C in plain LB broth or LB broth supplemented with glucose,

TABLE 2. Primers and probes used for qRT-PCR

Primer	Sequence[a]
MptAF	5′-CCTCGCAACTCACGGTGAAT-3′
MptAR	5′-TCTTGCTCGCCGAAAATCA-3′
MptA-Taq	5′-(6-FAM)TGCTGAAGGTATTTTGCAGT CCGGAACA(TAMRA)-3′
16S rRNAF	5′-GCGCAGGCGGTCTTTTAAG-3′
16S rRNAR	5′-CAATGACCCTCCCCGGTTA-3′
16S rRNA-Taq	5′-(6-FAM)CTGATGTGAAAGCCCCCGGC (TAMRA)-3′

[a] 6-FAM, 6-carboxyfluorescein (fluorophore); TAMRA, 6-carboxytetramethylrhodamine (quencher).

mannose, or cellobiose (0.5%, wt/vol) with shaking. A sample used for isolation of total RNA was taken at mid-exponential phase. RNA extraction was done using an RNeasy Protect bacterial mini kit (Qiagen) according to the manufacturer's recommendation with "on-column" digestion of genomic DNA. First-strand cDNA was synthesized from 300 ng of total RNA using random hexamers and SuperScript III reverse transcriptase as suggested by the manufacturer (Invitrogen). In order to assess DNA contamination, a control sample with all components of the reaction mixtures except SuperScript III was included.

The primers and probes for *mptA* (lmo0096) and the 16S rRNA gene (Table 2) were designed using *L. monocytogenes* EGDe genome sequences available in the ListiList database (http://genolist.pasteur.fr/ListiList) using Primer Express 2.0 (Applied Biosystems). Amplification and detection of the cDNA were done as described previously (40). Data analysis was done as described by Schmittgen and Livak (41). For each gene, two biological replicates were included, and each biological replicate was measured four times. Changes were calculated relative to corresponding wild-type strains grown on glucose. *P* values less than 0.05 and changes that were ≥3-fold were considered significant.

RESULTS

Development of sakacin P resistance. The natural IC_{50} of sakacin P for *L. monocytogenes* wild-type strain EGDe was high (Table 3), and this strain was assigned to the group containing *L. monocytogenes* L502 and L1037 (25). The frequency of development of sakacin P resistance for all strains ranged from 10^{-8} to 10^{-9}. The sakacin P-resistant strains displayed a wide range of levels of resistance to sakacin P (the IC_{50} were 20 to $>10^5$ times the IC_{50} for the wild-type strains). Based on the IC_{50} of sakacin P, the spontaneous mutants could be grouped into strains with high levels of resistance (IC_{50}, $\geq 10^4$ ng/ml) and strains with low levels of resistance (IC_{50}, $<10^4$ ng/ml). The available stock solution of sakacin P was not concentrated enough to determine the IC_{50} for some of the strains with high levels of resistance to sakacin P. The resistant mutants had the same serotype as the wild-type strains from which they were derived.

Characteristics of the resistant strains grown in the absence of sakacin P. (i) Stability of sakacin P resistance phenotype. The stability of the resistance phenotype differed substantially when the resistant strains were grown in the absence of selective pressure. As shown in Fig. 1, *L. monocytogenes* L40-6 started to revert after 50 generations, and *L. monocytogenes* L40-1 was stable for more than 125 generations.

(ii) Growth fitness on different carbon sources. Generally, the specific growth rates of the resistant strains on glucose, mannose, and lactose were lower than the growth rates of the corresponding wild-type strains. However, in LB broth supplemented with cellobiose, the growth of the wild-type strains and the growth of the resistant strains were similar (Fig. 2; see Table S1 in the supplemental material).

TABLE 3. IC_{50} of sakacin P for wild-type strains and spontaneous mutants of *L. monocytogenes* strains

Strain[a]	IC_{50} (ng ml^{-1})[b]
L502	0.32
L502-1[c]	$>10^5$
L502-2	$>10^5$
L502-3	$>10^5$
L502-4	10^3
L502-5	10^2
L502-6	10^2
L1037	0.22
L1037-1[c]	$>10^5$
L1037-2	10^3
L1037-3	10^2
L1037-4	10^2
L1037-5	10^2
L1037-6	10^2
EGDe	0.27
EGDe-1	$>10^5$
EGDe-2[c]	10^2
EGDe-3	10
EGDe-4	<10
EGDe-5	<10
EGDe-6	<10
L40	0.12
L40-1	10^4
L40-2	10^3
L40-3	10^3
L40-4	10^3
L40-5[c]	10^2
L40-6	10^2
L688	0.06
L688-1[c]	10^4
L688-2	10^3
L688-3	10^3
L688-4	10^3
L688-5	10^3
L688-6	10^3

[a] The designations of the sakacin P-resistant strains end with the suffixes 1 to 6.
[b] $>10^5$ represents concentrations higher than the highest sakacin P concentration used.
[c] Sakacin P-resistant strains derived from exposure to pure sakacin P.

In the presence of mannose or glucose spontaneous mutants of *L. monocytogenes* L40 and L502 could be placed in groups of resistant strains that grew fast or slow ($P < 0.05$) (Fig. 2). A positive correlation between the levels of sakacin P resistance and growth rates on glucose and mannose was observed for

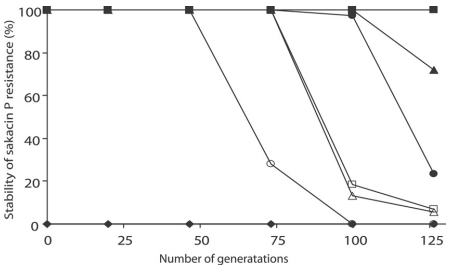

FIG. 1. Stability of sakacin P resistance phenotype in the absence of sakacin P. Symbols: ◆, L40; ■, L40-1; ▲, L40-2; □, L40-3; △, L40-4; ●, L40-5; ○, L40-6. Wild-type strain *L. monocytogenes* L40 was included as a control.

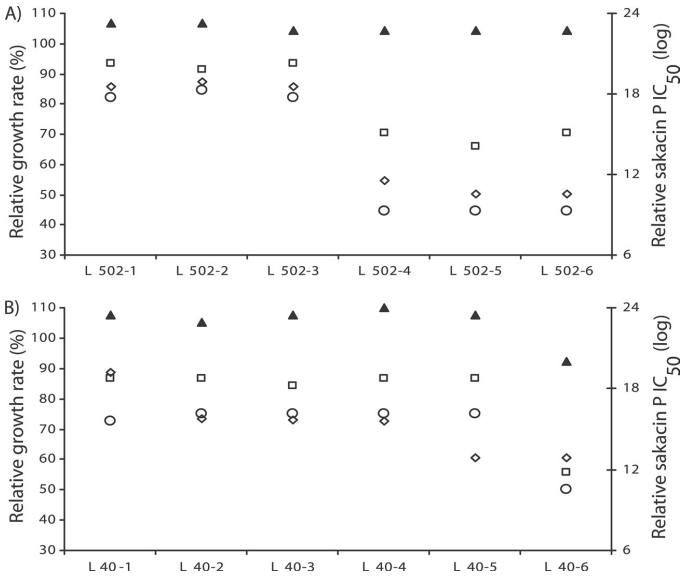

FIG. 2. Growth rates and levels of resistance to sakacin P of spontaneous bacteriocin-resistant mutants of *L. monocytogenes* L502 (A) and L40 (B). Symbols: ◇, sakacin P; □, glucose; ○, mannose; ▲, cellobiose. Values are expressed relative to the value for the wild-type strain (mutant/wild-type strain).

resistant strains derived from *L. monocytogenes* L502 ($r = 0.99$ and $P < 0.05$) (Fig. 2A). However, as shown for a representative strain in Fig. 2B, no correlation was observed for resistant strains derived from the other strains ($r = 0.3$ and $P > 0.05$). In general, the resistant strains derived from *L. monocytogenes* EGDe behaved differently than the other resistant strains (see Table S1 in the supplemental material).

The resistant and wild-type strains had similar maximum optical densities (total growth) on glucose and cellobiose (see Table S2 in the supplemental material). On mannose, with the exception of the slowly growing resistant strains of *L. monocytogenes* L502 (Fig. 2A), the total growth of the wild-type strains and the total growth of the resistant strains were similar ($P > 0.05$). Some of the resistant strains had unique growth fitness, particularly on rhamnose and lactose (see Tables S1 and S2 in the supplemental material).

(iii) Food-related stresses. Generally, the resistant strains were affected more by food-related stresses than the wild-type strains. When *L. monocytogenes* L502-6 was grown at room temperature in the presence of 2 mM acetic acid, the lag phase was prolonged by approximately 15 h and the specific growth rate was reduced by 50% compared to the lag phase and specific growth rate of *L monocytogenes* wild-type strain L502. Under extreme stress conditions (i.e., low pH, low temperature, and high concentration of organic acids), the difference in tolerance between the wild-type strains and the resistant strains was minimal.

Variation in tolerance among the resistant strains was observed; for instance, the lengths of the lag phase for *L. monocytogenes* L502-1 and L502-6 in BHI broth at 20°C (no-stress controls) were 40 and 80 min, respectively. In the control experiment, *L. monocytogenes* L502-1 grew 15% slower than *L. monocytogenes* L502-6. Addition of 5% NaCl prolonged the lag phases of *L. monocytogenes* L502-1 and L502-6 by 2 and 20 h, respectively. This salt also reduced the growth rate of *L. monocytogenes* L502-6 by 70% compared to that of *L. monocytogenes* L502-1. Generally, sakacin P-resistant strains showed less tolerance to salt and organic acid stresses than to stresses resulting from inorganic acid and low temperatures.

(iv) Biofilm formation. The resistant strains produced less biofilm than the corresponding wild-type strains, and substantial variation among the resistant strains was also observed. For example, the biofilm formation by *L. monocytogenes* L502-1 was greater than that by *L. monocytogenes* L502-6 ($P < 0.05$), and no difference was observed among resistant strains of *L. monocytogenes* L40 (data not shown).

(v) Electron microscopy. No significant difference in morphology was observed between the resistant and wild-type strains at either the exponential or stationary phase (data not shown).

(vi) FT-IR spectroscopy. PCA of the FT-IR spectra showed that the wild-type strains could be separated clearly from the corresponding mutants, with few exceptions (Table 4). Figure 3 shows the separation of *L. monocytogenes* wild-type strain L688 from the resistant strains in the polysaccharide region (1,200 to 900 cm^{-1}). The discriminatory power of the polysaccharide region was greater than those of the fatty acid and protein regions, in that order, and also varied from strain to strain. Surprisingly, the resistant strains derived from *L. monocytogenes* L502 clustered according to the level of resistance to sakacin P (high or low) (Fig. 4). The wild-type strain *L. mono-*

TABLE 4. FT-IR spectral regions that differentiate wild-type strains from sakacin P-resistant strains of *L. monocytogenes*

Spectral region[a]	L688	L502[c]	EGDe	L1037	L40[d]
Fatty acid (3,100-2,800 cm^{-1})	+[b]	+	+	−	+
Protein (1,800-1,500 cm^{-1})	+	+	−	−	−
Fatty acid and protein mixed (1,500-1,200 cm^{-1})	+	+	+	+	−
Polysaccharide (1,200-900 cm^{-1})	+	+	+	+	+
Fingerprint (900-700 cm^{-1})	+	+	+	+	−

[a] The assignment of bands is the assignment described by Naumann (34).
[b] +, wild-type strain forms a cluster distinct and separate from that formed by sakacin P-resistant strains (see Fig. 3); −, no visible separation.
[c] For *L. monocytogenes* L502, the clustering was according to the level of sakacin P resistance (see Fig. 4).
[d] *L. monocytogenes* L40-6 was frequently separated from the other strains (see text).

cytogenes L502 clustered together with the strains resistant to high levels of sakacin P, and all the spectral regions confirmed the separation (Table 4).

The resistant strains had FT-IR spectral band intensities distinct from those of the wild-type strains. For example, the intensities of bands at ~2,922 cm^{-1}, ~2,852 cm^{-1}, and ~1,230 cm^{-1} were higher for the resistant strains than for the wild-type strains (data not shown).

(vii) qRT-PCR. The expression of the *mptA* gene in *L. monocytogenes* strain L40-1 with a high level of resistance was downregulated at least 1,000-fold on all sugars tested compared to the expression in the corresponding wild-type strain grown on glucose ($P < 0.001$) (Fig. 5A). Interestingly, *mptA* gene expression was upregulated in *L. monocytogenes* strain L40-6 with a low level of resistance compared to the expression in the wild type ($P < 0.001$ and ≥3-fold change). Resistant strains derived from wild-type strain *L. monocytogenes* L502 (L502-1 and L502-6) showed patterns of *mptA* gene regulation with respect to the resistance level similar to those observed for *L. monocytogenes* L40 (Fig. 5B).

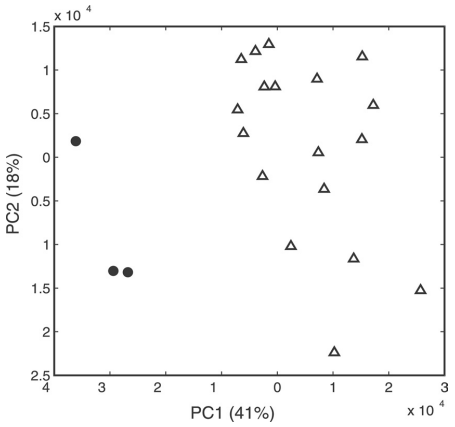

FIG. 3. PCA score plot using spectra in the polysaccharide region (1,200 to 900 cm^{-1}) as variables for wild-type and sakacin P-resistant strains of *L. monocytogenes* L688. The sakacin P-resistant strains (△) clustered separately from the wild-type (●). Three biological replicate samples were included in the PCA.

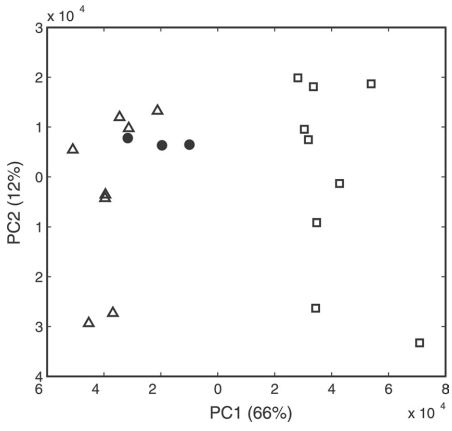

FIG. 4. PCA score plot using spectra in the polysaccharide region (1,200 to 900 cm^{-1}) as variables for wild-type and sakacin P-resistant strains of *L. monocytogenes* L502. The sakacin P-resistant strains of *L. monocytogenes* L502 clustered according to their levels of resistance to sakacin P as follows: low levels of resistance (□) and high levels of resistance (△). Wild-type strain L502 (●) clustered with the strains with high levels of resistance. Three biological replicate samples were included in the PCA.

(viii) Spontaneous mutants obtained with pure sakacin P and CFS. Resistant strains obtained after exposure to pure sakacin P had phenotypic and gene expression profiles similar to those of the resistant strains obtained after exposure to *L. sakei* fermentate.

DISCUSSION

Spontaneous mutants of *L. monocytogenes* with acquired resistance to class IIa bacteriocins restrict the potential use of these bacteriocins as food biopreservatives. The present study is a continuation of our previous work (25, 35), in which two wild-type strains with high levels of natural susceptibility to sakacin P and two strains with low levels of natural susceptibility to sakacin P (25), as well as strain EGDe, were further

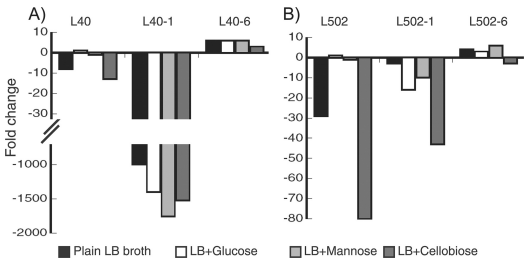

FIG. 5. Changes in *mptA* gene expression in wild-type and sakacin P-resistant strains of *L. monocytogenes* L40 (A) and L502 (B). The calculated changes are relative to the expression in the corresponding wild-type strains grown on glucose.

characterized. In order to obtain insight into the mechanisms and effects of acquired resistance to sakacin P, we included 30 spontaneous mutants derived from the five wild-type strains.

The frequency of development of resistance for all strains in the present study ranged from 10^{-8} to 10^{-9}. For class IIa bacteriocins frequencies between 10^{-3} and 10^{-6} have been reported (10, 11, 19, 25, 37, 38, 48). The observed divergence between the present study and previous studies could be due to strain variation (19, 48) and methodological differences. However, Gravesen et al. reported that the frequency of mutation to resistance to class IIa bacteriocins was not dependent on temperature, pH, salt, or the concentration of bacteriocin used (19).

In the present study, wide variation in the levels of acquired resistance was observed among the sakacin P-resistant strains (Table 3), and we divided the spontaneous mutants into strains with high levels of resistance and strains with low levels of resistance. The strains with high levels of resistance were over 10^5 times more resistant than the corresponding wild-type strains, and the strains with low levels of resistance were 20 to 10^4 times more resistant. Previous studies grouped mutants of *L. monocytogenes* that were resistant to class IIa bacteriocins into mutants with high levels of resistance (500 to 10^6 times more resistant than the wild-type strain) and mutants with intermediate levels of resistance (2 to 8 times more resistant than the wild-type strain) (2, 10, 20, 21, 44, 45).

In the present study, the sakacin P resistance phenotype was stable for at least for 50 generations (Fig. 1), and a class IIa bacteriocin-resistant strain with a stable phenotype for 20 generations has been described as a mutant (36). In addition, the resistant strains had different phenotypic and genotypic characteristics than the wild-type strains when they were tested in the absence of sakacin P (see below). Membrane adaptation has been described as a mechanism of resistance to class IIa bacteriocins, and the authors also highlighted the presence of extra resistance mechanisms among the mutants (44, 45). Taken together, these findings strongly suggest that the resistant strains obtained in the present study are mutants and that resistance due only to phenotypic adaptation is an unlikely explanation.

Sakacin P-resistant strains derived from the same strain showed differences in the stability of the sakacin P resistance phenotype. The strains derived from *L. monocytogenes* L40 (Fig. 1) and EGDe (data not shown) with high levels of sakacin P resistance had a more stable resistance phenotype than the strains with low levels of resistance. In contrast to previous studies (10, 11, 19, 33, 36, 38), the present study included several resistant strains, and the strains were grown for more than 120 generations. To our knowledge, this is the first evidence that links levels of resistance to sakacin P with the stability of the resistance phenotype in spontaneous mutants derived from the same wild-type strain. The observed stability and other differences (see below) in the two groups (high and low levels of resistance) derived from same strain may indicate that there are different modes of resistance to class IIa bacteriocins, as suggested previously (10, 36).

Resistant strains of *L. monocytogenes* L40 and L502 were the most thoroughly studied strains, and the spontaneous mutants derived from these strains with the two different levels of resistance also had different physiological and genetic characteristics. *L. monocytogenes* L502 strains, with a low level of resistance to sakacin P, generally grew slower (Fig. 2A), had reduced tolerance to stresses, and produced less biofilm than the strains with a high level of resistance to sakacin P. Representatives of the two groups of *L. monocytogenes* L40 strains showed similar tendencies. *L. monocytogenes* L40-6 was found to be the least stable strain (Fig. 1) and grew slower than *L. monocytogenes* L40-1 (Fig. 2B). In addition, the expression of the *mptA* gene was upregulated in *L. monocytogenes* L40-6 and L502-6 with low levels of resistance and downregulated in *L. monocytogenes* L40-1 and L502-1 with high levels of resistance compared to the expression in the wild-type strain (Fig. 5). Our FT-IR spectroscopy results also confirmed the grouping according to the level of resistance (Table 4 and Fig. 4).

Generally, the resistant strains had a reduced growth rate when they were grown on mannose and glucose compared to the growth on cellobiose (see Table S1 in the supplemental material). This is consistent with studies that showed the involvement of the mannose phosphotransferase (PTS) system in class IIa bacteriocin resistance (2, 21). In contrast, some of the resistant strains had growth rates on glucose (and to some extent growth rates on mannose) that were similar to those of the corresponding wild-type strains, and resistant strains with unique growth fitness on cellobiose, lactose, or rhamnose were observed (see Table S1 in the supplemental material). The relationship between the level of resistance to sakacin P and growth fitness was strain specific and sugar source dependent (Fig. 2). For example, spontaneous mutants derived from *L. monocytogenes* L502 with high levels of resistance to sakacin P grew significantly faster on glucose and mannose than the strains with low levels of resistance (Fig. 2A). However, no apparent relationship was observed among the resistant strains derived from the other wild-type strains. This substantial variation among the spontaneous mutants derived from the same strain may show that there are complex responses of the resistant strains due to alteration of the cell metabolism and/or different targets of the bacteriocin on the bacterial cell.

The results for reference strain EGDe, the only fully sequenced strain of *L. monocytogenes* (18), were often different from the results for the other four strains used. Strain Scott A, another laboratory reference strain, showed similar aberrant results (28). The EGDe strain may not be representative under the conditions of this study, and the generalizations that we made were based mainly on the results obtained with the other four strains.

In stress conditions that mimic the food environment, the resistant strains had less chance to grow, particularly in the presence of acids (organic and inorganic), salt, and low temperatures. We also found that the resistant strains had reduced abilities to form biofilms. This may suggest that the resistant strains do not compete well during harsh food processing and under storage conditions in foods when the bacteriocin is not present. More research is necessary to evaluate if resistant mutants are outcompeted in food or if their presence represents a bottleneck for use of bacteriocins in food.

Data obtained from FT-IR spectroscopy confirmed the grouping of the wild-type strains in the previously established groups (35) (data not shown). For both wild-type and resistant strains, the serotypes were accurately predicted by FT-IR spectroscopy irrespective of the observed high IC_{50} of sakacin P.

Resistance to sakacin P introduced distinct changes mainly in the polysaccharide, fatty acid, and protein regions, which represented the major cell membrane components (Table 4). The variations among the resistant strains observed by FT-IR spectroscopy also may suggest that there are pleiotropic effects of resistance to sakacin P in the intact cells.

Differences in band intensity between the wild-type and resistant strains were observed. All of the resistant strains had higher band intensities at ~2,922 cm^{-1}, ~2,852 cm^{-1}, and 1,230 cm^{-1} than the wild-type strains. Bands at ~2,922 cm^{-1} and ~2,852 cm^{-1} are assigned to carbon-hydrogen stretching of CH_2 in fatty acids, and bands around 1,230 cm^{-1} are due to double-bond stretching of the head group of phospholipids or phosphorus-containing carbohydrates, such as teichoic and lipoteichoic acids (34). It is often reported that strains of *L. monocytogenes* that are resistant to class IIa bacteriocins have alterations in the cell envelope, such as alterations in the fatty acid composition and cell surface charge (33, 44, 45). Taken together, FT-IR spectroscopy results mentioned above also strongly reflect the overall destabilized state of the cell envelope in the resistant strains.

With regard to expression of the *mptA* gene, upregulation and downregulation have previously been described for class IIa bacteriocin-resistant mutants (2, 20, 44). It has also been shown that components of the mannose PTS act as receptors for class IIa bacteriocins, thus explaining the mutant downregulation of *mptA* (8). This is consistent with our data for the resistant strains in the group with a high level of resistance to sakacin P (*L. monocytogenes* L40-1 and L502-1). However, in the characterized resistant strains belonging to the group of strains with low levels of resistance to sakacin P (*L. monocytogenes* L40-6 and L502-6), the *mptA* gene was upregulated. Gravesen et al. reported similar observations for a mutant with an intermediate level of resistance (20). In the present study, compared to the wild-type strains, the resistant strains with induced upregulation of the *mptA* gene were approximately 1,000 times more resistant, had reduced growth fitness, and had different FT-IR spectroscopy profiles. The present study and the report of Gravesen et al. (20) clearly showed that the level of *mptA* gene expression was not correlated to the level of resistance, in contrast to other findings (2, 44).

It is not clear whether the upregulation of *mptA* indicates a new mechanism of resistance. But since the growth in the presence of mannose and glucose still was significantly reduced in the resistant strains, it indicates that the mannose PTS system is somehow involved, although in a different manner than previously described. In bacteria, the PTS system in general (3) and the mannose PTS in particular (1) have a central role in a variety of regulatory events controlling carbon metabolism. Whether the observed upregulation of the *mptA* gene represents a new resistance mechanism itself or is due to indirect effects of an altered mannose PTS system remains to be investigated.

In conclusion, the present study showed substantial diversity among the spontaneous mutants. This finding emphasizes that care has to be taken when generalizations based on studies of a few strains and mutants are made. Spontaneous mutants with high levels of resistance to sakacin P tended to be more stable, had better growth fitness, and were more tolerant to food-related stresses than mutants with low levels of resistance. Previously, we showed that spontaneous mutants that were resistant to sakacin P were also resistant to other members of the class IIa bacteriocin group (25). In this respect, the results obtained in the present study may apply to other related class IIa bacteriocins, and this should create significant concern with regard to application of class IIa bacteriocins to food.

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Paper II

Global Transcriptional Analysis of Spontaneous Sakacin P-Resistant Mutant Strains of *Listeria monocytogenes* during Growth on Different Sugars

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Keywords: *Listeria monocytogenes*; class IIa bacteriocin; sakacin P; spontaneous mutants; resistance; genome wide transcriptome; microarray; qRT-PCR; mannose; cellobiose; PTS; *mpt*; virulence; bacteriophage P100.

Abstract

Subclass IIa bacteriocins have strong antilisterial activity and can control the growth of *Listeria monocytogenes* in food. However, *L. monocytogenes* may develop resistance towards such bacteriocins. In this follow-up study, the transcriptomes of a high level (L502-1) and a low level (L502-6) spontaneous sakacin P-resistant mutant strain of *L. monocytogenes* were compared to the wild-type (L502). The growth of the resistant strains was reduced on mannose but not affected on cellobiose and the transcriptomics was performed during growth on these sugars. The mannose phosphotransferase system (PTS) encoded by the *mptACD* operon (*mpt*) is known for transporting mannose and also is a receptor to class IIa bacteriocins. The *mpt* was repressed in L502-1, and this is in accordance with abolition of the bacteriocin receptor with resistance to class IIa bacteriocins. In contrast, the *mpt* was induced in L502-6. Despite the induction of the *mpt*, L502-6 showed 1,000 times resistance phenotype and reduced growth on mannose, suggesting the mannose-PTS may not be functional in L502-6. The microarray data suggests the presence of other transcriptional responses that may be linked to the sakacin P resistance phenotype particularly in L502-6. Most of commonly regulated genes encode proteins involved in transport and energy metabolism. The resistant strains displayed shift in general carbon catabolite control possibly mediated by the *mpt*. Our data suggest that the resistant strains may have a reduced virulence potential. Growth sugar- and mutant-specific responses were also revealed. The two resistant strains also displayed difference in the stability of the sakacin P resistance phenotype, growth in the presence of both the lytic bacteriophage P100 and activated charcoal. Taken together, the present study showed that a single time exposure to the class IIa bacteriocin sakacin P may elicit contrasting phenotypic and transcriptome responses in *L. monocytogenes* possibly mediated by the *mpt*.

Introduction

Listeriosis is a rare but potentially serious infectious disease. Consumption of food contaminated with *Listeria monocytogenes* is the major route of transmission [1]. In recent years, a resurgent trend in listeriosis has been reported in many European countries [2]. *L. monocytogenes* is also responsible for substantial food product recalls and considerable economic loss to food industry [3].

Bacteriocins produced by lactic acid bacteria are of major interest as natural and safe food preservatives targeting foodborne pathogens, including *L. monocytogenes* [4-7]. The lactic acid bacteria bacteriocins are grouped into several classes and subclasses [7]. Subclass IIa bacteriocins (also called pediocin-like) are considered the most important class II bacteriocins and have strong antilisterial activity [8].

Development of resistance by target cells after exposure to class IIa bacteriocins seriously hampers the use of these bacteriocins as biopreservatives [9,10]. The molecular mechanism behind the resistance is not fully known and is important to elucidate for optimal use of the bacteriocins. In *L. monocytogenes* and other related gram-positive bacteria, it is established that the mannose specific phosphotransferase system (PTS) encoded by the *mptACD* operon (hereafter *mpt*) is the receptor for class IIa bacteriocins, and abolition of the receptor is the most common mechanism behind resistance to class IIa bacteriocins [10-17]. A more recent transcriptome study [18] on class IIa bacteriocin-resistant *Enterococcus faecalis* indicated that, in addition to conferring resistance to class IIa bacteriocins, the mannose-PTS plays a key role in global carbon catabolite control. Interestingly, up-regulation of the *mpt* in three different class IIa bacteriocin-resistant mutants has been reported [19,20].

The virulence potential of class IIa bacteriocin-resistant strains is a concern that has to be addressed. A study by Gravesen et al. has investigated the expression of 13 selected virulence genes for two class IIa bacteriocin-resistant strains using targeted microarray (64 genes) and the study has shown that five of the virulence genes were significantly down-regulated in one of the resistant strains [19]. For the other resistant strain, the authors reported a non-significant induction of the tested virulence genes [19]. In another study, exposing of *L. monocytogenes* to a sakacin 1 producing strain [21] showed no apparent effect of this class IIa

bacteriocin on the hemolytic activity of *L. monocytogenes* [22]. Hence, more study is needed to understand the relationship between class IIa bacteriocin resistance with virulence potential.

We have recently shown that spontaneous sakacin P-resistant mutants of *L. monocytogenes* L502 can be divided into strains with high level of resistance and strains with low level resistance [20]. The high level resistant strain *L. monocytogenes* L502-1 (hereafter L502-1) shows a slight growth reduction on mannose, with down-regulation of the bacteriocin receptor gene (*mptA*), compared to that of the wild-type. In contrast, the low level sakacin P-resistant *L. monocytogenes* L502-6 (hereafter L502-6) shows dramatic growth reduction on mannose, and the *mptA* gene is up-regulated. The two resistant strains grow as the wild-type on cellobiose [20].

The main purpose of the present study was to identify possible genes that might be involved in sakacin P resistance particularly in the mutant strain that showed induction of the bacteriocin receptor. Genome-wide transcriptome profile of the two different sakacin P-resistant strains (L502-1 and L502-6) were compared to that of the wild-type (L502) upon growth on mannose or cellobiose. The data presented here independently confirm our previous report regarding with the repression and induction of the *mpt* operon in L502-1 and L502-6 respectively. Nevertheless, the transcriptomic and phenotypic results altogether suggest the *mpt* operon could be involved in the sakacin P resistance not only in L502-1 but also in L502-6. In addition, the transcriptomics suggests the presence of other transcriptional responses that may be linked to the sakacin P resistance in L502-6. To our knowledge, the present study reports the first genome-wide transcriptome profiling in spontaneous class IIa bacteriocin resistant mutant strains of *L. monocytogenes* and opens new possibilities for further studies.

Results and Discussion

The class IIa bacteriocins can inhibit unwanted microorganisms in food including the foodborne pathogen *L. monocytogenes* [23]. Development of spontaneous mutant strains with reduced susceptibility to the bacteriocin creates significant concern with regard to application of the bacteriocins as food preservative [24]. A deeper knowledge about the acquired resistance is crucial for optimal use of the bacteriocins in the food industry. Recently we have shown that substantial diversity among large number of *L. monocytogenes* spontaneous mutant strains obtained after a single time exposure to the class IIa bacteriocin sakacin P [20]. The present study is a continuation of the previous work [20], in which two different sakacin P-resistant strains of *L. monocytogenes* L502 were further characterized mainly using whole-genome DNA microarray.

Global gene expression profile and validation by quantitative real-time reverse transcriptase PCR

In the present study we compared the transcriptome profiles of two different spontaneous sakacin P-resistant mutant strains of *L. monocytogenes* with their wild-type strain (Table 1). The growth of the resistant strains was reduced on mannose but the resistance strains grow as wild-type on cellobiose (Table 1, [20]). For better understanding of the incidence(s) giving rise to the sakacin P resistance, the transcriptome analysis was performed during growth on mannose or cellobiose. During growth on mannose a total of 124 (87 up and 37 down) and 194 (152 up and 42 down) genes were regulated in L502-1 and L502-6 respectively, compared to the wild-type. The total number of genes affected on cellobiose were 39 (20 up and 19 down) in L502-1 and 36 (30 up and 6 down) in L502-6. The complete list of the differentially regulated genes in any of the growth conditions is available in Table S1. The result from Principal Component Analysis (PCA) indicates main variation in global transcriptional response was due to growth sugar effect followed by difference between the two resistant strains (Figure 1). These overall global transcriptional responses of the resistant strains grown on mannose and cellobiose was in line with the fitness of the resistant strains growing on the respective sugars (Table 1, [20]).

Analysis of the differentially expressed genes according to the CMR-JCVI (Comprehensive Microbial Resource of the J. Craig Venter Institute) role categories showed that the sakacin P-resistant strains had altered expression of genes belonging to the majority of the role categories (Figure S1 and Table S1). This indicates that the incidence(s) giving rise to the sakacin P resistance also affects the overall physiology of the resistant strains, which is in agreement with our earlier finding on intact cell profile of the sakacin P-resistant strains using Fourier transform infrared spectroscopy [20]. In general, genes belonging to the energy metabolism, transport and binding proteins, and amino acid biosynthesis of the CMR-JCVI role categories were overrepresented ($p < 0.001$ and odd ratios > 2) and most of them were induced (Figure S1).

The reliability of the microarray expression data was assessed by quantitative real-time reverse transcriptase PCR (qRT-PCR) analysis. The validation assay showed strong correlation of gene expression with the microarray results under all tested conditions ($r > 0.9$, Figure S2), confirming independently the reliability of the microarray data. The microarray probes used in the present study were based on the EGDe strain (Accession No. A-BUGS-19; http://bugs.sgul.ac.uk/A-BUGS-19). More than 94% of *L. monocytogenes* L502 genes were hybridized to the EGD-e based probes in any of experimental conditions tested so far (not shown). A recent study [25] has shown that the EGDe strain expresses comparable number of its genes (> 98% of its open reading frames) under different growth conditions. Taken together, the microarray analysis reported in the present study unambiguously reflects the global transcriptomic profiles of the sakacin P-resistant strains.

Transcriptional responses linked to resistance to sakacin P

The sakacin P-resistance phenotype was confirmed during growth on mannose and cellobiose using selective plates containing sakacin P at the time of harvesting. The growth fitness of the resistant strains was not affected in the presence of sakacin P when cultivated on mannose and cellobiose (not shown). Consequently, genes whose expression altered in both sugars were considered as potential genes that might be linked to the resistance. Genes differentially regulated in both sugars in L502-1 and L502-6 are listed in Table 2 and Table 3 respectively.

I) Abolition of the bacteriocin receptor (mpt)

In *L. monocytogenes,* it has been shown that the gene product of *mpt* is necessary for growth on mannose [26] and act as a receptor for the bacteriocin sakacin P [17,27]. The transcriptome assay showed that during growth on mannose the *mpt* was down-regulated in the high level sakacin P-resistant strain (L502-1) compared to the wild-type (Table 2). In contrast, under similar growth condition, the *mpt* was up-regulated in the low level sakacin P-resistant strain (L502-6) (Table 3). In both sakacin P-resistant strains the *mpt* was up-regulated during growth on cellobiose (Table 2 and Table 3). However, initial analysis of the spot intensities of the microarray in the two samples separately (mutant versus wild-type) suggests the relative up-regulation of the *mpt* on cellobiose might not be as such significant. To verify this, qRT-PCR assay was performed on *mptA* gene representing the *mpt* to investigate if cellobiose induces the *mpt* among the resistant strains. No significant induction of the *mptA* gene was observed in any of the strains during growth on cellobiose. In contrast, mannose induced the *mptA* gene in the following increasing order: L502-6 > wild-type > L502-1 (Figure S3). Previously it has been shown that in wild-type *L. monocytogenes* strain the *mpt* gene is induced by the presence of mannose but not by cellobiose [11,26].

The repression of *mpt* in L502-1 reported in the present study suggests that abolition of the *mpt* could be a part of sakacin P resistance (Table 2). Indeed, several seminal studies uncovered the role of mannose-PTS in resistance/susceptibility of target cells for the class IIa bacteriocin [10-15,17-19,27,28]. The induction of the *mpt* gene which encodes the bacteriocin receptor in L502-6 was intriguing (Table 3). Previously we have shown that another low level sakacin P-resistant strain (L40-6) also displayed induction of the *mptA* gene [20]. A similar trend in induction of the bacteriocin receptor in a carnobacteriocin B2-resistant strain both at gene and protein levels has been reported [19]. Despite the induction of the *mpt*, the two low level resistant strains studied in our laboratory showed 1,000 times more resistance phenotype than their respective wild-type strains. In addition, the growth of these resistant strains was reduced significantly particularly on mannose (Table 1). This may suggests the normal function of the gene product of *mpt* is affected not only in L502-1 but also in L502-6. Hence, abolition of the receptor (no expression or modification) could be the main resistance mechanism against the sakacin P in both resistant strains. Nevertheless, how the induced *mpt* expression in L502-6 confers resistance to sakacin P is not clear and requires further investigation. The transcriptome data also suggest the presence of other transcriptional

responses that may contribute to the sakacin P resistance phenotype particularly in L502-6 (see below).

Genes known to modulate the *mpt* expression and conferring resistance to a class IIa bacteriocin were not differentially expressed in any of the sakacin P-resistant strains when compared to the wild-type. This is in contrast to previous reports on defined mutants lacking functional σ^{54} factor [29], ResD (two-component response regulator) [30], Lmo0095 (*mpt* activator) [31], or PrfA (positive regulatory factor A) [19], and having increased resistance to class IIa bacteriocin possibly through inactivation of the mannose PTS. Deletion of the *manR* gene (encoding transcriptional activator for σ^{54}) resulted in resistance to a class IIa bacteriocin [11]. In the present study and in other spontaneous class IIa bacteriocin-resistant mutants studied [19], the expression of *manR* was generally up-regulated in the resistant strains compared to the wild-type (Table S1). The role of ManR in spontaneous class IIa bacteriocin resistance remains to be studied. Nevertheless, factors as type of strain used (defined versus spontaneous mutants) and the growth conditions (e.g. defined culture media versus complex media) may contribute for variation in regulation of genes encoding especially the PTS [19,26,32].

II) Modification of the cell envelope and antimicrobial translocation system

Genes encoding proteins associated with the cell envelope were induced in L502-6 (Table 3 and Table S1). This includes *srtB* which encodes a sortase protein that modifies the cell envelope and *lmo0366* encoding a putative surface lipoprotein (Table 3). The link between modification of the cell envelope and resistance to class IIa bacteriocins reported previously [15,33,34]. In the present study, *tatAC* operon encoding the Twin-Arginine Translocase (TAT) system was up-regulated in L502-6 (Table 3). In other bacteria as *Mycobacterium smegmatis* and *Campylobacter jejuni*, mutation of the *tat* specific genes increased susceptibility to antimicrobials indicating the TAT dependent translocation of antimicrobials [35,36]. Our transcriptional data together with results from the previous studies may suggest modification of the cell envelop and the TAT system may contribute to the sakacin P-resistance phenotype in strain L502-6. However, it is yet unclear whether these may also represent an indirect consequence of the altered *mpt* expression (see below).

The mannose PTS in global carbon catabolite control

In the present study genes associated with energy transport and metabolism were the most affected in the resistant strains particularly upon growth on mannose (Figure S1). *In-silico* analysis for catabolite responsive element (*cre*) sequence (WWTGNAARCGNWWWCAWW) described for *Bacillus subtilis* [37] indicated that most of the differentially regulated genes harbor potential *cre* sites important for carbon catabolite control *via* catabolite control protein A (CcpA) (not shown). Among the genes with potential *cre* site identified here some of them are previously described to be under carbon catabolite control in *L. monocytogenes* [31,38,39] and the closely related bacteria *B. subtilis* [40].

Known genes that are under catabolite control repression (CCR) and were induced in the resistant strains includes *lmo0027* (encode β-glucosides specific permase) [31], *lmo1254/lmo1255* (trehalose metabolism) [40], *glpK* and *glp*D (glycerol metabolism) [40], *citC*, *citZ* and *citB* (oxidation portion of tricarboxylic acid cycle) [39,40], and *lmo0847* (a putative glutamine ATP-binding cassette (ABC) transporter) [39]. In addition, a number of *pts* and other transporter and utilization genes for sugars that was not present in the growth media (e.g. fructose, galacitol, ascorbate, maltose and manitol) and their transcriptional activators and antiterminators were induced (Table S1). Conversely, genes *alsS* and *alsD* which involved in acetoin biosynthesis are under carbon catabolite activation [40] and here were found to be down-regulated (Table S1). The transcriptome data indicated that none of the genes encoding proteins mediating carbon catabolite control (e.g. *ptsI*, *hprK*, *ccpA*, *ccpC*, *cggr*) were differentially regulated (not shown).

Most of the transcriptome changes observed in the present study could be linked to global carbon catabolite control mediated by the altered *mpt* as shown in previous studies [18,26,31,41]. In addition to a role in carbon catabolite control, the mannose specific PTS is involved in a variety of cellular functions including, stress tolerance [42], biofilm formation [41] and virulence [31]. In fact, the sakacin P-resistant strains studied in the present study displayed low tolerance to food related stress [20], produce less biofilm [20] and may have reduced virulence potential (see below) compared to the wild-type. Therefore, it is possible that the regulatory role of the *mpt* affected other several genes including a number of known and putative genes encoding regulatory proteins mostly associated with the PTSs (Table S1) [43]. Further studies will be required to fully define the role of the *mpt* in *L. monocytogenes*.

Common and specific transcriptional response to the high- and low-level resistant strains

As it can be seen in Figure 2, common and unique genes were differentially expressed in the high level and the low level sakacin P-resistant strains upon growth on mannose and on cellobiose. Commonly down-regulated genes in the resistant strains mostly during on mannose include genes ascribed in virulence, antimicrobial resistance, pyrimidine metabolism and amino acid biosynthesis (Figure 2B, Table S1). The reduced expression of genes encoding pyrimidine and amino acid metabolism upon growth on mannose is inline with the growth fitness of the resistant strains on this sugar (Table 1). As mentioned earlier, mostly genes encoding proteins associated with energy transport and metabolism were commonly up-regulated in the sakacin P-resistant strains during growth on mannose (Figure 2B and Table S1).

The common genes up-regulated and down-regulated under all tested conditions were *lmo0848* and *lmo1251* respectively. The operon encoded by *lmo0847*and *lmo0848* is an ABC glutamine transporter and as mentioned earlier it is known to be under CCR [39]. The *lmo1251* is a PrfA-like putative CRP-FNR (cyclic AMP receptor protein-fumarate and nitrate reduction regulator) family transcriptional regulator. The CRP-FNR families mainly functions as positive transcriptional regulators and are known to control genes in various aspects as sugar and amino acid transport and metabolism and in pathogenesis [44].

A total of 49 genes (33 up-regulated and 16 down-regulated) were affected only in L502-1 upon growth on mannose (Table S1). The genes differentially regulated in L502-1 were mostly associated with transport and utilization of energy. In contrast, upon growth on mannose large number of genes (69 up-regulated and 28 down-regulated) encoding proteins associated with different cellular functions were differentially regulated in L502-6. In addition many genes encoding unknown proteins were induced in L2502-6. Despite the similar growth fitness of the two resistant strains on cellobiose, more genes specific to L502-6 were regulated than in L502-1 (Table S1). Together, these results may indicate that the incidence(s) giving rise to the sakacin P resistance in L502-6 resulted in a more complex trait(s) than in L502-1.

Virulence potential

In the present study, known and putative virulence determinant genes were significantly down-regulated in the resistant strains compared to that of the wild-type (Figure 3). The proteins encoded by the repressed genes have known roles in the infectious lifecycle of *L. monocytogenes* [25]. For example, the glutamate decarboxylase system (encoded by the *gadT2D2*) and the bile salt hydrolase (*bsh*) could help the bacterium to pass through the alimentary canal and the proteins encoded by *inlA, inlB, hly, actA, mpl,* and *plcA* could facilitate the adhesion, invasion and cell-to-cell spread of the bacterium [43]. The decreased expression in the *gadT2D2* operon (Figure 3) is partly in line with our earlier finding that showed an increased susceptibility of the sakacin P-resistant strains to low pH stress than the wild-type [20]. No expression data was available for *plcB* gene which encodes phospholipase C, however, the diminished lecithinase activity (Figure 4B) suggested a decrease in expression of the *plcB* gene. In the present study, the expression of *virR* (virulence regulator) and *srtB* genes were up-regulated in L502-6 compared to the wild-type (Figure 3). The VirR and SrtB are associated with modification of the cell surface [45,46], but the significance of the proteins encoded by these genes in the sakacin P-resistant strain remained to be investigated.

Although qualitative, the results in Figure 4 show that the hemolytic and lecithinase activity of L502-6 were apparently lower than that of the wild-type. In contrast, no difference was observed between L502-1 and the wild-type. The difference between the resistant strains could be attributed to the level of gene transcript (Figure 3) and/or growth fitness (Table 1, [20]). To rule out the latter possibility, an independent hemolytic assay was performed in the cell supernatant after the strains were grown in the presence of cellobiose, which gives a similar growth fitness (Table 1, [20]). In both sakacin P-resistant strains the hemolytic activity was slightly reduced compared to the wild-type, albeit statistically insignificant ($p= 0.3$) (not shown). A recent study [21,22] has shown no change in the hemolytic activity after subjecting a *L. monocytogenes* strain to the class IIa bacteriocin sakacin 1. Overall, it is possible that the reduced growth capacity of L502-6 might contribute to the reduced virulence. Further work on the virulence potential of the sakacin P-resistant strains using animal models will be highly informative.

In the present study, the addition of activated charcoal had no apparent effect on hemolytic and lecithinase activities of the strains (not shown). Surprisingly, activated charcoal inhibited the growth of L502-6 by approximately 95% compared to the wild-type (not shown). Removal of important culture components by activated charcoal and/or physical adsorption of the low level sakacin P-resistant strain to the activated charcoal could be the reasons. Thus, in contrast to the previous transcriptional study on class IIa bacteriocin-resistant mutants [19], the present transcriptome study was performed in the absence of activated charcoal.

Infection with the virulent phage P100

Bacteriophage P100 is a strict virulent phage of *L. monocytogenes* and infection inevitably leads to cell death [47]. We assessed if the resistance to sakacin P can cross-protect *L. monocytogenes* from phage infection using Listex P100, a commercial phage approved by the United States Food and Drug Administration as a food biopreservative for the control of *L. monocytogenes* [48]. At higher MOI all the strains were killed by the phage P100 (not shown). To our surprise, L502-6 showed reproducibly reduced susceptibility to low doses of phage P100 in contrast to the other strains. The killing effect of phage P100 was not dependent on the growth sugar (Figure 5). A similar leaky resistance type to lambda phage has been observed in *Escherichia coli* lacking the membrane components of the mannose PTS [49]. To obtain a complete understanding about the mechanism(s) that underline the reduced susceptibility in L502-6 to low doses of phage P100 requires further investigation.

Stability of the sakacin P resistance phenotype

During growth in the absence of selective pressure (sakacin P), the sakacin P resistant phenotype in L502-1 started to revert after approximately 20 generations. In contrast, the sakacin P resistance in L502-6 was stable up to 100 generations (not shown). This indicates that the low level resistant strain had a more stable sakacin P resistance phenotype than the high level resistant strain. This contradicts our previous observation which showed more stable phenotype of the high level sakacin P-resistant strains of *L. monocytogenes* L40 and EGDe than the low level resistant strains [20]. As described above, the low level sakacin P-resistant strain studied in the present study (L502-6) may have additional sakacin P-resistance mechanisms, indicating a possible factor that determines the phenotype stability.

The sakacin-P resistant strains are generated after the wild-type strains were exposed to a cell free supernatant (CFS) containing sakacin P prepared by heterologous expression of sakacin P in *Lactobacillus sake* Lb790(pMLS114) [20]. In the present study, CFS from the non-bacteriocinogenic *L. sakei* Lb790(pLPV111) was used as a negative control [50] and no antilisterial effect was observed in the presence of the CFS from the non-sakacin P producer strain culture (not shown).

Concluding remarks

The present study reports the first genome-wide transcriptome changes in two different spontaneous sakacin P-resistant mutant strains of *L. monocytogenes*. The experiment was done in the presence of mannose or cellobiose and this facilitated systematic identification of genes possibly related to the bacteriocin resistance. Our data suggested that, for both resistant strains, absence of a normal functional *mpt* as the major mechanism for the sakacin P resistance. In addition, modification of the cell envelope and efflux of the bacteriocin by TAT system might also contribute to the resistance phenotype in the low level sakacin P-resistant strain. The transcriptomics suggested a possible role of the mannose-PTS in global carbon catabolite control in *L. monocytogenes*.

The high level and the low level sakacin P-resistant strains show substantial difference in gene expression profile, stability of the sakacin P resistance phenotype, resistance to phage P100 infection, virulence potential, and growth in the presence of activated charcoal. In our previous study [20], it was shown that the resistant strains displayed different level of resistance to sakacin P, stress tolerance capacity, biofilm formation ability, and Fourier transform infrared spectroscopy profile. Overall, this indicates that the incidence(s) giving rise to the sakacin P resistance involves a complex regulatory gene network and have pleiotropic effects on the physiology of the resistant strains. It would be of interest to extend this study to link the resistance phenotype with mutation(s) in the DNA sequence. Further works on the virulence potential of spontaneous class IIa bacteriocin-resistant strains using animal models would have a practical importance.

Materials and Methods

Bacterial strains and growth conditions. The wild-type *L. monocytogenes* strain L502, a cheese isolate of serotype 1/2a and its two spontaneous sakacin P-resistant mutants have been described previously [20,51]. The mutants were derived after exposure to the class IIa bacteriocin sakacin P [20], and relevant characteristics are summarized in Table 1. The sakacin P producer *L. sakei* Lb790(pMLS114) and its isogenic non-sakacin P producer *L. sakei* Lb790(pLPV111) [50] were used to produce CFS.

Growth media as well as growth and storage conditions were as previously described [20]. Briefly, *L. monocytogenes* strains were cultivated aerobically at 30°C and liquid cultures were shaken at 200 r.p.m., unless stated otherwise. For hemolytic and lecithinase activity testing, culture media were modified by adding activated charcoal to a final concentration of 0.2 % (wt/ vol) (Sigma-Aldrich, Amersfoort, The Netherlands) to the Luria-Bertani (LB) broth supplemented with cellobiose. Trypticase soy agar (TSA) was modified by adding either 5% defibrinated horse blood (TCS Biosciences Ltd, Buckingham, UK), 10 % egg yolk or 30% CFS from a *L. sakei* culture. The production of CFS from *L. sakei* was done as previously described [20].

Sampling and RNA isolation. The transcriptome study was performed on *L. monocytogenes* strains grown in LB broth supplemented with mannose or cellobiose (0.5%, wt/vol) in the absence of bacteriocin. Sampling was done at mid-exponential phase and total RNA extraction was done using an RNeasy Protect bacterial mini kit [20]. The sakacin P-resistance phenotype was verified on selective plates containing sakacin P at the time of harvesting for RNA isolation. The quantity and quality of RNA was checked using NanoDrop ND-1000 (NanoDrop Technologies, Rockland, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). The isolated RNA sample was used both for the microarray and for validation assays by qRT-PCR. Two independently isolated RNA samples from each growth condition were used.

Microarray analysis. The PCR based DNA microarray slides were developed and printed by The Bacterial Microarray Group at St George's (BμG@S), University of London, UK. Protocols for the development and printing of the microarray slides have been described elsewhere [52,53]. The array design is available in BμG@Sbase (Accession No. A-BUGS-19; http://bugs.sgul.ac.uk/A-BUGS-19) and also ArrayExpress (Accession No. A-BUGS-19).

The cDNA synthesis and labeling were performed using 4 µg total RNA following guidelines from BµG@S [53]. The sakacin P-resistant mutants grown on mannose or cellobiose were compared to the wild type-strain grown on the respective sugar. Dye-swap hybridization was performed for each sample and the hybridization was done as described previously [52,53]. Scanning of the arrays was performed using Tecan LS scanner (Tecan, Männedorf, Switzerland). Spot-identification, -segmentation and -fluorescent intensity quantification were done using ImaGene 5.5 (BioDiversity, El Segundo, USA).

The microarray data analysis was done by the LIMMA package [54] in the R computing environment (http://www.r-project.org/). Preprocessing and normalization followed a standard procedure using methods described by Smyth and Speed [55]. Testing for differentially expressed genes was done using a linear mixed model as described [56]. A mixed-model approach was chosen to adequately describe between-array variation, and utilized the probe-replicates in each array. An empirical Bayes smoothing of gene-wise variances was conducted according to Smyth et al. [57]. For each gene, the p-value was adjusted to control the false discovery rate and the adjusted p-values were referred as q-values. Differentially expressed genes were selected using q-values less than 0.01 and with log ratio of ≥ 0.585 or ≤ -0.585 (equivalent to ≥ 1.5-fold changes).

The preprocessed data were also analyzed using The Unscrambler software (The Unscrambler v9.8; CAMO AS, Norway), GeneSpring GX7.3 (Agilent Technologies, Santa Clara, USA) and the LEGER database [58]. For the PCA, genes that were differentially expressed in any of the growth conditions were included. Hierarchical clustering (complete linkage, correlation distance metric) of all differentially expressed genes was used to generate a heatmap. Genes were grouped according to the role category of the CMR-JCVI (http://cmr.jcvi.org). A chi-square and odd ratios analysis was performed to determine if association exists between the deferentially expressed genes and the CMR-JCVI role category (http://faculty.vassar.edu/lowry/odds2x2.html). Known and putative genes involved in virulence were identified from the recent genome-wide transcriptional studies [25].

Hemolytic activity assay. An agar based hemolytic assay was performed on the overnight cultures grown in the LB broth supplemented with cellobiose. The blood agar was divided into four sections, and equal volume of bacterial suspensions of the wild-type and the sakacin P-resistant strains were spotted onto each quadrant. A no-growth control (only the broth) and

parallel inoculation of the bacterial suspensions onto TSA were included as a control. All the plates were incubated at 37°C and halos due to hemolytic activity were checked visually after 16-18 h. The assay was done twice on different days in duplicate. Hemolytic activity in the supernatant from the overnight cultures grown in the LB broth supplemented with cellobiose and activated charcoal was analyzed as described previously [38]. A total of six measurements were performed on two samples collected on different days. Viable cell count was performed to check if the addition of activated charcoal had effect on growth. Statistical significance test was performed using a t-test.

Lecithinase activity assay. A lecithinase activity assay was performed on agar containing egg-yolk [59]. The TSA egg-yolk agars were inoculated with the bacterial culture suspension following the same protocol described for the agar based hemolytic activity assay. Halos due to lecithinase activity were estimated after 72 h of incubation at 37°C. The assay was done in two biological duplicates each consisting at least two technical duplicates.

Infection with Listex P100. The *L. monocytogenes* strains were infected with bacteriophage P100 in LB broth supplemented with mannose or cellobiose. Phage titration of the stock solution of Listex P100 was performed following the instruction provided by the supplier (EBI food safety, Wageningen, The Netherlands). The *L. monocytogenes* strains were prepared as described earlier [20] and were infected with 10, 10^{-1}, 10^{-6} and 10^{-9} MOI (multiplicity of infection). Growth was monitored using a Bioscreen C instrument (Oy Growth Curves Ab Ltd., Helsinki, Finland) at 30°C for 18 h as described [20]. The assay was done twice on different days each consisting six replicates.

Stability of sakacin P resistance. The stability of the sakacin P resistance phenotype of L502-1 and L502-6 was performed as described before [20] with a slight modification. In the present study, CFS from the non-bacteriocinogenic *L. sakei* Lb790(pLPV111) used as a negative control [50]. The stability assay was performed in triplicates on different days.

Validation of microarray data by qRT-PCR assay. The microarray results were validated on six selected genes by qRT-PCR performed as previously described [20]. Selection of genes for the validation assay was following the guidelines suggested by Morey et al. [60]. The primer and probe sets for *actA* (encoding actin assembly-inducing protein), *lmo1251* (encoding a putative CRP-FNR family transcriptional regulator), *glpK* (glycerol kinase) and

kdpA (potassium-transporting ATPase A chain) were designed using Primer Express 3.0. The sets for the *mpt*A (mannose permease two, AB subunit) and 16S rRNA have been reported earlier [20] (Table S2).

Microarray data accession number. Microarray data are MIAME compliant. Fully annotated microarray data have been deposited in BμG@Sbase (accession number E-BUGS-110; http://bugs.sgul.ac.uk/E-BUGS-110) and also ArrayExpress (accession number E-BUGS-110).

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Table 1. The sakacin P-resistant strains of *L. monocytogenes* L502 used in this study.

Sakacin P resistant strains	Characteristics[a]			
	Level of resistance to sakacin P	Growth rate on mannose (%)	Growth rate on cellobiose (%)	*mptA* gene expression
L502-1	$>10^6$	82	107	repressed
L502-6	10^3	44	105	induced

[a] relative to the wild-type strain (mutant/wild-type) and detail can be found in reference [20].

Table 2. Differentially regulated genes in L502-1 during growth on both sugars.

Locus (gene)	Product (similar to)	Mannose[a]		Cellobiose	
		Log2	q-value	Log2	q-value
lmo0027	PTS, beta-glucosides specific enzyme IIABC	1.5	10^{-5}	4.1	10^{-7}
lmo0096 (*mptA*)	PTS mannose-specific, factor IIAB	-2.3	10^{-7}	1.4	10^{-4}
lmo0097 (*mptC*)	PTS mannose-specific, factor IIC	-2.4	10^{-9}	1.5	10^{-6}
lmo0098 (*mptD*)	PTS mannose-specific, factor IID	-2.4	10^{-11}	1.4	10^{-7}
lmo0204 (*actA*)	actin-assembly inducing protein precursor	-0.7	10^{-5}	-0.8	10^{-4}
lmo0781 (*mpoD*)	mannose-specific PTS component IID	1.2	10^{-6}	1	10^{-7}
lmo0782 (*mpoC*)	mannose-specific PTS component IIC	1.4	10^{-6}	1.7	10^{-8}
lmo0783 (*mpoB*)	mannose-specific PTS component IIB	1.2	10^{-6}	1.9	10^{-7}
lmo0784 (*mpoA*)	mannose-specific PTS component IIA	1.5	10^{-6}	2.1	10^{-9}
lmo0848	amino acid ABC transporter, ATP-binding protein	0.6	10^{-4}	0.7	10^{-6}
lmo1251	regulator of the Fnr CRP family (including PrfA)	-1.4	10^{-7}	-1.4	10^{-6}
lmo1254	alpha, alpha-phosphotrehalase	2.3	10^{-8}	0.6	10^{-5}
lmo1255	PTS trehalose specific enzyme IIBC	2.5	10^{-9}	0.6	10^{-7}
lmo2250 (*arpJ*)	amino acid ABC transporter, permease protein	-0.7	10^{-7}	-0.6	10^{-3}
lmo2683	cellobiose PTS enzyme IIB component	-1.5	10^{-6}	-1.4	10^{-8}
lmo2684	cellobiose PTS enzyme IIC component	-1.7	10^{-9}	-0.8	10^{-6}
lmo2685	cellobiose PTS enzyme IIA component	-1.3	10^{-6}	-1.4	10^{-8}

[a] Differentially regulated genes in the high level sakacin P-resistant strain (L502-1) during growth on mannose and on cellobiose. Log2 (expression ratio) is relative to the wild-type strain and q-value is a p-value adjusted to control false discovery rate.

Table 3. Differentially regulated genes in L502-6 during growth on both sugars.

Locus (gene)	Product (similar to)	Mannose[a]		Cellobiose	
		Log2	q-value	Log2	q-value
lmo0096 (*mptA*)	PTS mannose-specific, factor IIAB	1.6	10^{-7}	2.6	10^{-7}
lmo0097 (*mptC*)	PTS mannose-specific, factor IIC	1.6	10^{-6}	2.7	10^{-7}
lmo0098 (*mptD*)	PTS mannose-specific, factor IID	1.6	10^{-6}	2.6	10^{-9}
lmo0099	unknown	1.5	10^{-6}	0.8	10^{-4}
lmo0361 (*tatC*)	twin arginine translocase C	1.2	10^{-5}	1.2	10^{-4}
lmo0362 (*tatA*)	twin arginine translocase A	0.9	10^{-4}	1.0	10^{-3}
lmo0365	conserved hypothetical protein	1.4	10^{-4}	1.5	10^{-4}
lmo0366	putative lipoprotein	1.1	10^{-5}	1.2	10^{-3}
lmo0367	*B. subtilis* YwbN protein	1.4	10^{-5}	1.5	10^{-4}
lmo0433 (*inlA*)	internalin A	-0.8	10^{-6}	-0.6	10^{-5}
lmo0485	unknown	0.6	10^{-3}	0.8	10^{-3}
lmo0541	ABC transporter (binding protein)	0.8	10^{-3}	1.0	10^{-3}
lmo0847	glutamine ABC transporter (binding and transport protein)	0.7	10^{-5}	1.0	10^{-5}
lmo0848	amino acid ABC transporter, ATP-binding protein	0.7	10^{-5}	0.9	10^{-4}
lmo1007	unknown	0.7	10^{-4}	0.7	10^{-3}
lmo1251	regulator of the Fnr CRP family (including PrfA)	-2.0	10^{-6}	-1.6	10^{-5}
lmo1566 (*citC*)	isocitrate dehyrogenases	0.7	10^{-3}	0.7	10^{-3}
lmo1567 (*citZ*)	citrate synthase subunit II	0.8	10^{-5}	0.8	10^{-4}
lmo1641	aconitate hydratases	0.8	10^{-4}	0.9	10^{-5}
lmo1957 (*fhuG*)	ferrichrome ABC transporter (permease)	0.6	10^{-4}	0.7	10^{-3}
lmo1958 (*fhuB*)	ferrichrome ABC transporter (permease)	0.6	10^{-4}	0.7	10^{-3}
lmo1959 (*fhuD*)	ferrichrome binding protein	0.8	10^{-5}	1.0	10^{-3}
lmo1960 (*fhuC*)	ferrichrome ABC transporter (ATP-binding protein)	0.6	10^{-4}	0.6	10^{-3}
lmo2181 (*srtB*)	Sortase B protein	0.9	10^{-4}	1.0	10^{-3}
lmo2182	ferrichrome ABC transporter (ATP-binding protein)	0.9	10^{-4}	1.1	10^{-3}
lmo2183	ferrichrome ABC transporter (permease)	0.9	10^{-5}	1.0	10^{-3}
lmo2184	ferrichrome ABC transporter (binding protein)	1.2	10^{-4}	1.2	10^{-4}
lmo2185 *(svpA)*	surface virulence-associated protein, substrate for SrtB	1.1	10^{-4}	1.2	10^{-3}
lmo2186 *(isdC)*	iron-regulated surface determinants, substrate for SrtB	1.3	10^{-4}	1.4	10^{-4}
lmo2363 (*gadD2*)	glutamate decarboxylase	-0.8	10^{-3}	-1.1	10^{-3}
lmo2781	beta-glucosidase	0.8	10^{-6}	0.6	10^{-5}

[a] Differentially regulated genes in the low level sakacin P-resistant strain (L502-6) during growth on mannose and on cellobiose. Log2 (expression ratio) is relative to the wild-type strain and q-value is a p-value adjusted to control false discovery rate.

Figure Legends

Figure 1. PCA score plot of the differentially regulated genes in the sakacin P-resistant strains. PCA score plot displaying global transcriptome profile of L502-1 grown on mannose (♦), L502-1 grown on cellobiose (◊), L502-6 grown on mannose (●) and L502-6 grown on cellobiose (○). The two biological samples and their dye-swap replicates were included in the PCA. The explained variances for the two first components; PC1and PC2 are 53% and 27% respectively.

Figure 2. Presentation of the differentially regulated genes using a Venn diagram and heatmap. (**A**) A Venn diagram showing total number of common and specific genes differentially regulated in the sakacin P-resistant strains (L502-1 and L502-6) grown on mannose (M) or cellobiose (C). (**B**) A heatmap showing the differentially regulated genes in the sakacin P-resistant strains (L502-1 and L502-6) during growth on mannose (M) or on cellobiose (C). The designated biological role(s) of proteins encoded by the genes with similar pattern of regulations are shown in bracket. Color code: red, up-regulated; green, down-regulated; black, not differentially expressed.

Figure 3. Differentially regulated virulence determinant genes. Virulence determinant genes regulated in the sakacin P-resistant strains in any of the growth conditions. List of virulent determinate genes were taken from reference [25].

Figure 4. *In vitro* hemolytic and lecithinase activities. (**A**) Hemolytic activity on thin blood agar and (**B**) Lecithinase activity on egg-yolk agar of the wild-type (L502) and the sakacin P-resistant strains (L502-1 and L502-6). Images are a representative of two independent experiments each consisting of duplicates with reproducible results.

Figure 5. Infection with the lytic bacteriophage P100.
Growth of the sakacin P-resistant strains and the wild-type (**A**) not infected with the phage P100 and (**B**) infected with phage P100 at MOI of 10^{-9}. Symbols: L502 grown on mannose, ▲; L502 on cellobiose, △; L502-1 on mannose, ♦; L502-1 on cellobiose, ◊; L502-6 on mannose, ●; L502-1 on cellobiose, ○. Values shown are mean and standard error of the mean (error bars) of a representative of two independent experiments with six measurements each.

Figure 1

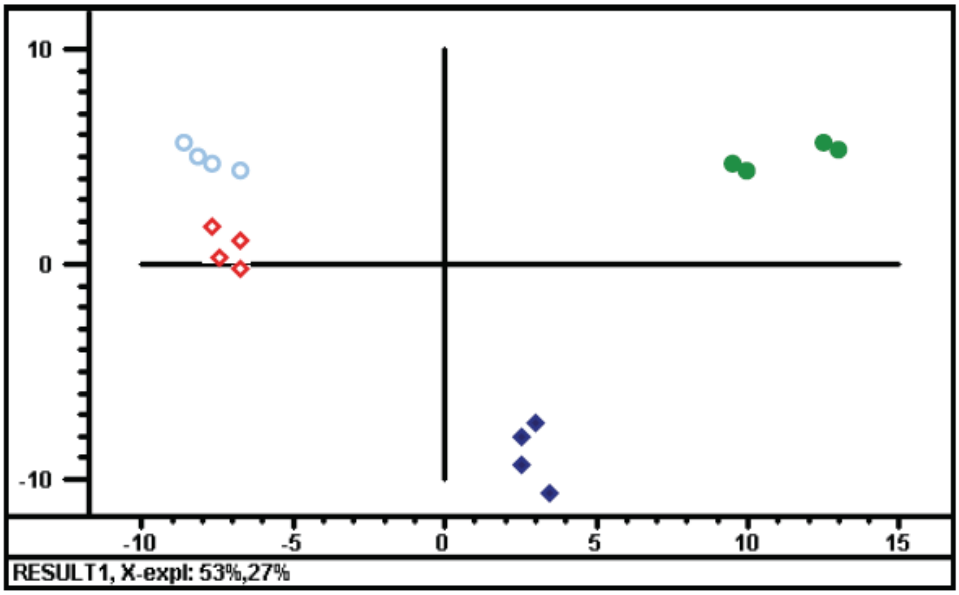


Figure 2

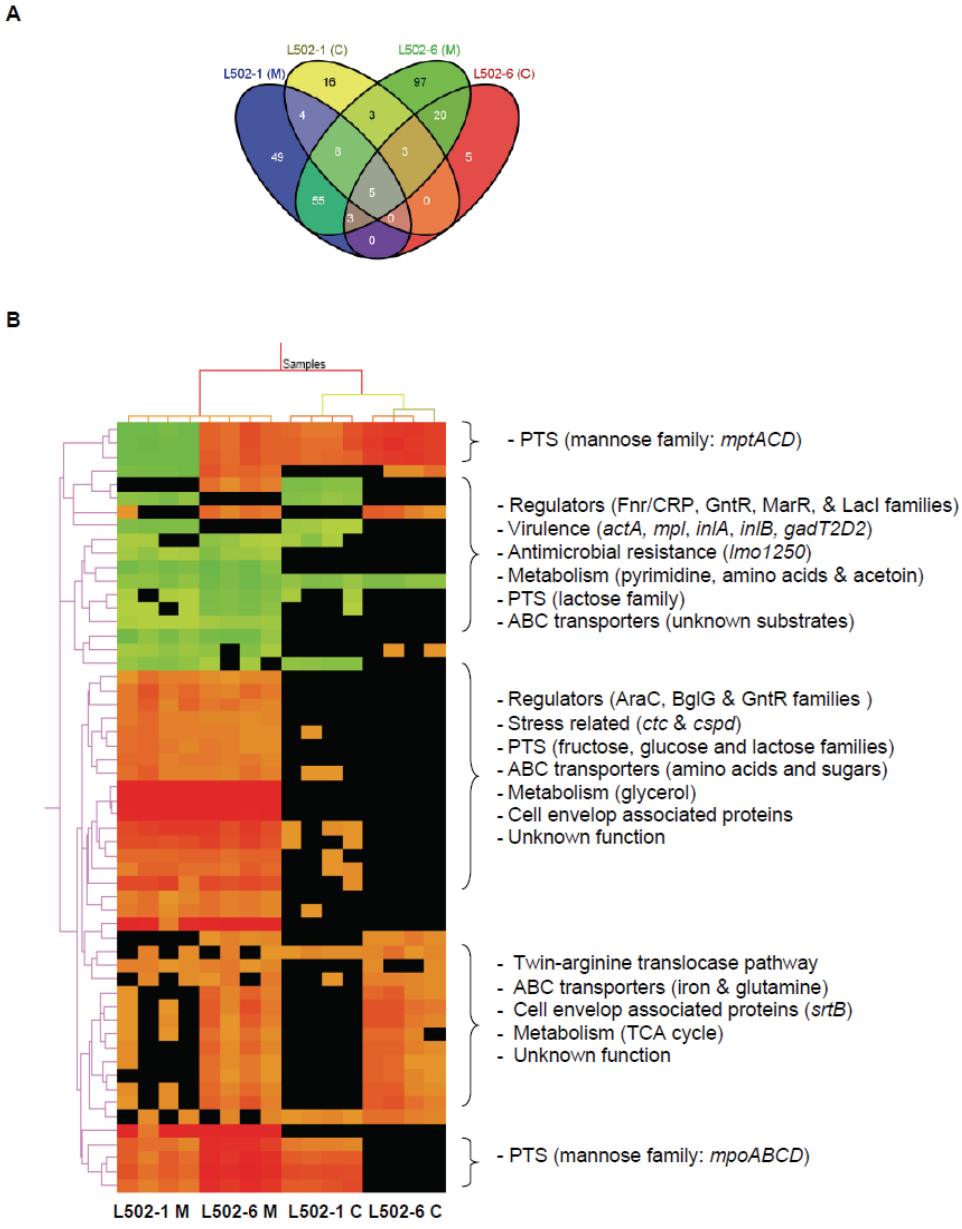

A
L502-1 (C)
L502-6 (M)
L502-1 (M)
L502-6 (C)
16
97
4
3
20
49
8
3
5
55
5
3
0
0
B
Samples
- PTS (mannose family: mptACD)
- Regulators (Fnr/CRP, GntR, MarR, & LacI families)
- Virulence (actA, mpl, inlA, inlB, gadT2D2)
- Antimicrobial resistance (lmo1250)
- Metabolism (pyrimidine, amino acids & acetoin)
- PTS (lactose family)
- ABC transporters (unknown substrates)
- Regulators (AraC, BglG & GntR families)
- Stress related (ctc & cspd)
- PTS (fructose, glucose and lactose families)
- ABC transporters (amino acids and sugars)
- Metabolism (glycerol)
- Cell envelop associated proteins
- Unknown function
- Twin-arginine translocase pathway
- ABC transporters (iron & glutamine)
- Cell envelop associated proteins (srtB)
- Metabolism (TCA cycle)
- Unknown function
- PTS (mannose family: mpoABCD)
L502-1 M
L502-6 M
L502-1 C
L502-6 C

Figure 3

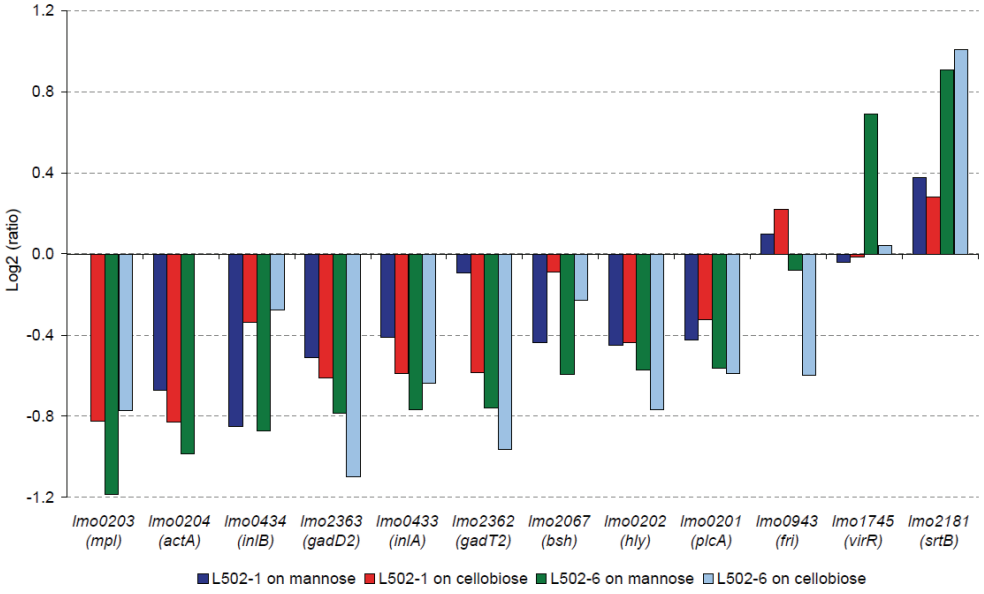

1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
Log2 (ratio)
lmo0203 (mpl)
lmo0204 (actA)
lmo0434 (inlB)
lmo2363 (gadD2)
lmo0433 (inlA)
lmo2362 (gadT2)
lmo2067 (bsh)
lmo0202 (hly)
lmo0201 (plcA)
lmo0943 (fri)
lmo1745 (virR)
lmo2181 (srtB)
L502-1 on mannose
L502-1 on cellobiose
L502-6 on mannose
L502-6 on cellobiose

Figure 4

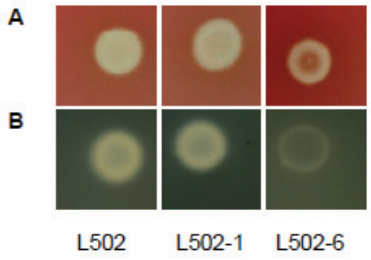
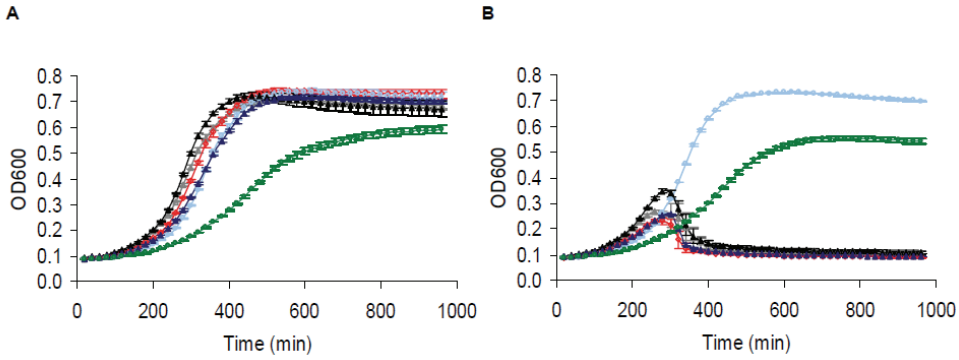
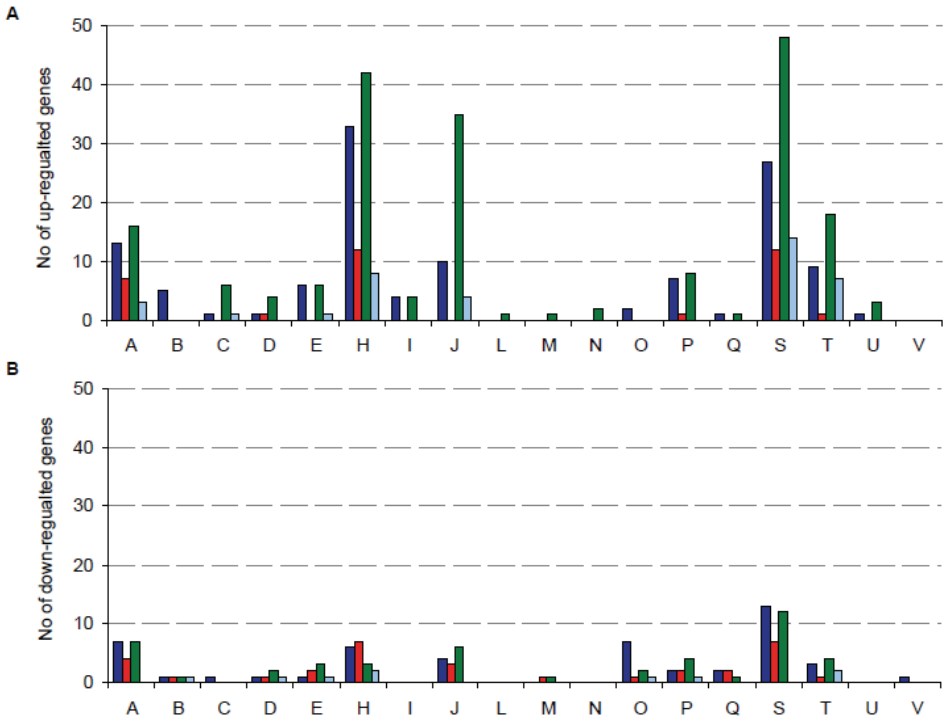


Figure 5



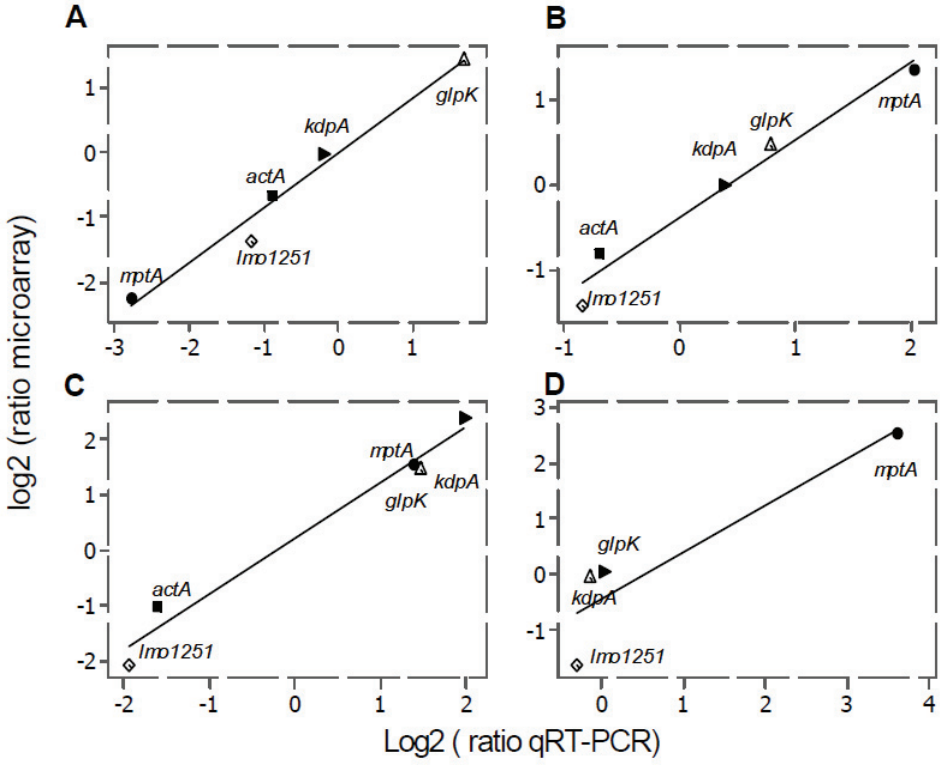
Supporting Information

Figure S1. Total number of regulated genes grouped according to their biological role.



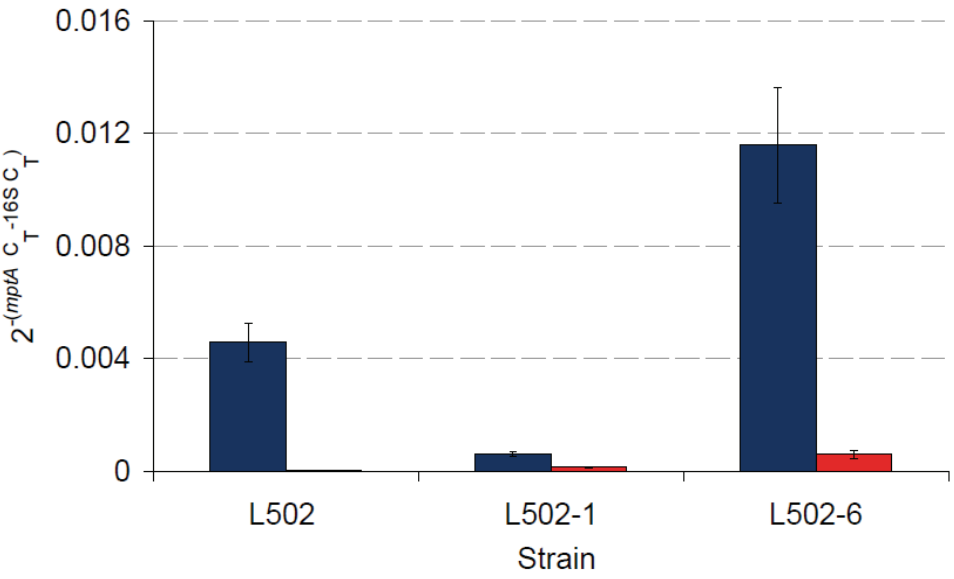
Total number of (**A**) up-regulated and (**B**) down-regulated genes in different functional role categories according to the primary annotation in CMR-JCVI *L. monocytogenes* EGDe genome database in the L502-1 grown on mannose (■), L502-1 grown on cellobiose (■), L502-6 grown on mannose (■) and L502-6 grown on cellobiose (■). A, Amino acid biosynthesis; B, Biosynthesis of cofactors, prosthetic groups, and carriers; C, Cell envelope; D, Cellular processes; E, Central intermediary metabolism; H, Energy metabolism; I, Fatty acid and phospholipid metabolism; J, Hypothetical proteins; L, Mobile and extrachromosomal element functions; M, Protein fate; N, Protein synthesis; O, Purines, pyrimidines, nucleosides, and nucleotides; P, Regulatory functions; Q, Signal transduction; S, Transport and binding proteins; T, Unclassified; U, Unknown function; V, Viral functions.

Figure S2. Validation of the microarray data by qRT-PCR analysis.



Validation of microarray data (y-axis) by qRT-PCR analysis (x-axis) on six genes. The transcriptional studies for the sakacin P-resistant strains (L502-1 and L502-6) and the wild-type (L502) was performed during growth on mannose or cellobiose. (**A**) L502-1 grown on mannose (**B**) L502-1 grown on cellobiose, (**C**) L502-6 grown on mannose, and (**D**) L502-6 grown on cellobiose and all the log2 ratio values are relative to the wild-type (L502) grown on the respective sugars. The data presented here represent results from two biological replicates.

Figure S3. Transcript level of the *mptA* gene.



Transcript level of the *mptA* gene extracted from qRT-PCR analysis in the sakacin P-resistant (L502-1 and L502-6) and the wild-type (L502) strain during growth on mannose (■) or cellobiose (■). Error bar indicates the standard error of the mean of two independent experiments.

Table S1. List of all differentially regulated genes.

Locus	Gene	Description (similar to)[a]	Role(s)[a]	L502-1 (M)[b]		L502-1 (C)		L502-6 (M)		L502-6 (C)	
				Log2	q-value	Log2	q-value	Log2	q-value	Log2	q-value
Amino acid biosynthesis											
lmo1588	*argD*	N-acetylornithine aminotransferase		-0.45	0.028	-0.26	0.046	**-0.92**	**0.001**	-0.11	0.462
lmo1589	*argB*	N-acetylglutamate 5-phosphotransferase		-0.35	0.167	-0.21	0.002	**-0.98**	**0.001**	0.02	0.862
lmo1590	*argJ*	ornithine acetyltransferase and amino-acid acetyltransferases		NA	NA	NA	NA	**-0.62**	**0.006**	-0.06	0.614
lmo2006	*alsS*	alpha-acetolactate synthase protein, AlsS		**-0.93**	**0.000**	-0.32	0.001	**-1.18**	**0.000**	0.17	0.183
lmo2090	*argG*	argininosuccinate synthase		-0.42	0.000	-0.53	0.011	**-1.27**	**0.000**	0.58	0.041
lmo2091	*argH*	argininosuccinate lyase		-0.29	0.308	-0.35	0.012	**-1.35**	**0.001**	0.18	0.422
lmo2252		aspartate aminotransferase		**-0.59**	**0.000**	-0.47	0.000	**-1.44**	**0.000**	-0.21	0.004
Biosynthesis of cofactors, prosthetic groups, and carriers											
lmo1042		molybdopterin biosynthesis protein moeA		**0.64**	**0.000**	0.12	0.220	0.52	0.000	0.02	0.840
lmo1043		molybdopterin-guanine dinucleotide biosynthesis MobB		**0.69**	**0.000**	0.15	0.036	0.51	0.000	0.04	0.493
lmo1045		molybdopterin converting factor (subunit 1).		**0.59**	**0.000**	0.11	0.152	0.39	0.000	0.00	0.971
lmo1046	*moaC*	molybdenum cofactor biosynthesis protein C		**0.60**	**0.000**	0.14	0.066	0.50	0.000	0.04	0.568
lmo1047		molybdenum cofactor biosynthesis protein A		**0.64**	**0.000**	0.15	0.028	0.49	0.000	-0.02	0.804
lmo1194	*cbiD*	cobalamin biosynthesis protein CbiD		**-0.68**	**0.001**	-0.39	0.054	0.06	0.233	-0.02	0.832
Cell envelope											
lmo0129		autolysin: N-acetylmuramoyl-L-alanine amidase		**-0.73**	**0.000**	-0.30	0.104	0.11	0.386	-0.23	0.220
lmo0195		membrane protein (putative ABC transporter)		-0.16	0.017	0.01	0.913	**1.90**	**0.000**	0.10	0.109
lmo0366		conserved hypothetical protein, putative lipoprotein		0.39	0.002	0.38	0.273	**1.14**	**0.000**	**1.23**	**0.001**
lmo0880		wall associated protein precursor (LPXTG motif)		0.12	0.155	0.06	0.883	**1.02**	**0.001**	-0.03	0.826
lmo1044		molybdopterin converting factor, subunit 2		**0.66**	**0.000**	0.13	0.298	0.53	0.000	0.00	0.990
lmo1215		N-acetylmuramoyl-L-alanine amidase (autolysin)		-0.10	0.065	-0.10	0.169	**1.10**	**0.000**	-0.10	0.066
lmo2229		penicillin-binding protein		-0.06	0.483	-0.04	0.683	**0.89**	**0.000**	-0.01	0.891
lmo2484		*B. subtilis* YvlD protein		-0.08	0.394	-0.09	0.214	**2.73**	**0.000**	-0.01	0.958
Cellular processes											
lmo0028		*E. coli* microcin C7 self-immunity protein (MccF)		0.07	0.398	**2.24**	**0.000**	-0.30	0.114	0.25	0.069
lmo0433	*inlA*	internalin A		-0.41	0.000	**-0.59**	**0.000**	**-0.77**	**0.000**	**-0.64**	**0.000**
lmo0434	*inlB*	internalin B		**-0.85**	**0.000**	-0.34	0.088	**-0.87**	**0.001**	-0.27	0.035
lmo0601		cell surface protein		-0.04	0.483	-0.09	0.430	**0.68**	**0.000**	-0.01	0.951
lmo1879	*cspD*	cold shock protein		**1.21**	**0.000**	0.36	0.002	**0.84**	**0.000**	0.06	0.773
lmo1967		toxic ion resistance proteins		-0.10	0.271	-0.06	0.556	**1.99**	**0.000**	0.20	0.397
lmo2230		arsenate reductase		-0.01	0.928	0.06	0.622	**0.90**	**0.000**	0.04	0.784
Central intermediary metabolism											
lmo0372		beta-glucosidase		**-1.85**	**0.000**	0.10	0.304	**-2.21**	**0.000**	0.06	0.818
lmo0574		beta-glucosidase		0.25	0.001	**-0.97**	**0.000**	0.15	0.038	-0.21	0.019
lmo0877		*B. subtilis* NagB protein		**0.61**	**0.001**	0.21	0.053	0.50	0.011	0.04	0.515
lmo0878		oxidoreductases		**0.68**	**0.000**	0.22	0.023	0.50	0.000	0.03	0.671
lmo2555		human N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein	N	-0.15	0.002	-0.09	0.215	**0.80**	**0.000**	0.03	0.620
lmo2721		glucosamine-6-phosphate isomerase		-0.50	0.001	-0.25	0.001	**-0.62**	**0.000**	-0.27	0.091
lmo2798		phosphatase		**0.84**	**0.000**	0.17	0.160	**0.87**	**0.001**	0.28	0.056
Energy metabolism											
lmo0105		chitinase B		0.56	0.033	**-1.61**	**0.000**	**1.02**	**0.000**	-0.09	0.166
lmo0261		phospho-beta-glucosidase		0.53	0.000	0.14	0.059	**0.63**	**0.000**	0.19	0.018
lmo0319		phospho-beta-glucosidase		0.31	0.000	**2.61**	**0.000**	0.25	0.010	0.49	0.014
lmo0343		transaldolase		**0.84**	**0.000**	0.32	0.003	**0.81**	**0.000**	0.25	0.006
lmo0344		dehydrogenase/reductase		**0.80**	**0.000**	0.36	0.005	**0.74**	**0.000**	0.30	0.002
lmo0345		sugar-phosphate isomerase		**0.96**	**0.000**	0.27	0.006	**0.75**	**0.000**	0.11	0.686
lmo0346		triosephosphate isomerase		0.60	0.013	0.37	0.090	**0.63**	**0.004**	0.14	0.342
lmo0347		dihydroxyacetone kinase	I	**0.81**	**0.001**	0.29	0.032	**0.77**	**0.007**	0.19	0.140
lmo0485			E	0.06	0.704	0.25	0.208	**0.65**	**0.007**	**0.79**	**0.002**
lmo0517		phosphoglycerate mutase		0.25	0.097	**2.45**	**0.000**	NA	NA	0.79	0.010
lmo0643		putative transaldolase		**0.78**	**0.000**	0.37	0.006	0.42	0.000	0.04	0.832
lmo0813		fructokinases		**1.10**	**0.000**	-0.20	0.238	**0.83**	**0.000**	0.29	0.013
lmo0917		beta-glucosidase		**0.89**	**0.000**	0.05	0.391	**1.01**	**0.000**	0.16	0.039
lmo0943	*fri*	non-heme iron-binding ferritin		0.10	0.496	0.22	0.067	-0.08	0.535	**-0.60**	**0.008**
lmo1254		alpha,alpha-phosphotrehalase		**2.30**	**0.000**	**0.59**	**0.000**	**2.01**	**0.000**	0.08	0.224
lmo1293	*glpD*	Glycerol-3-phosphate dehydrogenase		**1.98**	**0.000**	0.54	0.000	**2.11**	**0.000**	0.09	0.178
lmo1406	*pflB*	pyruvate formate-lyase		-0.20	0.091	**-0.64**	**0.000**	-0.15	0.018	0.10	0.092
lmo1538	*glpK*	glycerol kinase		**1.43**	**0.000**	0.46	0.000	**1.46**	**0.000**	-0.02	0.803
lmo1566	*citC*	isocitrate dehyrogenases		0.46	0.154	0.45	0.011	**0.68**	**0.006**	**0.71**	**0.005**
lmo1567	*citZ*	citrate synthase subunit II		0.55	0.037	0.40	0.000	**0.75**	**0.000**	**0.82**	**0.001**
lmo1579		alanine dehydrogenase		-0.04	0.440	-0.14	0.226	**0.80**	**0.009**	-0.33	0.309
lmo1641	*citB*	aconitate hydratases		**0.59**	**0.001**	0.47	0.003	**0.84**	**0.000**	**0.85**	**0.000**
lmo1992	*alsD*	alpha-acetolactate decarboxylase		-0.43	0.000	-0.24	0.001	**-0.70**	**0.000**	-0.07	0.506
lmo2122		maltodextrose utilization protein MalA	A	**0.61**	**0.000**	0.23	0.121	0.17	0.219	0.33	0.100
lmo2124		maltodextrin ABC-transport system (permease)		**0.68**	**0.000**	0.20	0.207	0.34	0.001	0.35	0.038
lmo2159		oxidoreductase	E	**1.11**	**0.000**	0.25	0.027	**0.82**	**0.000**	0.34	0.020
lmo2163		oxidoreductase	E	**1.28**	**0.000**	0.34	0.058	**1.07**	**0.000**	0.41	0.035
lmo2363	*gadD2*	glutamate decarboxylase	B,E,O	-0.51	0.021	**-0.61**	**0.006**	**-0.78**	**0.008**	**-1.10**	**0.001**
lmo2436		transcription antiterminator		0.32	0.000	**2.15**	**0.000**	0.18	0.014	0.44	0.047
lmo2584		formate dehydrogenase associated protein		**0.65**	**0.000**	0.19	0.061	0.41	0.000	-0.11	0.154
lmo2586		formate dehydrogenase alpha chain		**1.13**	**0.000**	0.25	0.018	**0.87**	**0.000**	-0.11	0.128
lmo2664		sorbitol dehydrogenase	E	**0.60**	**0.000**	0.15	0.015	0.14	0.033	-0.17	0.029
lmo2695		dihydroxyacetone kinase	I	**0.61**	**0.000**	0.16	0.070	**0.95**	**0.000**	0.05	0.370
lmo2696		hypothetical dihydroxyacetone kinase	I	0.57	0.000	0.15	0.027	**0.94**	**0.000**	0.11	0.103
lmo2720	*acs*	acetate-CoA ligase		**-0.66**	**0.000**	-0.36	0.004	**-1.02**	**0.000**	-0.21	0.011
lmo2743		transaldolase		**0.75**	**0.000**	0.09	0.447	**0.81**	**0.000**	0.15	0.061
lmo2771		beta-glucosidase		0.35	0.000	**0.86**	**0.000**	0.09	0.180	0.13	0.279
lmo2836		alcohol dehydrogenase	E	0.29	0.001	0.16	0.339	**0.65**	**0.002**	0.23	0.440
lmo2840		sucrose phosphorylase	O	**0.64**	**0.001**	0.26	0.057	0.47	0.000	0.34	0.028

Table S1. continued.

Locus	Gene	Description (similar to)[a]	Role(s)[a]	L502-1 (M)[b]		L502-1 (C)		L502-6 (M)		L502-6 (C)	
				Log2	q-value	Log2	q-value	Log2	q-value	Log2	q-value
Fatty acid and phospholipid metabolism											
lmo0110		lipase		**0.90**	**0.001**	0.36	0.041	**0.74**	**0.005**	0.06	0.858
lmo2175		dehydrogenase		**0.60**	**0.001**	0.39	0.004	0.45	0.030	-0.14	0.354
Hypothetical proteins											
lmo0099				**-1.96**	**0.000**	0.30	0.002	**1.49**	**0.000**	**0.80**	**0.001**
lmo0119				**-0.70**	**0.000**	-0.23	0.104	-0.20	0.478	NA	NA
lmo0120				-0.97	0.012	**-0.69**	**0.004**	0.09	0.442	-0.27	0.309
lmo0125				**-0.63**	**0.004**	-0.23	0.324	-0.24	0.160	-0.12	0.677
lmo0127		protein gp20 from Bacteriophage A118		**-0.82**	**0.000**	-0.17	0.491	-0.12	0.558	-0.09	0.748
lmo0193				-0.14	0.004	0.00	0.972	**2.21**	**0.000**	0.18	0.082
lmo0323		unknown proteins		**0.64**	**0.000**	0.41	0.000	**0.77**	**0.001**	0.25	0.046
lmo0335				NA	NA	NA	NA	**-0.65**	**0.007**	NA	NA
lmo0349				**0.66**	**0.004**	0.29	0.097	**1.00**	**0.000**	0.34	0.368
lmo0350				0.43	0.022	0.20	0.107	**0.59**	**0.000**	0.20	0.163
lmo0365		conserved hypothetical protein		0.41	0.009	0.46	0.212	**1.38**	**0.000**	**1.52**	**0.000**
lmo0391				0.50	0.000	0.16	0.018	**0.67**	**0.000**	0.07	0.354
lmo0397		unknown proteins		0.48	0.000	-0.07	0.305	**0.70**	**0.000**	0.07	0.309
lmo0412				-0.64	0.025	-0.39	0.002	**-0.94**	**0.000**	-0.08	0.719
lmo0477		putative secreted protein		0.45	0.000	0.18	0.009	**0.60**	**0.000**	-0.05	0.508
lmo0546		putative NAD(P)-dependent oxidoreductase		**0.78**	**0.003**	0.19	0.285	0.41	0.140	0.44	0.091
lmo0599		conserved hypothetical protein		-0.18	0.005	-0.14	0.054	**0.77**	**0.000**	0.09	0.272
lmo0600				-0.10	0.078	-0.05	0.630	**0.83**	**0.000**	0.09	0.237
lmo0864				0.27	0.049	0.15	0.100	**0.76**	**0.001**	0.13	0.141
lmo0879				**0.97**	**0.007**	0.45	0.009	**0.76**	**0.003**	-0.26	0.272
lmo0954				0.03	0.508	-0.07	0.333	**3.24**	**0.000**	0.00	0.957
lmo0955				0.06	0.573	-0.08	0.233	**2.70**	**0.000**	-0.03	0.801
lmo1007				0.20	0.084	0.17	0.283	**0.66**	**0.001**	**0.70**	**0.005**
lmo1020		*B. subtilis* YvqF protein		0.05	0.097	0.09	0.147	**1.39**	**0.000**	0.11	0.154
lmo1190				**0.66**	**0.000**	0.21	0.020	0.27	0.005	0.06	0.529
lmo1249				-0.72	0.017	-0.41	0.000	**-0.73**	**0.000**	-0.50	0.000
lmo1257				-0.30	0.002	**-1.11**	**0.009**	-0.18	0.036	-0.43	0.030
lmo1385		unknown protein		-0.08	0.074	-0.05	0.808	**-0.80**	**0.000**	-0.21	0.036
lmo1429		unknown proteins		-0.18	0.008	-0.23	0.228	**-0.95**	**0.009**	-0.23	0.111
lmo1637		membrane proteins		-0.07	0.051	-0.02	0.863	**2.27**	**0.000**	-0.05	0.604
lmo1690		hypothetical proteins		0.08	0.022	-0.25	0.001	**0.67**	**0.000**	-0.28	0.066
lmo1718		putative outer surface protein		-0.10	0.024	**-1.27**	**0.000**	-0.07	0.232	-0.16	0.043
lmo1728		cellobiose-phosphorylase		0.28	0.060	0.11	0.411	**0.69**	**0.001**	0.44	0.058
lmo1945		unknown protein		-0.29	0.007	-0.36	0.009	**-0.66**	**0.004**	-0.21	0.022
lmo2160		unknown proteins		**1.18**	**0.000**	0.34	0.006	**1.03**	**0.000**	0.40	0.011
lmo2161				**1.37**	**0.000**	0.49	0.008	**1.00**	**0.000**	0.39	0.049
lmo2162		unknown proteins		**1.28**	**0.000**	0.33	0.038	**1.02**	**0.000**	0.41	0.083
lmo2181		unknown protein		0.38	0.004	0.28	0.399	**0.91**	**0.000**	**1.01**	**0.002**
lmo2210				-0.12	0.295	-0.25	0.479	**1.38**	**0.000**	-0.21	0.372
lmo2224		unknown proteins		-0.09	0.082	-0.07	0.234	**1.67**	**0.000**	0.01	0.874
lmo2258				-0.06	0.286	-0.14	0.118	**1.01**	**0.000**	-0.32	0.044
lmo2486				-0.06	0.328	-0.06	0.703	**2.78**	**0.000**	0.17	0.030
lmo2487		*B. subtilis* YvlB protein		-0.01	0.863	-0.02	0.842	**3.27**	**0.000**	0.08	0.125
lmo2553		conserved hypothetical protein		-0.11	0.017	-0.04	0.646	**0.75**	**0.000**	0.04	0.565
lmo2567				-0.09	0.275	0.04	0.822	**2.86**	**0.000**	0.02	0.876
lmo2568				-0.08	0.355	-0.07	0.364	**4.20**	**0.000**	0.05	0.434
lmo2585		*B. subtilis* YrhD protein		**1.11**	**0.000**	0.40	0.001	**0.84**	**0.000**	-0.06	0.577
lmo2697				0.53	0.000	0.13	0.088	**0.69**	**0.000**	-0.02	0.839
lmo2835		an *E. coli* protein		**0.63**	**0.002**	0.23	0.063	**0.63**	**0.000**	0.38	0.010
Mobile and extrachromosomal element functions											
lmo2485		*B. subtilis* YvlC protein		-0.06	0.268	-0.03	0.774	**3.04**	**0.000**	0.04	0.690
Protein fate											
lmo0203	*mpl*	zinc metalloproteinase precursor		NA	NA	**-0.83**	**0.001**	**-1.19**	**0.001**	-0.77	0.011
lmo0963		putative heat shock protein HtpX, Listeria epitope LemB		-0.10	0.010	-0.09	0.060	**0.88**	**0.000**	-0.18	0.158
Protein synthesis											
lmo0211	*ctc*	*B. subtilis* general stress protein		0.16	0.004	-0.07	0.427	**0.70**	**0.000**	-0.14	0.023
Purines, pyrimidines, nucleosides, and nucleotides											
lmo0055	*purA*	adenylosuccinate synthetase		-0.38	0.000	0.01	0.921	**-0.60**	**0.000**	-0.14	0.560
lmo1831	*pyrE*	orotate phosphoribosyltransferases		**-1.18**	**0.000**	-0.12	0.414	-0.85	0.012	0.15	0.288
lmo1832	*pyrF*	orotidine 5'-phosphate decarboxylases		**-1.10**	**0.000**	-0.11	0.479	-0.82	0.012	0.04	0.790
lmo1833	*pyrD*	dihydroorotase dehydrogenase		**-1.02**	**0.000**	-0.08	0.817	-0.82	0.044	0.04	0.880
lmo1834	*pyrDII*	dihydroorotate dehydrogenase (electron transfer subunit)		**-1.32**	**0.000**	-0.12	0.580	-0.99	0.014	0.17	0.353
lmo1835	*pyrAB*	carbamoyl-phosphate synthetase (catalytic subunit)		**-1.28**	**0.000**	-0.03	0.914	-0.87	0.022	0.24	0.232
lmo1837	*pyrC*	dihydroorotase		**-1.28**	**0.000**	0.15	0.490	-0.96	0.109	0.34	0.296
lmo1838	*pyrB*	aspartate carbamoyltransferase		**-1.48**	**0.000**	0.13	0.610	-0.70	0.100	0.26	0.230
lmo1993	*pdp*	pyrimidine-nucleoside phosphorylase		**0.62**	**0.000**	0.28	0.022	0.19	0.011	0.13	0.154
Regulatory functions											
lmo0109		transcriptional regulatory proteins, AraC family		**0.92**	**0.000**	0.25	0.001	**0.75**	**0.000**	0.22	0.047
lmo0371		transcription regulator, GntR family		**-0.66**	**0.000**	-0.01	0.910	**-0.61**	**0.000**	-0.13	0.196
lmo0402		transcriptional antiterminator, BglG family		**4.03**	**0.000**	-0.04	0.643	**4.02**	**0.000**	0.07	0.407
lmo0425		transcription antiterminator, BglG family		**0.82**	**0.000**	0.12	0.093	0.25	0.007	-0.16	0.047
lmo0427		PTS fructose-specific enzyme IIB component		**0.73**	**0.000**	0.21	0.017	0.18	0.328	0.02	0.820
lmo0575		transcription regulator, GntR family		0.10	0.023	**-0.67**	**0.000**	0.09	0.181	-0.21	0.011
lmo0770		transcriptional regulator, LacI family		-0.33	0.002	-0.10	0.229	**-0.69**	**0.000**	-0.07	0.231
lmo0785	*manR*	transcriptional regulator, NifA/NtrC family		0.51	0.000	**0.65**	**0.000**	**0.82**	**0.000**	0.22	0.028
lmo0873		transcriptional regulator, antiterminator		**0.61**	**0.000**	0.30	0.127	0.52	0.015	0.02	0.796
lmo1021		sensor histidine kinase, *B. subtilis* YvqE protein		0.01	0.787	0.02	0.851	**1.45**	**0.000**	0.05	0.451
lmo1022		response regulator, *B. subtilis* YvqC protein		0.07	0.064	0.04	0.421	**1.39**	**0.000**	0.03	0.596
lmo1251		regulator of the Fnr CRP family (including PrfA)		**-1.39**	**0.000**	**-1.43**	**0.000**	**-2.04**	**0.000**	**-1.60**	**0.000**
lmo1618		transcription regulator MarR family		-0.21	0.061	-0.26	0.019	**-0.65**	**0.002**	-0.31	0.053
lmo1741		two-component sensor histidine kinase		-0.05	0.396	0.00	0.978	**0.59**	**0.000**	0.07	0.354
lmo1745		two-component response regulator		-0.04	0.456	-0.02	0.900	**0.69**	**0.000**	0.04	0.573
lmo2668		transcriptional antiterminator, BglG family		**0.86**	**0.000**	0.22	0.004	0.22	0.015	-0.12	0.039
lmo2851		AraC-type regulatory protein		**0.72**	**0.000**	0.02	0.936	**0.72**	**0.000**	0.12	0.224

Table S1. continued.

Locus	Gene	Description (similar to)[a]	Role(s)[a]	L502-1 (M)[b]		L502-1 (C)		L502-6 (M)		L502-6 (C)	
				Log2	q-value	Log2	q-value	Log2	q-value	Log2	q-value
Transport and binding proteins											
lmo0027		PTS, beta-glucosides specific enzyme IIABC	A,H	**1.49**	**0.000**	**4.09**	**0.000**	0.81	0.012	1.13	0.030
lmo0096	*mptA*	PTS mannose-specific, factor IIAB	A,H	**-2.27**	**0.000**	**1.37**	**0.000**	**1.58**	**0.000**	**2.56**	**0.000**
lmo0097	*mptC*	PTS mannose-specific, factor IIC	A,H	**-2.38**	**0.000**	**1.49**	**0.000**	**1.58**	**0.000**	**2.70**	**0.000**
lmo0098	*mptD*	PTS mannose-specific, factor IID	A,H	**-2.36**	**0.000**	**1.41**	**0.000**	**1.58**	**0.000**	**2.61**	**0.000**
lmo0194		ABC transporter, ATP-binding protein		-0.11	0.055	0.01	0.890	**2.04**	**0.000**	0.14	0.111
lmo0298		PTS beta-glucoside-specific enzyme IIC		0.74	0.035	-0.07	0.684	**0.95**	**0.001**	NA	NA
lmo0299		PTS beta-glucoside-specific enzyme IIB	A,H	0.76	0.013	NA	NA	**0.65**	**0.005**	0.13	0.411
lmo0301		PTS beta-glucoside-specific enzyme IIA	A,H	**1.02**	**0.000**	-0.35	0.105	**1.10**	**0.002**	-0.07	0.763
lmo0373		PTS beta-glucoside-specific enzyme IIC	Q	**-1.71**	**0.000**	0.28	0.126	**-1.81**	**0.000**	NA	NA
lmo0398		PTS enzyme IIA		**3.60**	**0.000**	NA	NA	**4.31**	**0.000**	NA	NA
lmo0399		fructose-specific PTS enzyme IIB		**4.65**	**0.000**	0.10	0.432	**4.60**	**0.000**	0.32	0.106
lmo0400		fructose-specific PTS enzyme IIC		**4.30**	**0.000**	0.22	0.083	**4.44**	**0.000**	0.18	0.327
lmo0426		PTS fructose-specific enzyme IIA		**0.59**	**0.000**	0.14	0.042	0.15	0.184	0.01	0.948
lmo0541		ABC transporter (binding protein)		0.23	0.098	0.15	0.592	**0.78**	**0.005**	**0.96**	**0.007**
lmo0573		conserved hypothetical protein		**-1.04**	**0.000**	0.05	0.795	**-1.06**	**0.000**	0.55	0.000
lmo0738		PTS beta-glucoside-specific enzyme IIABC	A,H	0.42	0.000	0.35	0.005	**0.59**	**0.001**	0.35	0.042
lmo0781	*mpoD*	mannose-specific PTS component IID	A,H	**1.24**	**0.000**	**0.96**	**0.000**	**2.97**	**0.000**	0.11	0.196
lmo0782	*mpoC*	mannose-specific PTS component IIC		**1.43**	**0.000**	**1.68**	**0.000**	**3.26**	**0.000**	0.23	0.017
lmo0783	*mpoB*	mannose-specific PTS component IIB		**1.21**	**0.000**	**1.86**	**0.000**	**3.06**	**0.000**	0.49	0.013
lmo0784	*mpoA*	mannose-specific PTS component IIA		**1.51**	**0.000**	**2.11**	**0.000**	**3.56**	**0.000**	0.40	0.000
lmo0798		lysine-specific permease		-0.25	0.001	-0.11	0.068	**-0.88**	**0.000**	-0.03	0.837
lmo0847		glutamine ABC transporter		0.56	0.001	**0.72**	**0.000**	**0.68**	**0.000**	**1.02**	**0.000**
lmo0848		amino acid ABC transporter, ATP-binding protein		**0.62**	**0.000**	**0.72**	**0.000**	**0.67**	**0.000**	**0.90**	**0.001**
lmo0859		sugar ABC transporter, periplasmic sugar-binding protein		**0.71**	**0.000**	0.26	0.003	**0.64**	**0.009**	0.19	0.089
lmo0860		sugar ABC transporter, permease protein		**0.60**	**0.006**	0.33	0.142	**0.95**	**0.000**	0.17	0.193
lmo0874		PTS enzyme IIA component		**0.63**	**0.004**	0.23	0.056	0.46	0.003	0.20	0.141
lmo0875		PTS, beta-glucoside enzyme IIB component	A,H	**0.72**	**0.008**	0.26	0.174	**0.81**	**0.002**	0.65	0.040
lmo0876		PTS, lichenan-specific enzyme IIC	A,H	**0.87**	**0.003**	0.17	0.324	0.38	0.176	NA	NA
lmo0897		transport proteins		-0.28	0.004	-0.25	0.016	**-0.68**	**0.000**	-0.08	0.306
lmo0914		PTS, IIB component	A,H	**0.91**	**0.002**	-0.03	0.807	**0.90**	**0.000**	0.17	0.206
lmo0915		PTS enzyme IIC	A,H	0.96	0.012	0.04	0.774	**1.30**	**0.002**	0.10	0.738
lmo0916		PTS enzyme IIA		**0.92**	**0.000**	0.03	0.822	**1.07**	**0.000**	0.14	0.138
lmo0997	*clpE*	ATP-dependent protease		-0.20	0.001	-0.01	0.934	**-0.88**	**0.000**	-0.17	0.156
lmo1023		a bacterial K(+)-uptake system	H	0.03	0.515	0.01	0.911	**1.37**	**0.000**	0.05	0.493
lmo1131		ABC transporters, ATP-binding proteins	H	-0.11	0.675	0.01	0.978	0.39	0.031	**0.94**	**0.003**
lmo1250		antibiotic resistance protein		**-0.62**	**0.003**	-0.47	0.000	**-0.86**	**0.000**	-0.48	0.043
lmo1255		PTStrehalose specific enzyme IIBC	A,H	**2.48**	**0.000**	**0.64**	**0.000**	**2.27**	**0.000**	0.13	0.048
lmo1539		glycerol uptake facilitator		**1.44**	**0.000**	0.53	0.000	**1.40**	**0.000**	-0.02	0.843
lmo1636		ABC transporter (ATP-binding protein)		-0.01	0.790	-0.01	0.916	**2.05**	**0.000**	0.06	0.284
lmo1719		PTS lichenan-specific enzyme IIA component	A,H	-0.03	0.426	**-1.38**	**0.000**	-0.03	0.610	-0.10	0.173
lmo1720		PTS lichenan-specific enzyme IIB component	A,H	-0.03	0.346	**-1.36**	**0.000**	-0.05	0.483	-0.18	0.015
lmo1730		sugar ABC transporter binding protein		**0.66**	**0.001**	0.13	0.417	**0.91**	**0.000**	0.29	0.046
lmo1731		sugar ABC transporter, permease protein		0.58	0.000	0.09	0.160	**0.72**	**0.000**	0.31	0.004
lmo1732		sugar ABC transporter, permease protein		0.66	0.024	0.33	0.128	**0.63**	**0.003**	0.37	0.126
lmo1746		ABC transporter (permease)		-0.06	0.274	-0.07	0.515	**1.16**	**0.000**	-0.05	0.440
lmo1747		ABC transporter (ATP-binding protein)		-0.02	0.700	0.00	0.977	**1.19**	**0.000**	-0.01	0.892
lmo1839	*pyrP*	uracil permease		**-1.66**	**0.000**	0.21	0.301	-1.06	0.025	0.41	0.088
lmo1846		conserved hypothetical proteins		-0.13	0.438	0.00	0.998	**-0.64**	**0.002**	-0.13	0.660
lmo1884		xanthine permeases		**-0.59**	**0.000**	0.24	0.057	-0.37	0.008	0.55	0.002
lmo1957	*fhuG*	ferrichrome ABC transporter (permease)		0.18	0.021	0.22	0.247	**0.63**	**0.000**	**0.65**	**0.001**
lmo1958	*fhuB*	ferrichrome ABC transporter (permease)		0.15	0.099	0.18	0.417	**0.60**	**0.000**	**0.73**	**0.002**
lmo1959		ferrichrome binding protein		0.24	0.052	0.22	0.259	**0.77**	**0.000**	**0.97**	**0.001**
lmo1960	*fhuC*	ferrichrome ABC transporter (ATP-binding protein)		0.20	0.079	0.06	0.677	**0.64**	**0.001**	**0.62**	**0.005**
lmo1973		PTS enzyme II A component	A,H	**0.60**	**0.000**	0.09	0.376	0.27	0.001	0.14	0.149
lmo2105		ferrous iron transport protein B		-0.42	0.115	-0.25	0.085	-0.44	0.016	**0.71**	**0.007**
lmo2183		ferrichrome ABC transporter (permease)		0.42	0.003	0.34	0.259	**0.94**	**0.000**	**1.04**	**0.001**
lmo2184		ferrichrome ABC transporter (binding protein)		0.41	0.005	0.39	0.222	**1.16**	**0.000**	**1.18**	**0.001**
lmo2250	*arpJ*	amino acid ABC transporter, permease protein		**-0.66**	**0.000**	**-0.59**	**0.002**	**-1.74**	**0.000**	0.08	0.588
lmo2251		amino acid ABC transporter (ATP-binding protein)		**-0.61**	**0.000**	-0.46	0.000	**-1.65**	**0.000**	0.00	0.968
lmo2254		unknown proteins		-0.37	0.000	0.04	0.519	**-0.64**	**0.000**	-0.06	0.359
lmo2362	*gadT2*	amino acid antiporter (acid resistance)		-0.09	0.258	-0.58	0.004	**-0.76**	**0.005**	-0.97	0.022
lmo2469		amino acid transporter		-0.47	0.000	-0.23	0.216	**-0.91**	**0.000**	-0.33	0.033
lmo2665		PTS galactitol-specific enzyme IIC component	A,H	**0.68**	**0.000**	0.14	0.015	0.18	0.069	-0.23	0.005
lmo2667		PTS galactitol-specific enzyme IIA component	A,H	**0.79**	**0.000**	0.19	0.058	0.29	0.001	-0.15	0.037
lmo2680	*kdpC*	potassium-transporting atpase c chain		-0.01	0.884	-0.05	0.502	**1.27**	**0.000**	-0.09	0.156
lmo2681	*kdpB*	potassium-transporting atpase b chain	H	-0.01	0.928	0.05	0.557	**2.19**	**0.000**	0.03	0.744
lmo2682	*kdpA*	potassium-transporting atpase a chain		-0.04	0.600	0.01	0.946	**2.40**	**0.000**	0.07	0.280
lmo2683		cellobiose PTS enzyme IIB	A,H	**-1.50**	**0.000**	**-1.42**	**0.000**	-0.36	0.075	-0.01	0.832
lmo2684		cellobiose PTS enzyme IIC	Q	**-1.71**	**0.000**	**-0.80**	**0.000**	-0.53	0.010	0.01	0.839
lmo2685		cellobiose PTS enzyme IIA	A,H	**-1.34**	**0.000**	**-1.37**	**0.000**	-0.58	0.006	-0.08	0.445
lmo2708		PTS, cellobiose-specific enzyme IIC	Q	-0.16	0.041	**-1.30**	**0.000**	0.22	0.076	0.23	0.005
lmo2762		PTS cellobiose-specific enzyme IIB	A,H	0.57	0.000	0.21	0.120	**0.84**	**0.003**	0.29	0.237
lmo2763		PTS cellobiose-specific enzyme IIC	Q	**0.62**	**0.000**	0.21	0.016	**0.79**	**0.001**	0.23	0.008
lmo2772		beta-glucoside-specific enzyme IIABC	A,H	0.30	0.010	**0.82**	**0.000**	-0.10	0.214	-0.02	0.952
lmo2780		cellobiose PTS enzyme IIA	A,H	NA	NA	0.07	0.510	**0.66**	**0.007**	0.23	0.242
lmo2782		PTS, cellobiose-specific IIB component	A,H	0.52	0.006	0.33	0.045	**0.76**	**0.002**	0.43	0.002
lmo2783		cellobiose PTS enzyme IIC	A,H	**0.71**	**0.000**	0.21	0.055	**0.86**	**0.000**	0.48	0.001
lmo2797		PTS mannitol-specific enzyme IIA	A,H	**1.05**	**0.000**	0.40	0.003	**1.06**	**0.000**	0.44	0.022
Unclassified (role category not yet assigned)											
lmo0118	*lmaA*	antigen A		**-0.95**	**0.000**	-0.41	0.099	-0.02	0.917	-0.42	0.046
lmo0130		5'-nucleotidase, putative peptidoglycan bound protein (LPXTG motif)		**0.77**	**0.000**	0.36	0.000	0.48	0.000	0.26	0.002
lmo0202	*hly*	listeriolysin O precursor		-0.45	0.019	-0.44	0.061	-0.57	0.003	**-0.77**	**0.004**
lmo0204	*actA*	actin-assembly inducing protein precursor		**-0.67**	**0.000**	**-0.83**	**0.000**	**-0.99**	**0.000**	NA	NA
lmo0361	*tatC*	twin arginine translocase C		0.40	0.002	0.32	0.300	**1.23**	**0.000**	**1.19**	**0.001**
lmo0362	*tatA*	twin arginine translocase A		0.37	0.000	0.28	0.471	**0.89**	**0.000**	**0.98**	**0.001**
lmo0367		conserved hypothetical protein B. subtilis YwbN protein		0.51	0.001	0.54	0.169	**1.39**	**0.000**	**1.46**	**0.000**

Table S1. continued.

Locus	Gene	Description (similar to)[a]	Role(s) [a]	L502-1 (M)[b]		L502-1 (C)		L502-6 (M)		L502-6 (C)	
				Log2	q-value	Log2	q-value	Log2	q-value	Log2	q-value
lmo0385		*B. subtilis* lolC protein and to fructokinase		**0.64**	**0.000**	0.23	0.038	**0.60**	**0.000**	0.15	0.247
lmo0401		*E. coli* YbgG protein, a putative sugar hydrolase		**4.30**	**0.000**	0.33	0.055	**4.33**	**0.000**	0.12	0.443
lmo0429		sugar hydrolase		**0.59**	**0.000**	0.21	0.037	0.18	0.011	-0.05	0.354
lmo0593		transport proteins (formate?)		**-0.70**	**0.000**	-0.22	0.104	**-0.83**	**0.000**	-0.10	0.195
lmo1369	*ptb*	phosphotransbutyrylase		-0.32	0.000	-0.13	0.073	**-0.60**	**0.000**	0.00	0.952
lmo1966		unknown proteins		0.02	0.863	0.04	0.666	**2.02**	**0.000**	-0.06	0.629
lmo1968		creatinine amidohydrolases		0.25	0.099	0.00	0.985	**0.92**	**0.002**	-0.09	0.719
lmo2067	*bsh*	conjugated bile acid hydrolase		-0.44	0.116	-0.09	0.442	**-0.59**	**0.010**	-0.23	0.321
lmo2125		maltose/maltodextrin ABC-transporter		**0.63**	**0.000**	0.23	0.065	0.41	0.000	0.68	0.031
lmo2158		*B. subtilis* YwmG protein		**0.66**	**0.003**	0.22	0.244	**0.80**	**0.001**	0.30	0.123
lmo2182		ferrichrome ABC transporter (ATP-binding protein)		0.34	0.016	0.36	0.217	**0.89**	**0.000**	**1.12**	**0.001**
lmo2185	*svpA*	surface virulence-associated protein, substrate for SrtB		0.48	0.003	0.47	0.180	**1.11**	**0.000**	**1.24**	**0.001**
lmo2186	*isdC*	iron-regulated surface determinants, substrate for SrtB		0.47	0.003	0.48	0.161	**1.25**	**0.000**	**1.41**	**0.001**
lmo2207		unknown protein		-0.07	0.271	0.00	0.974	**1.26**	**0.000**	0.02	0.875
lmo2257		hypothetical CDS		0.09	0.078	-0.03	0.748	**0.73**	**0.000**	-0.15	0.379
lmo2648		phosphotriesterase		0.33	0.021	0.21	0.067	**0.76**	**0.010**	0.45	0.166
lmo2679		the two components sensor protein kdpD		-0.04	0.463	-0.03	0.852	**0.93**	**0.000**	-0.08	0.296
lmo2742				0.58	0.000	0.08	0.425	**0.68**	**0.000**	0.08	0.601
lmo2745		ABC transporter (ATP-binding protein)		-0.05	0.279	-0.09	0.258	**2.02**	**0.000**	0.12	0.049
lmo2773		transcription antiterminator		0.46	0.000	**0.70**	**0.000**	-0.09	0.134	0.00	0.989
lmo2781		beta-glucosidase		**0.80**	**0.000**	0.20	0.067	**0.85**	**0.000**	**0.59**	**0.000**
lmo2785	*kat*	catalase		0.13	0.426	0.45	0.002	-0.51	0.002	**-0.73**	**0.007**
lmo2788	*bvrA*	transcription antiterminator		**0.61**	**0.000**	0.30	0.001	0.32	0.000	0.18	0.140
Unknown function											
lmo0042		*E. coli* DedA protein		-0.15	0.668	-0.10	0.099	**0.88**	**0.001**	-0.02	0.860
lmo0776		transcription regulator (repressor)		**0.69**	**0.000**	-0.20	0.004	0.39	0.000	-0.12	0.138
lmo0962	*lemA*	Listeria epitope LemA		-0.08	0.066	-0.11	0.046	**0.92**	**0.000**	-0.03	0.607
lmo1230		*B. subtilis* YshB protein		0.02	0.738	-0.01	0.900	**0.59**	**0.000**	-0.09	0.361
Viral functions											
lmo0115	*lmaD*	antigen D		**-0.61**	**0.004**	-0.03	0.900	-0.05	0.835	-0.05	0.839

[a] Description of genes according to the annotation of the Comprehensive Microbial Resource of the J. Craig Venter Institute (http://cmr.jcvi.org) and published literature resources. The functional role categories are also according to the primary annotation in CMR-JCVI *L. monocytogenes* EGDe genome database. A, Amino acid biosynthesis; B, Biosynthesis of cofactors, prosthetic groups, and carriers; E, Central intermediary metabolism; H, Energy metabolism; I, Fatty acid and phospholipid metabolism; J, Hypothetical proteins; N, Protein synthesis; O, Purines, pyrimidines, nucleosides, and nucleotides; Q, Signal transduction.

[b] Log2 expression ratio [a high level sakacin P-resistant strain (L502-1) and a low level sakacin P-resistant strains (L502-6) grown on mannose (M) or cellobiose (C) relative to the wild-type (L502) grown on the respective sugars]; values in bold face indicate differentially expressed genes as defined as log2 ratios ≥ 0.585 or ≤ -0.585 and with q-value < 0.01, p-value adjusted to control false discovery rate). NA, no data available. Fully annotated microarray data have been deposited in BμG@Sbase (accession number E-BUGS-110; http://bugs.sgul.ac.uk/E-BUGS-110) and also ArrayExpress (accession number E-BUGS-110).

Table S2. Primer and probe sets used for qRT-PCR.

Target	Primer and probe name	Sequence[a] (5′→3′)	Reference
mptA (lmo0096)	MptAF	CCTCGCAACTCACGGTGAAT	[20]
	MptAR	TCTTGCTCGCCGAAAATCA	
	MptA-Taq	TGCTGAAGGTATTTTGCAGTCCGGAACA	
actA (lmo0204)	lmo0204F	CGACCGACCAGCTATACAAGTG	This study
	lmo0204R	AATTTCCGCTGCGCTATCC	
	lmo0204-Taq	AGCGTCGTCATCCAGGATTGCCA	
lmo1251	lmo1251F	TGGAAATAGCGCCTGATCAAT	This study
	lmo1251R	TCCCGCAAAAATGACCAATT	
	lmo1251-Taq	TTTTCTAGTAAGCAATGAAG)	
glpK (lmo1538)	lmo1538F	GAAAGCAATGCTGCCAGAAGT	This study
	lmo1538R	GCAATACCTGCAACCGGAACT	
	lmo1538-Taq	ATCCTCTGAAGTATATGCGGACACAGTGCC	
kdpA (mo2682)	lmo2682F	GTTGGTGGTTCGTCGCTTTT	This study
	lmo2682R	TGTCGTGCATTGCGTTCAC	
	lmo2682-Taq	CGGCTTCCACAACGGCAGCC	
16S rRNA	16S rRNAF	GCGCAGGCGGTCTTTTAAG	[20]
	16S rRNAR	CAATGACCCTCCCCGGTTA	
	16S rRNA-Taq	CTGATGTGAAAGCCCCCGGC	

[a]Taq probes, 6-FAM, 6-carboxyfluorescein (fluorophore); TAMRA, 6-carboxytetramethylrhodamine (quencher).

Paper III

Global transcriptional responses of *Listeria monocytogenes* to hydrochloric acid, acetic acid and lactic acid stress

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ABSTRACT

This study presents global transcriptional responses of *Listeria monocytogenes* to low pH and acidulants commonly encountered by this foodborne pathogen in fermented foods and food environments. Growth in brain heart infusion broth acidified to pH 5 with hydrochloric acid (HCl), 10 mM acetic acid or 20 mM lactic acid was investigated. Microarray analyses showed a remarkable overlapping in transcriptional response among the acid stressed cultures. Despite the similarity, the overall transcriptional responses to organic acids were more pronounced than those of HCl. Transcripts associated with sugar uptake systems were reduced in the organic acids stressed cultures but not affected in the HCl stressed culture. The most highly up-regulated genes were *gadT2* and *gadD2* (> 38-folds) suggesting the glutamate decarboxylase (GAD) is the main resistance system against low pH under the studied growth conditions. In addition to the GAD system, other genes encoding known and putative proteins associated with acid stress survival including modification of the cell envelope, protection and repair of macromolecules, intracellular accumulation of histidine and osmolytes as well as general stress proteins were induced. The acid stress also induced genes encoding proteins described to be involved in virulence, protection against other types of stress and proteins with unknown functions. Genes encoding proteins associated with macromolecule synthesis and nutrient transporters were down-regulated and this corresponded well with growth inhibition under acidic conditions. In summary, this study identified large number of genes with direct and indirect role in acid stress defense and other types of stress which also opens the possibilities for more specific and in-depth future studies. Although the data confirmed numerous previous results on acid stress, this is, to our knowledge, the first study on the global transcriptional responses of *L. monocytogenes* to acetic acid and lactic acid stress.

INTRODUCTION

Listeria monocytogenes is a gram-positive foodborne pathogen that is responsible for severe and often life-threatening disease with a high economic burden to both public health services and the food industry (29). This foodborne pathogen grows optimally in the pH range of 6 to 7 (38). Acidic environment, however, is a common condition *L. monocytogenes* may encounter outside the host (e.g. low pH soil and foods) and inside the host (e.g. during gastric transit and in the phagosome of macrophages) (14).

Organic acids such as acetic- and lactic-acid may be naturally present in foods, but are also commonly used as food additives to extend shelf life and to control the growth of undesirable microorganisms, including *L. monocytogenes* (10, 46). Despite the widespread use of organic acids, the exact mode of their antimicrobial action still has not been fully elucidated (25, 41). It is presumed that the undissociated form of organic acids can transverse the cell membrane and once inside, the acids dissociate and affect acid and anion sensitive cellular functions (10). Organic acids are suggested to interfere with cell membrane integrity and functions, enzyme activities, macromolecules syntheses and may act as uncouplers (10, 41). In contrast, strong acids as hydrochloric acids (HCl), exert their antimicrobial effect by denaturing enzymes present on the cell surface and by lowering the cytoplasmic pH due to increased protons permeability caused by a large pH gradient (3).

In gram-positive bacteria a combination of constitutive and inducible strategies including consumption and efflux of protons (H^+), cell envelope modifications, protein and DNA repair and production of alkali can contribute survival during low pH stress [reviewed in (14, 45)]. *L. monocytogenes* has been shown to exhibit adaptive tolerance to low pH, termed as acid tolerance response, upon exposure to mild acid, which may protect the bacterium against extreme low pH and other adverse conditions (19, 33, 37). Spontaneous acid-tolerant mutants of *L. monocytogenes* that are constitutively resistant to low pH in the absence of adaption can be also recovered following exposure to extreme low pH (11, 37).

The global responses of *L. monocytogenes* to organic acids and HCl after shock stress and during adaptation have been investigated using a proteomic approach (38, 39, 58). These studies have indicated the presence of common as well as unique protein expression profiles under the acid stress conditions. However, most of the differentially expressed proteins were

not fully identified. A more recent genome wide transcriptional study on *L. monocytogenes* has analyzed the responses of this bacterium to the organic acid salt sodium diacetate and HCl (6). The global transcriptional responses of other gram-positive foodborne pathogens including *Bacillus cereus* (34) and *Staphylococcus aureus* (5, 42) to HCl, acetic acid and lactic acid stress have been investigated. These have given insight into how these foodborne pathogens combat stress due to the different acids. However, we are unaware of any reports that assess the global transcriptional responses of *L. monocytogenes* to acetic acid or lactic acid stress.

Microorganisms are commonly exposed to acetic- and lactic-acid, and a pH of 5 is representative of the mildly acidic environment bacteria may encounter in foods during production and processing (10, 22, 30). In the present exploratory descriptive study, growth characteristics and transcriptional responses of *L. monocytogenes* in medium acidified to pH 5 by addition of HCl, 10 mM acetic acid or 20 mM lactic acid are reported.

MATERIALS AND METHODS

Bacterial strain and culture media. *L. monocytogenes* L502 isolated from cheese, provided by The Norwegian Veterinary Institute, Oslo, Norway, has been described in detail earlier (53). The strain has the same serotype (1/2a) as the EGDe strain, which was used to generate the PCR based microarray slides used in this study (see below). *L. monocytogenes* L502 was grown in Brain Heart Infusion (BHI) broth or BHI agar plate (Oxoid Ltd., England) at 25°C. Growth in broth culture was with continuous shaking at 250 r.p.m. (Innova 4230, Refrigerated Incubator Shaker, New Brunswick Scientific, USA). The stock culture was stored frozen (−80°C) in BHI broth in the presence of 15% glycerol.

Stock solutions of 1M acetic acid (Merck, Germany) and DL-lactic acid (Sigma, Germany) were prepared and added to autoclaved BHI broth to a final total concentration of 2 mM to 50 mM. For the HCl stress, BHI broth was acidified using 6 M HCl (Merck, Germany). The acidified BHI broths were adjusted to pHs 4.0 to 5.5 at intervals of 0.5 pH units (PHM210, Standard pH Meter, Radiometer Analytical SAS, France) using the HCl and 6 M sodium hydroxide (Merck, Germany), and sterilized by filtration through 0.2 μm polyethersulfone membrane filters (Nalge Nunc International, USA). The reference BHI broth was adjusted to pH of 7.2. All broth cultures were freshly made and pre-warmed to 25°C before use.

Acid stress experiment. The *L. monocytogenes* L502 was streaked on a BHI agar plate and incubated at 25°C for 18-20 h. An overnight culture was prepared from the agar plate in 10 ml BHI broth (pH 7.2) and incubated for 18-20 h. The stationary-phase culture was diluted 1:100 (vol/vol) in four sterilized baffled Erlenmeyer flasks (500 ml) containing 99 ml of fresh BHI broth (pH 7.2). Exponentially growing cultures (OD_{600} of 0.4) were centrifuged at 5000g for 5 min at 25°C using a Sorvall model RC 5C Plus (Du Pont Instruments, USA). The cells were resuspended in 100 ml BHI broth containing (i) HCl (herein HCl stress), (ii) 10 mM acetic acid (herein acetic acid stress), (iii) 20 mM lactic acid (herein lactic acid stress) all at pH 5 and (iv) BHI pH 7.2 (herein reference) and transferred to Erlenmeyer flasks for further incubation. Harvesting for RNA isolation was performed when growth reached OD_{600} ~1.2. Schematic presentation of the experimental design is presented in supplementary materials (Supplementary Fig. S1).

Aliquot samples were regularly removed to monitor pH and growth using OD_{600} and total viable cells measurements. Cell viability was estimated on BHI agar plates using two biological replicates, each involving the analysis of two technical replicates. The plates were incubated at 30°C for 48 h and colonies were counted. The pH and OD were measured in three independent experiments.

RNA isolation. Total RNA extraction was done using an RNeasy Protect bacterial mini kit (53). The quantity and quality of RNA was checked using NanoDrop ND-1000 (NanoDrop Technologies, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The isolated RNA sample was used both for the microarray and for validation assay by quantitative real-time reverse transcriptase PCR (qRT-PCR). For the transcriptional studies, the experiments were done twice on different days with dye swapping in each microarray experiment.

Microarray analysis. The PCR based DNA microarray slides were developed and printed by The Bacterial Microarray Group at St George's (BµG@S), University of London, UK. Protocols for the development and printing of the microarray slides have been described previously (4, 24). The array design is available in BµG@Sbase (Accession No. A-BUGS-19; http://bugs.sgul.ac.uk/A-BUGS-19) and also ArrayExpress (Accession No. A-BUGS-19). The cDNA synthesis and labeling were performed using 4 µg total RNA following guidelines from BµG@S (24). Samples from each acid stress condition were compared with the reference sample as described previously (4, 24) and a total of at least four paired competitive hybridizations were performed. Scanning of the arrays was performed using Tecan LS scanner (Tecan, Switzerland). Spot-identification, -segmentation and -fluorescent intensity quantification were done using ImaGene 5.5 (BioDiversity, USA).

The microarray data analysis was done by the LIMMA package (48) in the R computing environment (http://www.r-project.org/). Preprocessing and normalization followed a standard procedure using methods described by Smyth and Speed (51). Testing for differentially expressed genes was done using a linear mixed model as described (49). A mixed-model approach was chosen to adequately describe between-array variation, and utilized the probe-replicates in each array. An empirical Bayes smoothing of gene-wise variances was conducted according to Smyth et al. (50). For each gene, the p-value was adjusted to control false discovery rate and the adjusted p-values were referred as q-values.

Differentially expressed genes were selected using q-values less than 0.01 and with log ratio of ≥ 0.585 or ≤ -0.585 (equivalent to ≥ 1.5-fold changes).

The Unscrambler software (The Unscrambler v9.8; CAMO AS, Norway), GeneSpring GX7.3 (Agilent Technologies, USA), and the LEGER database (16) were used for visualization, clustering and further analyses of the preprocessed microarray data. For the principal component analysis (PCA), genes that were differentially expressed in any of the growth conditions were included. Genes were grouped according to the category role of the Comprehensive Microbial Resource (CMR) of the J. Craig Venter Institute (JCVI) (http://cmr.jcvi.org). A chi-square and odd ratios analyses were performed to determine which role categories were significantly affected by the acid stress (http://faculty.vassar.edu/lowry/odds2x2.html). Genes belonging to a given operon were identified based on the recent *L. monocytogenes* operon map (54) and other literature sources were used to designate gene functions.

qRT-PCR. The microarray results were validated using qRT-PCR as previously described (53). The primer and probe sets for *actA* (encoding actin assembly-inducing protein), *lmo1251* [a putative CRP (cyclic AMP receptor protein)-FNR (fumarate and nitrate reduction regulator) family transcriptional regulator], *glpK* (glycerol kinase) and *kdpA* (potassium-transporting ATPase A chain) were designed using Primer Express 3.0. The sets for the *mpt*A (mannose permease two, AB subunit) and 16S rRNA have been reported earlier (53) (Supplementary Table S1).

Microarray data accession number. Fully annotated microarray data have been deposited in BµG@Sbase (accession number E-BUGS-109; http://bugs.sgul.ac.uk/E-BUGS-109) and also ArrayExpress (accession number E-BUGS-109).

RESULTS AND DISCUSSION

In this exploratory descriptive study, the growth characteristics as well as the global transcriptional responses of *L. monocytogenes* L502 to HCl, acetic acid and lactic acid stress were investigated.

Listeria monocytogenes growth in acidic conditions

Inhibitory acidic conditions leading to reduced growth rate for the strain were identified from growth studies using different pHs and concentrations of acetic acid, lactic acid and HCl. The growth of *L. monocytogenes* L502 was reduced but not completely inhibited at pH 5 when acidification was done by addition of HCl, 10 mM acetic acid or 20 mM lactic acid (Fig. 1). To obtain a similar growth rate of the strain in the organic acid treated cultures (Fig. 1), the concentration of total lactic acid was doubled compared to that of acetic acid. This is in line with the stronger antilisterial activity of acetic acid compared to lactic acid at equal pH and equimolar total acids (59). The pH of the reference culture decreased gradually from 7.2 to 6.3 during the course of growth. The pH of the HCl-, acetic acid-, and lactic acid-stressed cultures (herein acid stressed cultures), however, remained constant at pH 5 (Fig. 1C). According to the Henderson-Hasselbalch equation, the concentrations of the initial undissociated and anion forms of acetic acid (assuming $pK_a = 4.76$ and no effect from the BHI broth) were approximately 3.7 mM and 6.3 mM, respectively at pH 5. Under the same conditions, approximately 1.4 mM and 18.6 mM of the lactic acid ($pK_a = 3.86$) were expected to be in the undissociated and anion forms, respectively.

Following the acid treatments, the growth of the strain was arrested temporarily (Fig. 1). When the growth resumed, the specific growth rate (μ) of the strain in the reference culture ($0.54\ h^{-1}$) was higher than that of the HCl stressed culture ($0.3\ h^{-1}$, $p < 0.05$), which again was higher than in the organic acid stressed cultures ($\mu = 0.18\ h^{-1}$, $p < 0.05$). The observed difference in the antimicrobial potency among the three acids at a given pH is in agreement with results from previous studies (34, 59).

Global gene expression profile and validation by qRT-PCR

The transcriptome profiling was performed during similar growth states of the strain under the different acidic conditions. Both stressed and reference cells were harvested at OD_{600} ~1.2 (Fig. 1). The pH difference between the acid stressed cultures and the reference culture at the time of harvesting was approximately 1.7 pH units. A total of 511, 774 and 873 genes were differentially expressed in the HCl-, acetic acid-, and lactic acid-stressed cultures respectively, compared with the reference (q-values < 0.01 and with ≥ 1.5-fold changes).

The microarray probes used in the present study were based on the EGDe strain and more than 94% of *L. monocytogenes* L502 genes were hybridized to the EGD-e based probes in any of experimental conditions tested so far (not shown). A recent study by Toledo-Arana et al. (54) showed that the EGDe strain expresses comparable number of its genes (> 98% of its open reading frames) under different growth conditions. In the present study, the reliability of the microarray expression data was assessed by qRT-PCR analysis using six genes selected following the guidelines suggested by Morey et al. (35). The qRT-PCR validation assay showed a strong correlation of gene expression with the microarray results under all conditions tested (r > 0.9, Supplementary Fig. S2), confirming independently the reliability of the microarray data set.

As can be seen in Fig. 2, a large number of differentially expressed genes were common to all acid type stress; 204 up-regulated and 247 down-regulated compared with the reference. Further comparison of the transcriptional responses against each other revealed a high correlation between the acid treatments (r > 0.9, Supplementary Fig. S3). The similarity in the transcriptional response from the organic acids was highest (r = 0.98, Supplementary Fig. S3C). The overall striking similarity in transcriptional responses from the three acids may be attributed partly both to the equal pH effect (pH 5) and similar growth states of the strain at the time of harvesting. Despite the similarities, a PCA plot of the differentially expressed genes revealed a fairly high variation (47%) in transcriptional response between the HCl- and the organic acids-stressed cultures (Fig. 3). The next variation in the data set (12%) was mainly due to the transcriptional difference from the lactic acid and acetic acid. Our data is consistent with results from a recent study (34) that showed the presence of similar as well as distinct transcriptional response of *B. cereus* subjected to HCl, lactic acid and acetic acid stress.

Analysis of the differentially expressed genes according to the classification scheme of the CMR-JCVI role categories revealed that growth under acidic conditions triggered the expression of genes belonging to all functional categories (Fig. 4). The largest impact of the acid stress was on genes sets belonging to the cellular process and the transport and binding proteins functional groups ($p < 0.001$, odd ratios ≥ 1.5). Differentially expressed genes in the overrepresented role categories include genes associated with adaptations to atypical conditions (e.g. acid resistance, detoxification, and osmoregulatory) nutrient transport and virulence. These groups are described in greater detail below. In addition, a large number of genes coding for hypothetical proteins were also differentially regulated (Fig. 4).

Apart from genes differentially regulated in acid type-specific manner (see below), the key difference in the transcriptional responses from the three acids was on the level of expression. Thus, genes that showed identical or similar transcriptional profiles in the three acid stressed cultures were presented together.

Transcriptional responses to counteract the acidification

I) Glutamate Decarboxylase (GAD), Arginine Deiminase (ADI) and proton efflux systems

In *L. monocytogene*s, the Glutamate Decarboxylase (GAD) system comprises the glutamate decarboxylase enzymes (encoded by *gadD1*, *gadD2* or *gadD3*) and the membrane associated glutamate/ γ-aminobutyrate (GABA) antiporter (encoded by *gadT1* or *gadT2*) (13). Here we found that *gadT2* and *gadD2* were the most highly induced genes in the acid treated cultures (> 38-folds), suggesting glutamate decarboxylation to be major defense mechanism against low pH under the studied growth conditions (Table 1). A recent study (6) showed a similar induction of the *gadT2D2* operon for two different strains exposed to HCl (pH 5), albeit with low fold changes (4.2 to 9.8-folds). Our data set showed no change in the expression of other genes designated for the GAD system (not shown). The GAD system reduces acidification of the cell cytoplasm by consuming protons during decarboxylation of acidic glutamate to a natural GABA by the glutamic acid decarboxylase. The GABA may be exchanged for another extracellular glutamate by the antiporter (12, 26). In the present study, *lmo0913* encoding a putative succinate semialdehyde dehydrogenase enzyme which may further metabolize the GABA to succinate (13) was also significantly induced in the lactic acid stressed culture (Table 1). The role of the putative succinate semialdehyde dehydrogenase enzyme during

growth under acidic conditions has been demonstrated using a deletion mutant lacking *lmo0913* gene (1). Generally, the importance of the GAD system has also been proven by model studies including low pH foods (15), modified atmosphere-packed foods (17) and gastric fluid (12). Our transcriptional data together with results from the previous studies strongly suggest that the GAD system is a primary defense against low pH stress.

Listeria monocytogenes has the ability to extrude protons to maintain the cytoplasmic pH homeostasis (47). However, the expressions of genes associated with the F_0F_1-ATPase and the electron transport systems were not affected in the present study by the presence of the acids (not shown). In addition, genes encoding proteins for the Arginine Deiminase (ADI) system (*arcA*, *arcB*, *arcC* and *arcD*) showed inconsistent expression pattern (Supplementary Table S2). Ryan et al. (44) demonstrated simultaneous increase in transcription of all the *arc* genes during growth at acidic pH relative to the growth at pH 7. A recent study (6) showed strain dependent expression of genes associated with the F_0F_1-ATPase and the ADI systems in *L. monocytogenes* in responses to growth in acidic conditions. Overall, for the strain used in the present study and under the conditions tested, the proton efflux and the ADI systems appear to be of less importance.

II) Modification of the cell membrane

Exposure of the strain to acid stress resulted in differential regulation of a number of genes encoding proteins described to be associated with metabolism of the cell envelope, of fatty acids and of phospholipids (Fig. 4, Supplementary Table S2). Interestingly, *dltABCD* operon was 1.6 to 2.7-fold higher in the acid stressed cultures (Table 1). The Dlt proteins are required for the metabolism of cell wall-associated teichoic acids, and several studies on other gram-positive bacteria have shown that deletion of the *dlt* increased sensitivity towards to environmental stress including acid stress (7, 57). Previously it has been shown that *L. monocytogenes* reduce the amount of branched fatty acids in response to low pH stress (21, 32). Here we found that genes designated for the biosynthesis of branched chain fatty acids (*ptb-buk-lpd-bkdA1-bkdA2-bkdB*) were generally down-regulated (Table 1). The overall results suggest modification of the cell envelope architecture and composition may contribute to the acid response in the present study. Verification of the role of the *dlt* operon in *L. monocytogenes* deserves further investigations.

III) Protection and repair of macromolecules

Generally, several genes encoding proteins involved in DNA metabolism (specifically DNA repair and recombination) were up-regulated (Fig. 4, Table 1). Interestingly, the expression of *uvrB* and *uvrC* were significantly, and the *uvrA* moderately induced during growth under acidic conditions (Table 1). The UvrABC protein complex is involved in nucleotide excision repair and the importance of this protein complex during low pH stress has been demonstrated in *L. monocytogenes* and in other gram-positive bacteria (23, 27). The over-expressed genes associated with DNA metabolism in the present study may play a role in the repairing of acid-induced damage to DNA and are possibly also involved in protection against UV light stress (see below).

The proteases and the peptidases degrade aberrant proteins synthesized under stressful conditions (14) and a group of genes belonging to the protein fate functional category exhibited increase in transcripts during under acidic growth conditions (Fig. 4, Supplementary Table S2). For example, *htrA*, encoding a protease (Table 1) and other genes encoding different peptidases (*lmo1578, lmo1611, lmo1780, lmo2188, lmo2462,* and *lmo2338*) (Supplementary Table S2) were over-expressed in the acid stressed cultures. The induction of genes encoding proteases and peptidases during acid stress was shown in previous studies (5, 6, 52).

Chaperonins are protein complexes that assist folding of malformed proteins resulted from exposure to adverse conditions (31). The present study showed down-regulation of *groESL* genes encoding molecular chaperonin (Table 1), confirming a previous report on acid adapted strain of *Lactobacillus casei* (8). This contrasts numerous other studies that showed induction of genes encoding chaperonins (e.g. *groESL*) in response to acid stress [(20, 34) and others]. This may be due to a difference in sampling time point. Studies using our strain have shown that the expression of *groESL* and other chaperonins (the *dnaK* operon) was induced shortly after acid shock compared to a reference culture taken before the acid treatment (our unpublished result). This suggests that the transcriptional level of genes encoding chaperonins may vary after the onset of acid stress. Interestingly, the expression of *prsA1* (similar to a protein post-translocation molecular chaperonin) was induced (Table 1). The role of this extracellular, but cell-associated, folding chaperonin in acid adaptation remains to be elucidated.

IV) Intracellular histidine and osmolytes accumulation

Genes encoding proteins for histidine biosynthesis were generally up-regulated among the acid stressed cultures (Table 1). A contradicting microarray result from a recent acid stress study showed down-regulation of these genes in two different strains of *L. monocytogenes* (6). However, our data is consistent with the results of Broadbent et al. (8) that showed induction of genes associated with histidine metabolism in acid adapted *L. casei*. In addition, the authors demonstrated that supplementation of the growth medium with histidine conferred protection against acid challenge (8). The role of histidine in acid stress defense could be related to the basic nature of the amino acid. In the present study, genes encoding proteins implicated in osmotolerance including the *kdp* operon encoding a high affinity K^+ uptake system were over-expressed (Table 1). A recent study (6) showed that induction of the *kdp* operon in a strain-specific manner during growth under acidic conditions and *L. monocytogenes* tend to accumulate K^+ to counteract acid stress. A role of osmolytes in the cytoplasmic pH homeostasis of *Escherichia coli* has been shown previously (28). Verification of the role of histidine and osmolytes intracellular pool in acid tolerance in *L. monocytogenes* is thus of great interest.

V) Response involving general stress proteins

During growth under acidic conditions, induction of genes encoding general (universal) stress proteins was observed (Table 1). A recent study by Abram et al. (1) demonstrated the role of a general stress protein encoded by *lmo2391* in acid tolerance and interestingly our data showed induction of the *lmo2391* gene (Table 1). In addition, comparison of our data set with a previous heat shock response transcriptional study (55) revealed a total of 29 common up-regulated genes (not shown). This includes genes encoding universal stress proteins such as *lmo1580, lmo2673,* and *lmo2748* (Table 1), suggesting the contribution of general stress proteins in protection against acid as well as other non-specific stresses.

Genes associated with management of other stress conditions

It is well established that acid adapted *L. monocytogenes* exhibit cross-protection against other environmental stresses (19, 33, 37). In the present study, known and putative genes encoding

proteins associated with the management of stress conditions such as heavy metal toxicity, osmotic stress, oxidative stress, and UV light stress exhibited increases in expression (Table 1, Supplementary Table S3). Further research is necessary to elucidate a possible link between the induced genes and modulation in resistance to the stress conditions.

Influence of acid stress on virulence determinates

In order to investigate the effect of acid stress on virulence, a comprehensive list of known and potential virulence determinate genes were identified from a recent study (54). As shown in Fig. 5, an overall induction in virulence associated genes (24 up-regulated and 6 down-regulated) was observed. The proteins encoded by the up-regulated genes reported in the present study are known to have a role in adaptation in food, in the gastrointestinal tract as well as during the infection processes (18, 19, 56). Further studies using representative strains and appropriate models would give a better understanding on the effect of acid exposure on virulence properties.

Macromolecule syntheses and energy metabolism

The majority of genes encoding proteins involved in purine metabolism, pyrimidine metabolism, protein synthesis, sugar transport and citric acid cycle (particularly the oxidative portion) were down-regulated (Fig. 4, Supplementary Table S2) and this is in line with the reduced growth under acidic conditions (Fig. 1). Previous studies (8, 34) have demonstrated a similar repression of genes encoding proteins involved in active-energy consuming processes. The reason for this repression might be the need to use the available energy to maintain the internal pH homeostasis.

To elucidate the effect of acid stress on pyruvate metabolism, which is a key intersection in several pathways, genes encoding proteins involved in the pyruvate metabolism were examined. *L. monocytogenes* produce formate and ethanol under anaerobic growth condition but not during aerobic growth (43). However, the induction of *pflA* encoding the pyruvate formate-lyase that converts pyruvate to formate as well as the repression of *lmo2586* encoding a putative formate dehydrogenase that further catalyze the oxidation of formate to bicarbonate may suggest accumulation of formate during growth at acidic conditions (Supplementary Table S2). In addition, *lap* which encodes the enzyme alcohol-acetaldehyde dehydrogenase

that catalyzes the conversation of acetyl-CoA to ethanol *via* acetaldehyde exhibited a large increase in expression in the presence of the lactic acid (Supplementary Table S2). These results suggest that under acid stress, the pyruvate metabolism is shifted to a heterofermentative type resembling growth under "anaerobic" condition (43).

Synthesis of acetoin from pyruvate is suggested to alleviate acid stress (6). In the present study, however, the transcription of genes encoding the acetoin synthesis enzymes acetolactate synthase (*alsS*) and acetolactate decarboxylase (*alsD*) remained unchanged (not shown), and this contrast a recent study by Bowman et al. (6) that showed increase in the expression of *alsS* and *alsD* during growth under acidic conditions. Interestingly, these genes were induced for the same strain shortly after acid shock (our unpublished result), suggesting the transcriptional level of these genes may vary after the onset of acid stress. Further studies are necessary to support the transcriptional data with metabolic analyses.

Other responses

In addition to the genes described so far, genes encoding proteins involved in various roles such as transportation, signal transduction and regulation were influenced by the acid stress (Fig. 4, Supplementary Table S2). The expression of *sigL* (also known as *rpoN*) encoding σ^{54} (also called SigL) was found to be induced (Supplementary Table S2) but not the other four sigma factors described for *L. monocytogenes* (36). The induction of the *sigL* by lactic acid stress, as well as, the reported impaired growth of *sigL* null mutant during growth in the presence of this acid, particular at low temperature has suggested a possible role of σ^{54} in acid stress (40). In contrast to this, a more recent study has showed a higher survival rate of *sigL* null mutant under acidic stress compared to the parental strain (9). The σ^{54} is known to control the transcription of genes encoding proteins involved in carbohydrate and amino acid metabolisms (2). A number of two-component signal transduction system genes were also differentially regulated (Supplementary Table S2). Interestingly, several genes encoding proteins with unknown functions showed a marked increase in transcription under acidic growth conditions, suggesting more genes yet to be characterized related to acid stress response.

Transcriptional responses specific to the organic acids

Interestingly, the expression of genes encoding the mannose phosphotransferase system (encoded by *mptACD*), the maltose and maltodextrin ABC transporter (*lmo2121-lmo2126*) and a protein similar to beta-glucosidase (*lmo0917*) were down-regulated in the organic acid stressed cultures but were not affected in the HCl stressed culture (Supplementary Table S2). The reduced transcription of genes associated with sugar transport may be attributed to the pronounced growth inhibition of the strain particularly in the presence of the organic acids (Fig. 1). Comparison of transcriptional responses between the two organic acid stressed cultures showed difference only in six genes (Supplementary Table S2). Three of the genes, *lap* (encoding alcohol-acetaldehyde dehydrogenase), *lmo0989* (similar to a regulatory protein), and *lmo1257* (with unknown function) were up-regulated. *lmo2721* (similar to glucosamine-6-phosphate isomerase) was down-regulated in the lactic acid stressed culture. *lmo2145* and *lmo2742* (encoding proteins of unknown functions) were repressed in the presence of acetic acid. The exact roles of these specific genes in resistance to the organic acids remain to be investigated.

Concluding remarks

The growth study and the transcriptomic analyses provided insights regarding the response of the foodborne pathogen *L. monocytogenes* to acidulants and pH relevant to the food industry. This is, to our knowledge, the first study on the transcriptional responses of *L. monocytogenes* to acetic acid and lactic acid stress. The present study identified a number of known and putative genes that are linked to acid resistance including the GAD system, modification of the cell envelope, protection and repair of macromolecules, intracellular accumulation of histidine and osmolytes as well as general stress proteins. The cross-resistance of acid adapted cells to other stress conditions is of great concern and the present study identified possible genes that could be linked to acid induced cross-protection against a number of stress conditions. Low pH (in certain food and gastric juice) could be a signal to lead foodborne pathogens to a pathogenic lifestyle and both HCl and organic acids induced genes associated with virulence. The key differences between the HCl and the organic acid stress responses includes overall pronounced transcriptional responses in the presence of organic acids than in the HCl stressed culture and the transcripts associated with sugar uptake systems were reduced in the organic acids stressed cultures but not changed in the HCl stressed culture. In

conclusion, this study identified large number of genes with direct and indirect role in acid stress defense and other types of stress which also opens the possibilities for more specific and in-depth future studies.

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Table 1. Genes differentially regulated during growth in BHI broth acidified to pH 5 with hydrochloric acid (HCl), 10 mM acetic acid or 20 mM lactic acid.

Locus	Gene	Description (similar to)[a]	Log2 ratio[b]		
			HCl	AA	LA
GAD system					
lmo2362	*gadT2*	amino acid antiporter	**5.64**	**5.49**	**5.28**
lmo2363	*gadD2*	glutamate decarboxylase	**5.60**	**5.56**	**5.51**
lmo0913		conserved hypothetical protein	0.22	0.49	**0.90**
Modification of the cell envelope					
lmo0971	*dltD*	DltD protein	**0.86**	**1.11**	**1.12**
lmo0972	*dltC*	D-alanyl carrier protein	**0.65**	**0.85**	**1.05**
lmo0973	*dltB*	DltB protein	**0.84**	**1.15**	**1.42**
lmo0974	*dltA*	D-alanine-activating enzyme	**0.89**	**1.15**	**1.24**
lmo1369	*ptb*	phosphotransbutyrylase	**-0.69**	-0.16	**-1.37**
lmo1370	*buk*	branched-chain fatty-acid kinase	-0.48	-0.32	**-0.59**
lmo1371	*lpd*	branched-chain alpha-keto acid dehydrogenase E3 subunit	**-0.61**	-0.25	-0.55
lmo1372	*bkdA1*	branched-chain alpha-keto acid dehydrogenase E1 subunit	**-0.61**	-0.16	-0.57
lmo1374	*bkdB*	branched-chain alpha-keto acid dehydrogenase E2 subunit	**-0.74**	-0.25	-0.53
lmo1373	*bkdA2*	branched-chain alpha-keto acid dehydrogenase E1 subunit	-0.56	-0.16	-0.58
Protection and repair of macromolecules					
lmo0846		excinuclease ABC, chain C (UvrC)	**0.72**	**0.75**	**0.85**
lmo1234	*uvrC*	excinuclease ABC subunit C	**1.14**	**1.21**	**1.35**
lmo1248	*mutT*	8-oxo-dGTPase (mutT)	**0.71**	**1.10**	**2.07**
lmo1460	*recO*	*B. subtilis* RecO protein involved in DNA repair and homologous recombination	0.25	**0.60**	**0.57**
lmo1824	*priA*	primosomal replication factor Y	**0.59**	**0.84**	**0.90**
lmo1881		5-3 exonuclease	**1.06**	**1.40**	**1.60**
lmo2242		O^6-methylguanine-DNA methyltransferase	**0.41**	**0.82**	**1.01**
lmo2488	*uvrA*	excinuclease ABC (subunit A)	0.18	0.41	0.31
lmo2489	*uvrB*	excinuclease ABC (subunit B)	**0.59**	**0.94**	**0.95**
lmo2702	*recR*	recombination protein recR	**0.60**	**0.74**	**0.77**
lmo0292	*htrA*	heat-shock protein htrA serine protease	**1.07**	**1.12**	**1.26**
lmo1444	*prsA1*	protein secretion PrsA	**0.67**	**1.05**	**0.97**
lmo2068	*groEL*	class I heat-shock protein (chaperonin) GroEL	**-0.71**	**-0.74**	**-0.86**
lmo2069	*groES*	class I heat-shock protein (chaperonin) GroES	-0.50	-0.44	**-0.60**
Intracellular histidine and osmolytes accumulation					
lmo0565	*hisH*	amidotransferases	**0.77**	**0.86**	**0.72**
lmo0569	*hisZ*	histidyl-tRNA synthetase	**0.60**	**0.68**	**0.70**
lmo0561	*hisE*	phosphorybosil-AMP-cyclohydrolase (HisI2 protein)	0.47	**0.67**	**0.80**
lmo0562	*hisI*	phosphoribosyl-AMP cyclohydrolase (HisI1 protein)	0.50	0.48	0.55
lmo0563	*hisF*	cyclase HisF	0.55	**0.62**	**1.05**
lmo0564	*hisA*	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	0.52	**1.08**	**0.90**
lmo0566	*hisB*	imidazoleglycerol-phosphate dehydratase	0.55	0.57	**0.74**
lmo0567	*hisD*	histidinol dehydrogenases	0.47	0.56	**0.81**
lmo0568	*hisG*	ATP phosphoribosyltransferase	0.58	**0.82**	**0.75**

Table 1. Continued ..

Locus	Gene	Description (similar to)[a]	Log2 ratio[b]		
			HCl	AA	LA
lmo1425	*opuCD*	betaine/carnitine/choline ABC transporter (membrane p)	0.40	**0.75**	**0.98**
lmo1426	*opuCC*	glycine betaine/carnitine/choline ABC transporter (osmoprotectant-binding protein)	0.47	**0.95**	**0.95**
lmo1427	*opuCB*	glycine betaine/carnitine/choline ABC transporter (membrane protein)	0.47	**0.99**	**1.30**
lmo1428	*opuCA*	glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)	0.57	**0.99**	**1.36**
lmo2680	*kdpC*	potassium-transporting ATPase c chain	**0.91**	**1.52**	**2.09**
lmo2681	*kdpB*	potassium-transporting ATPase b chain	**1.01**	**2.18**	**3.28**
lmo2682	*kdpA*	highly potassium-transporting ATPase a chain	**1.19**	**2.58**	**3.53**
General stress proteins					
lmo0211	*ctc*	*B. subtilis* general stress protein	**1.12**	**1.74**	**2.36**
lmo1580		unknown protein	0.29	**0.69**	**0.87**
lmo2391		*B. subtilis* YhfK protein	**1.48**	**1.40**	**1.52**
lmo2398	*ltrC*	*B. subtilis* YutG protein	**0.95**	**0.93**	**0.95**
lmo2673		conserved hypothetical protein	0.22	0.49	**0.90**
lmo2748		*B. subtilis* stress protein YdaG	0.42	**0.86**	**1.37**

[a] Description of genes according to the annotation of the Comprehensive Microbial Resource of the J. Craig Venter Institute (http://cmr.jcvi.org) and published literature sources.

[b] Log2 expression ratio (HCl, hydrochloric acid; AA, acetic acid; or LA, lactic acid/reference culture); bold type values with log2 ratios ≥ 0.585 or ≤ -0.585 and with q-value < 0.01 (p-value adjusted to control false discovery rate). Extended list of genes with q-values are given in Supplementary Table S2.

CAPTIONS

Fig. 1. Growth of *L. monocytogenes* L502 **(A)** OD_{600} **(B)** total viable count and **(C)** the pH of the corresponding cultures in BHI broth acidified to pH 5 with hydrochloric acid (HCl) (◊), 10 mM acetic acid (Δ), or 20 mM lactic acid (■). Growth in BHI broth pH 7.2 (●) was used as reference. The solid and the dashed arrows indicate time of exposure to the acids and harvesting, respectively. The error bars represent standard errors of the mean.

Fig. 2. Venn diagram showing total number of genes **(A)** up-regulated and **(B)** down-regulated during growth in BHI broth acidified to pH 5 with HCl, 10 mM acetic acid (AA) or 20 mM lactic acid (LA).

Fig. 3. PCA score plot of genes differentially expressed in any of the acid stressed cultures during growth in BHI broth acidified to pH 5 with hydrochloric acid (HCl), 10 mM acetic acid (AA) or 20 mM lactic acid (LA). All the dye-swap and technical replicates from the two biological cultures were included in the PCA. The explained variances for PC1and PC2 are 47% and 12% respectively.

Fig. 4. Total number of **(A)** up-regulated and **(B)** down-regulated genes in different functional role categories according to the primary annotation in CMR-JCVI *L. monocytogenes* EGDe genome database during growth in BHI broth acidified to pH 5 with HCl (white bars), 10 mM acetic acid (gray bars) or 20 mM lactic acid (black bars).

Fig. 5. Virulence determinants expressed in any of the acid stressed cultures during growth in BHI broth acidified to pH 5 with hydrochloric acid (HCl), 10 mM acetic acid (AA) or 20 mM lactic acid (LA). List of virulence determinant genes were taken from reference (54).

Fig. 1

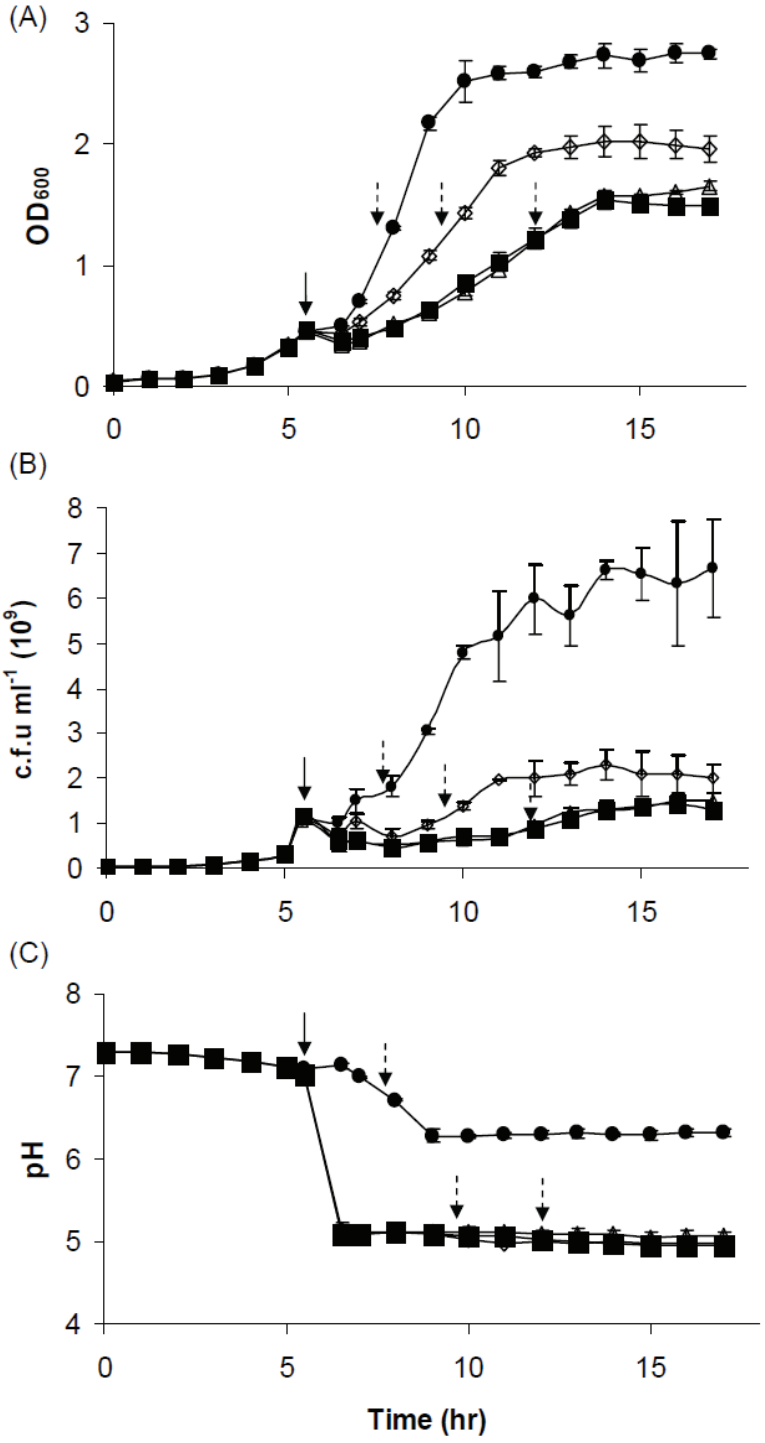

(A)
OD600
3
2
1
0
0
5
10
15
(B)
c.f.u ml-1 (10^9)
8
7
6
5
4
3
2
1
0
0
5
10
15
(C)
pH
8
7
6
5
4
0
5
10
15
Time (hr)

Fig. 2

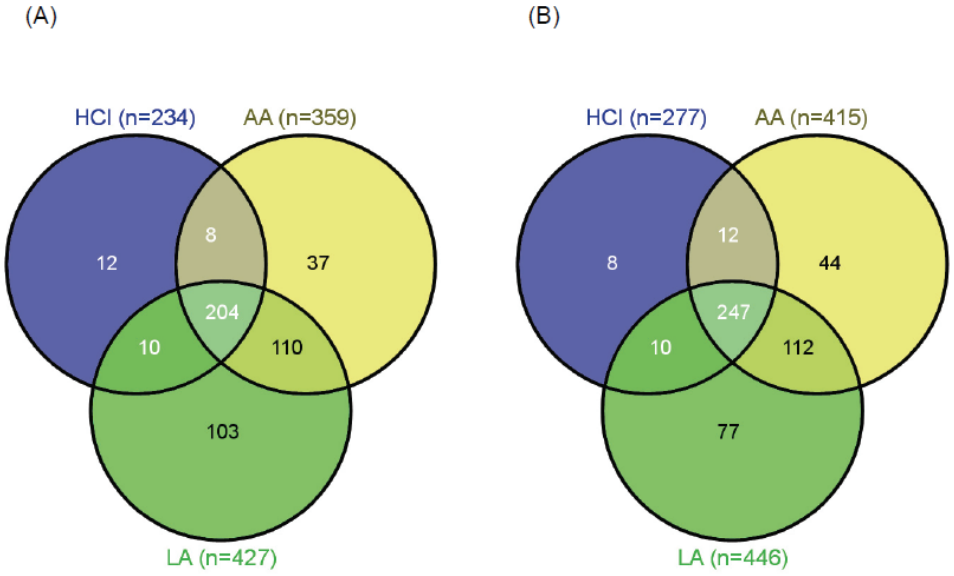

(A)
HCl (n=234)
AA (n=359)
12
8
37
204
10
110
103
LA (n=427)
(B)
HCl (n=277)
AA (n=415)
8
12
44
247
10
112
77
LA (n=446)

Fig. 3

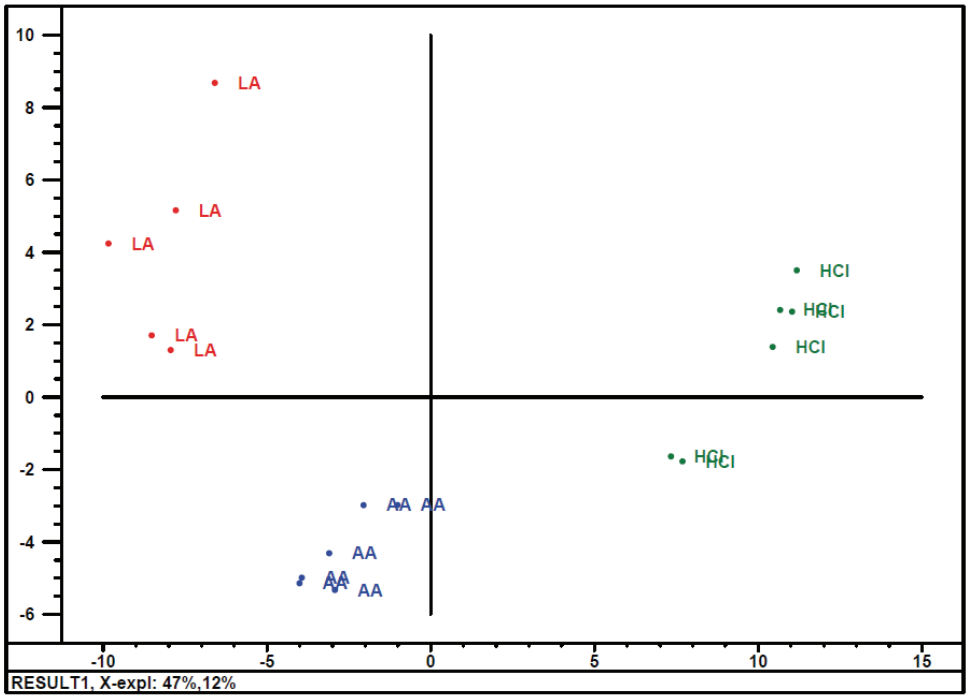

10
8
6
4
2
0
-2
-4
-6
-10
-5
0
5
10
15
LA
LA
LA
LA
LA
HCl
HCl
HCl
HCl
AA
AA
AA
AA
AA
RESULT1, X-expl: 47%,12%

Fig. 4

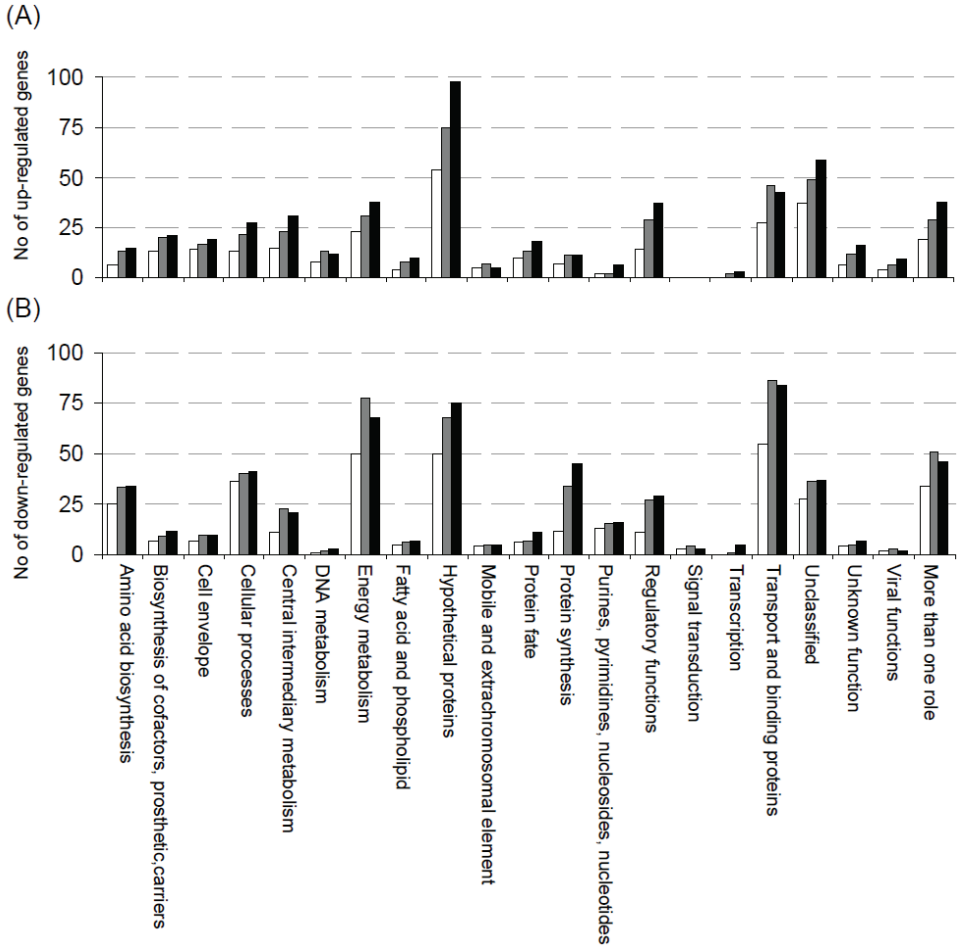
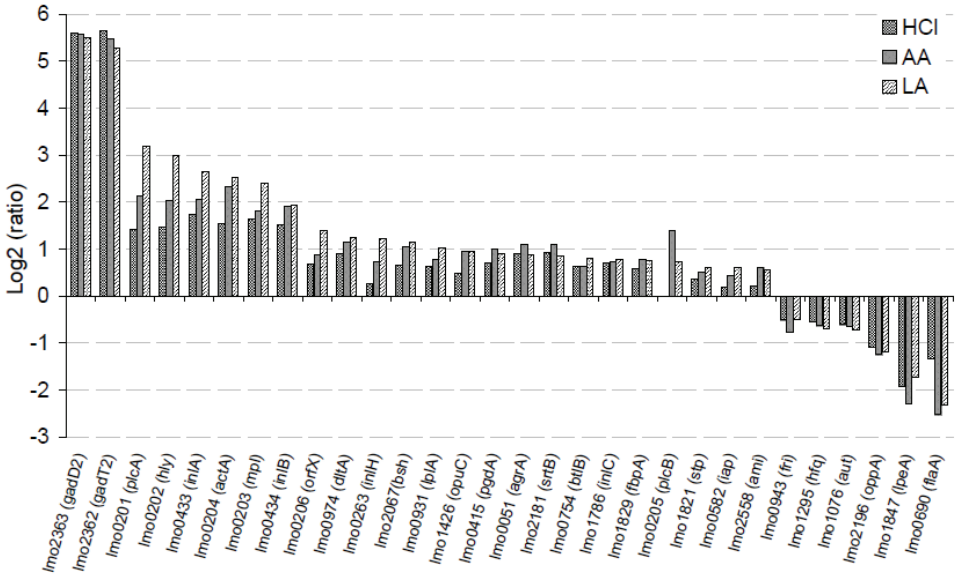

(A)
No of up-regulated genes
100
75
50
25
0
(B)
No of down-regulated genes
100
75
50
25
0
Amino acid biosynthesis
Biosynthesis of cofactors, prosthetic,carriers
Cell envelope
Cellular processes
Central intermediary metabolism
DNA metabolism
Energy metabolism
Fatty acid and phospholipid
Hypothetical proteins
Mobile and extrachromosomal element
Protein fate
Protein synthesis
Purines, pyrimidines, nucleosides, nucleotides
Regulatory functions
Signal transduction
Transcription
Transport and binding proteins
Unclassified
Unknown function
Viral functions
More than one role

Fig. 5


HCl
AA
LA
Log2 (ratio)
6
5
4
3
2
1
0
-1
-2
-3
lmo2363 (gadD2)
lmo2362 (gadT2)
lmo0201 (plcA)
lmo0202 (hly)
lmo0433 (inlA)
lmo0204 (actA)
lmo0203 (mpl)
lmo0434 (inlB)
lmo0206 (orfX)
lmo0974 (dltA)
lmo0263 (inlH)
lmo2067(bsh)
lmo0931 (lplA)
lmo1426 (opuC)
lmo0415 (pgdA)
lmo0051 (agrA)
lmo2181 (srtB)
lmo0754 (btlB)
lmo1786 (inlC)
lmo1829 (fbpA)
lmo0205 (plcB)
lmo1821 (stp)
lmo0582 (iap)
lmo2558 (ami)
lmo0943 (fri)
lmo1295 (hfq)
lmo1076 (aut)
lmo2196 (oppA)
lmo1847 (lpeA)
lmo0690 (flaA)

SUPPLEMENTAL MATERIALS

Table S1. Primer and probe sets used for qRT-PCR

Target	Primer and probe name	Sequence[a] (5′→3′)	Reference[b]
mptA (lmo0096)	MptAF	CCTCGCAACTCACGGTGAAT	Tessema et al.
	MptAR	TCTTGCTCGCCGAAAATCA	
	MptA-Taq	TGCTGAAGGTATTTTGCAGTCCGGAACA	
actA (lmo0204)	lmo0204F	CGACCGACCAGCTATACAAGTG	This study
	lmo0204R	AATTTCCGCTGCGCTATCC	
	lmo0204-Taq	AGCGTCGTCATCCAGGATTGCCA	
lmo1251	lmo1251F	TGGAAATAGCGCCTGATCAAT	This study
	lmo1251R	TCCCGCAAAAATGACCAATT	
	lmo1251-Taq	TTTTCTAGTAAGCAATGAAG)	
glpK (lmo1538)	lmo1538F	GAAAGCAATGCTGCCAGAAGT	This study
	lmo1538R	GCAATACCTGCAACCGGAACT	
	lmo1538-Taq	ATCCTCTGAAGTATATGCGGACACAGTGCC	
kdpA (mo2682)	lmo2682F	GTTGGTGGTTCGTCGCTTTT	This study
	lmo2682R	TGTCGTGCATTGCGTTCAC	
	lmo2682-Taq	CGGCTTCCACAACGGCAGCC	
16S rRNA	16S rRNAF	GCGCAGGCGGTCTTTTAAG	Tessema et al.
	16S rRNAR	CAATGACCCTCCCCGGTTA	
	16S rRNA-Taq	CTGATGTGAAAGCCCCCGGC	

[a]Taq probes, 6-FAM, 6-carboxyfluorescein (fluorophore); TAMRA, 6-carboxytetramethylrhodamine (quencher)

[b] Tessema et al. 2009. Appl. Environ. Microbiol. 75:6973-6980.

Table S2. Genes differentially regulated in *L. monocytogenes* L502 during growth in brain heart infusion broth acidified to pH 5 with hydrochloric acid (HCl), 10 mM acetic acid (AA) or 20 mM lactic acid (LA) compared to reference culture pH 7.2.

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
Amino acid metabolism up-regulated									
lmo0238	*cysE*	serine O-acetyltransferase		0.26	0.006	**0.59**	**0.000**	0.40	0.000
lmo0561		phosphorybosil-AMP-cyclohydrolase (HisI2 protein)		0.47	0.001	**0.67**	**0.001**	**0.80**	**0.000**
lmo0563	*hisF*	cyclase HisF		0.55	0.000	**0.62**	**0.000**	**1.05**	**0.000**
lmo0564	*hisA*	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase		0.52	0.000	**1.08**	**0.000**	**0.90**	**0.000**
lmo0565	*hisH*	amidotransferases		**0.77**	**0.000**	**0.86**	**0.000**	**0.72**	**0.000**
lmo0566	*hisB*	imidazoleglycerol-phosphate dehydratase		0.55	0.000	0.57	0.000	**0.74**	**0.000**
lmo0567	*hisD*	histidinol dehydrogenases		0.47	0.000	0.56	0.000	**0.81**	**0.000**
lmo0568	*hisG*	ATP phosphoribosyltransferase		0.58	0.000	**0.82**	**0.000**	**0.75**	**0.000**
lmo0594		homoserine O-acetyltransferase		**0.66**	**0.000**	**0.84**	**0.000**	**1.20**	**0.000**
lmo0978		branched-chain amino acid aminotransferase		0.49	0.000	0.53	0.000	**0.70**	**0.000**
lmo1387		pyrroline-5-carboxylate reductase		0.49	0.000	**0.66**	**0.000**	**0.90**	**0.000**
lmo1513		iron-sulfur cofactor synthesis protein	E	0.47	0.000	0.50	0.000	**0.79**	**0.000**
lmo1600	*aroA*	deoxy-D-arabino-heptulosonate 7-phosphate synthase		0.29	0.014	**0.61**	**0.002**	0.47	0.001
lmo1681		cobalamin-independent methionine synthase		**1.11**	**0.000**	**1.49**	**0.000**	**1.70**	**0.000**
Amino acid metabolism down-regulated									
lmo0036	*arcB*	ornithine carbamoyltransferase		**-0.82**	**0.004**	**-0.75**	**0.003**	NA	NA
lmo1588	*argD*	N-acetylornithine aminotransferase		**-1.06**	**0.000**	**-1.48**	**0.002**	**-1.47**	**0.001**
lmo1589	*argB*	N-acetylglutamate 5-phosphotransferase		**-1.92**	**0.000**	**-1.32**	**0.000**	**-1.02**	**0.009**
lmo1591	*argC*	N-acetylglutamate gamma-semialdehyde dehydrogenases		**-0.87**	**0.002**	-0.35	0.055	-0.73	0.015
lmo1627	*trpA*	tryptophan synthase (alpha subunit)		-0.45	0.016	**-0.65**	**0.008**	NA	NA
lmo1628	*trpB*	tryptophan synthase (beta subunit)		-0.47	0.000	-0.53	0.003	**-0.65**	**0.000**
lmo1983	*ilvD*	dihydroxy-acid dehydratase		-0.59	0.016	-0.77	0.038	**-0.86**	**0.005**
lmo1990	*leuD*	3-isopropylmalate dehydratase (small subunit)		**-0.74**	**0.000**	-0.41	0.019	**-1.10**	**0.000**
lmo2090	*argG*	argininosuccinate synthase		**-1.81**	**0.000**	**-1.92**	**0.000**	**-2.01**	**0.000**
lmo2091	*argH*	argininosuccinate lyase		**-2.00**	**0.000**	**-2.30**	**0.000**	**-2.01**	**0.000**
lmo2252		aspartate aminotransferase		**-1.31**	**0.000**	**-1.53**	**0.000**	**-1.73**	**0.000**
lmo2524		hydroxymyristoyl-(acyl carrier protein) dehydratase		-0.56	0.000	**-0.74**	**0.000**	**-0.79**	**0.000**
lmo2539	*glyA*	glycine hydroxymethyltransferase	B	-0.51	0.000	**-0.67**	**0.000**	**-0.68**	**0.000**
lmo2545	*thrB*	homoserine kinase		-0.50	0.000	-0.48	0.000	**-0.82**	**0.000**
lmo2546	*thrC*	threonine synthase		**-0.61**	**0.000**	-0.55	0.000	**-1.00**	**0.000**
lmo2547	*hom*	homoserine dehydrogenase		-0.23	0.000	-0.17	0.001	**-0.61**	**0.000**
Biosynthesis of cofactors, prosthetic groups, and carriers up-regulated									
lmo0316		hydroxyethylthiazole kinase (ThiM)		**0.91**	**0.000**	**0.91**	**0.000**	**1.58**	**0.000**
lmo0317		phosphomethylpyrimidine kinase (ThiD)		0.40	0.080	**0.93**	**0.000**	**1.27**	**0.000**
lmo0318		thiamin-phosphate pyrophosphorylase (ThiE)		**0.84**	**0.000**	**0.74**	**0.001**	**1.22**	**0.000**
lmo0884		protoporphyrinogen IX and coproporphyrinogen III oxidase (HemY)		**0.60**	**0.000**	**0.90**	**0.000**	**1.09**	**0.000**
lmo1038		molybdopterin-guanine dinucleotide biosynthesis protein A		**0.75**	**0.000**	**0.79**	**0.000**	**1.08**	**0.000**
lmo1147		bifunctional cobalamin biosynthesis protein CopB, (cobinamide kinase; cobinamide phosphatase guanylyltransferase)		**0.66**	**0.000**	**0.78**	**0.000**	**0.95**	**0.000**
lmo1148		cobalamin (5-phosphatase) synthetase		**0.69**	**0.000**	**0.97**	**0.000**	**0.91**	**0.000**
lmo1365	*tktB*	D-1-deoxyxylulose 5-phosphate synthase		0.56	0.000	**0.71**	**0.000**	**0.85**	**0.000**
lmo1553	*hemL*	glutamate-1-semialdehyde 2,1-aminotransferases		0.28	0.137	0.87	0.012	**0.84**	**0.005**
lmo1554	*hemB*	delta-aminolevulinic acid dehydratases (porphobilinogen synthase)		**0.98**	**0.000**	**0.74**	**0.000**	**0.78**	**0.000**
lmo1555		uroporphyrinogen III cosynthase (HemD)		0.31	0.000	0.52	0.000	**0.68**	**0.000**
lmo1556	*hemC*	porphobilinogen deaminases (hydroxymethylbilane synthase)		0.30	0.000	0.40	0.000	**0.62**	**0.000**
lmo1557	*hemA*	glutamyl-tRNA reductase		0.31	0.000	0.33	0.000	**0.59**	**0.000**
lmo1676	*menF*	menaquinone-specific isochorismate synthase		0.41	0.000	0.56	0.000	**0.59**	**0.000**
lmo1901	*panC*	panthotenate synthetases		0.57	0.000	**0.60**	**0.000**	0.57	0.000
lmo2211	*hemH*	ferrochelatase		0.51	0.000	**0.61**	**0.000**	0.57	0.000
lmo2212	*hemE*	uroporphyrinogen III decarboxylase		0.52	0.000	**0.68**	**0.000**	0.55	0.000
lmo2256		unknown protein		**0.62**	**0.000**	0.40	0.000	0.51	0.000
lmo2571		nicotinamidase		0.29	0.000	**0.60**	**0.000**	0.47	0.000
lmo2770		gamma-glutamylcysteine synthetase (for the N-terminal part) and to cyanophycin synthetase (C-terminal part)		0.43	0.000	**0.61**	**0.000**	0.44	0.000
Biosynthesis of cofactors, prosthetic groups, and carriers down-regulated									
lmo0637		methyltransferase		-0.33	0.003	-0.44	0.000	**-0.61**	**0.001**
lmo1042		molybdopterin biosynthesis protein moeA		-0.37	0.000	-0.50	0.000	**-0.66**	**0.000**
lmo1043		molybdopterin-guanine dinucleotide biosynthesis MobB		-0.40	0.000	-0.54	0.000	**-0.67**	**0.000**
lmo1045		molybdopterin converting factor (subunit 1).		-0.41	0.000	**-0.65**	**0.000**	**-0.78**	**0.000**
lmo1046	*moaC*	molybdenum cofactor biosynthesis protein C		**-0.66**	**0.000**	**-1.08**	**0.000**	**-0.78**	**0.000**
lmo1047	*moaA*	molybdenum cofactor biosynthesis protein A		**-0.68**	**0.000**	**-0.93**	**0.000**	**-0.83**	**0.000**
lmo1048	*moaB*	molybdenum cofactor biosynthesis protein B		**-0.65**	**0.000**	**-0.91**	**0.000**	**-0.84**	**0.000**
lmo1315		undecaprenyl diphosphate synthase		**-0.63**	**0.000**	**-1.01**	**0.000**	**-0.86**	**0.000**
lmo1930		heptaprenyl diphosphate synthase component II (menaquinone biosynthesis)		**-0.76**	**0.000**	**-1.03**	**0.000**	**-0.88**	**0.000**
lmo2046		ketopantoate reductase involved in thiamin biosynthesis		**-0.61**	**0.000**	**-1.04**	**0.000**	**-0.97**	**0.000**
lmo2101		protein required for pyridoxine synthesis		**-0.72**	**0.000**	**-0.98**	**0.000**	**-1.04**	**0.000**
Cell envelope up-regulated									
lmo0366		conserved hypothetical protein, putative lipoprotein		**0.85**	**0.000**	**0.92**	**0.000**	**0.80**	**0.000**
lmo0415		endo-1,4-beta-xylanase		**0.70**	**0.000**	**1.01**	**0.000**	**0.89**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo0481		unknown protein		0.19	0.168	**0.72**	**0.001**	**0.60**	**0.000**
lmo0603		unknown		0.40	0.000	0.42	0.000	**0.60**	**0.000**
lmo0610		internalin proteins, putative peptidoglycan bound protein (LPXTG motif)		**2.05**	**0.000**	**1.84**	**0.000**	**1.60**	**0.000**
lmo0880		wall associated protein precursor (LPXTG motif)		**0.73**	**0.000**	**1.15**	**0.000**	**1.51**	**0.000**
lmo0886	*dal*	alanine racemase		0.43	0.000	**0.65**	**0.000**	**0.69**	**0.000**
lmo0971	*dltD*	DltD protein for D-alanine esterification of lipoteichoic acid and wall teichoic acid		**0.86**	**0.000**	**1.11**	**0.000**	**1.12**	**0.000**
lmo0972	*dltC*	D-alanyl carrier protein		**0.65**	**0.000**	**0.85**	**0.000**	**1.05**	**0.000**
lmo0973	*dltB*	DltB protein for D-alanine esterification of lipoteichoic acid and wall teichoic acid		**0.84**	**0.000**	**1.15**	**0.000**	**1.42**	**0.000**
lmo1062		ABC transporters (permease protein)		**0.72**	**0.000**	**0.61**	**0.000**	NA	NA
lmo1216		N-acetylmuramoyl-L-alanine amidase (autolysin)		**0.96**	**0.000**	**0.95**	**0.000**	**1.10**	**0.000**
lmo1290		internalin proteins, putative peptidoglycan bound protein (LPXTG motif)		**0.81**	**0.000**	**1.33**	**0.000**	**0.98**	**0.000**
lmo1413		putative peptidoglycan bound protein (LPXTG motif)		**1.21**	**0.000**	**1.48**	**0.000**	**1.53**	**0.000**
lmo1438		penicillin-binding protein		**0.65**	**0.000**	0.53	0.000	0.56	0.000
lmo1605	*murC*	UDP-N-acetyl muramate-alanine ligases		0.22	0.001	0.49	0.000	**0.60**	**0.000**
lmo1713		cell-shape determining proteins		**0.62**	**0.000**	0.49	0.000	**0.82**	**0.000**
lmo1799		putative peptidoglycan bound protein (LPXTG motif)		**0.64**	**0.000**	0.37	0.000	0.41	0.000
lmo1829		fibronectin binding proteins		0.57	0.000	**0.79**	**0.000**	**0.76**	**0.004**
lmo2043		integral membrane proteins		0.52	0.000	0.56	0.030	**0.73**	**0.004**
lmo2691		autolysin, N-acetylmuramidase		0.26	0.000	**0.60**	**0.000**	0.44	0.000
Cell envelope down-regulated									
lmo0514		internalin protein, putative peptidoglycan bound protein (LPXTG motif)		**-0.62**	**0.000**	**-0.65**	**0.000**	-0.57	0.004
lmo0688		unknown protein		**-0.88**	**0.000**	**-1.12**	**0.000**	**-1.29**	**0.000**
lmo0695		unknown	N,V	**-0.94**	**0.000**	**-1.52**	**0.000**	**-1.52**	**0.000**
lmo0789		conserved hypothetical proteins		-0.25	0.000	-0.52	0.000	**-0.75**	**0.000**
lmo1044		molybdopterin converting factor, subunit 2		**-0.88**	**0.000**	**-1.24**	**0.000**	**-1.21**	**0.000**
lmo1076		autolysin (EC 3.5.1.28) (N-acetylmuramoyl-L-alanine amidase)		**-0.61**	**0.000**	**-0.64**	**0.000**	**-0.72**	**0.000**
lmo1521		N-acetylmuramoyl-L-alanine amidase		-0.34	0.000	-0.53	0.000	**-0.62**	**0.000**
lmo2130		unknown protein		-0.46	0.000	**-0.97**	**0.000**	**-1.26**	**0.000**
lmo2147		unknown protein		-0.05	0.076	-0.46	0.000	**-0.72**	**0.000**
lmo2312		unknown		-0.14	0.279	**-0.59**	**0.007**	-0.38	0.004
lmo2590		ATP binding proteins		-0.35	0.015	**-1.00**	**0.001**	NA	NA
lmo2854		*B. subtilis* SpoIIIJ protein		**-0.77**	**0.000**	**-1.06**	**0.000**	**-1.26**	**0.000**
Cellular process up-regulated									
lmo0153		probable high-affinity zinc ABC transporter (Zn(II)-binding lipoprotein)	C,M	0.11	0.245	**0.60**	**0.000**	**0.70**	**0.003**
lmo0201	*plcA*	phosphatidylinositol-specific phospholipase c	I	**1.41**	**0.000**	**2.14**	**0.000**	**3.19**	**0.000**
lmo0205	*plcB*	phospholipase C	I	NA	NA	**1.40**	**0.002**	**0.72**	**0.008**
lmo0263	*inlH*	internalin H		0.27	0.067	**0.74**	**0.000**	**1.22**	**0.001**
lmo0291		conserved hypothetical protein *B. subtilis* YycJ protein	U	**1.01**	**0.000**	**1.33**	**0.000**	**1.30**	**0.000**
lmo0389	*ltrA*	low temperature requirement protein A	M	**0.61**	**0.000**	**0.59**	**0.000**	**0.63**	**0.002**
lmo0433	*inlA*	Internalin A		**1.74**	**0.000**	**2.06**	**0.000**	**2.66**	**0.000**
lmo0434	*inlB*	Internalin B		**1.50**	**0.000**	**1.90**	**0.000**	**1.92**	**0.000**
lmo0669		oxidoreductase		0.54	0.000	**0.88**	**0.002**	**1.32**	**0.000**
lmo0906		glutathione reductase	B	**0.62**	**0.000**	**0.81**	**0.000**	**1.05**	**0.000**
lmo0983		glutathione peroxidase		0.35	0.000	0.39	0.000	**0.70**	**0.000**
lmo1400		N-acetyltransferase		0.58	0.000	**1.13**	**0.000**	**1.11**	**0.000**
lmo1433		glutathione reductase	B	**2.02**	**0.000**	**2.09**	**0.000**	**1.84**	**0.000**
lmo1580		unknown protein		0.29	0.000	**0.69**	**0.000**	**0.87**	**0.000**
lmo1694		CDP-abequose synthase		**0.65**	**0.000**	**1.11**	**0.000**	**1.44**	**0.000**
lmo1786	*inlC*	internalin C		**0.71**	**0.000**	**0.72**	**0.000**	**0.79**	**0.000**
lmo2167		unknown protein	U	0.41	0.000	0.36	0.000	**0.61**	**0.000**
lmo2189		putative competence protein from streptococcus pneumoniae		0.58	0.000	**0.86**	**0.000**	**0.92**	**0.000**
lmo2190	*mecA*	competence negative regulator mecA		0.33	0.010	0.34	0.003	**0.66**	**0.000**
lmo2230		arsenate reductase		0.34	0.000	**0.81**	**0.000**	**1.30**	**0.000**
lmo2398	*ltrC*	low temperature requirement C protein, also *B. subtilis* YutG protein	M	**0.95**	**0.000**	**0.93**	**0.000**	**0.95**	**0.000**
lmo2399		conserved hypothetical proteins		**0.89**	**0.000**	**0.96**	**0.000**	**0.71**	**0.000**
lmo2512	*comFC*	late competence protein comFC		0.30	0.021	NA	NA	**1.27**	**0.001**
lmo2673		conserved hypothetical protein		0.22	0.000	0.49	0.001	**0.90**	**0.000**
Cellular process down-regulated									
lmo0152		oligopeptide ABC transporter-binding protein	L	**-1.56**	**0.000**	**-1.59**	**0.000**	**-2.18**	**0.000**
lmo0196		*B. subtilis* SpoVG protein		-0.42	0.000	**-0.65**	**0.000**	**-0.67**	**0.000**
lmo0197		*B. subtilis* SpoVG protein		**-0.59**	**0.000**	**-0.79**	**0.000**	**-0.79**	**0.000**
lmo0275		C-terminal part *B. subtilis* ComEC protein		-0.56	0.000	-0.58	0.000	**-0.60**	**0.000**
lmo0331		internalin, putative peptidoglycan bound protein (LPXTG motif)		-0.31	0.003	-0.54	0.001	**-0.66**	**0.000**
lmo0601		cell surface protein		**-0.65**	**0.000**	-0.45	0.001	**-0.79**	**0.000**
lmo0682		flagellar hook-basal body protein FlgG		-0.56	0.000	**-0.75**	**0.000**	**-0.93**	**0.000**
lmo0683		chemotactic methyltransferase CheR		**-0.84**	**0.000**	**-1.17**	**0.000**	**-0.97**	**0.002**
lmo0685		motility protein (flagellar motor rotation) MotA	C	**-0.65**	**0.000**	**-0.95**	**0.000**	**-1.23**	**0.000**
lmo0686	*motB*	motility protein (flagellar motor rotation) MotB		**-0.70**	**0.000**	**-0.96**	**0.000**	**-1.21**	**0.000**
lmo0689		CheA activity-modulating chemotaxis protein CheV		**-0.95**	**0.000**	**-1.24**	**0.000**	**-1.51**	**0.000**
lmo0690	*flaA*	flagellin protein		**-1.33**	**0.000**	**-2.53**	**0.000**	**-2.32**	**0.000**
lmo0691	*cheY*	Chemotaxis response regulator CheY	P	**-0.88**	**0.000**	**-1.41**	**0.000**	**-1.37**	**0.000**
lmo0692	*cheA*	two-component sensor histidine kinase CheA		-0.58	0.000	**-1.05**	**0.000**	**-0.98**	**0.000**
lmo0693		flagellar motor switch protein FliY C-terminal part		**-1.04**	**0.000**	**-1.65**	**0.000**	**-1.65**	**0.000**
lmo0696		flagellar hook assembly protein		**-1.12**	**0.000**	**-1.81**	**0.000**	**-1.91**	**0.000**
lmo0697		flagellar hook protein FlgE		**-0.91**	**0.000**	**-1.65**	**0.000**	**-1.80**	**0.000**
lmo0698		flagellar switch protein		**-1.16**	**0.000**	**-1.79**	**0.000**	**-1.89**	**0.000**
lmo0699		flagellar switch protein FliM		**-0.96**	**0.000**	**-1.73**	**0.000**	**-1.85**	**0.000**
lmo0700		flagellar motor switch protein FliY		**-1.33**	**0.000**	**-1.99**	**0.000**	**-2.09**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo0705		flagellar hook-associated protein FlgK		**-1.01**	**0.000**	**-1.54**	**0.000**	**-1.58**	**0.000**
lmo0706		flagellar hook-associated protein 3 FlgL		**-1.51**	**0.000**	**-2.12**	**0.000**	**-2.09**	**0.000**
lmo0707		flagellar hook-associated protein 2 FliD		**-1.61**	**0.000**	**-2.28**	**0.000**	**-2.16**	**0.000**
lmo0708		hypothetical flagellar protein		**-1.61**	**0.000**	**-2.20**	**0.000**	**-2.22**	**0.000**
lmo0710		flagellar basal-body rod protein FlgB		**-1.59**	**0.000**	**-2.22**	**0.000**	**-2.24**	**0.000**
lmo0711		flagellar basal-body rod protein FlgC		**-1.70**	**0.000**	**-2.34**	**0.000**	**-2.18**	**0.000**
lmo0712		flagellar hook-basal body complex protein FliE		**-1.53**	**0.000**	**-2.11**	**0.000**	**-2.12**	**0.000**
lmo0713		flagellar basal-body M-ring protein FliF		**-1.42**	**0.000**	**-1.98**	**0.000**	**-1.91**	**0.000**
lmo0714		flagellar motor switch protein FliG		**-1.32**	**0.000**	**-1.72**	**0.000**	**-1.72**	**0.000**
lmo0723		metyl-accepting chemotaxis protein		**-1.59**	**0.000**	**-2.42**	**0.000**	**-2.11**	**0.000**
lmo1245		unknown		**-0.71**	**0.000**	**-1.05**	**0.000**	**-1.26**	**0.000**
lmo1364	*cspL*	cold shock protein		**-0.65**	**0.000**	**-0.74**	**0.000**	**-1.18**	**0.000**
lmo1625		putative transporters	M	**-0.67**	**0.000**	-0.56	0.000	-0.44	0.001
lmo1638		unknown protein		-0.58	0.000	**-0.65**	**0.000**	**-1.08**	**0.000**
lmo1699		some similarities to methyl-accepting chemotaxis proteins		**-1.11**	**0.000**	**-1.60**	**0.000**	**-1.43**	**0.000**
lmo1700		unknown		**-1.17**	**0.000**	**-1.72**	**0.000**	**-1.52**	**0.000**
lmo1729		beta-glucosidases	E,H,I	**-0.80**	**0.001**	**-1.32**	**0.000**	**-0.98**	**0.005**
lmo1879	*cspD*	cold shock protein		**-1.84**	**0.000**	**-2.46**	**0.000**	**-2.06**	**0.000**
lmo2016	*cspB*	major cold-shock protein		**-0.84**	**0.000**	**-0.88**	**0.001**	**-0.91**	**0.001**
lmo2196	*oppA*	pheromone ABC transporter (binding protein)	L	**-1.08**	**0.000**	**-1.25**	**0.000**	**-1.20**	**0.000**
lmo2569		dipeptide ABC transporter (dipeptide-binding protein)	L	**-3.03**	**0.000**	**-4.17**	**0.000**	**-3.80**	**0.000**
lmo2687		cell division protein FtsW		-0.50	0.000	**-0.65**	**0.000**	-0.56	0.000
lmo2688		cell division protein FtsW		-0.43	0.000	**-0.59**	**0.000**	-0.52	0.000
Central intermediary metabolism up-regulated									
lmo0030		conserved hypothetical protein		0.28	0.000	**0.65**	**0.001**	**0.65**	**0.001**
lmo0050	*agrC*	sensor histidine kinase (AgrC from *Staphylococcus*)		**1.01**	**0.000**	**1.44**	**0.000**	**1.56**	**0.000**
lmo0078		phosphoglycerate dehydrogenase		0.36	0.001	0.56	0.000	**0.80**	**0.000**
lmo0134		*E. coli* YjdJ protein		0.57	0.000	**0.89**	**0.000**	**0.88**	**0.003**
lmo0271		phospho-beta-glucosidase		0.27	0.000	0.51	0.000	**0.60**	**0.000**
lmo0339		inorganic pyrophosphatase		0.29	0.000	0.45	0.000	**0.60**	**0.000**
lmo0493		acylase		0.38	0.000	0.43	0.000	**0.71**	**0.000**
lmo0574		beta-glucosidase		0.48	0.000	**1.11**	**0.000**	**1.36**	**0.000**
lmo0609		*E. coli* phage shock protein E		**0.72**	**0.000**	**0.82**	**0.000**	**0.85**	**0.000**
lmo0663		conserved hypothetical proteins		**0.63**	**0.000**	**0.76**	**0.000**	**0.82**	**0.000**
lmo0792		conserved hypothetical protein		**0.62**	**0.000**	**0.86**	**0.000**	**0.85**	**0.000**
lmo0823		oxydoreductases		**0.83**	**0.000**	**0.69**	**0.000**	**1.10**	**0.000**
lmo0849		amidases		**0.63**	**0.000**	0.36	0.002	0.40	0.019
lmo1006		aminotransferases (*B. subtilis* PatA protein)		0.44	0.000	**0.59**	**0.000**	0.56	0.000
lmo1145		*Salmonella* enterica PduV protein		NA	NA	0.75	0.019	**0.75**	**0.008**
lmo1258		unknown		0.32	0.000	**0.69**	**0.000**	**0.77**	**0.000**
lmo1477		oxidoreductase		**1.03**	**0.000**	**1.24**	**0.000**	**0.91**	**0.001**
lmo1684		glycerate dehydrogenases		**0.83**	**0.000**	**1.18**	**0.000**	**1.47**	**0.000**
lmo1961		oxidoreductases		**0.68**	**0.000**	**0.80**	**0.000**	**0.71**	**0.000**
lmo2370		aminotransferase		0.39	0.000	0.57	0.000	**0.71**	**0.000**
lmo2400		acetyltransferase		**0.92**	**0.000**	**1.15**	**0.000**	**1.04**	**0.000**
lmo2453		lipolytic enzyme		**0.72**	**0.000**	**0.95**	**0.000**	**1.11**	**0.000**
lmo2540		phosphatases		0.29	0.000	0.55	0.000	**0.65**	**0.000**
lmo2592		oxidoreductase, aldo/keto reductase family		0.54	0.000	**0.74**	**0.000**	**0.90**	**0.000**
Central intermediary metabolism down-regulated									
lmo0018		beta-glucosidase		-0.16	0.265	**-0.59**	**0.000**	-0.37	0.046
lmo0536		6-phospho-beta-glucosidase		**-0.61**	**0.000**	**-1.06**	**0.000**	**-1.10**	**0.000**
lmo0727		L-glutamine-D-fructose-6-phosphate amidotransferase		-0.28	0.000	-0.42	0.000	**-1.01**	**0.000**
lmo0877		*B. subtilis* NagB protein (glucosamine-6-phosphate isomerase)		**-1.06**	**0.000**	**-1.08**	**0.000**	**-0.73**	**0.001**
lmo0878		oxidoreductases		**-0.68**	**0.000**	**-0.91**	**0.000**	**-0.73**	**0.000**
lmo0956		N-acetylglucosamine-6P-phosphate deacetylase (EC 3.5.1.25)		-0.41	0.000	**-0.78**	**0.000**	-0.41	0.000
lmo0957		glucosamine-6-Phoasphate isomerase (EC 5.3.1.10)		-0.31	0.000	**-0.60**	**0.000**	-0.24	0.001
lmo1384		unknown protein		-0.54	0.000	**-0.90**	**0.000**	**-0.83**	**0.000**
lmo2350		*B. subtilis* YtmI protein		**-1.50**	**0.000**	**-2.31**	**0.000**	**-2.59**	**0.000**
lmo2721		glucosamine-6-phosphate isomerase		-0.06	0.353	0.15	0.129	**-0.89**	**0.000**
lmo2761		beta-glucosidase		**-0.95**	**0.000**	**-1.51**	**0.000**	**-1.58**	**0.000**
lmo2786	*bvrC*	bvrC		**-2.54**	**0.000**	**-3.36**	**0.000**	**-3.41**	**0.000**
lmo2798		phosphatase		**-1.52**	**0.000**	**-1.74**	**0.000**	**-0.84**	**0.000**
lmo2831		phosphoglucomutase		-0.57	0.000	**-1.12**	**0.000**	**-1.19**	**0.000**
DNA metabolism up-regulated									
lmo0846		excinuclease ABC, chain C (UvrC)		**0.72**	**0.000**	**0.75**	**0.000**	**0.85**	**0.000**
lmo1234	*uvrC*	excinuclease ABC subunit C		**1.14**	**0.000**	**1.21**	**0.000**	**1.35**	**0.000**
lmo1248		8-oxo-dGTPase (MutT)		**0.71**	**0.000**	**1.10**	**0.000**	**2.07**	**0.000**
lmo1460		*B. subtilis* RecO protein involved in DNA repair and homologous recombination		0.25	0.000	**0.60**	**0.000**	0.57	0.000
lmo1509		exodeoxyribonuclease V	V	0.14	0.000	0.50	0.000	**0.59**	**0.000**
lmo1824	*priA*	primosomal replication factor Y		**0.59**	**0.000**	**0.84**	**0.000**	**0.90**	**0.000**
lmo1825		pantothenate metabolism flavoprotein homolog	B	0.57	0.000	**0.82**	**0.000**	**0.91**	**0.000**
lmo1872		methyltransferases	A,B,E,N	**0.75**	**0.000**	**0.85**	**0.000**	**0.75**	**0.000**
lmo1881		5-3 exonuclease		**1.06**	**0.000**	**1.40**	**0.000**	**1.60**	**0.000**
lmo2231		unknown protein		0.57	0.002	**0.74**	**0.005**	**0.81**	**0.001**
lmo2242		O^6-methylguanine-DNA methyltransferase		0.41	0.000	**0.82**	**0.000**	**1.01**	**0.000**
lmo2489	*uvrB*	excinuclease ABC (subunit B)		**0.59**	**0.000**	**0.94**	**0.000**	**0.95**	**0.000**
lmo2702	*recR*	recombination protein RecR		**0.60**	**0.000**	**0.74**	**0.000**	**0.77**	**0.000**
lmo2757		ATP-dependent DNA helicases		0.57	0.000	**0.71**	**0.000**	0.57	0.000

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
DNA metabolism down-regulated									
lmo0045	*ssb*	single-strand binding protein (SSB)		-0.48	0.000	**-0.71**	**0.000**	**-0.96**	**0.000**
lmo1639		dna-3-methyladenine glycosidase		-0.42	0.000	-0.52	0.000	**-0.83**	**0.000**
lmo2308		single-stranded DNA-binding protein		**-0.63**	**0.000**	**-0.77**	**0.000**	**-0.99**	**0.000**
Energy metabolism up-regulated									
lmo0031		transcriptional regulator LacI family		0.30	0.012	**0.62**	**0.000**	**0.61**	**0.000**
lmo0043	*arcA*	arginine deiminase		**0.61**	**0.000**	0.48	0.020	0.38	0.010
lmo0295		FMN-containing NADPH-linked nitro/flavin reductase	E	0.58	0.000	**0.83**	**0.000**	**0.70**	**0.000**
lmo0406		*B. subtilis* YyaH protein		0.48	0.000	**0.63**	**0.000**	0.58	0.000
lmo0539		tagatose-1,6-diphosphate aldolase		0.37	0.000	0.56	0.000	**0.82**	**0.000**
lmo0773		alcohol dehydrogenase	E	**0.87**	**0.000**	**1.02**	**0.000**	**1.39**	**0.000**
lmo0788		unknown	P	**0.94**	**0.000**	**0.94**	**0.000**	**1.64**	**0.000**
lmo0817		*E. coli* PhnB protein		**0.69**	**0.000**	**1.11**	**0.000**	**1.30**	**0.000**
lmo0913		succinate semialdehyde dehydrogenase		**0.99**	**0.000**	**2.32**	**0.000**	**2.48**	**0.000**
lmo0934		*B. subtilis* YhbA protein		**0.69**	**0.000**	**1.05**	**0.000**	**0.87**	**0.000**
lmo0936		Nitroflavin-reductase		**0.85**	**0.000**	**1.27**	**0.000**	**1.12**	**0.000**
lmo0975		ribose 5-phosphate isomerase		**1.07**	**0.000**	**1.33**	**0.000**	**1.46**	**0.000**
lmo1233	*trxA*	thioredoxin		0.50	0.000	**0.64**	**0.000**	**0.87**	**0.000**
lmo1511		unknown protein		0.23	0.000	0.50	0.000	**0.73**	**0.000**
lmo1534		L-lactate dehydrogenase		0.55	0.000	**0.59**	**0.000**	**0.81**	**0.000**
lmo1579		alanine dehydrogenase		**0.96**	**0.000**	**1.23**	**0.000**	**1.77**	**0.000**
lmo1609		thioredoxin		0.43	0.000	**0.61**	**0.000**	**0.83**	**0.000**
lmo1678		5-methyltetrahydrofolate-homocysteine methyltransferase (MetH)		**1.04**	**0.000**	**1.56**	**0.000**	**1.90**	**0.000**
lmo1679		cystathionine beta-lyase		**1.04**	**0.000**	**1.55**	**0.000**	**1.85**	**0.000**
lmo1800		protein-tyrosine phosphatase		**1.29**	**0.000**	**1.01**	**0.000**	**0.91**	**0.000**
lmo1917	*pflA*	pyruvate formate-lyase		**1.23**	**0.000**	**1.23**	**0.000**	**1.63**	**0.000**
lmo1935		protein-tyrosine/serine phosphatase		**0.75**	**0.000**	0.37	0.035	**0.70**	**0.002**
lmo2110		mannnose-6 phospate isomerase		0.19	0.179	**0.60**	**0.001**	0.55	0.001
lmo2363	*gadD2*	glutamate decarboxylase	B,E,O	**5.60**	**0.000**	**5.56**	**0.000**	**5.51**	**0.000**
lmo2404		conserved hypothetical proteins		**0.76**	**0.000**	**0.72**	**0.000**	**0.61**	**0.000**
lmo2424		thioredoxin		**1.03**	**0.000**	**1.08**	**0.000**	**1.31**	**0.000**
lmo2457	*tpi*	triose phosphate isomerase		0.32	0.000	**0.64**	**0.000**	**0.80**	**0.000**
lmo2475		phosphomannomutase and phosphoglucomutase		0.55	0.000	**0.65**	**0.000**	**0.70**	**0.000**
lmo2477	*galE*	UDP-glucose 4-epimerase		0.50	0.000	0.57	0.000	**0.64**	**0.000**
lmo2478	*trxB*	thioredoxin reductase		**0.64**	**0.000**	**0.64**	**0.000**	**0.85**	**0.000**
lmo2494		negative regulator of phosphate regulon		0.22	0.044	0.39	0.019	**0.77**	**0.002**
lmo2573		zinc-binding dehydrogenase	E	0.47	0.000	**0.64**	**0.000**	**0.91**	**0.000**
lmo2695		dihydroxyacetone kinase	I	0.11	0.006	0.36	0.000	**0.68**	**0.000**
lmo2696		hypothetical dihydroxyacetone kinase	I	0.20	0.000	0.34	0.000	**0.74**	**0.000**
lmo2712		gluconate kinase		**0.84**	**0.000**	**1.02**	**0.000**	**0.99**	**0.000**
Energy metabolism down-regulated									
lmo0039	*arcC*	carbamate kinase		-0.37	0.003	-0.47	0.001	**-0.79**	**0.002**
lmo0105		chitinase B		**-1.43**	**0.000**	**-1.99**	**0.000**	**-2.05**	**0.000**
lmo0183		alpha-glucosidase		-0.31	0.000	**-0.65**	**0.000**	**-0.75**	**0.000**
lmo0342		transketolase		**-1.25**	**0.000**	**-1.75**	**0.000**	**-1.80**	**0.000**
lmo0343		transaldolase		**-1.95**	**0.000**	**-2.54**	**0.000**	**-2.48**	**0.000**
lmo0344		dehydrogenase/reductase		**-1.81**	**0.000**	**-1.93**	**0.000**	**-2.40**	**0.000**
lmo0345		sugar-phosphate isomerase		**-2.00**	**0.000**	**-2.50**	**0.000**	**-2.16**	**0.000**
lmo0346		triosephosphate isomerase		**-1.34**	**0.000**	**-1.83**	**0.001**	-0.71	0.053
lmo0347		dihydroxyacetone kinase	I	**-1.46**	**0.000**	**-1.76**	**0.000**	**-1.53**	**0.000**
lmo0348		dihydroxyacetone kinase	I	**-1.34**	**0.002**	**-1.89**	**0.000**	**-1.74**	**0.000**
lmo0499		ribulose-5-phosphate 3 epimerase		-0.31	0.001	**-0.93**	**0.000**	-0.45	0.126
lmo0500		transaldolase		NA	NA	**-0.75**	**0.008**	NA	NA
lmo0517		phosphoglycerate mutase		NA	NA	**-1.60**	**0.001**	NA	NA
lmo0557		phosphoglycerate mutase		-0.28	0.000	-0.55	0.000	**-0.61**	**0.000**
lmo0643		putative transaldolase		**-0.70**	**0.000**	**-1.16**	**0.000**	**-0.83**	**0.001**
lmo0735		Ribulose-5-Phosphate 3-Epimerase		**-0.87**	**0.000**	**-1.33**	**0.000**	**-1.29**	**0.000**
lmo0736		ribose 5-phosphate isomerase		**-1.02**	**0.000**	**-1.33**	**0.000**	**-1.23**	**0.000**
lmo0813		fructokinases		**-0.69**	**0.000**	**-1.08**	**0.000**	**-0.66**	**0.000**
lmo0837	*uhpT*	hexose phosphate transport protein		-0.29	0.000	-0.45	0.000	**-1.04**	**0.000**
lmo0862		oligo-1,6-glucosidase		**-0.87**	**0.000**	**-1.30**	**0.006**	-0.47	0.003
lmo0865		phosphomannomutase		**-0.79**	**0.000**	**-0.87**	**0.000**	**-0.69**	**0.000**
lmo0917		beta-glucosidase		0.40	0.010	**-0.70**	**0.000**	**-0.63**	**0.000**
lmo0943	*fri*	non-heme iron-binding ferritin		-0.51	0.000	**-0.77**	**0.000**	-0.50	0.000
lmo0948		transcription regulator		**-0.62**	**0.000**	**-0.78**	**0.000**	**-1.03**	**0.000**
lmo1032		transketolase		-0.58	0.000	**-0.82**	**0.000**	**-0.96**	**0.000**
lmo1033		transketolase		-0.52	0.000	**-0.95**	**0.000**	**-0.69**	**0.000**
lmo1142		Salmonella enterica PduS protein		-0.48	0.000	-0.58	0.000	**-0.96**	**0.001**
lmo1254		alpha,alpha-phosphotrehalase		**-4.64**	**0.000**	**-4.84**	**0.000**	**-4.49**	**0.000**
lmo1293	*glpD*	glycerol 3 phosphate dehydrogenase		**0.68**	**0.001**	**-0.78**	**0.000**	-0.43	0.000
lmo1305	*tkt*	transketolase		**-1.34**	**0.000**	**-1.65**	**0.000**	**-1.14**	**0.000**
lmo1349		glycine dehydrogenase (decarboxylating) subunit 1	E	-0.37	0.000	**-0.61**	**0.000**	**-0.63**	**0.000**
lmo1350		glycine dehydrogenase (decarboxylating) subunit 2	E	-0.46	0.000	**-0.62**	**0.000**	**-0.69**	**0.000**
lmo1371		branched-chain alpha-keto acid dehydrogenase E3 subunit		**-0.61**	**0.000**	-0.25	0.000	-0.55	0.000
lmo1374		branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase)		**-0.74**	**0.000**	-0.25	0.000	-0.53	0.000
lmo1538	*glpK*	glycerol kinase		-0.40	0.000	**-1.44**	**0.000**	**-1.08**	**0.000**
lmo1566	*citC*	isocitrate dehyrogenases		**-0.78**	**0.000**	**-0.82**	**0.002**	-0.64	0.012

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo1581	*ackA*	acetate kinase	E	-0.46	0.000	**-0.65**	**0.000**	**-0.68**	**0.000**
lmo1634		Alcohol-acetaldehyde dehydrogenase		-0.38	0.009	-0.35	0.007	**1.52**	**0.007**
lmo1641	*citB*	aconitate hydratases		**-1.39**	**0.000**	**-1.35**	**0.000**	**-1.42**	**0.000**
lmo1789		Nad(P)h Oxidoreductase chain B	E	-0.47	0.000	**-0.60**	**0.000**	-0.46	0.000
lmo1867		pyruvate phosphate dikinase		**-0.86**	**0.000**	**-1.05**	**0.000**	**-0.89**	**0.000**
lmo1868		conserved hypothetical proteins		**-0.65**	**0.001**	**-0.76**	**0.000**	-0.20	0.032
lmo1883		chitinases		**-0.64**	**0.000**	**-1.26**	**0.000**	**-1.59**	**0.000**
lmo1936	*gpsA*	NAD(P)H-dependent glycerol-3-phosphate dehydrogenase		-0.58	0.000	**-0.67**	**0.000**	**-0.73**	**0.000**
lmo2094		L-fuculose-phosphate aldolase		-0.43	0.000	**-0.75**	**0.000**	**-0.64**	**0.003**
lmo2122		maltodextrose utilization protein MalA	A	0.48	0.014	**-2.28**	**0.000**	**-1.67**	**0.000**
lmo2123		maltodextrin ABC-transport system (permease)		0.14	0.478	**-2.71**	**0.000**	**-2.09**	**0.000**
lmo2124		maltodextrin ABC-transport system (permease)		0.39	0.049	**-2.89**	**0.000**	**-2.37**	**0.000**
lmo2126		maltogenic amylase		0.55	0.001	**-1.79**	**0.000**	**-1.64**	**0.000**
lmo2159		oxidoreductase	E	**-2.63**	**0.000**	**-3.28**	**0.000**	**-3.13**	**0.000**
lmo2163		oxidoreductase	E	**-2.40**	**0.000**	**-3.07**	**0.000**	**-2.90**	**0.000**
lmo2436		transcription antiterminator		-0.43	0.000	**-0.71**	**0.000**	-0.52	0.000
lmo2437		unknown		-0.55	0.000	**-0.69**	**0.000**	-0.23	0.015
lmo2584		formate dehydrogenase associated protein		**-0.71**	**0.000**	**-0.93**	**0.000**	**-0.78**	**0.000**
lmo2586		formate dehydrogenase alpha chain		**-1.38**	**0.000**	**-2.12**	**0.000**	**-1.90**	**0.000**
lmo2664		sorbitol dehydrogenase	E	-0.49	0.000	**-0.68**	**0.000**	**-0.78**	**0.000**
lmo2720		acetate-CoA ligase		**-0.99**	**0.000**	-0.35	0.000	**-1.87**	**0.000**
lmo2730		phosphatase		**-0.87**	**0.007**	**-0.96**	**0.001**	**-0.67**	**0.002**
lmo2771		beta-glucosidase		**-0.63**	**0.000**	**-1.05**	**0.000**	**-0.89**	**0.000**
lmo2834		oxidoreductases	E	**-0.88**	**0.004**	**-0.87**	**0.002**	**-0.78**	**0.003**
lmo2836		alcohol dehydrogenase	E	-0.57	0.010	**-0.69**	**0.001**	**-0.77**	**0.001**
Fatty acid and phospholipid metabolism up-regulated									
lmo0786		acyl-carrier protein phosphodiesterase and to NAD(P)H dehydrogenase		**0.78**	**0.000**	**0.86**	**0.000**	**0.85**	**0.000**
lmo0885		holo-acyl-carrier protein synthase		0.48	0.000	**0.74**	**0.000**	**0.77**	**0.000**
lmo1381		unknown		0.51	0.000	**0.88**	**0.000**	**1.07**	**0.000**
lmo2452		carboxylesterase		**0.62**	**0.000**	**0.70**	**0.000**	**0.91**	**0.000**
lmo2471		NADH oxidase		0.48	0.000	**0.77**	**0.000**	**0.87**	**0.000**
Fatty acid and phospholipid metabolism down-regulated									
lmo0110		lipase		**-0.98**	**0.000**	**-1.81**	**0.000**	**-1.62**	**0.000**
lmo1186		ethanolamine utilization protein EutH - Escherichia coli		-0.28	0.004	**-0.60**	**0.000**	-0.34	0.001
lmo1806	*acpA*	acyl carrier proteins		-0.44	0.000	-0.41	0.000	**-0.64**	**0.000**
lmo2175		dehydrogenase		-0.49	0.003	**-0.99**	**0.002**	-0.72	0.020
lmo2201		3-oxoacyl-acyl-carrier protein synthase		-0.22	0.000	-0.28	0.002	**-0.75**	**0.000**
lmo2202		3-oxoacyl- acyl-carrier protein synthase		**-0.70**	**0.000**	-0.46	0.000	**-1.08**	**0.000**
Hypothetical protein up-regulated									
lmo0049		unknown		0.39	0.000	**0.65**	**0.000**	**0.72**	**0.000**
lmo0095		unknown		0.55	0.012	**0.97**	**0.000**	NA	NA
lmo0119		unknown		0.33	0.007	**0.59**	**0.000**	NA	NA
lmo0120		unknown		0.26	0.049	**0.82**	**0.000**	0.49	0.004
lmo0138		unknown		**0.90**	**0.002**	**1.23**	**0.000**	**0.99**	**0.000**
lmo0140		unknown		NA	NA	**0.62**	**0.007**	0.43	0.023
lmo0149		unknown		**0.76**	**0.000**	**1.29**	**0.000**	**1.15**	**0.000**
lmo0170		unknown		**0.63**	**0.000**	**0.79**	**0.000**	**0.96**	**0.000**
lmo0206		unknown		**0.67**	**0.000**	**0.86**	**0.000**	**1.39**	**0.000**
lmo0209		unknown		**1.13**	**0.000**	**1.32**	**0.000**	**1.48**	**0.000**
lmo0247		unknown		**1.08**	**0.000**	**0.83**	**0.000**	0.57	0.000
lmo0260		unknown		0.55	0.000	0.49	0.000	**0.60**	**0.000**
lmo0270		unknown		-0.06	0.549	0.40	0.027	**0.67**	**0.000**
lmo0335		unknown		**0.81**	**0.000**	**1.35**	**0.000**	**1.62**	**0.000**
lmo0337		unknown		**0.87**	**0.000**	0.88	0.052	**1.56**	**0.000**
lmo0338		unknown		**0.66**	**0.000**	**0.84**	**0.000**	**1.22**	**0.000**
lmo0340		unknown		0.31	0.054	**0.82**	**0.000**	**1.08**	**0.000**
lmo0363		*Salmonella* typhimurium peptidase E		**0.63**	**0.000**	**0.85**	**0.000**	**1.05**	**0.000**
lmo0364		transcription regulator		0.24	0.002	0.54	0.000	**0.61**	**0.000**
lmo0365		conserved hypothetical protein		**0.88**	**0.000**	**1.00**	**0.000**	**0.87**	**0.000**
lmo0375		unknown		**0.89**	**0.000**	**1.27**	**0.000**	**1.49**	**0.000**
lmo0376		putative transcription regulator		**0.81**	**0.000**	**1.24**	**0.000**	**1.33**	**0.000**
lmo0377		unknown		**0.66**	**0.000**	**0.87**	**0.000**	**1.04**	**0.000**
lmo0380		unknown		**0.67**	**0.007**	**1.23**	**0.000**	**1.25**	**0.000**
lmo0381		unknown		0.44	0.000	**0.64**	**0.000**	0.39	0.003
lmo0439		module of peptide synthetase		NA	NA	NA	NA	**0.99**	**0.003**
lmo0457		unknown		0.19	0.207	0.70	0.025	**0.59**	**0.009**
lmo0478		putative secreted protein		-0.12	0.020	0.42	0.000	**0.62**	**0.000**
lmo0479		putative secreted protein		-0.13	0.060	0.49	0.000	**0.60**	**0.000**
lmo0551		unknown		**0.64**	**0.000**	0.58	0.000	**0.64**	**0.000**
lmo0552		unknown		**1.84**	**0.000**	**1.51**	**0.000**	**1.95**	**0.000**
lmo0578		putative conserved membrane protein		0.54	0.000	**0.67**	**0.000**	**0.68**	**0.000**
lmo0596		unknown protein		0.07	0.702	**0.68**	**0.002**	**0.77**	**0.004**
lmo0604		*B. subtilis* YvlA protein		**0.87**	**0.000**	**0.89**	**0.000**	**1.41**	**0.000**
lmo0628		unknown		NA	NA	NA	NA	**1.15**	**0.000**
lmo0654		unknown		**0.70**	**0.000**	**0.90**	**0.000**	**1.18**	**0.000**
lmo0670		unknown		NA	NA	**0.84**	**0.002**	**1.15**	**0.000**
lmo0794		*B. subtilis* YwnB protein		**1.18**	**0.000**	**1.07**	**0.000**	**0.83**	**0.000**
lmo0804		unknown		**0.63**	**0.000**	**0.94**	**0.000**	**1.01**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo0805		unknown		0.54	0.000	**0.82**	**0.000**	**0.95**	**0.000**
lmo0851		unknown		**0.87**	**0.000**	**1.11**	**0.000**	**1.38**	**0.000**
lmo0868		unknown		**0.64**	**0.001**	0.57	0.003	0.09	0.606
lmo0869		unknown		**0.94**	**0.000**	**0.79**	**0.000**	**1.04**	**0.000**
lmo0870		unknown		**0.80**	**0.000**	**0.72**	**0.000**	**0.78**	**0.000**
lmo0905		unknown		0.57	0.000	**0.69**	**0.000**	**0.96**	**0.000**
lmo0920		*B. subtilis* YcgR protein		**1.46**	**0.000**	**1.36**	**0.000**	**1.74**	**0.000**
lmo0921		*B. subtilis* YcgQ protein		**1.46**	**0.000**	**1.37**	**0.000**	**1.72**	**0.000**
lmo0932		conserved hypothetical protein		**0.87**	**0.000**	**0.93**	**0.000**	**1.02**	**0.000**
lmo0953		unknown		**1.01**	**0.000**	**1.28**	**0.000**	**1.44**	**0.000**
lmo0964		*B. subtilis* YjbH protein		0.43	0.000	0.55	0.000	**0.80**	**0.000**
lmo0977		*B. subtilis* YjcH protein		0.50	0.000	0.55	0.000	**0.74**	**0.000**
lmo0982		glucanase and peptidase		0.48	0.000	**0.60**	**0.000**	**0.75**	**0.000**
lmo0984		two-component response regulator		0.57	0.000	**0.78**	**0.000**	**1.16**	**0.000**
lmo0985		unknown		0.52	0.000	**0.81**	**0.000**	**0.91**	**0.000**
lmo0994		unknown		0.30	0.000	**0.63**	**0.000**	**0.89**	**0.000**
lmo0995		*B. subtilis* YkrP protein		0.31	0.001	**0.60**	**0.000**	0.47	0.002
lmo1056		unknown		**0.99**	**0.000**	**0.84**	**0.000**	**0.92**	**0.000**
lmo1123		unknown		**0.84**	**0.000**	**0.70**	**0.009**	0.80	0.010
lmo1137		unknown		**1.05**	**0.000**	**0.96**	**0.000**	**0.92**	**0.000**
lmo1140		unknown		**0.71**	**0.000**	**1.05**	**0.000**	**1.52**	**0.000**
lmo1146		unknown		0.48	0.008	**0.72**	**0.000**	**0.93**	**0.002**
lmo1241		unknown		**0.72**	**0.000**	**1.64**	**0.000**	**1.91**	**0.000**
lmo1249		unknown		**2.80**	**0.000**	**3.64**	**0.000**	**4.81**	**0.000**
lmo1257		unknown		0.01	0.950	0.07	0.757	**0.85**	**0.000**
lmo1281		*B. subtilis* YneP protein		0.53	0.000	0.53	0.000	**0.71**	**0.000**
lmo1401		conserved hypothetical protein		0.43	0.000	**0.87**	**0.000**	**0.89**	**0.000**
lmo1402		*B. subtilis* YmcA protein		0.45	0.000	**0.92**	**0.000**	**0.87**	**0.000**
lmo1535		unknown protein		0.45	0.000	0.48	0.000	**0.68**	**0.000**
lmo1608		unknown protein		0.35	0.000	**0.61**	**0.000**	**0.78**	**0.000**
lmo1610		hypothetical gene		**0.65**	**0.000**	**0.98**	**0.000**	**1.33**	**0.000**
lmo1650		hypothetical proteins		0.39	0.000	0.43	0.000	**0.68**	**0.000**
lmo1656		unknown		**0.71**	**0.000**	**0.79**	**0.000**	**0.59**	**0.000**
lmo1670		conserved hypothetical proteins		0.41	0.002	0.49	0.000	**0.66**	**0.000**
lmo1690		hypothetical proteins		**0.83**	**0.000**	**0.83**	**0.000**	**1.47**	**0.000**
lmo1776		unknown protein		0.51	0.000	0.43	0.000	**0.63**	**0.000**
lmo1841		unknown		0.41	0.002	**0.84**	**0.009**	**1.24**	**0.000**
lmo2049		unknown protein		0.56	0.000	0.53	0.023	**0.97**	**0.000**
lmo2115		ABC transporter (permease)		**0.89**	**0.000**	**0.63**	**0.000**	**0.82**	**0.000**
lmo2120		unknown protein		0.40	0.000	0.57	0.000	**0.74**	**0.000**
lmo2156		unknown		0.57	0.000	0.54	0.001	**0.85**	**0.000**
lmo2169		unknown		0.15	0.127	0.64	0.014	**0.60**	**0.001**
lmo2177		unknown protein		**0.98**	**0.000**	**0.83**	**0.000**	**1.17**	**0.000**
lmo2181		unknown protein		**0.91**	**0.000**	**1.10**	**0.000**	**0.86**	**0.000**
lmo2187		unknown		**1.07**	**0.000**	**1.16**	**0.000**	**1.41**	**0.000**
lmo2204		unknown protein		0.56	0.000	**0.79**	**0.000**	**0.72**	**0.000**
lmo2210		unknown		**0.97**	**0.000**	0.27	0.046	**0.96**	**0.003**
lmo2213		unknown protein		**1.03**	**0.000**	**1.25**	**0.000**	**1.95**	**0.000**
lmo2258		unknown		0.36	0.000	**0.64**	**0.000**	**0.81**	**0.000**
lmo2339		conserved hypothetical protein		0.52	0.000	**0.67**	**0.000**	**1.14**	**0.000**
lmo2340		*Erwinia chrysanthemi* IndA protein		**0.62**	**0.000**	0.32	0.006	0.42	0.000
lmo2364		Hypothetical protein		0.29	0.002	0.43	0.006	**0.86**	**0.001**
lmo2375		unknown		0.58	0.000	0.33	0.000	**0.61**	**0.001**
lmo2387		conserved hypothetical protein		**1.41**	**0.000**	**1.34**	**0.000**	**1.28**	**0.000**
lmo2388		*B. subtilis* YwqG protein		**1.06**	**0.000**	**1.00**	**0.000**	**1.03**	**0.000**
lmo2391		conserved hypothetical protein *B. subtilis* YhfK protein		**1.48**	**0.000**	**1.40**	**0.000**	**1.52**	**0.000**
lmo2420		unknown		0.37	0.000	0.51	0.000	**0.76**	**0.000**
lmo2432		unknown		0.55	0.000	**0.77**	**0.002**	**0.80**	**0.001**
lmo2454		unknown		**0.78**	**0.000**	**1.11**	**0.000**	**0.97**	**0.000**
lmo2472		conserved hypothetical protein		0.56	0.000	**0.88**	**0.000**	**0.92**	**0.000**
lmo2474		conserved hypothetical protein		**0.65**	**0.000**	**0.92**	**0.000**	**1.02**	**0.000**
lmo2570		unknown		0.34	0.000	0.49	0.000	**0.83**	**0.000**
lmo2572		Chain A, Dihydrofolate Reductase		0.36	0.000	0.53	0.000	**0.71**	**0.000**
lmo2639		unknown		**0.60**	**0.000**	0.53	0.000	0.54	0.000
lmo2669		unknown		**0.59**	**0.000**	0.56	0.000	**0.70**	**0.000**
lmo2697		unknown		0.18	0.003	0.26	0.001	**0.74**	**0.000**
lmo2701		*B. subtilis* YaaL protein		**0.64**	**0.000**	**0.82**	**0.000**	**0.90**	**0.000**
lmo2709		unknown		0.42	0.000	0.45	0.000	**0.77**	**0.000**
lmo2729		unknown protein		**0.66**	**0.000**	0.53	0.000	**0.75**	**0.000**
lmo2748		*B. subtilis* stress protein YdaG		0.42	0.000	**0.86**	**0.000**	**1.37**	**0.000**
lmo2808		hypothetical secreted protein		NA	NA	**0.65**	**0.007**	NA	NA
Hypothetical protein down-regulated									
lmo0038		conserved hypothetical protein		-0.16	0.170	-0.52	0.000	**-0.76**	**0.004**
lmo0099		unknown		0.21	0.092	-0.50	0.000	**-0.78**	**0.000**
lmo0269		transporter		**-0.60**	**0.000**	-0.31	0.003	-0.49	0.023
lmo0322		unknown protein		-0.26	0.001	**-0.90**	**0.000**	-0.57	0.001
lmo0323		unknown protein		-0.53	0.000	**-1.28**	**0.000**	**-0.95**	**0.000**
lmo0349		unknown		**-1.26**	**0.000**	**-1.51**	**0.001**	**-1.31**	**0.003**
lmo0350		unknown		**-1.51**	**0.000**	**-1.60**	**0.001**	**-1.53**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo0351		unknown protein		-0.61	0.015	**-0.90**	**0.008**	-0.54	0.005
lmo0391		unknown		**-0.83**	**0.000**	**-1.22**	**0.000**	**-1.44**	**0.000**
lmo0392		*B. subtilis* YqfA protein		**-0.90**	**0.000**	**-1.38**	**0.000**	**-1.58**	**0.000**
lmo0393		unknown		**-0.80**	**0.000**	**-1.13**	**0.000**	**-1.28**	**0.000**
lmo0546		putative NAD(P)-dependent oxidoreductase		**-0.96**	**0.000**	**-1.66**	**0.000**	**-1.44**	**0.001**
lmo0589		unknown		**-0.59**	**0.000**	**-0.75**	**0.000**	**-0.70**	**0.000**
lmo0599		conserved hypothetical protein		-0.58	0.000	**-0.61**	**0.000**	**-1.02**	**0.000**
lmo0600		unknown		**-0.73**	**0.000**	**-0.72**	**0.000**	**-1.02**	**0.000**
lmo0634		*E. coli* putative tagatose 6-phosphate kinase		-0.39	0.002	-0.54	0.024	**-0.68**	**0.002**
lmo0642		unknown		-0.23	0.000	**-0.65**	**0.000**	**-0.65**	**0.000**
lmo0684		unknown		**-0.86**	**0.000**	**-1.12**	**0.000**	**-1.42**	**0.000**
lmo0687		unknown		**-0.73**	**0.000**	**-0.95**	**0.000**	**-1.18**	**0.000**
lmo0701		unknown		**-1.09**	**0.000**	**-1.80**	**0.000**	**-1.90**	**0.000**
lmo0702		unknown		**-1.24**	**0.000**	**-1.83**	**0.000**	**-1.96**	**0.000**
lmo0703		unknown		**-1.13**	**0.000**	**-1.73**	**0.000**	**-1.88**	**0.000**
lmo0704		unknown		**-1.31**	**0.000**	**-1.87**	**0.000**	**-1.97**	**0.000**
lmo0709		unknown		**-1.63**	**0.000**	**-2.29**	**0.000**	**-2.25**	**0.000**
lmo0715		unknown		**-1.28**	**0.000**	**-1.69**	**0.000**	**-1.86**	**0.000**
lmo0718		unknown		**-1.39**	**0.000**	**-1.53**	**0.000**	**-1.70**	**0.000**
lmo0724		*B. subtilis* YvpB protein		**-1.68**	**0.000**	**-2.54**	**0.000**	**-2.27**	**0.000**
lmo0726		Hypothetical CDS		-0.09	0.086	-0.11	0.086	**-0.61**	**0.000**
lmo0731		unknown		**-1.34**	**0.000**	**-1.34**	**0.000**	**-1.48**	**0.000**
lmo0737		unknown		**-1.25**	**0.000**	**-1.49**	**0.000**	**-1.60**	**0.000**
lmo0748		unknown		-0.48	0.000	**-0.59**	**0.000**	**-0.72**	**0.000**
lmo0751		unknown		-0.46	0.001	-0.40	0.005	**-0.62**	**0.001**
lmo0765		unknown		**-0.71**	**0.005**	**-0.79**	**0.000**	-0.66	0.049
lmo0780		unknown		**-0.73**	**0.000**	-0.49	0.004	**-0.60**	**0.009**
lmo0791		unknown		**-0.98**	**0.000**	**-0.96**	**0.000**	**-1.00**	**0.000**
lmo0796		conserved hypothetical protein		-0.37	0.000	**-0.61**	**0.000**	**-0.61**	**0.000**
lmo0863		unknown		**-1.03**	**0.000**	**-1.57**	**0.000**	**-0.88**	**0.001**
lmo0864		unknown		**-0.91**	**0.000**	**-1.43**	**0.000**	**-1.23**	**0.000**
lmo0867		unknown		-0.45	0.000	**-0.84**	**0.000**	**-0.64**	**0.000**
lmo0879		unknown		**-0.94**	**0.000**	**-1.00**	**0.000**	**-0.78**	**0.008**
lmo0882		*B. subtilis* YdbS protein		**-0.78**	**0.000**	**-0.98**	**0.000**	**-0.89**	**0.000**
lmo0883		*B. subtilis* YbtB protein		**-0.82**	**0.000**	**-1.08**	**0.000**	**-1.09**	**0.000**
lmo0903		conserved hypothetical protein		-0.54	0.000	-0.55	0.000	**-0.98**	**0.000**
lmo1031		hypothetical proteins		**-0.62**	**0.000**	**-0.65**	**0.000**	**-0.69**	**0.000**
lmo1188		unknown		**-0.65**	**0.000**	**-0.79**	**0.000**	**-0.66**	**0.000**
lmo1190		unknown		-0.44	0.000	**-0.80**	**0.000**	-0.52	0.000
lmo1210		unknown protein		-0.14	0.006	-0.50	0.000	**-0.68**	**0.000**
lmo1211		unknown protein		-0.23	0.000	-0.47	0.000	**-0.75**	**0.000**
lmo1242		*B. subtilis* YdeI protein		-0.19	0.001	**-0.61**	**0.000**	**-0.59**	**0.000**
lmo1306		*B. subtilis* YneF protein		-0.32	0.000	-0.39	0.000	**-0.71**	**0.000**
lmo1310		*E. coli* YbdN protein		-0.48	0.000	**-0.76**	**0.000**	**-0.72**	**0.001**
lmo1312		unknown		-0.40	0.000	**-0.63**	**0.000**	**-0.75**	**0.000**
lmo1323		*B. subtilis* YlxR protein		-0.36	0.000	**-0.61**	**0.000**	**-0.83**	**0.000**
lmo1326		conserved hypothetical protein *B. subtilis* YlxP protein		**-0.70**	**0.000**	**-0.78**	**0.000**	**-0.97**	**0.000**
lmo1452		conserved hypothetical protein		-0.58	0.000	-0.47	0.000	**-0.67**	**0.000**
lmo1453		conserved hypothetical protein		-0.56	0.000	-0.56	0.000	**-0.81**	**0.000**
lmo1470		unknown protein		-0.32	0.000	-0.40	0.000	**-0.66**	**0.000**
lmo1489		unknown protein		-0.39	0.000	-0.40	0.000	**-0.62**	**0.000**
lmo1541		unknown protein		-0.52	0.000	**-0.78**	**0.000**	**-0.97**	**0.000**
lmo1584		unknown protein		**-0.86**	**0.000**	-0.39	0.044	**-0.84**	**0.001**
lmo1626		unknown		-0.38	0.000	**-0.67**	**0.001**	-0.35	0.010
lmo1637		membrane proteins		-0.34	0.000	**-0.66**	**0.000**	-0.51	0.000
lmo1728		some similarities to cellobiose-phosphorylase		**-0.66**	**0.002**	**-0.77**	**0.000**	-0.90	0.014
lmo1762		unknown		-0.37	0.000	-0.47	0.000	**-0.61**	**0.000**
lmo1972		pentitol PTS system enzyme II B component		-0.49	0.000	**-0.86**	**0.000**	-0.58	0.012
lmo2079		unknown		**-0.62**	**0.000**	**-0.61**	**0.000**	**-0.84**	**0.000**
lmo2160		unknown protein		**-2.71**	**0.000**	**-3.41**	**0.000**	**-3.15**	**0.000**
lmo2161		unknown		**-2.56**	**0.000**	**-2.50**	**0.000**	**-2.85**	**0.000**
lmo2162		unknown protein		**-2.48**	**0.000**	**-2.90**	**0.000**	**-2.44**	**0.000**
lmo2223		unknown protein		-0.38	0.000	**-0.74**	**0.000**	**-0.80**	**0.000**
lmo2226		unknown protein		**-0.75**	**0.000**	**-0.78**	**0.000**	**-0.88**	**0.000**
lmo2343		nitrilotriacetate monooxygenase		**-1.69**	**0.000**	**-2.38**	**0.000**	**-2.65**	**0.000**
lmo2344		*B. subtilis* YtnI protein		**-1.80**	**0.000**	**-2.52**	**0.000**	**-2.76**	**0.000**
lmo2351		NADH-dependent FMN reductase		**-1.70**	**0.000**	**-2.37**	**0.000**	**-2.22**	**0.000**
lmo2416		unknown		**-0.96**	**0.000**	**-1.03**	**0.000**	**-1.30**	**0.000**
lmo2567		unknown		**-0.62**	**0.000**	**-0.73**	**0.000**	-0.58	0.027
lmo2568		unknown		**-1.10**	**0.000**	**-1.22**	**0.000**	**-1.27**	**0.000**
lmo2585		*B. subtilis* YrhD protein		**-1.16**	**0.000**	**-1.61**	**0.000**	**-1.64**	**0.000**
lmo2646		unknown		**-1.89**	**0.000**	**-2.13**	**0.000**	**-2.31**	**0.000**
lmo2686		unknown		-0.34	0.007	**-0.68**	**0.000**	**-0.66**	**0.001**
lmo2731		transcription regulator (RpiR family)		**-0.93**	**0.000**	**-0.98**	**0.000**	**-0.77**	**0.000**
lmo2775		hypothetical membrane protein		-0.57	0.000	**-0.70**	**0.000**	**-0.62**	**0.000**
lmo2832		unknown protein		-0.57	0.000	**-1.10**	**0.000**	**-1.23**	**0.000**
lmo2835		*E. coli* protein		**-0.98**	**0.000**	**-1.38**	**0.000**	**-1.15**	**0.000**
lmo2852		unknown		**-0.78**	**0.000**	**-1.01**	**0.000**	**-1.34**	**0.000**
Mobile and extrachromosomal element up-regulated									

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo0174		transposase		NA	NA	**1.06**	**0.002**	NA	NA
lmo0329		transposase		0.19	0.100	**0.85**	**0.002**	0.46	0.028
lmo0330		transposase		**0.72**	**0.000**	**0.81**	**0.000**	**1.13**	**0.000**
lmo0660		transposases		**0.73**	**0.000**	**0.65**	**0.000**	**1.08**	**0.000**
lmo0827		transposases		**0.63**	**0.000**	**0.83**	**0.000**	**0.76**	**0.000**
lmo0828		transposases		**0.65**	**0.000**	**1.08**	**0.000**	**1.02**	**0.000**
lmo2360		transmembrane protein	V	**2.97**	**0.000**	**3.47**	**0.000**	**3.44**	**0.000**
Mobile and extrachromosomal element down-regulated									
lmo1295		host factor-1 protein	P	-0.54	0.000	**-0.63**	**0.000**	**-0.69**	**0.000**
lmo1955		integrase/recombinase		**-0.70**	**0.000**	**-1.18**	**0.000**	**-1.06**	**0.000**
Protein fate up-regulated									
lmo0203	*mpl*	Zinc metalloproteinase precursor		**1.63**	**0.000**	**1.82**	**0.000**	**2.40**	**0.000**
lmo0292		heat-shock protein htrA serine protease		**1.07**	**0.000**	**1.12**	**0.000**	**1.26**	**0.000**
lmo0655		phosphoprotein phosphatases	P	0.50	0.000	0.54	0.001	**0.79**	**0.000**
lmo0931		lipoate protein ligase A	B,I	**0.63**	**0.000**	**0.78**	**0.000**	**1.01**	**0.000**
lmo1217		endo-1,4-beta-glucanase and to aminopeptidase		0.46	0.000	0.58	0.000	**0.82**	**0.000**
lmo1578		X-Pro dipeptidase		**0.64**	**0.000**	**0.76**	**0.000**	**1.05**	**0.000**
lmo1611		aminopeptidase		**0.61**	**0.000**	**0.88**	**0.000**	**1.01**	**0.000**
lmo1780		aminotripeptidase (peptidase T)		0.54	0.000	**0.84**	**0.000**	**1.08**	**0.000**
lmo1821		putative phosphoprotein phosphatase		0.36	0.000	0.50	0.000	**0.62**	**0.000**
lmo1864		hemolysinIII proteins, putative integral membrane protein		0.47	0.000	**0.74**	**0.000**	**0.69**	**0.000**
lmo2157	*sepA*	sepA		**1.32**	**0.000**	**1.14**	**0.000**	**1.53**	**0.000**
lmo2188		oligoendopeptidase		0.44	0.000	0.42	0.000	**0.62**	**0.000**
lmo2338	*pepC*	aminopeptidase C		**0.94**	**0.000**	**1.04**	**0.000**	**1.30**	**0.000**
lmo2462		dipeptidases		**0.78**	**0.000**	**1.06**	**0.000**	**1.40**	**0.000**
lmo2510	*secA*	translocase binding subunit (ATPase)		0.14	0.113	0.31	0.005	**0.63**	**0.001**
Protein fate down-regulated									
lmo0245	*secE*	preprotein translocase subunit		-0.56	0.000	-0.43	0.000	**-0.71**	**0.000**
lmo0680		flagella-associated protein flhA		-0.52	0.000	**-0.89**	**0.000**	**-1.25**	**0.000**
lmo0681		flagellar biosynthesis protein FlhF		-0.51	0.000	**-0.77**	**0.000**	**-0.96**	**0.000**
lmo0942		heat shock protein HtpG		-0.23	0.000	-0.58	0.000	**-0.61**	**0.000**
lmo1267	*tig*	trigger factor (prolyl isomerase)		**-0.59**	**0.000**	**-0.90**	**0.000**	**-0.94**	**0.000**
lmo1375		aminotripeptidase		**-0.98**	**0.000**	**-0.79**	**0.000**	**-1.00**	**0.000**
lmo1529		unknown protein		**-0.68**	**0.000**	**-0.83**	**0.000**	**-0.98**	**0.000**
lmo1585		proteases		**-0.62**	**0.000**	**-0.60**	**0.000**	**-0.64**	**0.000**
lmo1666		peptidoglycan linked protein (LPxTG)		-0.53	0.001	-0.47	0.000	**-0.68**	**0.000**
lmo2068	*groEL*	class I heat-shock protein (chaperonin) GroEL		**-0.71**	**0.000**	**-0.74**	**0.000**	**-0.86**	**0.000**
lmo2069	*groES*	class I heat-shock protein (chaperonin) GroES		-0.50	0.000	-0.44	0.000	**-0.60**	**0.000**
Protein synthesis up-regulated									
lmo0211	*ctc*	*B. subtilis* general stress protein		**1.12**	**0.000**	**1.74**	**0.000**	**2.36**	**0.000**
lmo0239	*cysS*	cysteinyl-tRNA synthetase		0.22	0.003	**0.70**	**0.000**	0.50	0.000
lmo0486	*rpmF*	ribosomal protein L32		**0.74**	**0.000**	**0.82**	**0.000**	**1.05**	**0.000**
lmo0569	*hisZ*	histidyl-tRNA synthetase		**0.60**	**0.000**	**0.68**	**0.000**	**0.70**	**0.000**
lmo0933		sugar transferase	E	0.41	0.000	**0.67**	**0.000**	**0.69**	**0.000**
lmo0935		*B. subtilis* CspR protein, rRNA methylase homolog		**0.78**	**0.000**	**1.10**	**0.000**	**1.01**	**0.000**
lmo1512		putative tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase		0.42	0.000	0.54	0.000	**0.59**	**0.000**
lmo1822		RNA-binding Sun protein		0.48	0.000	**0.67**	**0.000**	**0.73**	**0.000**
lmo1823	*fmt*	methionyl-tRNA formyltransferase		0.42	0.000	**0.61**	**0.000**	**0.63**	**0.000**
lmo1882		ribosomal protein S14		**1.23**	**0.000**	**1.89**	**0.000**	**1.81**	**0.000**
lmo2555		human N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein	E	**0.82**	**0.000**	**0.92**	**0.000**	**0.90**	**0.000**
Protein synthesis down-regulated									
lmo0044	*rpsF*	ribosomal protein S6		**-0.72**	**0.000**	**-0.92**	**0.000**	**-1.18**	**0.000**
lmo0046	*rpsR*	ribosomal protein S18		**-0.59**	**0.000**	**-0.82**	**0.000**	**-1.23**	**0.000**
lmo0248	*rplK*	ribosomal protein L11		-0.57	0.000	**-1.04**	**0.000**	**-1.11**	**0.000**
lmo0249	*rplA*	ribosomal protein L1		**-0.65**	**0.000**	**-1.02**	**0.000**	**-1.18**	**0.000**
lmo0250	*rplJ*	ribosomal protein L10		**-0.81**	**0.000**	**-1.24**	**0.000**	**-1.45**	**0.000**
lmo0251	*rplL*	ribosomal protein L12		**-0.73**	**0.000**	**-1.12**	**0.000**	**-1.26**	**0.000**
lmo0716		H^+-transporting ATP synthase alpha chain FliI, flagellar-specific, -		**-1.45**	**0.000**	**-1.84**	**0.000**	**-1.90**	**0.000**
lmo1314	*frr*	ribosome recycling factors		-0.45	0.000	-0.47	0.000	**-0.60**	**0.000**
lmo1324		conserved hypothetical protein, *B. subtilis* YlxQ protein		-0.54	0.000	**-0.76**	**0.000**	**-0.85**	**0.000**
lmo1325	*infB*	translation initiation factor IF-2		-0.44	0.000	**-0.62**	**0.000**	**-0.82**	**0.000**
lmo1328	*truB*	tRNA pseudouridine 55 synthase		**-0.73**	**0.000**	**-1.01**	**0.000**	**-1.19**	**0.000**
lmo1330	*rpsO*	ribosomal protein S15		**-0.60**	**0.000**	**-0.78**	**0.000**	**-0.92**	**0.000**
lmo1458	*glyS*	glycyl-tRNA synthetase beta chain		-0.51	0.000	**-0.71**	**0.000**	**-0.92**	**0.000**
lmo1459	*glyQ*	glycyl-tRNA synthetase alpha chain		-0.48	0.000	**-0.62**	**0.000**	**-0.71**	**0.000**
lmo1471		ribosomal protein L11 methyltransferase		-0.40	0.000	-0.49	0.000	**-0.65**	**0.000**
lmo1480	*rpsT*	ribosomal protein S20		-0.42	0.000	-0.55	0.000	**-0.73**	**0.000**
lmo1540	*rpmA*	ribosomal protein L27		-0.50	0.000	**-0.70**	**0.000**	**-0.92**	**0.000**
lmo1542	*rplU*	ribosomal protein L21		-0.50	0.000	**-0.79**	**0.000**	**-0.89**	**0.000**
lmo1596	*rpsD*	ribosomal protein S4		-0.47	0.000	**-0.64**	**0.000**	**-0.92**	**0.000**
lmo1657	*tsf*	translation elongation factor		-0.52	0.000	**-0.73**	**0.000**	**-0.87**	**0.000**
lmo1658	*rpsB*	30S ribosomal protein S2		-0.39	0.000	**-0.61**	**0.000**	**-0.65**	**0.000**
lmo1783	*rplT*	ribosomal protein L20		**-0.61**	**0.000**	**-1.01**	**0.000**	**-1.27**	**0.000**
lmo1784	*rpmI*	ribosomal protein L35		**-0.64**	**0.000**	**-1.11**	**0.000**	**-1.42**	**0.000**
lmo1785	*infC*	translation initiation factor IF-3		-0.05	0.466	-0.44	0.000	**-0.63**	**0.000**
lmo1787	*rplS*	ribosomal protein L19		-0.38	0.000	**-0.63**	**0.000**	**-0.74**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo1797	*rpsP*	ribosomal protein S16		**-0.78**	**0.000**	**-1.14**	**0.000**	**-1.43**	**0.000**
lmo1816	*rpmB*	ribosomal protein L28		-0.57	0.000	-0.57	0.000	**-0.80**	**0.000**
lmo1949		unknown protein		-0.38	0.000	-0.42	0.000	**-0.76**	**0.000**
lmo2121		maltosephosphorylase	E	0.16	0.498	**-2.99**	**0.000**	**-2.41**	**0.000**
lmo2548	*rpmE*	ribosomal protein L31		-0.51	0.000	**-0.86**	**0.000**	**-1.07**	**0.000**
lmo2596	*rpsI*	ribosomal protein S9		-0.45	0.000	**-0.66**	**0.000**	**-0.84**	**0.000**
lmo2605	*rplQ*	ribosomal protein L17		-0.32	0.000	-0.51	0.000	**-0.59**	**0.000**
lmo2614	*rpmD*	ribosomal protein L30		-0.48	0.000	**-0.66**	**0.000**	**-0.67**	**0.000**
lmo2619	*rpsN*	ribosomal protein S14		-0.51	0.000	**-0.82**	**0.000**	**-0.90**	**0.000**
lmo2623	*rpsQ*	ribosomal protein S17		-0.38	0.000	**-0.79**	**0.000**	**-0.85**	**0.000**
lmo2624	*rpmC*	ribosomal protein L29		-0.28	0.000	**-0.72**	**0.000**	**-0.74**	**0.000**
lmo2625	*rplP*	ribosomal protein L16		-0.29	0.000	**-0.67**	**0.000**	**-0.73**	**0.000**
lmo2628	*rpsS*	ribosomal protein S19		-0.26	0.000	**-0.61**	**0.000**	**-0.80**	**0.000**
lmo2630	*rplW*	ribosomal protein L23		-0.16	0.001	-0.45	0.000	**-0.66**	**0.000**
lmo2631	*rplD*	ribosomal protein L4		-0.29	0.000	-0.55	0.000	**-0.74**	**0.000**
lmo2632	*rplC*	ribosomal protein L3		-0.18	0.000	-0.40	0.000	**-0.60**	**0.000**
lmo2633	*rpsJ*	ribosomal protein S10		-0.42	0.000	**-0.85**	**0.000**	**-1.02**	**0.000**
lmo2656	*rpsL*	ribosomal protein S12		-0.50	0.000	**-0.83**	**0.000**	**-1.05**	**0.000**
lmo2856	*rpmH*	ribosomal protein L34		-0.31	0.000	-0.37	0.000	**-0.66**	**0.000**
Purines, pyrimidines, nucleosides, and nucleotides up-regulated									
lmo0812		unknown	P	0.26	0.000	0.38	0.000	**0.86**	**0.000**
lmo1770	*purL*	phosphoribosylformylglycinamidine synthetase II		0.50	0.000	0.56	0.000	**0.65**	**0.000**
lmo1772	*purC*	phosphoribosylaminoimidazole succinocarboxamide synthetase		0.50	0.000	0.56	0.000	**0.73**	**0.000**
lmo1774	*purK*	phosphoribosylaminoimidazole carboxylase II		0.42	0.000	0.50	0.000	**0.68**	**0.000**
lmo2694		lysine decarboxylase	B,E,H	0.42	0.000	0.58	0.000	**0.73**	**0.000**
lmo2758	*guaB*	inosine-monophosphate dehydrogenase		**0.66**	**0.000**	**0.81**	**0.000**	0.29	0.001
Purines, pyrimidines, nucleosides, and nucleotides down-regulated									
lmo0199	*prs*	phosphoribosyl pyrophosphate synthetase		-0.45	0.000	-0.55	0.000	**-0.78**	**0.000**
lmo0812		unknown	P	0.26	0.000	0.38	0.000	**0.86**	**0.000**
lmo1770	*purL*	phosphoribosylformylglycinamidine synthetase II		0.50	0.000	0.56	0.000	**0.65**	**0.000**
lmo1772	*purC*	phosphoribosylaminoimidazole succinocarboxamide synthetase		0.50	0.000	0.56	0.000	**0.73**	**0.000**
lmo1774	*purK*	phosphoribosylaminoimidazole carboxylase II		0.42	0.000	0.50	0.000	**0.68**	**0.000**
lmo1831	*pyrE*	orotate phosphoribosyltransferases		**-1.41**	**0.000**	**-2.15**	**0.000**	**-3.06**	**0.000**
lmo1832	*pyrF*	orotidine 5-phosphate decarboxylases		**-1.30**	**0.000**	**-1.99**	**0.000**	**-3.14**	**0.000**
lmo1833	*pyrD*	dihydroorotase dehydrogenase		**-1.22**	**0.000**	**-2.05**	**0.000**	**-2.89**	**0.000**
lmo1834	*pyrDII*	dihydroorotate dehydrogenase (electron transfer subunit)		**-1.23**	**0.000**	**-2.22**	**0.000**	**-3.71**	**0.000**
lmo1835	*pyrAB*	carbamoyl-phosphate synthetase (catalytic subunit)		**-1.04**	**0.000**	**-2.18**	**0.000**	**-3.95**	**0.000**
lmo1836	*pyrAa*	carbamoyl-phosphate synthetase (glutaminase subunit)		**-0.87**	**0.006**	**-1.67**	**0.001**	**-1.27**	**0.000**
lmo1837	*pyrC*	dihydroorotase		**-0.89**	**0.000**	**-1.87**	**0.000**	**-3.24**	**0.000**
lmo1838	*pyrB*	aspartate carbamoyltransferase		**-0.71**	**0.000**	**-1.94**	**0.000**	**-3.49**	**0.000**
lmo1840	*pyrR*	pyrimidine operon regulatory protein		**-0.65**	**0.000**	**-1.39**	**0.000**	**-2.49**	**0.000**
lmo1885		xanthine phosphoribosyltransferase		**-0.59**	**0.000**	**-1.23**	**0.000**	**-1.66**	**0.000**
lmo1929	*ndk*	nucleoside diphosphate kinase		-0.55	0.000	**-0.77**	**0.000**	**-0.85**	**0.000**
lmo1953	*pnp*	purine-nucleoside phosphorylase		**-0.75**	**0.000**	**-1.20**	**0.000**	**-1.24**	**0.000**
lmo1954	*drm*	phosphopentomutase		**-0.86**	**0.000**	**-1.37**	**0.000**	**-1.32**	**0.000**
lmo1993	*pdp*	pyrimidine-nucleoside phosphorylase		-0.44	0.000	**-0.73**	**0.000**	**-0.95**	**0.000**
lmo2694		lysine decarboxylase	B,E,H	0.42	0.000	0.58	0.000	**0.73**	**0.000**
lmo2758	*guaB*	inosine-monophosphate dehydrogenase		**0.66**	**0.000**	**0.81**	**0.000**	0.29	0.001
lmo2840		Sucrose phosphorylase	H	**-1.02**	**0.000**	**-1.34**	**0.000**	**-0.92**	**0.002**
Regulatory functions up-regulated									
lmo0048		*Staphylococcus* two-component sensor histidine kinase AgrB		**0.60**	**0.000**	**0.91**	**0.000**	**1.17**	**0.000**
lmo0051	*agrA*	two-components response regulator protein (AgrA from *Staphylococcus*)		**0.91**	**0.000**	**1.11**	**0.000**	**0.88**	**0.000**
lmo0255		unknown protein	V	**1.02**	**0.000**	**0.92**	**0.000**	**0.78**	**0.000**
lmo0315		thiamin biosynthesis protein		**0.70**	**0.000**	**0.87**	**0.000**	**1.08**	**0.000**
lmo0325		transcriptional regulators		0.56	0.000	0.30	0.001	**0.70**	**0.000**
lmo0430		transcriptional regulator (LysR family)		0.44	0.001	**0.60**	**0.000**	**0.79**	**0.000**
lmo0480		putative transcriptional regulator		0.36	0.000	**0.68**	**0.001**	**0.84**	**0.000**
lmo0526		transcription regulator (TipA from Streptomyces coelicolor)		0.36	0.043	**0.66**	**0.001**	0.76	0.025
lmo0575		transcription regulator GntR family		0.39	0.000	**0.78**	**0.000**	**1.13**	**0.000**
lmo0606		transcription regulator MarR family		0.56	0.000	**0.82**	**0.000**	**1.18**	**0.000**
lmo0639		transcription regulator (surface protein PAg negative regulator par)		0.24	0.021	0.54	0.000	**0.88**	**0.002**
lmo0659		transcription regulator (Rgg type)		**0.97**	**0.000**	**1.13**	**0.000**	**1.64**	**0.000**
lmo0740		putative transcription regulator		**0.61**	**0.000**	**0.74**	**0.000**	**0.91**	**0.000**
lmo0753		transcription regulator Crp/Fnr family		0.51	0.000	0.50	0.000	**0.69**	**0.000**
lmo0770		transcriptional regulator (LacI family)		0.29	0.000	0.51	0.000	**0.65**	**0.000**
lmo0799		unknown		**0.65**	**0.000**	**0.77**	**0.000**	**0.91**	**0.000**
lmo0802		GTP-pyrophosphokinase		0.47	0.000	**0.77**	**0.000**	**0.61**	**0.004**
lmo0822		transcriptional regulators		**0.81**	**0.000**	**1.17**	**0.000**	**1.55**	**0.000**
lmo0989		regulatory proteins (MarR family)		0.17	0.006	0.10	0.158	**0.81**	**0.000**
lmo1251		regulator of the Fnr CRP family (including PrfA)		**1.51**	**0.000**	**1.47**	**0.000**	**1.28**	**0.000**
lmo1517		nitrogen regulatory PII protein		0.57	0.001	0.50	0.004	**0.81**	**0.000**
lmo1693		hypothetical proteins		0.28	0.000	**0.68**	**0.001**	**0.63**	**0.000**
lmo1721		transcriptional regulator (NifA/NtrC family)		0.09	0.243	**0.59**	**0.000**	0.45	0.004
lmo1820		putative serine/threonine-specific protein kinase		0.33	0.000	0.56	0.000	**0.61**	**0.000**
lmo1962		transcription regulators (TetR family)		0.31	0.003	0.47	0.013	**1.14**	**0.000**
lmo2021		unknown protein		0.45	0.000	0.47	0.003	**0.79**	**0.001**
lmo2176		transcriptional regulator (tetR family)		0.47	0.018	**0.80**	**0.002**	0.73	0.029
lmo2178		putative peptidoglycan bound protein (LPXTG motif)		0.58	0.000	**0.69**	**0.000**	0.36	0.056

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo2179		putative peptidoglycan bound protein (LPXTG motif)		**1.23**	**0.000**	**1.59**	**0.000**	**0.66**	**0.002**
lmo2366		transcription regulator DeoR family		**0.68**	**0.000**	**0.83**	**0.000**	**0.85**	**0.000**
lmo2421	*cesK*	two-component sensor histidine kinase		0.56	0.000	**0.76**	**0.000**	**0.93**	**0.000**
lmo2460		*B. subtilis* CggR hypothetical transcriptional regulator		0.48	0.000	**0.81**	**0.000**	**0.82**	**0.000**
lmo2593		transcription regulators (MerR family)		**0.81**	**0.000**	**1.10**	**0.000**	**1.35**	**0.000**
lmo2678		two components response regulator		0.57	0.000	**1.06**	**0.000**	**1.54**	**0.000**
lmo2728		transcription regulator MerR family		**0.68**	**0.000**	**0.74**	**0.000**	**0.84**	**0.000**
lmo2784		lichenan operon transcription antiterminator licR		0.58	0.000	**0.59**	**0.000**	**0.62**	**0.000**
lmo2792		unknown		0.31	0.000	0.38	0.000	**0.68**	**0.000**
Regulatory functions down-regulated									
lmo0109		transcriptional regulatory proteins, AraC family		**-0.71**	**0.000**	**-1.29**	**0.000**	**-1.15**	**0.000**
lmo0192		PurR, transcription repressor of purine operon of *B. subtilis*		-0.40	0.000	**-0.71**	**0.000**	**-0.76**	**0.000**
lmo0297		transcriptional antiterminator (BglG family)		-0.34	0.000	-0.50	0.000	**-0.67**	**0.000**
lmo0425		transcription antiterminator BglG family		**-0.89**	**0.000**	**-1.08**	**0.000**	**-1.32**	**0.000**
lmo0427		PTS fructose-specific enzyme IIB component		**-1.03**	**0.000**	**-1.27**	**0.000**	**-0.70**	**0.005**
lmo0785	*manR*	transcriptional regulator (NifA/NtrC family)		-0.19	0.037	-0.44	0.000	**-0.78**	**0.000**
lmo0806		transcription regulator		-0.18	0.000	**-1.13**	**0.000**	**-1.46**	**0.000**
lmo0873		transcriptional regulator (antiterminator)		**-0.73**	**0.000**	**-1.29**	**0.000**	**-0.67**	**0.001**
lmo0958		transcription regulator (GntR family)		-0.42	0.000	**-0.62**	**0.000**	-0.41	0.000
lmo1150		Regulatory protein Salmonella typhimurium PocR protein		-0.55	0.000	**-1.16**	**0.000**	**-0.89**	**0.000**
lmo1172		two-component response regulator		-0.54	0.000	**-0.82**	**0.000**	**-0.73**	**0.000**
lmo1173		two-component sensor histidine kinase		-0.53	0.000	**-0.76**	**0.000**	**-0.67**	**0.000**
lmo1280	*codY*	*B. subtilis* CodY protein		-0.35	0.000	**-0.60**	**0.000**	**-0.68**	**0.000**
lmo1311		unknown		-0.28	0.000	**-0.79**	**0.000**	**-0.74**	**0.000**
lmo1788		transcription regulator		-0.47	0.000	**-0.62**	**0.000**	-0.50	0.000
lmo1994		transcription regulators (LacI family)		-0.49	0.000	**-0.71**	**0.000**	**-0.65**	**0.000**
lmo2003		transcription regulator GntR family		**-0.86**	**0.000**	**-0.90**	**0.000**	**-0.89**	**0.000**
lmo2004		transcription regulator GntR family		-0.46	0.000	-0.55	0.001	**-0.75**	**0.000**
lmo2088		transcription regulators		**-0.84**	**0.000**	**-1.05**	**0.000**	**-1.20**	**0.000**
lmo2099		transcription antiterminator		-0.66	0.017	-0.61	0.017	**-0.62**	**0.004**
lmo2100		transcriptional regulator (GntR family) and to aminotransferase (MocR-like)		**-0.88**	**0.000**	**-1.09**	**0.000**	**-0.89**	**0.000**
lmo2144		transcription regulator GntR family		-0.52	0.000	**-0.62**	**0.000**	**-0.69**	**0.000**
lmo2145		unknown protein		-0.25	0.007	**-0.71**	**0.000**	0.07	0.564
lmo2146		transcription regulator LysR family		-0.50	0.000	**-0.63**	**0.000**	**-0.71**	**0.000**
lmo2173		sigma-54-dependent transcriptional activator		-0.52	0.011	**-0.67**	**0.007**	-0.27	0.164
lmo2233		transcriptional regulators (LysR family)		-0.43	0.000	-0.55	0.001	**-0.61**	**0.000**
lmo2447		transcription regulator		**-0.75**	**0.000**	**-0.99**	**0.000**	**-0.94**	**0.000**
lmo2668		transcriptional antiterminator (BglG family)		-0.53	0.000	**-0.65**	**0.000**	**-0.82**	**0.000**
lmo2690		transcription regulator, TetR family		-0.48	0.000	-0.56	0.000	**-0.73**	**0.000**
lmo2764		xylose operon regulatory protein and to glucose kinase		**-1.19**	**0.000**	**-1.59**	**0.000**	**-1.68**	**0.000**
lmo2851		AraC-type regulatory protein		**-1.10**	**0.000**	**-1.63**	**0.000**	**-1.79**	**0.000**
Transcription up-regulated									
lmo1706		transport proteins		0.44	0.000	**0.63**	**0.000**	0.46	0.000
lmo1880		RNase HI		0.25	0.000	0.27	0.001	**0.68**	**0.000**
lmo2275		Portein gp28 [Bacteriophage A118]	V	0.31	0.000	0.57	0.000	**0.62**	**0.000**
lmo2461	*sigL*	RNA polymerase sigma-54 factor (sigma-L)		0.50	0.000	**0.79**	**0.000**	**0.93**	**0.000**
Transcription down-regulated									
lmo0246	*nusG*	transcription antitermination factor		-0.33	0.000	-0.58	0.000	**-0.67**	**0.000**
lmo0258	*rpoB*	RNA polymerase (beta subunit)		-0.35	0.000	-0.52	0.000	**-0.67**	**0.000**
lmo1322	*nusA*	N utilization substance protein A (NusA protein)		-0.32	0.000	-0.49	0.000	**-0.73**	**0.000**
lmo1327	*rbfA*	ribosome-binding factor A		-0.58	0.000	**-0.83**	**0.000**	**-0.96**	**0.000**
lmo2855	*rnpA*	ribonuclease P protein component		-0.29	0.000	-0.57	0.000	**-0.78**	**0.000**
Transport and binding up-regulated									
lmo0003		conserved hypothetical protein		**0.83**	**0.000**	**1.17**	**0.000**	**0.77**	**0.000**
lmo0169		glucose uptake protein		**0.98**	**0.000**	**1.28**	**0.000**	**1.17**	**0.000**
lmo0301		PTS beta-glucoside-specific enzyme IIA component	A,H	**0.61**	**0.010**	0.50	0.002	0.60	0.014
lmo0341		unknown	D	0.51	0.000	**0.71**	**0.000**	**1.43**	**0.000**
lmo0405		phosphate transport protein		**0.70**	**0.000**	**0.86**	**0.000**	**0.86**	**0.000**
lmo0641		heavy metal-transporting ATPase		0.58	0.000	**0.65**	**0.000**	0.51	0.000
lmo0667		ABC transporter (ATP-binding protein)		**0.68**	**0.000**	**0.75**	**0.000**	**0.83**	**0.000**
lmo0668		putative ABC transporter, permease protein		0.51	0.000	**0.62**	**0.000**	**0.65**	**0.000**
lmo0787		amino acid transporter		0.54	0.000	**0.64**	**0.000**	0.32	0.000
lmo0841		cation (calcium) transporting ATPase		0.22	0.000	0.40	0.000	**0.59**	**0.000**
lmo0876		PTS system, Lichenan-specific enzyme IIC component	A,H	**-1.34**	**0.001**	NA	NA	NA	NA
lmo0986		antibiotic ABC transporter, ATP-binding protein		0.26	0.000	0.45	0.000	**0.63**	**0.000**
lmo1039		ABC transporter, ATP-binding protein		**0.67**	**0.000**	**0.92**	**0.000**	**1.03**	**0.000**
lmo1040		molybdenum ABC transporters (permease)		0.47	0.000	**0.70**	**0.000**	**0.87**	**0.000**
lmo1063		ABC transporter (ATP binding protein)		**1.22**	**0.000**	**1.16**	**0.000**	**1.07**	**0.000**
lmo1095		PTS system, cellobiose-specific IIB component (cel A)	A,H	**0.60**	**0.000**	**0.71**	**0.000**	**0.65**	**0.000**
lmo1250		antibiotic resistance protein		**3.42**	**0.000**	**4.02**	**0.000**	**4.49**	**0.000**
lmo1300		arsenic efflux pump protein	D	**1.10**	**0.000**	**1.16**	**0.000**	**1.55**	**0.000**
lmo1425	*opuCD*	betaine/carnitine/choline ABC transporter (membrane p)		0.40	0.000	**0.75**	**0.000**	**0.98**	**0.000**
lmo1426	*opuCC*	glycine betaine/carnitine/choline ABC transporter (osmoprotectant-binding protein)		0.47	0.000	**0.95**	**0.000**	**0.95**	**0.000**
lmo1427	*opuCB*	glycine betaine/carnitine/choline ABC transporter (membrane protein)		0.47	0.000	**0.99**	**0.000**	**1.30**	**0.000**
lmo1428	*opuCA*	glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)		0.57	0.000	**0.99**	**0.000**	**1.36**	**0.000**
lmo1446	*zurM*	metal (zinc) transport protein (ABC transporter, permease protein)		0.45	0.000	**0.62**	**0.000**	**0.76**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo1447	*zurA*	metal (zinc) transport protein(ABC transporter, ATP-binding protein)		0.57	0.000	**0.78**	**0.000**	**0.82**	**0.000**
lmo1514		unknown protein	H	0.57	0.000	**0.95**	**0.000**	**0.90**	**0.000**
lmo1738		amino acid ABC transporter (binding protein)		**0.94**	**0.000**	**1.12**	**0.000**	**1.27**	**0.000**
lmo1739		amino acid (glutamine) ABC transporter (ATP-binding protein)		**0.65**	**0.000**	**0.71**	**0.000**	**1.05**	**0.000**
lmo1740		amino acid (glutamine) ABC transporter, permease protein		**0.81**	**0.000**	**0.89**	**0.000**	**1.01**	**0.000**
lmo1889		conserved hypothetical proteins		**0.66**	**0.001**	**0.71**	**0.009**	0.58	0.001
lmo1960	*fhuC*	ferrichrome ABC transporter (ATP-binding protein)		**0.89**	**0.000**	**1.00**	**0.000**	**0.90**	**0.000**
lmo1963		unknown protein		0.29	0.063	**0.75**	**0.001**	0.65	0.010
lmo2059		potassium channel subunit		0.42	0.036	**0.75**	**0.000**	0.57	0.006
lmo2105		ferrous iron transport protein B		0.54	0.000	**1.14**	**0.000**	**1.03**	**0.000**
lmo2114		ABC transporter (ATP-binding protein)		**1.05**	**0.000**	**0.81**	**0.000**	**1.07**	**0.000**
lmo2183		ferrichrome ABC transporter (permease)		**0.76**	**0.000**	**0.99**	**0.000**	**0.65**	**0.000**
lmo2184		ferrichrome ABC transporter (binding protein)		**0.77**	**0.000**	**0.97**	**0.000**	**0.62**	**0.000**
lmo2259		phosphotransferase system (PTS) beta-glucoside-specific enzyme IIA		0.41	0.000	**0.59**	**0.000**	**0.84**	**0.000**
lmo2362	*gadT2*	amino acid antiporter (acid resistance)		**5.64**	**0.000**	**5.49**	**0.000**	**5.28**	**0.000**
lmo2369		*B. subtilis* general stress protein 13 containing a ribosomal S1 protein domain	C,U	0.54	0.000	**0.80**	**0.000**	**0.84**	**0.000**
lmo2470		internalin proteins	D,V	**0.64**	**0.000**	**0.73**	**0.000**	**0.66**	**0.004**
lmo2483		HPr-P(Ser) kinase/phosphatase	P	0.48	0.000	**0.71**	**0.000**	**0.80**	**0.000**
lmo2497		phosphate ABC transporter (permease protein)		NA	NA	**0.63**	**0.007**	0.41	0.038
lmo2499		phosphate ABC transporter (binding protein)		NA	NA	0.43	0.023	**0.76**	**0.001**
lmo2558	*ami*	autolysin, amidase		0.22	0.000	**0.60**	**0.000**	0.56	0.000
lmo2580		ABC transporter, ATP-binding protein		**1.05**	**0.000**	**1.65**	**0.000**	**2.32**	**0.000**
lmo2581		conserved hypothetical protein		**1.23**	**0.000**	**1.99**	**0.000**	**2.56**	**0.000**
lmo2652		transcriptional antiterminator	A,H,	0.56	0.000	**0.75**	**0.000**	**0.77**	**0.000**
lmo2680	*kdpC*	potassium-transporting atpase c chain		**0.91**	**0.000**	**1.52**	**0.000**	**2.09**	**0.000**
lmo2681	*kdpB*	potassium-transporting atpase b chain	H,	**1.01**	**0.000**	**2.18**	**0.000**	**3.28**	**0.000**
lmo2682	*kdpA*	potassium-transporting atpase a chain		**1.19**	**0.000**	**2.58**	**0.000**	**3.53**	**0.000**
lmo2799		phosphotransferase system mannitol-specific enzyme IIBC		NA	NA	NA	NA	**-0.80**	**0.006**
lmo2816		transport protein		**0.77**	**0.002**	0.28	0.018	**0.60**	**0.002**
lmo2845		transmembrane efflux proteins		**0.90**	**0.000**	**1.15**	**0.000**	**0.94**	**0.000**
Transport and binding down-regulated									
lmo0022		PTS system, fructose-specific IIB component	A,H	NA	NA	**-0.72**	**0.010**	NA	NA
lmo0027		PTS system, beta-glucosides specific enzyme IIABC	A,H	**-1.88**	**0.000**	**-3.24**	**0.000**	**-2.76**	**0.000**
lmo0096	*mptA*	PTS system mannose-specific, factor IIAB	A,H	0.16	0.317	**-0.65**	**0.000**	**-1.02**	**0.000**
lmo0097	*mptC*	PTS system mannose-specific, factor IIC	A,H	0.06	0.721	**-0.87**	**0.000**	**-1.22**	**0.000**
lmo0098	*mptD*	PTS system mannose-specific, factor IID	A,H	0.07	0.600	**-0.92**	**0.000**	**-1.28**	**0.000**
lmo0135		oligopeptide ABC transport system substrate-binding proteins		**-1.25**	**0.000**	**-1.85**	**0.000**	**-2.54**	**0.000**
lmo0136		oligopeptide ABC transporter, permease protein		**-0.63**	**0.000**	**-1.14**	**0.000**	**-1.76**	**0.000**
lmo0137		oligopeptide ABC transporter, permease protein		-0.51	0.000	**-0.83**	**0.000**	**-1.48**	**0.000**
lmo0179		sugar ABC transporters, permease proteins		**-0.77**	**0.000**	**-1.00**	**0.000**	**-1.14**	**0.000**
lmo0180		sugar ABC transporter, permease protein		-0.56	0.012	**-0.74**	**0.001**	NA	NA
lmo0181		sugar ABC transporter, sugar-binding protein		-0.42	0.000	-0.69	0.013	**-0.68**	**0.003**
lmo0278		sugar ABC transporter, ATP-binding protein		-0.20	0.080	**-1.16**	**0.000**	**-1.26**	**0.000**
lmo0399		fructose-specific phosphotransferase enzyme IIB		0.17	0.279	**-0.77**	**0.000**	-0.31	0.077
lmo0400		fructose-specific phosphotransferase enzyme IIC		0.23	0.190	**-0.59**	**0.002**	-0.46	0.009
lmo0426		PTS fructose-specific enzyme IIA component		**-1.22**	**0.000**	**-1.37**	**0.000**	**-1.56**	**0.000**
lmo0428		PTS fructose-specific enzyme IIC component		**-1.00**	**0.000**	**-1.09**	**0.000**	**-1.52**	**0.000**
lmo0519		multidrug resistance protein		-0.43	0.000	**-0.86**	**0.000**	**-1.18**	**0.000**
lmo0544		PTS system, glucitol/sorbitol-specific enzyme II CII component	A,H	NA	NA	**-0.93**	**0.005**	NA	NA
lmo0559		putative membrane protein		**-0.99**	**0.000**	**-1.55**	**0.000**	**-1.62**	**0.000**
lmo0573		conserved hypothetical protein		**-1.13**	**0.000**	**-2.07**	**0.000**	**-2.48**	**0.000**
lmo0738		phosphotransferase system (PTS) beta-glucoside-specific enzyme IIABC component	A,H	**-1.71**	**0.000**	**-2.01**	**0.000**	**-2.20**	**0.000**
lmo0767		ABC transporter, permease protein		-0.47	0.001	**-0.74**	**0.007**	-0.48	0.014
lmo0781	*mpoD*	mannose-PTS component IID	A,H	-0.54	0.000	**-1.16**	**0.000**	**-1.06**	**0.000**
lmo0782	*mpoC*	mannose-PTS component IIC		-0.22	0.163	**-0.84**	**0.000**	**-0.72**	**0.000**
lmo0783	*mpoB*	mannose-PTS component IIB		-0.31	0.049	**-1.01**	**0.000**	**-0.67**	**0.000**
lmo0784	*mpoA*	mannose-PTS component IIA		-0.32	0.064	**-0.91**	**0.000**	**-0.75**	**0.000**
lmo0798		lysine-specific permease		-0.46	0.000	**-0.69**	**0.000**	-0.40	0.001
lmo0807		spermidine/putrescine ABC transporter, ATP-binding protein		-0.11	0.032	**-0.71**	**0.000**	**-1.11**	**0.000**
lmo0808		spermidine/putrescine ABC transporter, permease protein		-0.16	0.002	**-0.94**	**0.000**	**-1.44**	**0.000**
lmo0809		spermidine/putrescine ABC transporter, permease protein		-0.28	0.000	**-0.93**	**0.000**	**-1.39**	**0.000**
lmo0810		spermidine/putrescine-binding protein		-0.20	0.000	**-1.00**	**0.000**	**-1.41**	**0.000**
lmo0847		Glutamine ABC transporter (binding and transport protein)		**-1.99**	**0.000**	**-2.26**	**0.000**	**-2.63**	**0.000**
lmo0848		amino acid ABC transporter, ATP-binding protein		**-2.17**	**0.000**	**-2.44**	**0.000**	**-2.75**	**0.000**
lmo0859		putative sugar ABC transporter, periplasmic sugar-binding protein		**-1.23**	**0.000**	**-1.93**	**0.000**	**-1.54**	**0.000**
lmo0860		sugar ABC transporter, permease protein		**-0.86**	**0.000**	-0.59	0.012	**-0.83**	**0.001**
lmo0874		PTS system enzyme IIA component		**-1.11**	**0.000**	**-1.19**	**0.000**	**-0.93**	**0.000**
lmo0875		PTS system, beta-glucoside enzyme IIB component	A,H	-0.50	0.002	**-0.69**	**0.001**	**-0.70**	**0.002**
lmo0901		PTS system, cellobiose-specific IIC component	Q	-0.58	0.000	**-0.77**	**0.000**	-0.51	0.002
lmo0916		phosphotransferase system enzyme IIA		0.34	0.001	**-0.68**	**0.000**	-0.40	0.000
lmo0947		hypothetical transport protein		-0.53	0.000	**-0.76**	**0.000**	**-0.89**	**0.000**
lmo0981		efflux transporter		-0.53	0.000	-0.53	0.000	**-0.69**	**0.000**
lmo1073		metal binding protein (ABC transporter)		-0.58	0.000	**-0.83**	**0.000**	**-1.24**	**0.000**
lmo1255		PTS system trehalose specific enzyme IIBC	A,H	**-5.69**	**0.000**	**-5.82**	**0.000**	**-5.55**	**0.000**
lmo1370		branched-chain fatty-acid kinase		-0.48	0.000	-0.32	0.000	**-0.59**	**0.000**
lmo1372		branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha subunit)		**-0.61**	**0.000**	-0.16	0.009	-0.57	0.000
lmo1389		sugar ABC transporter, ATP-binding protein		-0.21	0.000	-0.36	0.000	**-0.60**	**0.000**
lmo1390		ABC transporter (permease proteins)		-0.36	0.000	-0.47	0.000	**-0.72**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo1391		sugar ABC transporter, permease protein		-0.38	0.000	-0.55	0.000	**-0.73**	**0.000**
lmo1409		multidrug-efflux transporter		-0.41	0.000	**-0.82**	**0.000**	**-0.95**	**0.000**
lmo1424		manganese transport proteins NRAMP		**-1.23**	**0.000**	**-1.47**	**0.000**	**-1.78**	**0.000**
lmo1431		ABC transporter (ATP-binding protein)		**-0.89**	**0.000**	**-1.00**	**0.000**	**-1.12**	**0.000**
lmo1539		glycerol uptake facilitator		-0.29	0.000	**-1.25**	**0.000**	**-1.02**	**0.000**
lmo1682		transmembrane transport proteins		-0.23	0.087	-0.28	0.027	**-0.86**	**0.002**
lmo1730		sugar ABC transporter binding protein		**-1.31**	**0.000**	**-2.03**	**0.000**	**-1.48**	**0.002**
lmo1731		sugar ABC transporter, permease protein		**-1.08**	**0.000**	**-1.64**	**0.000**	**-1.35**	**0.000**
lmo1732		sugar ABC transporter, permease protein		**-1.03**	**0.000**	**-1.41**	**0.000**	**-1.48**	**0.000**
lmo1761		putative sodium-dependent transporter		-0.46	0.000	**-0.64**	**0.000**	**-0.65**	**0.000**
lmo1839	*pyrP*	uracil permease		**-0.88**	**0.000**	**-2.49**	**0.000**	**-3.79**	**0.000**
lmo1848		similar metal cations ABC transporter (permease protein)		**-2.25**	**0.000**	**-2.62**	**0.000**	**-2.55**	**0.000**
lmo1849		metal cations ABC transporter, ATP-binding proteins		**-2.46**	**0.000**	**-2.89**	**0.000**	**-3.03**	**0.000**
lmo1884		xanthine permeases		**-0.77**	**0.000**	**-1.43**	**0.000**	**-1.74**	**0.000**
lmo1973		PTS system enzyme II A component	A,H	**-0.82**	**0.000**	**-0.68**	**0.000**	**-0.86**	**0.000**
lmo1997		PTS mannose-specific enzyme IIA component		**-1.27**	**0.000**	**-1.24**	**0.004**	**-0.95**	**0.001**
lmo2001		PTS mannose-specific enzyme IIC component	A,H	**-0.68**	**0.000**	**-1.04**	**0.000**	-0.50	0.005
lmo2002		PTS mannose-specific enzyme IIB component	A,H	**-1.00**	**0.000**	**-1.15**	**0.002**	**-1.12**	**0.000**
lmo2084		unknown	D	**-0.73**	**0.000**	**-1.24**	**0.000**	**-1.09**	**0.000**
lmo2087		unknown protein		**-0.92**	**0.000**	**-0.99**	**0.000**	**-1.01**	**0.001**
lmo2097		PTS system galactitol-specific enzyme IIB component	A,H	**-0.62**	**0.003**	**-0.78**	**0.000**	-0.43	0.001
lmo2227		ABC transporter (ATP-binding protein)		**-0.70**	**0.000**	**-0.80**	**0.000**	**-0.83**	**0.000**
lmo2238		transport system permease protein		-0.39	0.001	**-0.72**	**0.000**	**-0.66**	**0.000**
lmo2250	*arpJ*	amino acid ABC transporter, permease protein		**-1.84**	**0.000**	**-2.19**	**0.000**	**-2.50**	**0.000**
lmo2251		amino acid ABC transporter (ATP-binding protein)		**-1.78**	**0.000**	**-2.06**	**0.000**	**-2.39**	**0.000**
lmo2346		amino acid ABC-transporter, ATP-binding protein		**-1.71**	**0.000**	**-2.34**	**0.000**	**-2.39**	**0.000**
lmo2347		amino acid ABC transporter (permease)		**-1.85**	**0.000**	**-2.64**	**0.000**	**-2.98**	**0.000**
lmo2349		amino acid ABC transporter (binding protein)		**-1.80**	**0.000**	**-1.91**	**0.000**	**-2.61**	**0.000**
lmo2372		ABC-transporter ATP binding proteins		-0.40	0.000	**-0.78**	**0.000**	**-0.67**	**0.000**
lmo2575		cation transport protein (efflux)		**-0.86**	**0.000**	**-0.93**	**0.000**	**-1.53**	**0.000**
lmo2591		surface protein (GW repeat) N-acetylmuramidase		-0.47	0.000	**-0.60**	**0.000**	-0.56	0.000
lmo2650		hypothetical PTS enzyme IIB component	A,H	**-1.49**	**0.000**	**-1.50**	**0.000**	**-1.98**	**0.000**
lmo2651		mannitol-specific PTS enzyme IIA component		**-1.77**	**0.000**	**-2.16**	**0.000**	**-2.33**	**0.000**
lmo2665		PTS system galactitol-specific enzyme IIC component	A,H	**-0.63**	**0.000**	**-0.84**	**0.000**	**-1.04**	**0.000**
lmo2666		PTS system galactitol-specific enzyme IIB component	A,H	**-0.60**	**0.000**	**-0.72**	**0.000**	**-0.82**	**0.000**
lmo2667		PTS system galactitol-specific enzyme IIA component	A,H	-0.45	0.000	-0.49	0.000	**-0.64**	**0.000**
lmo2683		cellobiose phosphotransferase enzyme IIB component	A,H	**-1.62**	**0.000**	**-1.99**	**0.000**	**-2.92**	**0.000**
lmo2684		cellobiose phosphotransferase enzyme IIC component	Q	**-2.21**	**0.000**	**-2.91**	**0.000**	**-3.67**	**0.000**
lmo2685		cellobiose phosphotransferase enzyme IIA component	A,H	**-1.76**	**0.000**	**-2.16**	**0.000**	**-2.84**	**0.000**
lmo2689		Mg2+ transport ATPase		**-0.66**	**0.000**	**-0.77**	**0.000**	**-0.60**	**0.000**
lmo2708		PTS system, cellobiose-specific enzyme IIC	Q	**-2.85**	**0.000**	**-3.23**	**0.000**	**-3.16**	**0.000**
lmo2762		PTS cellobiose-specific enzyme IIB	A,H	**-1.15**	**0.000**	**-1.60**	**0.000**	**-1.45**	**0.000**
lmo2763		PTS cellobiose-specific enzyme IIC	Q	**-0.84**	**0.000**	**-1.01**	**0.000**	**-1.26**	**0.000**
lmo2765		PTS cellobiose-specific enzyme IIA		**-0.82**	**0.000**	**-1.17**	**0.000**	**-1.45**	**0.000**
lmo2772		beta-glucoside-specific enzyme IIABC	A,H	-0.51	0.000	**-1.02**	**0.000**	**-0.90**	**0.000**
lmo2787	*bvrB*	beta-glucoside-specific phosphotransferase enzyme II ABC component	A,H	**-2.52**	**0.000**	**-3.14**	**0.000**	**-2.99**	**0.000**
lmo2797		phosphotransferase system mannitol-specific enzyme IIA	A,H	**-1.67**	**0.000**	**-2.19**	**0.000**	**-2.39**	**0.000**
lmo2837		sugar ABC transporter permease protein		-0.32	0.072	**-0.73**	**0.000**	**-0.70**	**0.001**
lmo2838		sugar ABC transporter permease protein		**-1.11**	**0.001**	**-1.25**	**0.000**	**-1.12**	**0.000**
Unclassified (role category not yet assigned) up-regulated									
lmo0102		unknown		**0.78**	**0.000**	**0.71**	**0.000**	**0.78**	**0.001**
lmo0139		unknown		**0.64**	**0.000**	**1.10**	**0.000**	**0.98**	**0.000**
lmo0172		transposase C-terminal part		**0.64**	**0.008**	0.48	0.016	NA	NA
lmo0173		transposase (N-terminal part)		**0.83**	**0.000**	**1.38**	**0.000**	NA	NA
lmo0175		putative peptidoglycan bound protein (LPXTG motif)		0.52	0.003	**0.91**	**0.002**	**1.08**	**0.000**
lmo0185		conserved hypothetical proteins		0.38	0.000	0.55	0.000	**0.65**	**0.000**
lmo0189		*B. subtilis* Veg protein		0.25	0.000	**0.70**	**0.000**	0.52	0.000
lmo0202	*hly*	listeriolysin O precursor		**1.46**	**0.000**	**2.04**	**0.000**	**3.00**	**0.000**
lmo0204	*actA*	actin-assembly inducing protein precursor		**1.54**	**0.000**	**2.32**	**0.000**	**2.52**	**0.000**
lmo0208		conserved hypothetical protein		**0.64**	**0.000**	**0.81**	**0.000**	**0.95**	**0.000**
lmo0265		succinyldiaminopimelate desuccinylase		NA	NA	0.46	0.133	**1.09**	**0.002**
lmo0336		unknown		**0.80**	**0.000**	**1.48**	**0.000**	**1.47**	**0.000**
lmo0354		fatty-acid--CoA ligase		0.38	0.000	**0.66**	**0.000**	0.53	0.000
lmo0361		conserved hypothetical integral membrane protein		**1.17**	**0.000**	**1.36**	**0.000**	**1.37**	**0.000**
lmo0362		conserved hypothetical protein		**1.30**	**0.000**	**1.28**	**0.000**	**1.28**	**0.000**
lmo0367		conserved hypothetical protein *B. subtilis* YwbN protein		**0.75**	**0.000**	**0.91**	**0.000**	**0.83**	**0.000**
lmo0368		different proteins		0.57	0.000	**0.62**	**0.000**	0.55	0.000
lmo0473		pseudogene		**0.65**	**0.000**	1.15	0.011	**1.22**	**0.000**
lmo0553		unknown		0.37	0.000	**0.62**	**0.000**	**1.14**	**0.000**
lmo0582	*iap*	P60 extracellular protein, invasion associated protein Iap		0.20	0.000	0.43	0.000	**0.60**	**0.000**
lmo0586		unknown		**0.67**	**0.000**	**0.76**	**0.000**	**0.80**	**0.000**
lmo0595		O-acetylhomoserine sulfhydrylase		**0.71**	**0.000**	**1.01**	**0.000**	**1.24**	**0.000**
lmo0607		ABC transporter, ATP-binding protein		**0.77**	**0.000**	**1.26**	**0.000**	**1.54**	**0.000**
lmo0608		ABC transporter, ATP-binding protein		0.38	0.000	**0.67**	**0.000**	**1.05**	**0.000**
lmo0621		conserved hypothetical protein		**0.94**	**0.000**	**1.08**	**0.000**	**1.16**	**0.000**
lmo0625		unknown		0.37	0.000	**0.64**	**0.000**	**0.72**	**0.000**
lmo0661		unknown protein		**2.52**	**0.000**	**2.67**	**0.000**	**2.69**	**0.000**
lmo0664		acetyl transferase		0.47	0.000	0.44	0.000	**0.63**	**0.000**
lmo0754		bile acid 7-alpha dehydratase		**0.64**	**0.000**	**0.63**	**0.000**	**0.79**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo0800		*B. subtilis* YqkB protein		**1.68**	**0.000**	**2.07**	**0.000**	**2.27**	**0.000**
lmo0829	*nifJ*	pyruvate-flavodoxin oxidoreductase		**0.73**	**0.000**	**0.95**	**0.000**	**1.17**	**0.000**
lmo0871		*B. subtilis* YtcD protein		0.38	0.000	0.48	0.000	**0.59**	**0.000**
lmo0930		conserved hypothetical protein, *B. subtilis* YhfI protein		**0.70**	**0.000**	**0.89**	**0.000**	**1.17**	**0.000**
lmo0974	*dltA*	D-alanine-activating enzyme (dae), D-alanine-D-alanyl carrier protein ligase (dcl)		**0.89**	**0.000**	**1.15**	**0.000**	**1.24**	**0.000**
lmo1018		*E. coli* copper homeostasis protein CutC		0.49	0.000	0.52	0.000	**0.64**	**0.000**
lmo1252		*B. subtilis* YxkD protein		**0.87**	**0.000**	**1.46**	**0.000**	**1.17**	**0.000**
lmo1336		5-formyltetrahydrofolate cyclo-ligase		**0.64**	**0.000**	0.50	0.000	0.43	0.000
lmo1380		unknown		0.20	0.000	0.48	0.000	**0.69**	**0.000**
lmo1397	*cinA*	competence-damage inducible protein CinA		**0.78**	**0.000**	**0.73**	**0.000**	**0.66**	**0.000**
lmo1430		unknown protein		0.30	0.000	0.55	0.000	**0.59**	**0.000**
lmo1510		unknown protein		0.26	0.000	**0.61**	**0.000**	**0.63**	**0.000**
lmo1655		unknown protein		**0.91**	**0.000**	**1.03**	**0.001**	**0.67**	**0.003**
lmo1680		cystathionine gamma-synthase		**0.95**	**0.000**	**1.29**	**0.000**	**1.63**	**0.000**
lmo1705		deoxyguanosine kinase/deoxyadenosine kinase(I) subunit		**0.75**	**0.000**	**0.97**	**0.000**	**1.16**	**0.000**
lmo1895	*dnaD*	chromosome replication initiation protein		0.17	0.001	0.31	0.000	**0.69**	**0.004**
lmo1968		creatinine amidohydrolases		0.50	0.000	**0.69**	**0.000**	**0.74**	**0.000**
lmo2067		conjugated bile acid hydrolase		**0.67**	**0.001**	**1.06**	**0.000**	**1.14**	**0.002**
lmo2170		unknown protein		**0.78**	**0.002**	**0.98**	**0.003**	**0.72**	**0.003**
lmo2180		unknown protein		**1.19**	**0.000**	**1.40**	**0.000**	**1.08**	**0.002**
lmo2182		ferrichrome ABC transporter (ATP-binding protein)		**0.77**	**0.000**	**1.00**	**0.000**	**0.67**	**0.000**
lmo2287		putative tape-measure [Bacteriophage A118]		**0.71**	**0.000**	**0.75**	**0.001**	0.53	0.011
lmo2300		putative terminase large subunit from Bacteriophage A118		0.58	0.000	**0.90**	**0.000**	**0.95**	**0.000**
lmo2304		Bacteriophage A118 gp65 protein		NA	NA	NA	NA	**0.79**	**0.001**
lmo2317		protein gp49 [Bacteriophage A118]		0.56	0.015	**0.88**	**0.000**	**1.04**	**0.000**
lmo2403		*B. subtilis* YunD protein		0.53	0.000	**0.72**	**0.000**	**0.84**	**0.000**
lmo2406		*B. subtilis* YunF protein		0.57	0.000	**0.86**	**0.000**	**1.12**	**0.000**
lmo2439		unknown		**0.66**	**0.000**	0.53	0.000	**0.86**	**0.000**
lmo2473		conserved hypothetical protein		0.42	0.000	**0.61**	**0.000**	**0.70**	**0.000**
lmo2522		hypothetical cell wall binding protein from *B. subtilis*		**1.59**	**0.000**	**2.13**	**0.000**	**3.28**	**0.000**
lmo2541		yeast translation initiation protein		0.38	0.000	**0.69**	**0.000**	**0.67**	**0.000**
lmo2602		conserved hypothetical protein		**0.60**	**0.000**	**1.01**	**0.000**	**1.43**	**0.000**
lmo2671		unknown		0.25	0.000	0.56	0.000	**0.63**	**0.000**
lmo2679		the two components sensor protein kdpD		**0.66**	**0.000**	**1.20**	**0.000**	**1.70**	**0.000**
lmo2740		unknown		0.53	0.000	**0.78**	**0.000**	**0.75**	**0.000**
lmo2759		unknown protein		0.40	0.000	0.32	0.000	**0.61**	**0.000**
lmo2817		peptidases		0.32	0.027	0.20	0.319	**0.75**	**0.001**
lmo2850		sugar transport proteins		NA	NA	**-0.80**	**0.008**	NA	NA
Unclassified (role category not yet assigned) down-regulated									
lmo0107		ABC transporter, ATP-binding protein		**-0.84**	**0.000**	**-0.75**	**0.005**	-0.61	0.022
lmo0108		ABC transporter, ATP-binding protein		**-1.04**	**0.000**	**-1.06**	**0.000**	**-1.12**	**0.000**
lmo0130		5-nucleotidase, putative peptidoglycan bound protein (LPXTG motif)		**-2.50**	**0.000**	**-3.39**	**0.000**	**-3.70**	**0.000**
lmo0385		*B. subtilis* IolC protein and to fructokinase		**-0.69**	**0.000**	**-0.84**	**0.000**	**-0.91**	**0.000**
lmo0591		unknown membrane proteins		**-0.82**	**0.000**	**-1.17**	**0.000**	**-1.22**	**0.000**
lmo0768		sugar ABC transporter, periplasmic sugar-binding protein		**-0.71**	**0.000**	**-0.85**	**0.000**	**-0.84**	**0.000**
lmo0769		alpha-1,6-mannanase		-0.53	0.000	-0.58	0.000	**-0.62**	**0.001**
lmo1001		*B. subtilis* protein YkvS		-0.55	0.000	**-0.70**	**0.000**	**-0.65**	**0.000**
lmo1075		teichoic acid translocation ATP-binding protein TagH (ABC transporter)		-0.36	0.000	**-0.62**	**0.000**	-0.47	0.000
lmo1143		Salmonella enterica PduT protein		-0.41	0.000	**-0.64**	**0.000**	**-0.66**	**0.001**
lmo1303		*B. subtilis* YneA protein		-0.55	0.000	-0.48	0.000	**-0.61**	**0.000**
lmo1321		conserved hypothetical protein, *B. subtilis* YlxS protein		-0.27	0.000	-0.45	0.000	**-0.64**	**0.000**
lmo1369		phosphotransbutyrylase		**-0.69**	**0.000**	-0.16	0.031	**-1.37**	**0.000**
lmo1388	*tcsA*	CD4+ T cell-stimulating antigen, lipoprotein		**-0.67**	**0.000**	**-0.83**	**0.000**	**-0.62**	**0.000**
lmo1651		ABC transporter (ATP-binding protein)		**-0.95**	**0.000**	**-1.08**	**0.000**	**-1.32**	**0.000**
lmo1652		ABC transporter (ATP-binding protein)		**-1.14**	**0.000**	**-1.39**	**0.000**	**-1.77**	**0.000**
lmo1790		unknown protein		-0.54	0.000	**-0.60**	**0.000**	-0.48	0.000
lmo1796		unknown protein		**-0.76**	**0.000**	**-1.11**	**0.000**	**-1.33**	**0.000**
lmo1847		adhesion binding proteins and lipoproteins with multiple specificity for metal cations (ABC transporter)		**-1.94**	**0.000**	**-2.30**	**0.000**	**-1.72**	**0.005**
lmo1970		putative phosphotriesterase related proteins		**-0.69**	**0.000**	-0.68	0.016	**-0.61**	**0.006**
lmo1998		opine catabolism protein		**-1.02**	**0.000**	**-1.60**	**0.000**	**-1.30**	**0.000**
lmo1999		glucosamine-fructose-6-phosphate aminotransferase		**-0.99**	**0.000**	**-1.55**	**0.000**	**-1.44**	**0.001**
lmo2102		unknown		-0.57	0.000	**-0.82**	**0.000**	**-0.79**	**0.001**
lmo2125		maltose/maltodextrin ABC-transporter (binding protein)		0.17	0.321	**-2.88**	**0.000**	**-2.10**	**0.000**
lmo2158		*B. subtilis* YwmG protein		**-1.17**	**0.000**	-0.54	0.000	-0.19	0.095
lmo2207		unknown protein		-0.44	0.000	-0.49	0.000	**-0.69**	**0.000**
lmo2264		unknown protein		-0.56	0.000	**-0.76**	**0.000**	**-0.80**	**0.000**
lmo2333		pseudogene		**-0.91**	**0.000**	**-1.00**	**0.001**	**-0.95**	**0.000**
lmo2345		conserved hypothetical protein		**-1.97**	**0.000**	**-2.58**	**0.000**	**-2.96**	**0.000**
lmo2348		amino acid ABC-transporter (permease)		**-1.85**	**0.000**	**-2.54**	**0.000**	**-2.95**	**0.000**
lmo2433		acetylesterase		**-1.34**	**0.000**	**-1.43**	**0.000**	**-1.90**	**0.000**
lmo2647		creatinine amidohydrolase		**-1.27**	**0.000**	**-1.50**	**0.000**	**-1.36**	**0.001**
lmo2648		Phosphotriesterase		**-1.44**	**0.000**	**-1.81**	**0.000**	**-1.65**	**0.000**
lmo2732		unknown		-0.58	0.000	**-1.10**	**0.000**	**-1.17**	**0.000**
lmo2742		unknown		-0.07	0.310	**-0.60**	**0.000**	-0.06	0.503
lmo2751		ABC transporter, ATP-binding protein		-0.32	0.000	**-0.72**	**0.000**	**-0.82**	**0.000**
lmo2752		ABC transporter, ATP-binding protein		**-0.65**	**0.000**	**-0.98**	**0.000**	**-0.95**	**0.000**
lmo2773		transcription antiterminator		**-1.45**	**0.000**	**-2.11**	**0.000**	**-2.21**	**0.000**
lmo2785	*kat*	catalase		**-1.31**	**0.000**	**-1.59**	**0.000**	**-1.35**	**0.000**

Locus	Gene	Description (similar to)[a]	Role(s)[a]	HCl[b]		AA		LA	
				Log2	q-value	Log2	q-value	Log2	q-value
lmo2788	*bvrA*	transcription antiterminator		**-1.23**	**0.000**	**-1.82**	**0.000**	**-1.74**	**0.000**
lmo2801		putative N-acetylmannosamine-6-phosphate epimerase		**-1.62**	**0.000**	**-1.99**	**0.000**	**-1.23**	**0.004**
lmo2853		*B. subtilis* Jag protein		**-0.67**	**0.000**	**-0.93**	**0.000**	**-1.12**	**0.000**
Unknown function up-regulated									
lmo0212		unknown		0.38	0.000	0.55	0.000	**0.80**	**0.000**
lmo0602		transcription regulator		**0.61**	**0.000**	**0.71**	**0.000**	**0.96**	**0.000**
lmo0624		unknown protein		0.24	0.000	0.47	0.000	**0.62**	**0.000**
lmo0795		conserved hypothetical protein		**0.85**	**0.000**	**0.67**	**0.000**	0.38	0.000
lmo0919		ABC transporter ATP-binding protein (antibiotic resistance)		0.56	0.000	**0.75**	**0.000**	**0.70**	**0.000**
lmo0976		*B. subtilis* YjcF protein		0.42	0.000	0.45	0.000	**0.63**	**0.000**
lmo1569		unknown protein		0.40	0.000	**0.60**	**0.000**	**0.81**	**0.000**
lmo2005		oxidoreductase		**1.33**	**0.001**	**1.98**	**0.000**	**1.82**	**0.000**
lmo2109		hydrolase		0.23	0.088	**0.59**	**0.004**	**0.79**	**0.000**
lmo2127		unknown		**0.82**	**0.000**	**0.87**	**0.000**	**1.05**	**0.000**
lmo2165		transcription regulator CRP/FNR family		0.52	0.002	**0.90**	**0.000**	**0.63**	**0.001**
lmo2260		unknown protein		0.54	0.000	**0.80**	**0.000**	**0.89**	**0.000**
lmo2361		conserved hypothetical protein		**2.61**	**0.000**	**3.78**	**0.000**	**3.51**	**0.000**
lmo2397		NifU protein		0.44	0.003	0.43	0.035	**0.61**	**0.000**
Unknown function down-regulated									
lmo0590		fusion of two types of conserved hypothetical proteinconserved hypothetical		-0.54	0.001	-0.50	0.014	**-0.86**	**0.001**
lmo0694		unknown		**-0.89**	**0.000**	**-1.27**	**0.000**	**-1.18**	**0.000**
lmo0776		transcription regulator (repressor)		**-0.83**	**0.000**	**-1.20**	**0.000**	**-1.23**	**0.000**
lmo1049		molybdopterin biosynthesis protein MoeB		-0.33	0.000	**-0.64**	**0.000**	-0.51	0.000
lmo1937		unknown protein		-0.41	0.001	-0.46	0.000	**-0.63**	**0.000**
lmo2649		hypothetical PTS enzyme IIC component		**-1.91**	**0.000**	**-2.37**	**0.000**	**-2.34**	**0.000**
lmo2796		transcription regulator		**-0.85**	**0.000**	**-1.39**	**0.000**	**-1.23**	**0.001**
lmo2829		yeast protein Frm2p involved in fatty acid signaling		-0.13	0.074	-0.43	0.000	**-0.77**	**0.000**
Viral functions up-regulated									
lmo0255		unknown protein	P	**1.02**	**0.000**	**0.92**	**0.000**	**0.78**	**0.000**
lmo1444		protein secretion PrsA (post-translocation molecular chaperone)		**0.67**	**0.000**	**1.05**	**0.000**	**0.97**	**0.000**
lmo2112		unknown		0.58	0.000	**0.68**	**0.000**	**0.78**	**0.000**
lmo2275		protein gp28 [Bacteriophage A118]	R	0.31	0.000	0.57	0.000	**0.62**	**0.000**
lmo2283		protein gp20 [Bacteriophage A118]		0.55	0.000	**0.68**	**0.005**	**0.91**	**0.000**
lmo2286		protein gp17 [Bacteriophage A118]		0.21	0.051	0.35	0.107	**0.74**	**0.007**
Viral functions down-regulated									
lmo0717		transglycosylase		**-1.48**	**0.000**	**-1.83**	**0.000**	**-2.08**	**0.000**
lmo2072		putative DNA binding proteins		-0.37	0.000	**-0.63**	**0.000**	-0.54	0.000

[a] Description of genes according to the annotation of the Comprehensive Microbial Resource of the J. Craig Venter Institute (http://cmr.jcvi.org). The functional role categories are also according to the primary annotation in CMR-JCVI *L. monocytogenes* EGDe genome database. A, Amino acid biosynthesis; B, Biosynthesis of cofactors, prosthetic groups, and carriers; C, Cell envelope; D, Cellular processes; E, Central intermediary metabolism; G, DNA metabolism; H, Energy metabolism; I, Fatty acid and phospholipid metabolism; J, Hypothetical proteins; L, Mobile and extrachromosomal element functions; M, Protein fate; N, Protein synthesis; O, Purines, pyrimidines, nucleosides, and nucleotides; P, Regulatory functions; Q, Signal transduction; R, Transcription; S, Transport and binding proteins; T, Unclassified; U, Unknown function; V, Viral functions.

[b] Log2 expression ratio (HCl, hydrochloric acid; AA, acetic acid; or LA, lactic acid/reference culture); values in bold face indicate differentially expressed genes as defined as log2 ratios ≥ 0.585 or ≤ -0.585 and with q-value < 0.01 (p-value adjusted to control false discovery rate). NA, no data available. Fully annotated microarray data have been deposited in BμG@Sbase (accession number E-BUGS-109; http://bugs.sgul.ac.uk/E-BUGS-109) and also ArrayExpress (accession number E-BUGS-109).

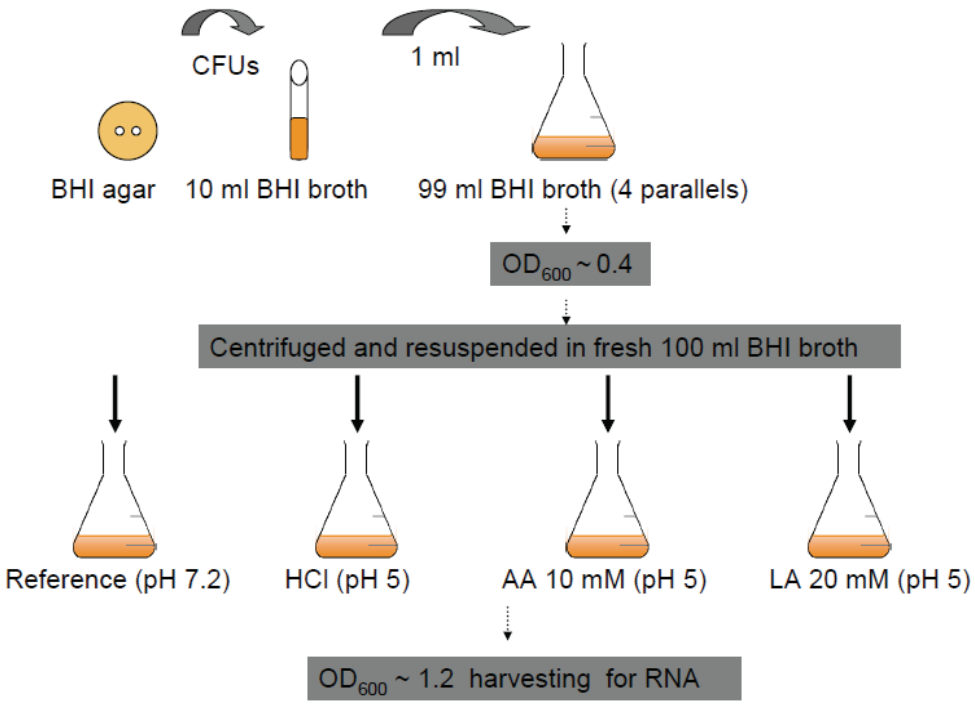
Table S3. Up-regulated known and putative genes encoding proteins implicated in cross-protection against other stress conditions in *L. monocytogenes* L502 grown in Brain Heart Infusion broth acidified to pH 5 with HCl, 10 mM acetic acid (AA) or 20 mM lactic acid (LA).

Locus	Gene	Product (similar to)[a]	Log2 ratio[b]		
			HCl	AA	LA
Heavy metal resistance					
lmo0641		heavy metal-transporting ATPase	0.58	**0.65**	0.51
lmo1300		arsenic efflux pump protein	**1.10**	**1.16**	**1.55**
lmo2230		arsenate reductase	0.34	**0.81**	**1.30**
lmo2231		divalent cations efflux	0.57	**0.74**	**0.81**
Oxidative stress					
lmo0906		glutathione reductase	**0.62**	**0.81**	**1.05**
lmo0983		glutathione peroxidase	0.35	0.39	**0.70**
lmo1233	*trxA*	thioredoxin	0.50	**0.64**	**0.87**
lmo1433		glutathione reductase	**2.02**	**2.09**	**1.84**
lmo1609		thioredoxin	0.43	**0.61**	**0.83**
lmo2424		thioredoxin	**1.03**	**1.08**	**1.31**
lmo2478	*trxB*	thioredoxin reductase	**0.64**	**0.64**	**0.85**

[a] Description of genes according to the annotation of the Comprehensive Microbial Resource of the J. Craig Venter Institute (http://cmr.jcvi.org) and published literature sources.

[b] Log2 expression ratio (HCl, hydrochloric acid; AA, acetic acid; or LA, lactic acid/reference culture); bold type values log2 ratios ≥ 0.585 and with q-value < 0.01 (p-value adjusted to control false discovery rate).

(A)


CFUs
1 ml
BHI agar
10 ml BHI broth
99 ml BHI broth (4 parallels)
OD600 ~ 0.4
Centrifuged and resuspended in fresh 100 ml BHI broth
Reference (pH 7.2)
HCl (pH 5)
AA 10 mM (pH 5)
LA 20 mM (pH 5)
OD600 ~ 1.2 harvesting for RNA

(B)

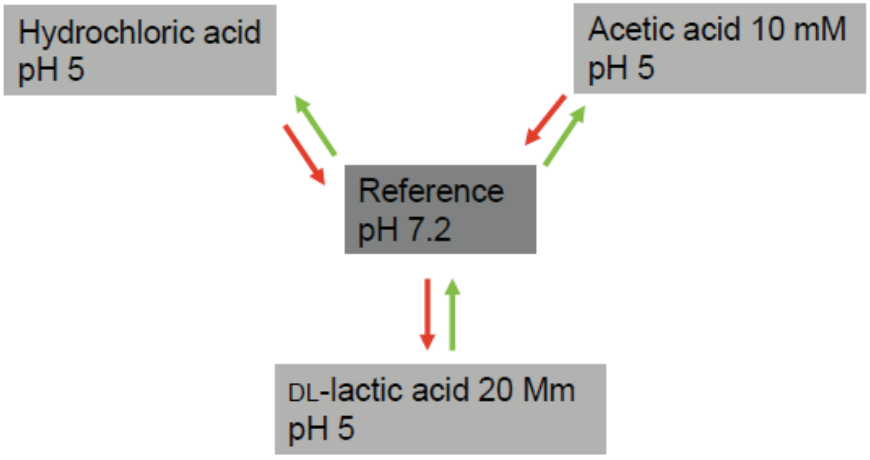

Hydrochloric acid pH 5
Acetic acid 10 mM pH 5
Reference pH 7.2
DL-lactic acid 20 Mm pH 5

Fig. S1. (A) Schematic presentation of the experimental design. The *L. monocytogenes* L502 was streaked on a BHI agar plate and incubated at 25°C for 18-20 h. An overnight culture was prepared from the agar plate in 10 ml BHI broth (pH 7.2) and incubated for 18-20 h. The stationary-phase culture was diluted 1:100 (vol/vol) in four sterilized baffled Erlenmeyer flasks (500 ml) containing 99 ml of fresh BHI broth (pH 7.2). Exponentially growing cultures (OD_{600} of 0.4) were centrifuged at 5000g for 5 min at 25°C and the cells were resuspended in 100 ml BHI broth containing: BHI pH 7.2 reference, (flask no 1), HCl pH 5 (flask no 2), 10 mM acetic acid pH 5 (AA) (flask no 3), and 20 mM lactic acid pH 5 (LA) (flask no 4). Harvesting for RNA isolation was performed when growth reached OD_{600} ~1.2. **(B)** The microarray hybridization scheme. The double arrows indicate dye swapping hybridizations. The microarray was repeated twice on different days, each experiment consisting of dye-swap and at least one technical replicate (a minimum of four hybridizations per a given acid stress condition).

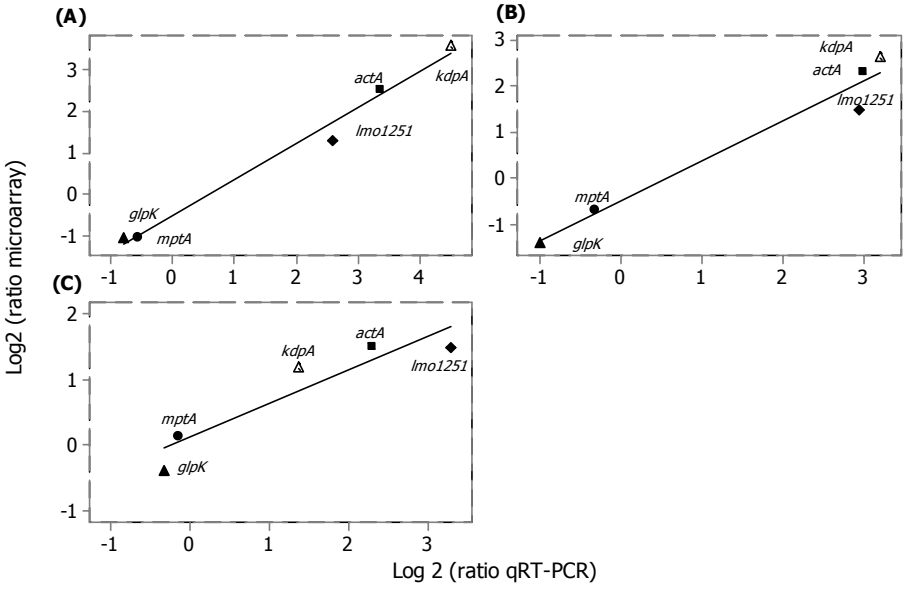


Fig. S2. Validation of microarray data (y-axis) by qRT-PCR analysis (x-axis). The transcriptional studies were performed during growth in brain heart infusion broth acidified to pH 5 with hydrochloric acid (HCl), 10 mM acetic acid (AA) or 20 mM lactic acid (LA). **(A)** LA **(B)** AA and **(C)** HCl stressed culture and the log2 ratio values are relative to the reference culture (initial pH 7.2).

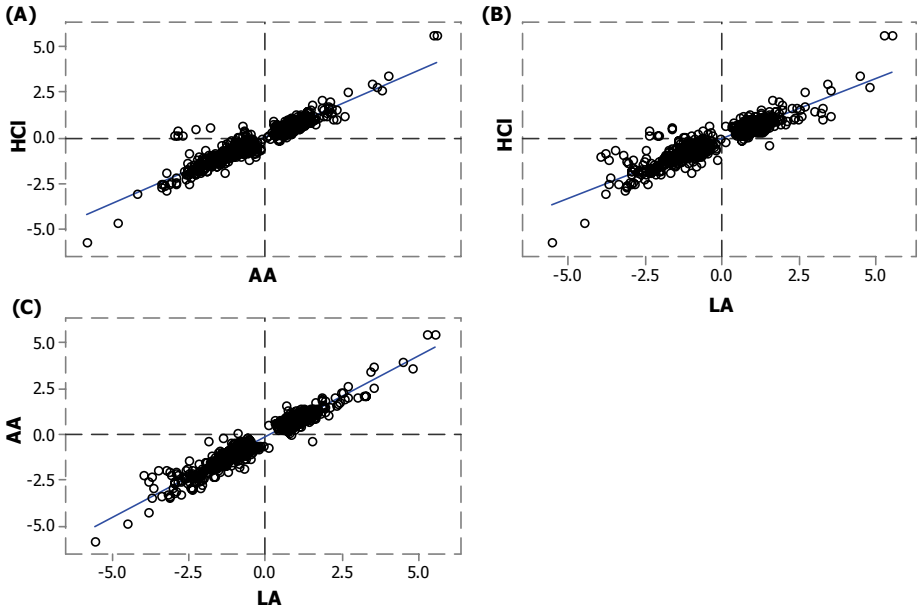


Fig. S3. Scatter plot on microarray data set using genes differentially expressed in set any of the acid growth conditions **(A)** hydrochloric acid (HCl) versus acetic acid (AA); **(B)** HCl versus lactic acid and (LA) **(C)** AA versus LA. The transcriptional studies were performed during growth in brain heart infusion broth acidified to pH 5 with HCl, 10 mM acetic acid or 20 mM lactic acid and the log2 ratio values are relative to the reference culture (pH 7.2).