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Microarray-Analyse der pH-Stressantwort von *Listeria monocytogenes* und *Corynebacterium glutamicum*

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Abbreviations

ATCC	American Type Culture Collection
ATP	adenosine triphosphate
ATR	Acid Tolerance Response
bp	base pairs
BCECF	2`, 7`-bis-(2-carboxyethyl)-5(and-6)-carboxyfluorescein
BHI	brain heart infusion broth
BSA	bovine serum albumin
°C	degree Celsius
cDNA	complementary DNA
cfu	colony forming unit
Clp	caseinolytic protease
Cy	cyanine dye
DEPC	diethyl cyanophosphonate
DMSO	dimethylsulfoxid
DNA	deoxyribonucleic acid
dNTP	deoxynucleotid-5'-triphosphate
EDTA	ethylendiaminetetraacetic acid
e. g.	for example
et al.	and others
GAD	glutamate decarboxylase
h	hour
l	Liter
LB agar	Luria-Bertrani agar
M	Molar
mg	milligram
ml	milliliter
mM	millimolar
MOPS	4-Morpholinepropanesulfonic acid
mRNA	messenger RNA
µ	micron
µl	microliter
µM	micromolar
nM	nanomolar
ORF	open reading frames
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pH	negative decadic logarithm of the molar concentration of hydrogen ions
PTS	phosphotransferase system
RNA	ribonucleic acid
ROS	reactive oxygen species
RT	reverse transcription
RT-PCR	reverse transcription polymerase chain reaction
rpm	rounds per minute
SDS	sodium dodecyl sulfate
TAE	tris-acetate-EDTA
TIFF	tagged image file format
TSA	tryptic soy agar

TSB	tryptic soy broth
UV	ultra violet
v/v	volume per volume
wt	wild type
w/v	weight per volume
www	world wild web

Abstract

Bacteria respond to acidic environments by producing a large set of specific and general stress proteins. In this study, the acid tolerance response of two neutrophile bacteria, *Corynebacterium glutamicum* and *Listeria monocytogenes,* were analyzed using DNA-microarray technology. To verify the microarray results the regulation of some pH stress response genes were confirmed by real time quantitative RT-PCR.

In *C. glutamicum* we identified 116 up- and 90 down-regulated genes in response to acid adaptation. Only a minority of these genes has been described to play a role in acid stress response of other bacteria. Gene disruption analysis of up-regulated genes, showed no significant phenotypic changes except for *sigB*, encoding a general stress response regulator. These results suggest a complex and redundant response of *C. glutamicum* to low pH. The acid sensitive phenotype of the *sigB* disruption mutant revealed, that in *C. glutamicum* the acid adaptation system is, at least partially, SigB-dependent.

In *L. monocytogenes,* a genome-wide transcription profiling was derived in response to hydrochloric acid, which may mimic the environmental signal encountered during transit of the acidic fluid in the stomach of a host. The results of the transcriptomic analysis of the acid shock and acid adaptation responses were compared to the effect of a temperature shift (from 25 to 37°C). The analysis of the data revealed a strong correlation between the acid stress response and virulence gene expression.

The DNA microarray measurements allowed the comparison of the acid adaptation response of these two Gram-positive bacteria with each other. There is evidence suggesting, that SigB is essential in both *C. glutamicum* and *L. monocytogenes* for induction of acid stress response genes.

In summary, these results expand our understanding by showing new described genes contributing and reacting to the acidic stress response.

Zusammenfassung

Bakterien reagieren auf saure Umweltbedingungen mit der Produktion einer großen Anzahl allgemeiner und spezifischer Stressproteine. Im Rahmen dieser Arbeit wurde in den beiden neutrophilen Bakterien *Corynebacterium glutamicum* und *Listeria monocytogenes* unter Einsatz der *DNA-microarray* Technologie die zur Säuretoleranz führende Antwort untersucht. Die durch den *microarray* erhaltenen Ergebnisse wurden verifiziert in dem die Regulation einiger durch pH-Stress induzierten Gene mit Hilfe der quantitativen *real time RT-PCR* überprüft wurde.

In *C. glutamicum* konnten 116 induzierte sowie 90 reprimierte Gene nach pH-Erniedrigung identifiziert werden. Nur wenige dieser Gene wurden bislang in anderen Bakterien im Zusammenhang mit der Säurestress-Antwort beschrieben. Einige dieser induzierten Gene, wurden durch Insertionsmutagenese inaktiviert. Jedoch konnte keine phänotypischen Veränderungen gefunden werden, mit Ausnahme bei dem Gens *sigB*, welches für ein Regulatorprotein der allgemeinen Stress-Antwort kodiert. Diese Ergebnisse deuten auf eine komplexe und redundante Säure Schock-Antwort von *C. glutamicum* hin. Der säure-sensitive Phänotyp der *sigB*-Insertionsmutante zeigt, dass das Säure-Adaptationssystem in *C. glutamicum* - zumindest teilweise - von SigB abhängig ist.

In *L. monocytogenes* wurde ein genomweites Transkriptionsprofil nach einem durch Salzsäure induzierten Stress erstellt. Salzsäure imitiert das Umweltsignal, welchem Bakterien während der Passage durch das saure Milieu des Magens eines Wirtsorganismus begegnen. Die Ergebnisse der Transkriptom-Analyse des Säureschocks als auch der Säureadaptation wurden mit dem Effekt, der durch eine bloße Temperaturveränderung (von 25°C auf 37°C) hervorgerufen wird, verglichen. Die Untersuchungen deckten eine enge Korrelation zwischen der Säurestress-Antwort und der Induktion von Virulenzgenen auf.

Die *DNA-microarray* Messungen ermöglichten es, einen Vergleich zwischen den beiden Gram-positiven Bakterien anzustellen. Es gibt Hinweise darauf, dass SigB sowohl in *C. glutamicum* als auch in *L. monocytogenes* für die Induktion von Säurestress-Genen essentiell ist.

Zusammengefasst erweitern die Ergebnisse dieser Arbeit unser Verständnis darüber, welche Gene, mit z. T. unbekannter Funktion, in die Säurestress-Antwort involviert sind und darauf reagieren.

1 Introduction

1.1 Adaptation to acidic environment

Bacteria show amazing ability to survive and multiply under extreme environmental conditions. Consequently, these organisms are equipped with several redundant and complex systems for coping with a variety of stress situation. Stress response tolerance to high (122) or low temperature (116), high salt concentration (13) or oxidative stress (17) are widely known and well studied, lesser investigated is the bacterial adaptation to acidic environments. Most of our limited knowledge concerning acid adaptation is derived from studies involving Gram-negative pathogens, therefore our study focused on Gram-positive acid stress response in *Corynebacterium glutamicum* and *Listeria monocytogenes*.

Gram-positive bacteria include ubiquitous gastric commensals and potentially beneficial probiotic bacteria. They play a considerable role in many food processing and fermantation technologies. On the other hand Gram-positive pathogens can cause severe gastrointestinal diseases and other species are etiologic agents of the dental caries disease (90). All these bacteria are challenged in survival due to constantly changing environmental pH. For example the lactic acid producing bacteria involved in the manufacturing of dairy, vegetable and meat products convert glucose to lactate leading to a gradual reduction in pH of the matrix (46). Gastrointestinal pathogens, as well as probiotic bacteria, must survive and overcome physiological barriers in the host organism, the highly acidic gastric juice in the stomach, and the presence of organic acids found in the small intestine (41). After phagocytosis of pathogenic bacteria by macrophages the phagosome begin acidify rapidly. Despite of that, mycobacteria can survive and multiply in this hostile environment (42).

Acidic cytoplasmic pH negatively affects metabolic flow and damage macromolecules. Many bacteria can adapt to low pH by a phenomena, called induced acid tolerance (4, 16, 67). The bacterial response encompasses also the process of the pH homeostasis (6, 43, 91), whereby a cell maintains a relatively constant intracellular pH (pH_i) over a broad range of outer (pH_o) values (121). The regulation of the cytoplasmic pH implies a combination of active and passive pH homeostasis systems. These systems result in the induction of acid stress response transcriptional regulators, export of protons, changes in the fatty acid composition of the cell envelope, synthesis of shock and repair proteins, chaperons, and production of basic compounds.

1.2 *Corynebacterium glutamicum*

C. glutamicum is an aerobic Gram-positive soil bacterium with high G+C content (30). It has a significant industrial importance in the production of amino acids. In particular for L-glutamate and L-lysine are used as nutritious additives in food and feed (1). It belongs to the actinomycete taxon, which also includes mycobacteria and nocardiae. All these bacteria share the property of having mycolic acids (α-alkyl-β-hydroxyl fatty acids) in their cell wall (87). Due to the importance of *C. glutamicum* for amino acid production, most studies on this organism focused on amino acid biosynthesis. However, in recent years, driven by the establishment of the *C. glutamicum* genome sequence, novel possibilities are provided to use *C. glutamicum* as a non-pathogenic model organism to study common features in other high GC-content Gram-positive pathogens, such as pathogenic corynebacteria and mycobacteria (14).

1.3 *Listeria monocytogenes*

The intracellular pathogen *L. monocytogenes* is a Gram-positive of low G+C content, micro-aerophilic, non-sporeforming motile rod. The degree of motility is temperature dependent, where the production of flagellin at temperatures higher than 37°C is considerably reduced, and thereby reducing motility. The natural habitat of this bacteria is the decomposing plant material, but it is well equipped to survive extreme environmental conditions. *Listeria* tolerates high concentrations of salts and a relatively low pH. The pH for optimum growth ranges between pH 5.5 and 9.0. Optimum growth temperature is 35-37°C, although they are able to multiply at refrigeration temperatures (131). *L. monocytogenes* is potentially pathogenic; it is the causative agent of the foodborne listeriosis causing serious localized as well as systemic infections in humans.

1.4 Gram-positive acid adaptation response

1.4.1 Active transport of H^+, the proton pumps

The active export of H^+ ions is one if the main mechanisms to control the cytoplasmic pH. A bacterial F_1F_0-ATPase has a fundamental role in this maintenance of the pH homeostasis.

In bacteria having a respiratory chain the primary role the F_1F_0-ATPase multisubunit enzyme complex is to synthesize ATP from the proton gradient build by the respiratory chain. In bacteria without a respiratory chain, this enzyme complex is responsible for the

maintenance of the pH homeostasis via export of protons through ATP hydrolysis (89). For example *Streptococcus pneumoniae* does not contain a respiratory chain; thereby the sole function of this enzyme complex is the establishment of an ambient cytoplasmic pH. The ATPase activity in *Enterococcus hirae* is regulated by the acid dependent assembling of the subunits responding to the acidic cytoplasmic pH (3). In *Streptococcus mutans* the ATPase operon is up-regulated in response to the acidification of the medium. The importance of the ATPase in the acid tolerance was shown by an acid sensitive mutant of a *Streptococcus* strain with a defect in this enzyme. Cotter *et al.* (27) Reported that *Listeria* cells were sensitive to acid stress after treatment with ATPase inhibitor.

Other cation transport ATPases, such as K^+-ATPase, can also participate to the maintenance of the cytoplasmic pH, through the K^+-H^+ antiport function. This system couples H^+ export to the import of K^+(55). For example, in *Lactococcus lactis* a lower cytoplasmic pH was measured in the absence of K^+ compared to the growth in K^+ rich medium when the cells were grown at low pH.

1.4.2 Amino acid decarboxylases

Decarboxylase systems lower the H+ ion concentration and regulate the cytoplasmic pH through proton consuming decarboxylation reaction. The arginine, glutamate and lysine decarboxylases use intracellular substrates consuming a proton in each reaction. The product (cadaverine, agmatine, or γ-aminobutyrate) is then exchanged for amino acid from the medium via an antiporter. The net effect of the decarboxylation reaction is on one hand the decrease of the intracellular proton concentration, and on the other hand alkalization of the environment through the exported γ-aminobutyrate, which is less acidic than glutamate (28). In a small subset of bacteria this glutamate decarboxylase (GAD) acid resistance has been found to be the most important system for the survival of the acidic challenge, such as *Escherichia coli, Listeria monocytogenes, Shigella flexneri, Clostridium perfringens, Bacteroides* ssp*., Fusobacterium* ssp. and *Eubacterium* ssp. who transit the acidic barrier of the stomach for invading or colonizing the human gut. Summarizing, two proteins are involved in the GAD system; a cytoplasmic glutamate decarboxylase and the cell membrane protein glutamate- γ-aminobutyrate antiporter. Both enzymes show a low optimal pH, suggesting their function in acidic habitat.

1.4.3 Changes in the outher membrane composition

Alterations in the cell membrane result in major reduction in permeability of intact cells to protons, providing an additional protection against acidic pH (107). The physiologically acid adapted cells of *Streptococcus mutans* were found to be enriched in mono-unsaturated and long fatty acid chains compared with unadapted cells grown at neutral pH. *Streptococcus* cells treated with the fatty acid biosynthesis inhibitor cerulenin were unable to alter their membrane fatty acid profiles and therefore unable to survive severe acidification (45). In *L. monocytogenes* production of C14:0 and C16:0 straight-chain fatty acids significantly increases in cells adapting to acid, whereas C18:0 levels decrease. Proton permeability assays revealed that the adapted cell membranes were less permeable to protons.

1.4.4 Repair and protection systems for DNA and protein

DNA repair systems and chaperon molecules have been implicated in the acid resistance of bacteria to low pH (56). Intracellular acidification increases the depurination of the DNA (83). Depurination manifests in chain breaks and cross links, causing a local loss of genetic information. In bacteria, under acid stressed conditions, several DNA repair mechanism are potentially involved in the acid induced DNA repair, including recombinational repair (RecA), mismatch repair, base excision repair and the nucleotide excision repair (NER) pathway, which has the capacity of locating and excising all types of DNA lesions. For example *uvrABC* in *Bacillus subtilis*, belong to the NER pathways. These enzymes work in a concerted manner to excise the lesion within a fragment (114).

Production of molecular chaperons, such as GroEL and DnaK, is a central feature of bacterial stress responses. Chaperons are involved in various stresses, promoting protein folding and renaturation, refolding denatured proteins, and eliminating damaged proteins. In *S. mutans* induced expression of the DnaK operon as well as GroESL was demonstrated in response to acid shock (78). At low pH induction of heat shock proteins was observed in *L. lactis* including members of the CtsR regulon (like ClpE and ClpP) and the members of HrcA regulated chaperones (like GroEL, GroES, DnaK and GrpE). Studies have shown that both CtsR and HrcA respond initially to the presence of misfolded proteins, which is due to intracellular pH decrease (46).

1.4.5 Regulation of the gram-positive acid tolerance response

Alternative and ECF (extra cytoplasmic function) sigma factors, plus two component systems are involved in sensing stress signals and coordinating the bacterial gene

expression. Sigma B is a "general" stress sigma factor, its role in acid tolerance response has been established in *B. subtilis, Brevibacterium flavum, L. monocytogenes* (41) and *Staphylococcus aureus*. In *Bacillus subtilis* SigB regulates a large general stress response regulon, contributing to the transcription of more than 100 genes. The regulation of SigB occurs through the function of the Rsb ("regulator of sigma B") proteins. Environmental stress as low pH and others are sensed trough RsbU, whereas energy stress sensed through the RsbP and RsbQ, by a regulatory cascade (132).

In may bacteria the link between the alterations in environmental conditions and the adaptive response has been shown to result from the sensing and regulatory activities of two component signal transduction systems. In *L. monocytogenes* the genes *lisRK*, encoding for a two-component regulatory system, has an important role in the in the acid stress response. This system is also required for the full virulence of this pathogen (26). The membrane associated histidine kinase *lisK* observes changes in environmental parameters, and the cytoplasmic response regulator *lisR* regulates subsequently the gene expression.

1.4.6 Production of alcalic compounds

Urease and the arginine deiminase (ADI) pathway are the most common ways in which bacteria alkalify their environment. Neutralization of acids results from the production of NH_3, which combines with protons in cytoplasm to produce NH_4^+, thereby raising the internal pH.

Urease catalyses the hydrolysis of urea to two molecules of ammonia and one molecule of carbon dioxide. This system has been described best in *Helicobacter pylori.* However, some gram-positive bacteria, such as the oral bacteria *Streptococcus salivarius* and *Actinomycetes naeslundii*, also produce urease, using urea contained in the saliva. This raises the pH of the biofilm in the oral cavity and improves bacterial survival.

Another mechanism for ammonia production and therefore suggested with a function in pH homeostasis is the arginine deiminase pathway. The ADI pathway has been identified in a broad range of bacteria, including *Bacillus* spp*., L. lactis, Streptococcus* spp*., and Pseudomonas* spp*.,* catabolizing arginine to ornithine, ammonia and carbon dioxide. For *Streptococcus sanguis* enhanced viability might be attributed to the pH rise due to ammonia production by the ADI pathway. However, as demonstrated by Champomier *et al.* (20)*,* arginine degradation by *Lactobacillus sakei* is associated to general environmental adaptation and can not be related to pH protection only.

1.5 The specific acid stress responses of *Corynebacterium glutamicum* and *Listeria monocytogenes*

1.5.1 Acid stress response of *Corynebacterium glutamicum*

C. glutamicum is one of the most important bacteria in industrial fermentation processes. For these bioprocesses complete understanding and description of stress responses is fundamental. For example increase in osmotic pressure, as the direct result of product accumulation, is able to disrupt bacterial growth and production of the desirable compound ceases (130). Therefore, the osmotic stress response has been described in detail for this bacteria. In this setting, the media pH is strictly controlled, which could explain the fact, that the acid stress response of *C. glutamicum* has not been investigated yet.

1.5.2 Acid stress response of *Listeria monocytogenes*

L. monocytogenes, a foodborne pathogen has not only to withstand the pH changes in the environment, but also in fermented low-pH foods, and at a number of stages in its infectious cycle. *L. monocytogenes* responds to environmental acidity by regulating the synthesis of a number of proteins. Following exposure to mild acid, *Listeria* has been shown to exhibit a significant acid tolerance response (ATR) system, which is capable of protecting cells from subsequent lethal acid stress (49). The ability of these bacteria to survive acidic challenge was found to be growth phase dependent, cells in the stationary phase are more resistant than cells in the logarithmic phase (101). Proteom analysis using 2-D gel electrophoresis during acid adaptation revealed changes in the levels of 53 proteins (103). Significant differences were also observed between the non-adapted *L. monocytogenes* LO28 and a constitutively acid tolerant mutant. There were also differences in protein expression observed between the effect of weak organic and strong inorganic acids. *Listeria* has to survive the strong acidic gastric fluid as a natural barrier during transit of the stomach. The glutamate decarboxylase acid resistance is the most important component of the listerial acid tolerance during both, logarithmic and stationary phases of growth (28). In *L. monocytogenes* EGD-e three decarboxylase encoding homologs, *gadA, gadB* and *gadD* and two antiporter homologs, *gadC* and *gadE* have been characterized (25). *ΔgadA, ΔgadB*, and *ΔgadC* mutants were used to verify the importance of the GAD system in the survival of *L. monocytogenes* in the gastric fluid and in the low-pH food: The elimination of the GAD system resulted in a higher sensitivity to low pH and more rapid killing. Furthermore acid resistance is subject to strain variation. Cotter *et al.*

(28) demonstrated that resistance variation correlates with the levels of the GAD activity of these strains.

The role of SigB seems to be well defined in the stress responses of gram-positive bacteria including *B. subtilis* and *S. aureus*. The SigB operon comprises *rsbRSTUVWX* and *sigB*. The Rsb proteins (rsb – regulator of sigma-B) are all involved in the posttranslational regulation of activity: In *Listeria* the effect of SigB was demonstrated using a *ΔsigB* mutant strain (32). Ferreira *et al.* (40) has shown that σ^B contributes to cellular survival through two mechanisms: a general acid tolerance to which σ^B contributes throughout all growth phases and the pH-inducible ATR mechanism as mentioned above that is at lest partially SigB dependent in exponential-phase cells.

As mentioned above, the success of *Listeria* as a pathogen requires the ability to sense and respond to environmental changes. Cotter *et al.* (26) described a two-component signal transduction system LisRK and demonstrated that it functions in acid stress response and plays a role in vivo survival.

1.6 Aims of this work

While the Gram-negative acid tolerance systems are well characterized in details, the Gram-positive acid stress response is lesser investigated. The purpose of this project is to investigate by physiological and molecular biological approaches the stress response of *Corynebacterium glutamicum*. The transcription analysis of the corynebacterial acid stress response revealed that the pH homeostasis system of this bacteria appears highly complex and redundant. Only a minor overlap was found with other described pH homeostasis systems. These findings led us to the aim to compare the whole genome transcriptomic changes of *C. glutamicum* with another gram-positive bacteria due to the acid challenge. For this investigation we chose the relevant food borne pathogen *Listeria monocytogenes EGDe*.

2 Materials and Methods

2.1 Bacterial strains and culture conditions

Bacterial strains and plasmids used in this study are listed in Table 2-1 *C. glutamicum* and derivatives were grown at 30°C in tryptic soy broth (TSB, Becton Dickinson, Heidelberg, Germany). When appropriate, 50 µg/ml kanamycin (Becton Dickinson, Heidelberg, Germany) was added. *L. monocytogenes* was grown at temperatures of 25°C, 30°C or at 37°C in brain heart infusion medium (BHI; Becton Dickinson, Heidelberg, Germany). *E. coli* strains were grown in LB medium at 37°C with shaking. Medium used for the acid adaptation experiments of *C. glutamicum* was acidified with the addition of 90% lactic acid (Merck Eurolab, Ismaning, Germany), for *L. monocytogenes* HCl was used (see below).

Tab. 2-1: Bacterial strains and plasmids used in this study

Strain/plasmid	Relevant characteristics[a]	Source/reference
Strains		
Corynebacterium glutamicum	Type strain	ATCC 13032
Listeria monocytogenes EGDe	Serotype 1/2a	Goebel, W.
Escherichia coli TOP 10	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80 *lacZ*Δ.M15 *ΔlacX*74 *recA*1 *deoR araD*139 Δ(*ara-leu*)7697 *galU galK rpsL* (Str^R) *endA*1 *nupG*	Invitrogen
Escherichia coli DH5α	F^- Φ80*lacZ*Δ(*lacZYA-arg*F)*U*169 *deoR recA*1 *endA1 hsdR17*(r_k^-, m_k^+) *phoA supE*44 *thi*-1 *gyrA*96 *relA*1 *tonA*	Invitrogen
Plasmids		
pCR2.1 TOPO	Ap^r, Km^r, *E. coli* cloning vector for PCR products	Invitrogen
pWLQ2	Ap^r (E), Km^r (E, C), $lacI^q$, P*tac*	Liebl *et al.* (81)
pK19mobsacB	Km^r (E, C), mobilisable *E. coli* vector used for the construction of *C. glutamicum* deletion mutants	Schäfer *et al.* (115)

a: Abbreviations: Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Cm^r, chloramphenicol resistance; letters in parentheses indicate if the resistance functions in *E. coli* (E) or *C. glutamicum* (C), $lacI^q$, *lac* repressor gene; P*tac*, *tac* promoter

2.2 Fermentation of *Corynebacterium glutamicum*

Continuous cultures of *C. glutamicum* ATCC 13032 were grown in a 2L turbidostat fermentor (Biostat B; B. Braun Biotech, Melsungen) at 30°C. The oxygen concentration was kept at 50% of saturation by continuous aeration (2L/min) with a mixture of air and nitrogen. The fermentor was inoculated with 2% overnight culture. Once the optical

density at 600 nm reached 0.5 it was kept constant by continuous addition of TSB. During the fermentation the pH was kept constant at pH 7.5 or pH 5.7 by addition of 1 M NaOH or 1 M lactic acid, if necessary. Samples (50 ml) were drawn after five generations of growth at pH 7.5 or pH 5.7. The generation times were calculated from the consumption of medium. Cells were harvested by centrifugation at 5000 rpm for 5 minutes at 4°C. Pellets were frozen in liquid nitrogen and stored at -70°C until RNA extraction.

2.3 Internal pH measurement

Internal pH was determined with the fluorescent pH indicator 2', 7'-bis- (2-carboxyethyl)-5[and-6]-carboxyfluorescein (BCECF) as described by Negulescu *et al.* (98) with some modifications. Six ml of *C. glutamicum* suspension grown in TSB at pH 7.5 were harvested at exponentional growth phase (OD_{600} = 0.5) by centrifugation (5000 g, 22°C, 3min). The cells were washed two times with 50 mM potassium phosphate buffer at pH 7.5 and resuspended in 3 ml of the same buffer. Thirty µl of 0,1mM lipophylic acethoxymethyl ester of BCECF (BCECF-AM, Sigma-Aldrich, Taufkirchen, Germany) dissolved in DMSO was added to the cell suspension (final concentration 1µM). The culture was incubated for 30 min at 30°C, and 200 rpm in the dark. The cells were harvested by centrifugation, washed two times with phosphate buffer and resuspend in phosphate buffer energized by 50 mM glucose at appropriate pH value.

A fluorescence excitation of intracellular BCECF was recorded using VICTOR™ multilabel counter (EG&G Wallac, Turku, Finland). The dual-excitations wavelengths for BCECF are 450 nm (pH independent isosbestic point) and 490nm. The emission wavelength is 535 nm, measured in time intervals of 1 s. The normalization with the fluorescent intensity ratio at the isosbestic point eliminated the fluorescent measurement artifacts including photobleaching, leakage and non-uniform loading of the pH-indicator. *In situ* calibration was performed by using a mixture of ionophores nigericin, gramicidin, and valinomycin to the final concentration of 10, 20, and 10 µM, respectively, to equilibrate the intracellular pH with the controlled extracellular medium between pH 3.5 and pH 7.5. Finally the cytoplasmic pH was estimated using a calibration curve obtained from the normalized emission values correlated to the outer pH values.

2.4 Standard DNA manipulations

2.4.1 DNA isolation

C. glutamicum was grown overnight in 10 ml TSB, harvested, and incubated in 3 ml solution B1, containing 25 mM Tris-HCL, 10 mM EDTA, 50 mM glucose, pH 8.0 and 20 mg/ml lysosyme, at 37°C for 2 h. The cells were lysed with 200µl 20% (w/v) SDS. After addition of 5 mg pronase E from *Steptococcus griseus* (Serva, Heidelberg, Germany) the mixture was incubated at 37°C for 1 h. DNA was extracted with 3 ml phenol:chloroform (1:1) and centrifugation for 20 min at 10,000g. The DNA in the supernatant was precipitated with ethanol and resuspended (127).

Chromosomal DNA from *E. coli* and *L. monocytogenes* was isolated according to the instructions of the NucleoSpin Food DNA isolation kit (Macherey-Nagel, Düren, Germany).

2.4.2 Isolation of plasmid DNA

Plasmid DNA was isolated from plasmid-carrying *E. coli* cultures, after overnight incubation in 5 ml antibiotic containing LB culture media according to the instructions of the NucleoBond AX 20 Plasmid Mini-Prep Kit (Macherey-Nagel, Düren, Germany).

2.4.3 DNA digestion with restriction enzymes

DNA restriction was carried out following standard procedures (113) and according to instructions from manufacturers for restriction enzymes (Promega, Mannheim; New England Biolabs, Frankfurt; MBI Fermentas Molecular Biology, St Leon-Rot; Roche, Mannheim, Germany).

2.5 Electrotransformation

Transformation of *E. coli* was carried out by electroporation according to the protocols by Dower *et al.* (35). For *C. glutamicum* the protocol from van der Rest *et al.* (129), was used with a BioRad Gene Pulser (BioRad, Münnchen, Germany) and 2 mm electroporation cuvettes (EquiBio Ltd., Kent, UK).

2.6 Polymerase Chain Reaction (PCR) products

Oligonucleotides used for PCR reactions were obtained from MWG Biotech (Ebersberg, Germany). PCR amplification was performed using standard PCR protocols as described automated thermocycler with 0.2-ml thin-walled PCR tubes (Advanced Biotechnologies, Hamburg, Germany). The annealing temperature was adjusted to the melting temperature

of the primers and the extension time to the size of the expected fragment. PCR products were purified using spin columns with the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Freiburg, Germany) according to the instructions of the manufacturer.

2.7 Blotting and Southern hybridization

2.7.1 Construction of specific DIG-labeled gene probes by random primed DNA labeling

Digoxigenin labeled TOPO cloning vector was used as specific probe. The labeled probe was obtained by random primed labeling reaction. Three µg *Eco*RI digested DNA was denatured at 100°C for 10 min in 15 µl final volume. Following the incubation the samples were chilled on ice for 10 min and the following reaction mix was added to the denaturated DNA: 2 µl hexanucleotide mix, 2 µl dNTP labeling mix and 1µl Klenow enzyme (labeling grade). The labeling mix was incubated for 20 hours at 37°C and heating to 65°C for 10 min stopped the reaction.

2.7.2 Southern-Blotting

Restricted DNA was fractionated on 0.8 % agarose gel and transferred onto positively charged nylon membrane (Hybond-N+, Amersham Pharmacia Biotech, Freiburg, Germany) by vacuumblotting (2016 Vacugene XL, Pharmacia) at 45-50 mbar for 1 h with 20 x SSC. Following the blot, the membrane was washed twice in 2 x SSC and UV-crosslinked at 302 nm (0.3 J/cm2) for 0.5 min (Fluo-Link). Table 2-2 summarizes the reaction steps and buffers used in the blotting reaction.

Tab. 2-2: Blotting Solutions and buffers:

Reaction step	Buffer solution	time
1**. Depurination**	0.25 M HCl MilliQ	15 min 2 x 5 min
2. Denaturation	0.5M NaOH 1.5 M NaCl MilliQ	30 min 2 x 5 min
4. Neutralization	1 M Tris-Base 1.5 M NaCl pH 7.5 MilliQ	30 min 2 x 5 min
5. Washing	2 x SSC	2 x 5 min

Hybridization was carried out overnight at 42°C with the specific DIG-labeled probe using a Micro-4 hybridization oven (Hybaid, Heidelberg, Germany). The hybridized probe was detected by chemiluminescence using AGFA Curix X-Ray film (AGFA-Gevaert, Mortstel, Belgium) which was exposed for different time intervals (5-60 min). The reaction steps and buffers of the Southern hybridization are summarized in the Table 2-3.

Tab. 2-3: Reaction steps, solutions and buffers for hybridization

Reaction step	Solutions / Buffers	Time	Temperature
I. Hybridization	1. Prehybridization buffer 2. Hybridization with Dig-labelled probe	1–2 h overnight	42°C 42°C
II. Washing			
1. Low stringent 2x SSC 0.5% SDS	50 ml 20x SSC 12.5 ml 20% SDS ad 500 ml	2 x 15 min	42°C
2. High stringent 0.1 x SSC 0.5% SDS	5 ml 20x SSC 12.5 ml 20% SDS ad 500 ml	1 x 15 min	42°C
3. Washing buffer 1 x maleic acid buffer	100 ml 5 x maleic acid buffer ad 500 ml	1 x 1 min	RT
4. Blocking reagent (Boehringer Mannheim) 1x PBS 0.5% SDS 0.2% I-Block (Casein)	50 ml PBS 12.5 ml 20% SDS 1g Block powder ad 500 ml	1 x 1 h	RT
III. Immuno reaction 1. Conjugate buffer	10 ml Blocking buffer 2 µl Anti-Digoxigenin-AP Fab fragment (Roche)	1 h	RT
IV. Washing buffer 1. Washing buffer 1 x maleic acid buffer	100 ml 5 x maleic acid buffer ad 500 ml	2 x 15 min	RT
V. Detection 1. Reaction buffer (0.1 M DEA pH 9.5) 2. Substrate buffer	5g DEA (Diethanolamine) 0.5ml 1M MgCl2 ad 500 ml 1 ml reaction buffer DEA 10 µl Tropix CDP-Star (Stratagene)	1 x 2min 7 min	RT RT

20 x SSC:

NaCl	3 M	175,3 g/L
$Na_3(H_2O)_2$ citrate	0.3 M	88.2 g/L

(pH 7.0 adjusted with 1 M HCl)

Maleic acid buffer:

Maleic acid	0.1 M	16 g/L
NaCl	0.15M	8.8 g/L

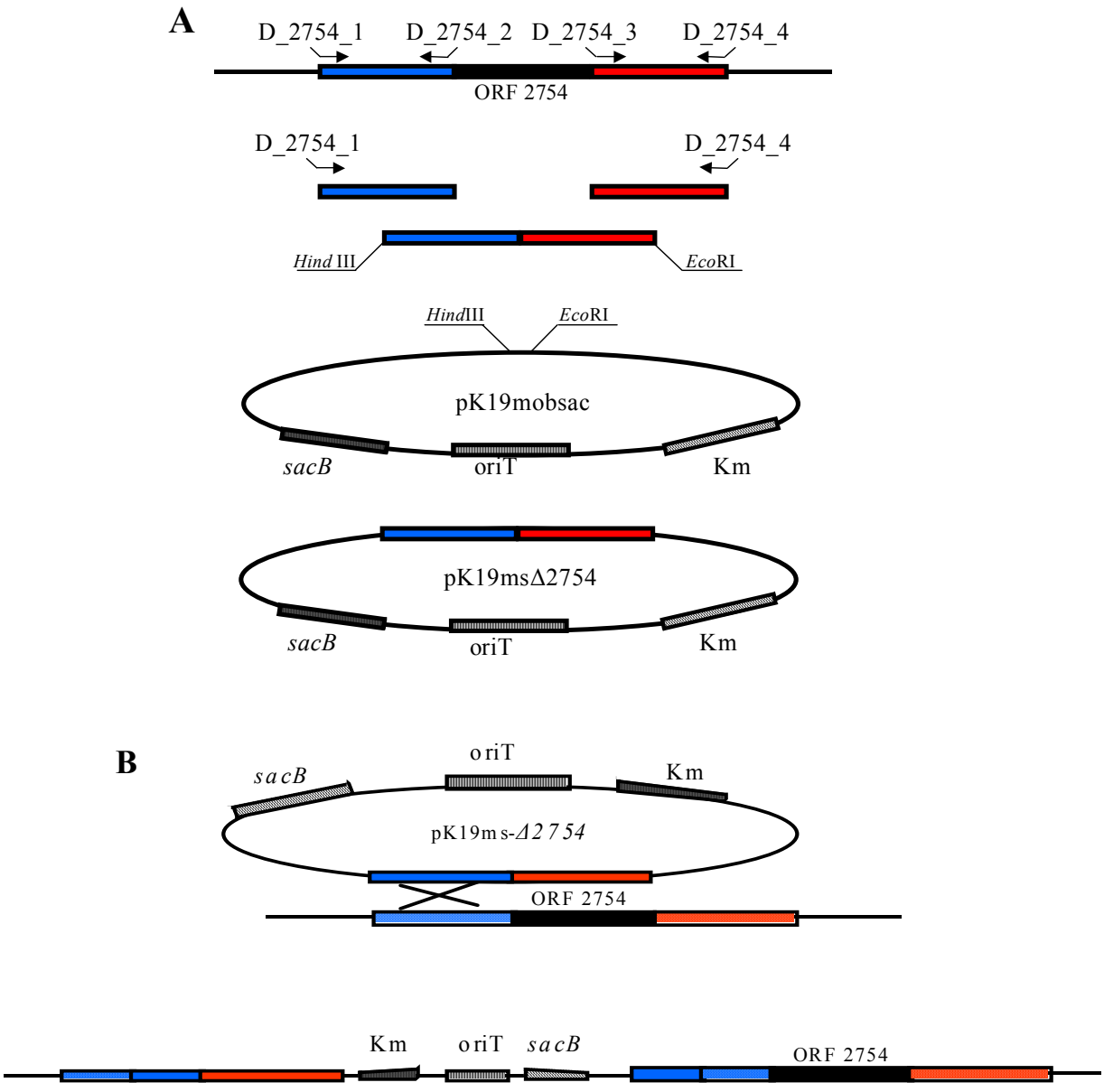
(pH 7.5 adjusted with 1 M HCl)

Standard hybridization solution:

5 x SSC
0.1 % N-laurylsarcosine
0.02 % SDS
50 % Formamide
2 % Blocking Reagent (Roche, Penzberg, Germany)

2.8 Construction of *Corynebacterium glutamicum* mutant strains

2.8.1 Construction of the ORF2754 deletion mutant The in-frame deletion mutant strain of *C. glutamicum* involved crossover PCR and using the suicide vector pK19*mobsac* (Figure 2-1 A, B, C). This plasmid i) can not replicate in *C. glutamicum*, ii) contains a kanamycin resistance gene, and iii) contains *sacB* coding for a levansucrase, which is responsible for the sucrose sensitivity. In the first step two PCR products were generated representing the 5´and 3´flanking region of ORF2757 (see Figure 2-1 A). The primers D_2754_1 and D_2754_4 (Table 2-5) contained *Eco*RI and *Hind*III restriction sites at the 5´end. The primers D_2754_2 and D_2754_3 (Table 2-4) included complementary 21 bp tag sequences at their 5´end. In the second step the resulting PCR products were used as template for crossover PCR with primers 1 and 4. The amplified fusion product was cloned into the digested pK19*mobsac*, resulting in pK19*ms*-ΔORF2754. The plasmid was transferred into *C. glutamicum* by electroporation, and the transformation mixture was plated on 25µg/ml kanamycin containing agar plate to select recombinants (Figure 2-1 B). To select for a second recombination event, one of the kanamycin resistant clones was grown under non-selective conditions without kanamycin and plated onto a 10% sucrose containing agar plates. Sucrose resistant clones should have excised the plasmid by a second crossover event or restored the wild type situation (Figure 2-1 C-1, -2). The deletion of the target gene in the sucrose-resistant and kanamycin-sensitive clones was confirmed by PCR.


A
D_2754_1
D_2754_2
D_2754_3
D_2754_4
ORF 2754
D_2754_1
D_2754_4
Hind III
EcoRI
HindIII
EcoRI
pK19mobsac
sacB
oriT
Km
pK19msΔ2754
sacB
oriT
Km
B
oriT
sacB
Km
pK19ms-Δ2754
ORF 2754
Km
oriT
sacB
ORF 2754

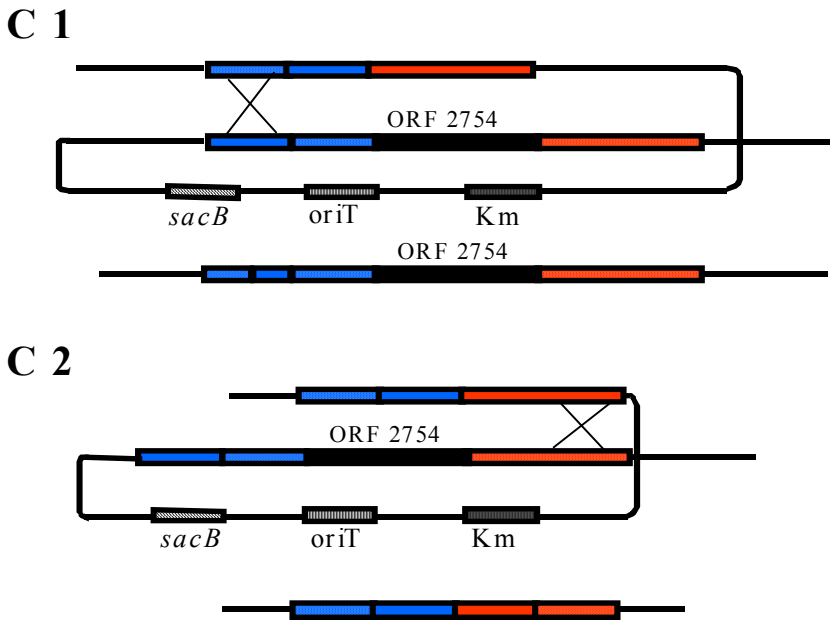


Fig. 2-1 A, B, C: Construction of ORF2754 deletion mutant strain.

2.8.2 Construction of the ORF2754 over expression mutant

The pWLQOE2754 expressions plasmid was constructed, for over expression of ORF2754 respectively in *C. glutamicum* (Figure 2-2.). In the first step the target gene were amplified using a PCR with the PCR primers OE_2754_F and OE_2754_R (Table 2-4) contained *Bam*HI and *Sma*I restriction sites at the 5´-terminus. The resulting product was cloned into pWLQ2 (Table 2-1), resulting in the expression plasmid pWLQOE2754. The expression vector was transferred into *C. glutamicum* by electroporation.

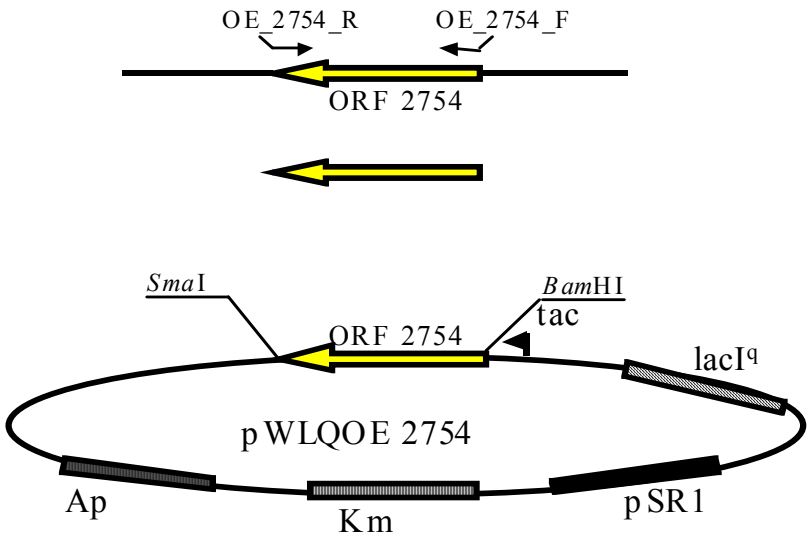


Fig. 2-2: Construction of ORF2754 overexpression mutant strain.

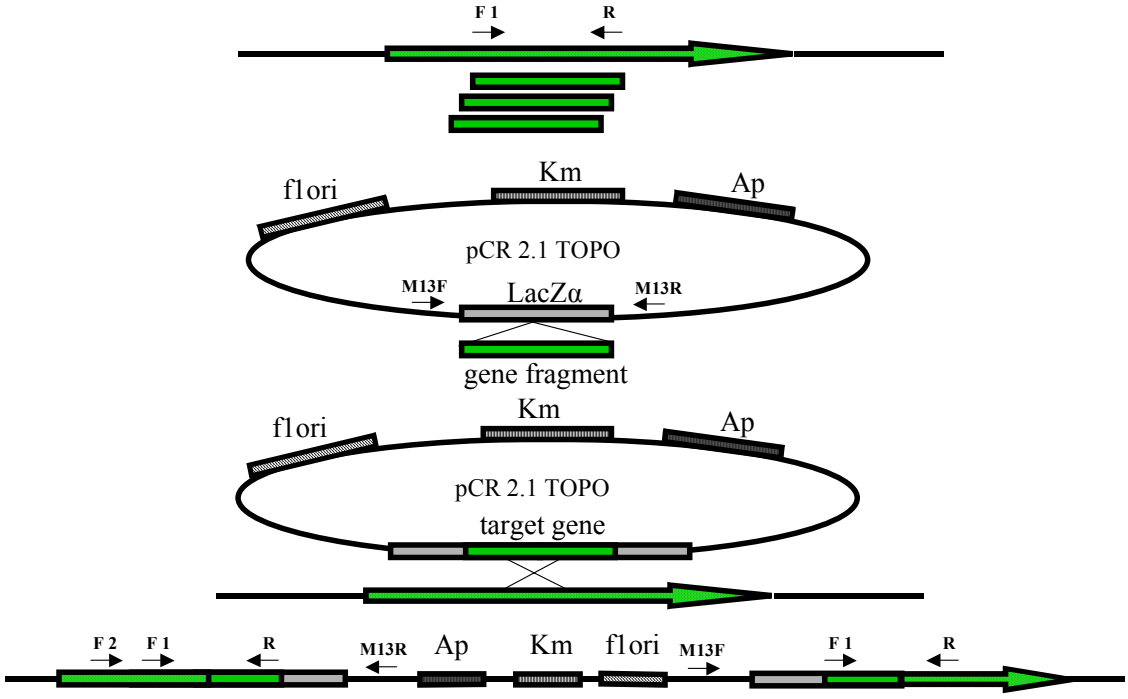


Fig.: 2-3: Construction of gene disruption mutants.

2.8.3 Construction of gene disruption mutants

With specific primers (Table 2-5) the inner region of the target gene was amplified (Figure 2-3). The amplified fragment was cloned into pCR2.1 TOPO (Invitrogen) cloning vector. This plasmid is unable to replicate in *C. glutamicum* and contains a kanamycin resistance gene. The plasmid was transformed into *C. glutamicum* by electroporation. Kanamycin resistant clones should have integrated the plasmid into the chromosome by homologous recombination. Integration of the plasmid was confirmed by PCR amplification using the F2 forward primer (located upstream from the F1 forward primer) and either M13_REV(-29) or M13_UNI(-21) standard primers (see oligonucleotides in Table 2-5). Southern hybridization was performed to verify the position of the plasmid integration using digoxigenin labeled pCR2.1 as specific hybridization probe.

Tab. 2-4: Oligonucleotides used in the construction of the ORF2754 mutant strains

Oligonucleotides	**Sequence**	**T_m (°C)**
OE_2754_F	5′-ATCCCCGGGTCATAGCCGATCACCAACAAGTTGTTG-3′	72.3
OE_2754_R	5′-ATCGGATCCAATGTCGGGGCGAGCTCCTGA-3′	69,3
D_2754_2	5′-TGTTTAAGTGGATCCGATGGGGACACATCGTTGCAGTCAACT-3′	>75
D_2754_1	5′-ATCAAGCTTACGTGAAATGACGAATACCG-3′	68,1
D_2754_4	5′-ATCGAATTCAGCTGGATAAACAATTGATCG-3′	69,2
D_2754_3	5′-CCCATCGGATCCACTTAAACAACTTCTCTGTATGAACCTGACTTTA-3′	>75

Tab. 2-5: List of the ORFs targeted for the gene disruption experiments, and the used oligonucleotides

ORF Nr	Oligonucleotide	Sequence	T_m (°C)*
ORF21	ORF21_F	5′-GCGGCTCGTAAGAAGGCTGT-3′	62.0
	ORF21_R	5′-ATCAGCATGGTTACCGGCAG-3′	60.8
	ORF21_F2	5′-TTTGTTAACATCACGGCTCT-3′	53.3
ORF517	ORF517_F	5′-GCTGCCCATTTTGCTCAGTG-3′	61.5
	ORF517_R	5′-CGTTGTCCGGCGTGACAGTC-3′	64.3
	ORF517_F2	5′-GCTTTGAAGGCGTCAAGCAC-3′	60.8
ORF851	ORF851_F	5′-TCGAGCGCAAACTGAACAAG-3′	59.8
	ORF851_R	5′-TCTCAAACCAGCGTCCAAGC-3′	61.0
	ORF851_F2	5′-CTCAGACACCCGCCAAAATC-3′	60.2
ORF1058	ORF1058_F	5′-AGATCATGTGGACTCTGCGG-3	57.9
	ORF1058_R	5′-CTGTTTTGAGTTTGCTGCTG-3	54.4
	ORF1058_F2	5′-CATTATGAGTAACGCCGTAG-3′	51.4
ORF1152	ORF1152_F	5′-TGAACCGCCGACTAAAGTTG-3′	58.8
	ORF1152_R	5′-TGCTGAGCCATCTTTCTGCT-3′	58,3
	ORF1152_F2	5′-TTTGCTACCACGCTAGTCGC-3′	59.2
ORF1173	ORF1173_F	5′-TCTTCAGCAGCATCCCAAAG-3′	58.6
	ORF1173_R	5′-CGATGAGATCCTTGCCTTCT-3′	56.7
	ORF1173_F2	5′-GTACGTCTCGCGTTCTAGCC-3′	57.7
ORF1293	ORF1293_F	5′-AAAGGAGTTATCGAAATGGA-3	51.8
	ORF1293_R	5′-CGACGTTCGTCTTCGCTCAT-3	63.6
	ORF1293_F2	5′-GCCCGGACCAGTGACAACAA-3′	63.7
ORF1347	ORF1347_F	5′-GAATTCACAGACGCTCACGG-3′	58.7
	ORF1347_R	5′-TTTCTCGGATTACTTCGGGG-3′	59.3
	ORF1347_F2	5′-CTCGCAGCAGCACTACCTCT-3′	58.1
ORF1518	ORF1518_F	5′-GATCCCCGTGGTGTTGCTGT-3′	62.6
	ORF1518_R	5′-GAGGGAGGCTTCCAATTCGC-3′	63.4
	ORF1518_F2	5′-ACCGCCCCACTGAACTCTTG-3′	51.8
ORF1558	ORF1558_F	5′-CCCAGGTTGCCGATGATTCT-3′	62.0
	ORF1558_R	5′-TTCCCCATTCGTCGAGTCCA-3′	63.3
	ORF1558_F2	5′-CTTGTTGTGGTGCCATTCCG-3′	61.9

ORF1567	ORF1567_F	5′-GTGGGACCTTGCTCAGTTCA-3′	58.0
	ORF1567_R	5′-CTGCGATTTCACGAACAACC-3′	58.9
	ORF1567_F2	5′-TCAAGAACGGAACTGACGGA-3′	58.7
ORF1642	ORF1642_F	5′-CCCGGTATGGTGTCCAAAAC-3′	59.9
	ORF1642_R	5′-GCAAGAAGCTCCTGCTGCAG-3′	61.1
	ORF1642_F	5′-ACCCTCGATCAACTCGCGTA-3′	60.5
ORF1703	ORF1703_F	5′-CGCCTCGCGTACCGTCTT-3	56.3
	ORF1703_R	5′-TTGAGCCCAGACTTCCG-3	54.9
	ORF1703_F2	5′-GTCCCGAGATGACGCACC-3′	58.7
ORF194	ORF1941_F	5′-TGGGATACATCGTCAACATC-3′	53.3
	ORF1941_R	5′-CTGGAACTGCTCCTGAATTT-3′	54.4
	ORF1941_F2	5′-CACCGTCGCTGGCACACCTT-3′	66.3
ORF2000	ORF2000_F	5′-GGCATCGGCAAAACTGCCTT-3′	64.7
	ORF2000_R	5′-CTTGTTCACCTGCTCCACAA-3′	56,5
	ORF2000_F2	5′-CCAAACCAACGACAATCCCT-3′	59.6
ORF2146	ORF2146_F	5′-TTCATCCTCAGCGGAATCAA-3′	58.8
	ORF2146_R	5′-CCACAATGGGGTGTCTTCAG-3′	58.2
	ORF2146_F2	5′-ATTCCTCGGCACACTGCTCG-3′	63.3
ORF2433	ORF2433_F	5′-GGGTTCATCCAAGAGGGCAA-3′	61.7
	ORF2433_R	5′-GAATTCAACCGGCGCGTAAT-3′	62.5
	ORF2433_F2	5′-GGGTTCATCCAAGAGGGCAA-3′	62.0
ORF2607	ORF2607_F	5′-AGGTTTACGCCTGTTATCCG-3	52,2
	ORF2607_R	5′-CAGAGGCTGACTCCTGGGAT-3	58.7
	ORF2607_F2	5′-GCTCGTAGACATTGCTATTGAGAA-3′	58.5
ORF2703	ORF2703_F	5′-GCATCGGCTTAACCACAATA-3′	56.4
	ORF2703_R	5′-GTCCCCGATTGTCTCGTGGT-3′	61.7
	ORF2703_F2	5′-GTCAGTCGTATTGCAGGCGC-3′	61.5
ORF2779	ORF2779_F	5′-TGTCCGACTCCACTGGCTTG-3	61.5
	ORF2779_R	5′-GGGACTTTACGAAGGGCTGC-3	61.3
	ORF2779_F2	5′-GACGGACTCGGACACTCACA-3′	58.5
ORF2919	ORF2919_F	5′-ACACCTCCAGGATGTCTTCG-3	56.9
	ORF2919_R	5′-TTCGTTGCCGTAGTTTTTGA-3	57.1
	ORF2919_F2	5′-AGAAATTCGTGCACCGTACT-3′	54.9
ORF2930	ORF2930_F	5′-AAAGCTCGATTTACCCAACG-3′	57.4
	ORF2930_R	5′-CTGCGGTTGTGTTCATCATT-3′	56.5
	ORF2930_F2	5′-ATCGTGTATTTTTCCTCGGC-3′	57.0
ORF3458	ORF3458_F	5′-CGGCTTTGCCCTCGAGACCG-3′	67.8
	ORF3458_R	5′-TCTGGGAAGCGACGCCGAAC-3′	69.5
	ORF3458_F2	5′-TTGGCTTTGGGTATCGCAGG-3′	63.3
ORF3467	ORF3467_F	5′-TGGTCGCCCTCACCCTCGCT-3′	69.4
	ORF3467_R	5′-GTTTGAGTGGGTGATGCGAC-3′	58.3
	ORF3467_F2	5′-CCGTCTTCTTCCACAACCTG-3′	57.8
ORF3470	ORF3470_R	5′-CTCCAGTGAGGTACCGAACC-3′	57.3
	ORF3470_F	5′-CACTTTTCCGTGCAGAAGAA-3′	56.7
	ORF3470_F2	5′-CACTTTTCCGTGCAGAAGAA-3′	56.3
ORF3543	ORF3543_F	5′-CCGACTGGTCAGTGCGTGAA-3′	62.4
	ORF3543_R	5′-GCTGCTGTTGCTTCCGTCAT-3′	61.0
	ORF3543_R2	5′-ACCGTAGTCTCGGCTAATGC-3′	51.1

	M13_REV(-29)	5′-CAGGAAACAGCTATGACC-3′	53.7
	M13_UNI(-21)	5′-TGTAAAACGACGGCCAGT-3′	53.7

*Tm: melting point, calculated by the manufacturer (MWG Biotech AG, Ebersberg, Germany)

2.9 Generation of *Corynebacterium glutamicum* DNA microarrays**

**This work was performed by Dr. Volker F. Wendisch (Institute of Biotechnology Research Center Jülich, Jülich, Germany).

DNA microarrays based on PCR products of *C. glutamicum* were generated for use in global gene expression (77, 137). The genes were amplified in 96-well plates with genomic DNA of *C. glutamicum* ATCC 13032 as the template and gene-specific primers purchased from Degussa (Frankfurt, Germany). The identities and quality of the PCR products were checked by gel electrophoresis, and the PCR products were precipitated with isopropanol, resuspended in 3x SSC (1x SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), and transferred to 384-well plates. PCR products were printed onto poly-L-lysine-coated glass slides by using an arraying robot. The DNA microarrays were rehydrated in a 1x SSC atmosphere, UV cross-linked (650 µJ), and blocked in 230 ml of methyl pyrrolidinone containing 15 ml of 1 M boric acid (titrated to pH 8.0 with sodium hydroxide) and 4.4 g of succinic anhydride. The *C. glutamicum* whole-genome DNA microarray contained 3,673 PCR products covering 2,860 of the 2,994 genes (506 genes in duplicate) described for the genome according to the National Center for Biotechnology Information (NCBI) and 284 additional putative coding sequences (23 sequences in duplicate). In general, the PCR products were 500 ± 50 bp long and represented regions of the genes which facilitate specific hybridization. Additionally, 100 spots of *C. glutamicum* genomic DNA were used as normalization controls, and 16 spots of λDNA, 16 spots of *E. coli* DNA, and one spot of the *E. coli aceK* gene were used as negative controls.

2.9.1 Total RNA preparation and cDNA synthesis

Aliquots (50ml) of exponentially growing (OD_{600nm} ~ 0.5) *C. glutamicum* cultures were harvested by centrifugation (5 min; 3,500 x g; at RT). The cells were resuspended in 350 µl of RNeasy RLT buffer (Qiagen, Hilden, Germany) and mechanically disrupted by 30 s of bead beating with 0.5 g of 0.1-mm-diameter zirconium-silica beads (Roth, Karlsruhe, Germany) using a Silamat S5 (Vivadent, Ellwangen, Germany). After centrifugation (2 min; 14,500 x g), the supernatant was processed using the RNeasy system (Qiagen Hilden, Germany) with DNase on-column treatment according to the manufacturer's instructions

for RNA extraction. The quantity and quality of the extracted total RNA were determined by UV spectroscopy (at 260, 280, and 230 nm) and visually by denaturing formaldehyde agarose gel electrophoresis. Twenty to 25 µg of total RNA were used for random hexamer-primed synthesis of fluorescently labeled cDNA by reverse transcription with Superscript II (GibcoBRL-Life Technologies, Gaithersburg, Md.) using the fluorescent nucleotide analogue FluoroLink Cy3-dUTP (green) or Cy5-dUTP (red, Amersham Pharmacia, Little Chalfont, United Kingdom) as described before (104). Labeled cDNA probes were purified and concentrated using Microcon YM-30 filter units (Millipore, Bedford, Massachusetts).

2.9.2 DNA-microarray hybridization and washing

Combined Cy5- and Cy3-labeled cDNA probes containing 1.2 µg/µl of poly(A) (Sigma, Taufkirchen, Germany) as a competitor, 30 mM HEPES, and 0.3% sodium dodecyl sulfate (SDS) in 3x SSC, were hybridized to the arrays for 5 to 16 h at 65°C. After hybridization, the arrays were washed in 1x SSC containing 0.03% SDS for and finally dipped in 0.05x SSC. The microarrays were dried by brief centrifugation (5 min; 45 x g).

2.9.3 Data normalization and gene expression analysis

Immediately after stringent wash of the arrays, fluorescence intensities at 635 and 532 nm were acquired using a GenePix 4000 laser scanner (Axon Inc., Union City, Calif.) and processed as TIFF images. Raw fluorescence data were analyzed quantitatively using GenePix version 3.0 software (Axon Inc.). Data were normalized to the average ratio of *C. glutamicum* genomic DNA. The normalized ratio of the median (GenePix) was taken to reflect the relative RNA abundance for spots whose green or red fluorescence signal was at least threefold above the background fluorescence. When both fluorescence signals were less than threefold above background, the signals were considered too weak to be analyzed quantitatively. For statistical analysis, P values from independent replicate experiments were calculated based on Student's t test using log-transformed gene ratios and genomic DNA ratios, which were normalized to zero. Only genes showing significantly changed RNA levels (P values of <0.05) were considered for further analysis. Analysis of gene expression data was performed by selecting genes showing at least twofold-increased or -decreased average RNA levels.

2.9.4 Validation of expression profile of fermentation *Corynebacterium glutamicum* via real time RT-PCR

Hundred ml TSB pH 7.5 or 5.7 was inoculated with 0.5 ml overnight culture. At an OD_{600} of 0.5 10 ml of culture was centrifuged at 5000 rpm for 5 minutes and cell pellets were immediately frozen in liquid nitrogen. Total RNA was isolated using RNeasy Mini kit (Qiagen, Hilden, Germany) according to manufacturer recommendation with the following modifications. Frozen cells were resuspended in 700µl RTL buffer containing mercaptoethanol and disrupted in Ribolyzer (Hybaid) using Lysing Matrix B beads (Qbiogene, Heidelberg, Germany) for 3 times 45 s at a speed of 6.5 m/s. Cells were cooled on ice between runs. Finally, the tubes were centrifuged for 2 min at full speed and the supernatant was removed into a fresh tube for further RNA extraction. On column DNAse digestion was performed for 15 min at 30°C and the RNA was eluted in 50µl water. Despite optimization of the DNase treatment some DNA contamination remained, which was removed by a ssubsequent DNAse digestion using Ambion's DNA-free™ (Ambion, Huntingdon, UK) according to manufacturer's instruction.

A two step real time RT-PCR was performed. One µg of total RNA was transcribed to cDNA by Superscript™ III (Invitrogen, Karlsruhe, Germany) in a 20 µl reaction using random primers, for 1.5 h at 50°C followed by an enzyme inactivation step for 15 min at 70°C. The cDNA was 10-fold serially diluted and real time PCR was performed. Changes in gene expression were quantified in an iCycler (BioRad, München, Germany). For a 25 µl reaction 12.5 µl ABsolute™ QPCR SYBR® Green Fluorescein mix, (Abgene, Hamburg, Germany) 0.5 µM of each primer (Table 2-6) and 2 µl of cDNA were used. The cycling conditions were 40 cycles of 95°C for 15 s, 55°C 15 s and 72°C for 20 s preceded with a 15 min enzyme activation at 95°C amplifying a fragment of 100-150 bp. For each amplification run the calculated threshold cycle (C_t) of the 16S rRNA was used for normalization. Formation of unspecific products was analyzed using the melting curve function of the iCycler.

Tab. 2-6: List of the oligonucleotides used in the validation of the *C. glutamicum* DNA array experiments

Oligonucleotide	Sequence	T_m (°C)
16S_F	5′-GTAGGGTGCGAGCGTTGTCC-3′	63.5
16S_R	5′-CGCCATTGGTGTTCCTCCTG-3′	61.4
NCgl377F	5′-GAAACTTGCACCTCGTATGC-3′	57.3
NCgl377R	5′-TTCGATACTCGGTTTGAGCT-3′	55.3
ORF857RT_F1	5′-ATCAGCCACCAAGAACAAAC-3′	55.3

ORF857RT_R1	5′-GGTAAATTCGCCTCAGAACG-3′	57.3
1168RT_F1	5′-ACGGTGAATTGTTGATGGAA-3′	53.2
1168RT_R1	5′-CCTGAACGTGGGTTGGAT-3′	56.0
sigE_F	5′-GTCCCGAGATGACGCACCCG-3′	67.9
sigE_R	5′-GGCATGTCTGCCTGTCCAGC-3′	63.5
sigB F	5′-CGCAGGATCTCGCAACGA-3′	63.4
sigB R	5′-GCCGATGCCGTTGAGGTAAA-3′	63.3
ORF2779RT_F1	5′-AGCCCTTCGTAAAGTCCC-3′	56.0
ORF2779RT_R1	5′-GATCCAACACGCCACAAC-3′	56.0
3549RT_F1	5′-ACATCGCCCACCAATACG-3′	56.0
3549RT_R1	5′-TTGAACCATCTCGGCAGTT-3′	54.5

*Tm: melting point, calculated by the manufacturer (MWG Biotech AG, Ebersberg, Germany)

2.10 Characterization of the mutant strains

2.10.1 Determination of the growth characteristic of the mutant strains

Growth curves of mutant strains were obtained using a Bioscreen C (Thermo Labsystems, Engelsbach, Germany). A 5 ml overnight culture was diluted 1:50 in TSB (pH 7.2) and 10 µl were used to inoculate honeycomb microtiter plates containing 240 µl of TSB pH 7.5 and pH 5.7. The plates were incubated with continuous shaking at 30°C and OD_{600} measurements were obtained every 15 minutes for a period of 24 h.

2.10.2 Acid shock assay

Acid shock was performed to determine the role of the disrupted gene on the survival capabilities under acidic conditions. Hundred ml TSB pH 7.5 was inoculated with 1ml of an overnight culture. At an OD_{600} of 0.5 50 ml cells were harvested by centrifugation at 5000 g for 5 minutes. The cells were suspended in 50 ml TSB pH 4 and incubated at 30°C for 30 minutes. Hundred µl samples were removed before and after acid shock, serially diluted in TSB pH 7.5 and plated on TSA plates. The percent survival was calculated by comparing the cell counts obtained following acid shock to those in the original pH 7.5 cell suspension prior to acid shock.

2.11 Iron availability assay

An iron assay was performed to assess the availability of iron at low pH in undefined complex growth media such as TSB and BHI compared to a defined minimal medium like CGXII. Four different growth media were prepared; TSB, TSB+Fe containing 10 mg/L of $FeSO_4$• $7H_2O$ and 0.1mM protocatechuic acid, BHI, and CGXII. CGXII was prepared according to Keilhauer et al. (68) except, protocatechuic acid was added to a final

concentration of 0.1mM. Protocatechuic acid acts as a chelator, facilitating the bioavailability of iron in the growth medium (80). For all of the media pH was adjusted to 7.5 and 5.7. Hundred ml of medium at pH 7.5 or 5.7 was inoculated with 1 ml of overnight culture. At mid-exponential growth phase (OD_{600} of 0.5), cells from 10 ml medium were harvested by centrifugation at 5000xg for 5 minutes. Pellets were immediately frozen in liquid nitrogen. The RNA isolated from the cells was subjected to real time PCR analysis as described above.

2.12 Acid shock and adaptation of *Listeria monocytogenes*

Exponentially phase (OD_{600} 0.5) cells of *L. monocytogenes* EGD-e, grown in BHI media at 25°C or 37°C respectively, were exposed to sublethal acid stress (pH 5.0, BHI adjusted with 5M HCl), after habituation for 120 min fresh BHI broth (at pH 5.2) was inoculated, to perform an acid adaptation phase. Probes were taken after acid shock 0 (control), 15, 30, 60, and 120 minutes and additionally after adaptation in the mid log phase (OD_{600} = 0.5). Five ml of the cell suspension was added to 10 ml RNA protect reagent (Qiagen, Hilden, Germany) and harvested by centrifugation (5min, 5000 x g) the cells were stored at -70°C. The cell density at neutral and acidic condition was photometrically determined.

2.13 Generation of *Listeria monocytogenes* EGDe DNA microarray

2.13.1 Total RNA isolation

Total RNA was isolated as described above for *C. glutamicum*.

2.13.2 Preparation of labeled cDNA

For each comparative hybridization labeled cDNA was synthesized by reverse transcription from experimental RNA (acid shock at pH 5.0 after 15, 30, 60 and 120 min and acid adaptation sample at 25 or 37°C) in the presence of Cy5-dCTP, and from control RNA (acid shock at pH 5.0 0 min, and nonadapted cell samples) with Cy3-dCTP (Amersham Biosciences). For each reverse transcription reaction 40 µg of total RNA were mixed with 9 µg random hexamer primer (Invitrogen) in a final volume of 18 µl, heated to 70°C for 5 min and chilled on ice. 22 µl Mastermix (containing 50 mM Tris-HCl, pH 8.3), 75mM KCl, 3mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM dATP, 0.5mM dGTP, 0.5 mM dTTP, 0.2 mM dCTP, 40 U RNaseOUT Ribonuclease Inhibitor and 200U Superscript III Rnase H^- Reverse Transcriptase (Gibco, Life Technologies), 2 µl either Cy- or Cy5-conjugated dCTP (Amersham Biosciences, Freiburg, Germany) were added. The reaction

mix was incubated for 10 min at room temperatures, and hold at 42°C for 2 hours. Following reverse transcription, the RNA template was degraded by RNase digestion using 2 µl of Rnase, Dnase free (Roche, Mannheim, Germany) at 37°C for 30 min. The labeled cDNA was purified prior to hybridization using AutoSeq G-50 columns (Amersham Biosciences, Freiburg, Germany).

2.13.3 Microarray hybridization and post washing

The combined fluorescent labeled control and treatment cDNA probes were concentrated by speed vacuum at 45°C and combined in a final volume of 50 µl containing 7.5µl 20x SSC and 5µl 1% SDS. The samples were heated to 95°C for 2 min, cooled down to room temperature and pipetted direct onto microarray slides. A glass coverslip was applied, and the arrays were incubated over night at 50ºC in a humidified hybridization chamber (Corning, Schiphol, Nederland) in a water bath.

After hybridization the slides were placed in the staining dish containing the first wash buffer, and the cover slip was gently removed. The first washing step was performed in 2x SSC containing 0.2 % SDS for 5 min, followed by washing for 10 min in 2x SSC, 10 min in 0.2x SSC respectively and finally the slides were rinsed in 0.05x SSC. The slides were dried by centrifugation at 1500 rpm for 5 min at room temperature (Megafuge 1.0R, Heraeus).

2.13.4 Data collection and analysis

Following hybridization, the array slides were scanned using a Affimertix GMS 418 scanner (MWG Biotech, Ebersberg, Germany). Separate images were acquired for Cy3 and Cy5 at a resolution of 10 µm per pixel. The spots were identified and distinguished from the background using ImaGene (Biodiscovery, El Segundo, CA, USA). Signal intensity was background subtracted using the local background value. The data were normalized by total intensity, to adjust for differences in labeling and detection efficiencies for the fluorescent labels and for differences in the quantity of starting RNA from the two samples examined in the assay. Only the data whose signal intensity was at least threefold above the background were further processed. For statistical analyses of the gene expression data P values for the independent replicate experiments were calculated based on the student t test by using $\log_2$-transformed fluorescent ratios for the individual replicates compared with the constant expressed control genes. Genes that showed significantly changed RNA levels ($P<0.05$) with at least twofold increased or decreased average RNA levels were

considered further and analysed with a k-means cluster using the average linkage clustering method.

2.13.5 K-means clustering

Microarrays generates thousands of data points for every experiment. For better understanding of the data, systematic methods for their organization are required. K-means clustering uses partitions with reference vectors attached, the reference vectors are initialized randomly and genes are partitioned to their most similar reference vector. Than each reference vector is recalculated as the average of the genes that mapped to it. Last these steps are repeated until convergence, that is, all genes map to the same partition on consecutive iterations (123). (38). K-means clustering method was performed with the use of the Cluster and TreeView software (The Institute for Genomic Research, TIGR), currently available on the Eisen Lab web server (http://rana.lbl.gov/).

2.14 Real time RT-PCR to validate the expression profile of *Listeria monocytogenes* in the acid adaptation experiment

The two-step real time RT-PCR experiments were carried out the as described for *C. glutamicum* with some modification. Total RNA samples were DNAse digested using Ambion's DNA-free™ Kit (Ambion, Huntingdon, UK), according to manufacturer's instruction, for complete DNA removal. The processed RNA samples were purified using the Rneasy Mini Kit (Qiagen, Hilden, Germany).

Genes were chosen for validation of the DNA array are summarized in the Table 2-7.

Tab. 2-7: List of the genes used for the validation of the *L. monocytogenes* DNA-microarray experiments

DNA-microarray experiment	Identification Nummer	Putative function
37°C acid stress, adaptation	Lmo0200	PrfA
	Lmo0202	Hly
	Lmo0889	RsbR positive regulator of sigB activity
	Lmo1389	Sugar ABC transporter, ATP binding protein
	Lmo2748	Similar to B. subtilis stress protein YdaG
25°C acid stress, adaptation	Lmo0200	PrfA
	Lmo0679	FlhB flagellar biosynthetic protein
	Lmo0847	Glutamine ABC transporter
	Lmo1740	AA (glutamine) ABC transporter, permease
	Lmo2434	Glutamate decarboxylase

Temperature shift 25°C to 37°C	Lmo0109	Similar to transcriptional regulator protein AraC family
	Lmo0200	PrfA
	Lmo0679	FlhB flagellar biosynthetic protein
	Lmo0692	CheA two component sensor histidine kinase
	Lmo1997	PTS mannose specific enzyme IIA component

The Table 2-8 illustrates the oligonucleotides sequences used in the real time RT-PCR experiments.

Tab. 2-8: Oligonucleotides used in the validation of the *L. monocytogenes* DNA array experiments

Oligonucleotides	**Sequence**	**T_m (°C)***
16S_LmonF1	5′-AGACACGGCCCAGACTCCT-3′	64,8
16S_LmonR1	5′-GATCCGAAAACCTTCTTCATACA-3′	59,2
lmo0109_F	5′-TTAATCCTGGCGTGAGTC-3′	57,6
lmo0109_R	5′-GGGAACGTATTTCGAGTG-3′	57,2
lmo0200F3	5′-GTATCACAAAGCTCACGAGT-3′	58,3
lmo0200R3	5′-TGTATCAATAAAGCCAGACAT-3′	54,7
lmo0202_F	5′-CAAACTGAAGCAAAGGAT-3′	55,3
lmo0202_R	5′-CTTAGGACTTGGAGGCGGAG-3′	61,4
lmo0679_F	5′-TTGATGCCGATTATGGTG-3′	55,3
lmo0679_R	5′-TCAGCCGTTTGAATTGTG-3′	55,3
lmo0692_F	5′-ATGGTGCCAGTGGACAGT-3′	59,9
lmo0692_R	5′-ATCTGCGCCTTCAATCAC-3′	57,6
lmo0847_F	5′-CTTGAACGCTGGTGCCTAT-3′	60,1
lmo0847_R	5′-TTGCTGCTTCCATTTGTCCT-3′	58,3
lmo0889_F	5′-AAAGCAGACTTACTGAATGA-3′	46,7
lmo0889_R	5′-TGCTAGTTTCTTCGTACATT-3′	46,2
lmo1389_F	5′-GTTAGGAATCAGGAAGGAAT-3′	48,4
lmo1389_R	5′-AATAGCGTAGATTTACCTGC-3′	48,3
lmo1740_F	5′-TGCGAGGCACGCCATTAC-3′	62,1
lmo1740_R	5′-ACGGCATCCATTCGGTCA-3′	59,9
lmo1997_F	5′-GAAGCGTTCCTCAGTCGT-3′	59,9
lmo1997_R	5′-CGGCATATTCATAAATCCCTAA-3′	57,0
lmo2434_F	5′-AGAAAGCACGAGTATCCC-3′	57,6
lmo2434_R	5′-ATTTCCCTCATCCATCAA-3′	53,0
lmo2748_F	5′-TGGGTGTATTGACATCCGTA-3′	50,3
lmo2748_F	5′-GCCAGATGGTGTATAAAGGG-3′	52,1

*Tm: melting point, calculated by the manufacturer (MWG Biotech AG, Ebersberg, Germany)

3 Results

3.1 Results of the acid adaptation response of *Corynebacterium glutamicum*

3.1.1 ATR response of *Corynebacterium glutamicum*

The ATR response of *C. glutamicum* was investigated under different pH conditions. Cells growing exponentially at pH 7.0 (control) and at an adaptive pH 6.0 or 5.7, were exposed to pH 3.5 for 90 minutes. Survival was determined by plating serial dilutions. In the non-adapted culture no viable cells were detected after 30 and 90 minutes exposure whereas in the adapted cultures 10% viability at pH 6 and 80% viability at pH 5.7 was observed after 30 minutes exposure (Figure 3-1). Therefore, cells adapted to pH 5.7 demonstrate an effective ATR response of *C. glutamicum* when shocked at pH 3.5.

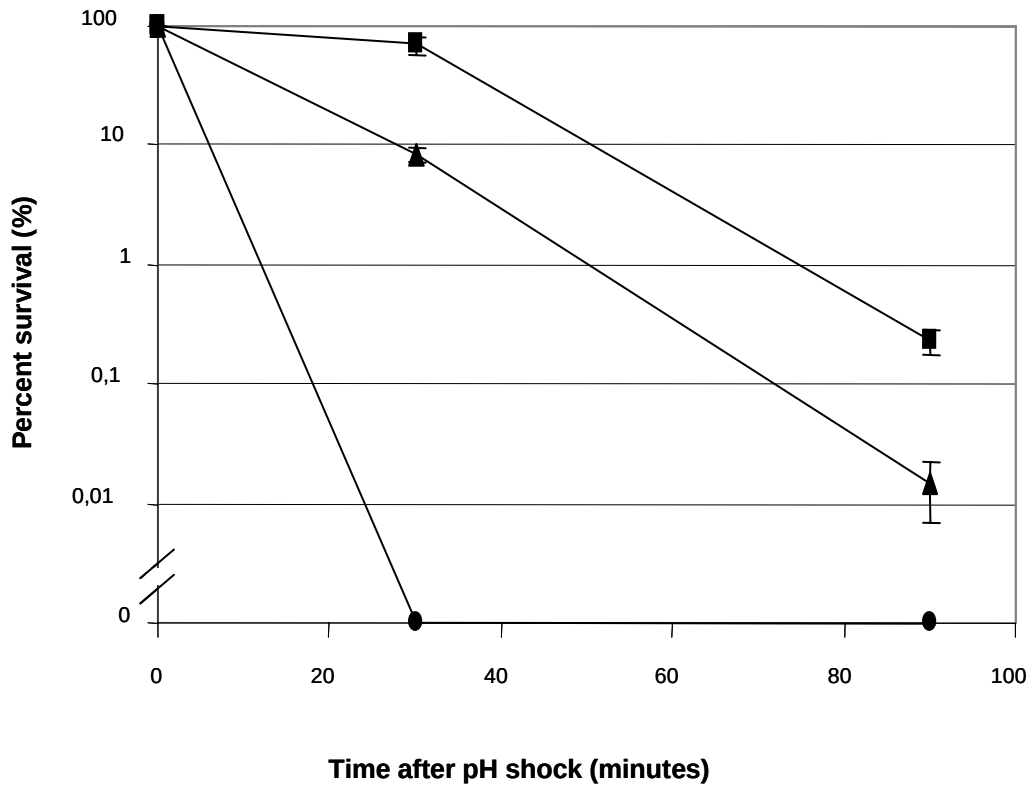


Fig. 3-1: Acid tolerance response of *C. glutamicum*. Adapted and nonadapted cells of *C. glutamicum* were investigated in acid shock experiments. Exponential phase cells grown at pH 7.0 (●), pH 6.0 (▲) or pH 5.7 (■) were exposed to pH 3.5. Survivors were determined after 30 and 90 minutes by serial plating.

3.1.2 Acid-induced changes of cytoplasmic pH

Changes of the cytoplasmic pH induced by external low pH were determined using the pH indicator dye BCECF (Figure 3-2). The cytoplasmic pH remained relatively constant up to mildly acidic external pH. Below an external pH of 5.5, a dramatic decrease in cytoplasmic pH was observed indicating a collapse of the internal pH homeostasis system of the bacterial cell. This observation correlates well with the fact that the pH minimum of growth of *C. glutamicum* is at pH 5.5 (data not shown).

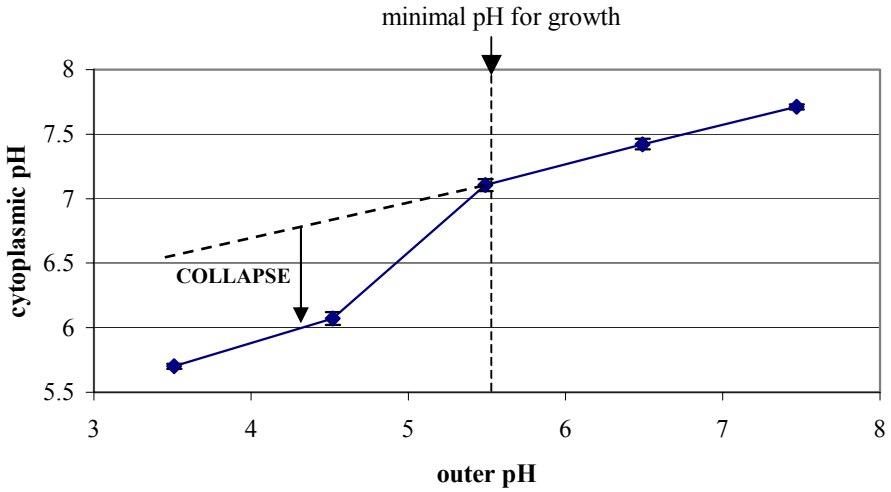


Fig. 3-2: Cytoplasmic pH Changes in comparison to outer pH alterations. Cytoplasmic pH changes were measured in *C. glutamicum* ATCC 13032 which were grown in pH 7.5 to an OD_{600} of 0.5 using a fluorescent dye BCECF.

3.1.3 Comparison of gene expression at acidic and neutral pH

To identify differentially expressed genes in response to acid adaptation, the global gene expression patterns of exponential phase *C. glutamicum* cells, fermented in a continuous turbidostat fermentor at neutral and acidic conditions, were analyzed using DNA microarrays (61). Starting from two independent fermentation experiments four DNA microarray analyses were carried out. Genes, whose signal intensity showed at least a 3 fold intensity increase above the background signal, who were differentially expressed in at least three of the experiments, and exhibited a median ratio of ≥ 2 were examined. A total of 116 up-regulated and 90 down-regulated genes could be identified representing about 6 % of the ORFs in the genome. Based on their putative function, the up-regulated genes can be classified in three main categories: transporters (30 ORFs), transcriptional regulators/proteins (6 ORF'S) and cell metabolism proteins (27 ORFs). Hypothetical proteins of unknown function (i.e., 53 putative genes) comprised as much as 38% of the up-regulated ORFs.

Thirty ORFs encode putative proteins belonging to different transport system, of which 20 are organized in 5 putative operons. Four of these operons (ORF 855-857, 3549-3551, 1168-1173, 1346-1352) were similar to an ABC type cobalamin/Fe^{3+} siderophore transport system and one operon (ORF 1516 and 1517) codes for a predicted ABC type multidrug transport system. Another group comprises putative proteins involved in the transport of ionic compounds (ORF 851, 2433 and 2905) cation transport ATPase; (ORF 2779) Co/Zn/Cd efflux; (ORF 2583), divalent heavy metal cation transporter).

Six putative transcriptional regulators have been found among the up-regulated ORFs: Two sigma factors, *sigB* and *sigE,* the sensory component of histidine kinase (ORF 1518), a Mn-dependent transcriptional regulator (ORF 3470) as well as two putative transcriptional regulators (ORF 927, 1703).

The remainders of the up-regulated ORFs represent proteins involved in numerous cell metabolic pathways such as carbohydrate metabolism and components of respiratory metabolims. Subunits of F_0F_1 ATP synthase, (ORF 1807-1813) and the heat shock proteins GroES and GroEL (ORF 1088 and 1092, respectively), whose role in temperature stress is well documented, exhibited a 2 fold down regulation. Further proteins showing a down regulation were the 25 ribosomal proteins and several transport system involved in amino acid and cation transport.

3.1.4 Validation of expression profile of fermentation *via* real time quantitative RT-PCR

The fermentor harbors numerous metallic parts and during the extended fermentation process trace amounts of metals could have dissolved in the medium due to the low pH. These metal ions may in turn trigger gene expression. To test the possible effect of such a phenomenon on the transcription profile, the expression of some genes was analyzed by real time PCR in cells grown in batch cultures in glass vessels. An overnight culture of *C. glutamicum* was inoculated in fresh medium at pH 7.5 and 5.7. RNA isolated from exponentially growing cells was reverse transcribed and subjected to real time PCR analysis using the iCycler. The genes analyzed included the two sigma factors (s*igB* and sig*E*), ORFs from putative iron transport operons and a cation efflux transporter. The genes examined by real time PCR (Figure 3-3) show the same up-regulated trend as was observed in the microarray analysis, thereby disproving any suspicion that transcription might have been triggered by acid-dissolved metal ions rather than the pH decrease.

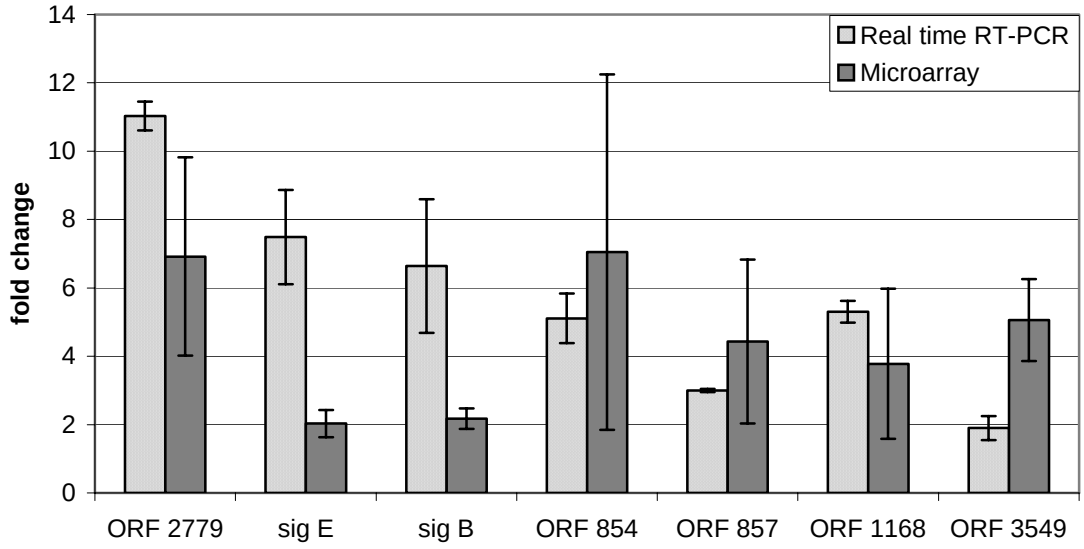


Fig. 3-3: Comparison between microarray analysis and real time PCR results of *C. glutamicum* gene expression profiling. Fold changes of genes expressed in cells adapted to pH 5.7 in a fermentor as analyzed by microarray were compared to real time RT PCR of *C. glutamicum* cells grown in batch culture

3.1.5 Effect of the deletion and overexpression of the ORF2754 on the acid resistance of *Corynebacterium glutamicum*

The acid tolerance of the mutant strains was investigated in growth curve experiments at neutral (pH 7.5) and acidic (pH 5.7). The growth characteristic of the deletion mutant strain (*C. glutamicum ΔORF2754)* and the overexpressing strain (*C. glutamicum* OE2754) at acidic pH showed no significant differences compared to the wild type. The deletion in the strain *C. glutamicum ΔORF2754* was verified using a PCR technology; the overexpression of the putative protein encoded by ORF2754 in the strain pWLQOE2754 was not confirmed.

3.1.6 Gene disruption mutants of *Corynebacterium glutamicum*

Mutant strains of *C. glutamicum* which were kanamycin resistant were confirmed by PCR reactions with a primer based on the chromosome of *C. glutamicum* and a primers based upon the plasmid. In addition, Southern hybridization was conducted to confirm that chromosomal integration took place at a central gene location in all mutants. Although, it cannot be ruled out that the interrupted genes are capable of producing a functional products.

3.1.7 Influence of acidic pH on the growth of disruption mutants

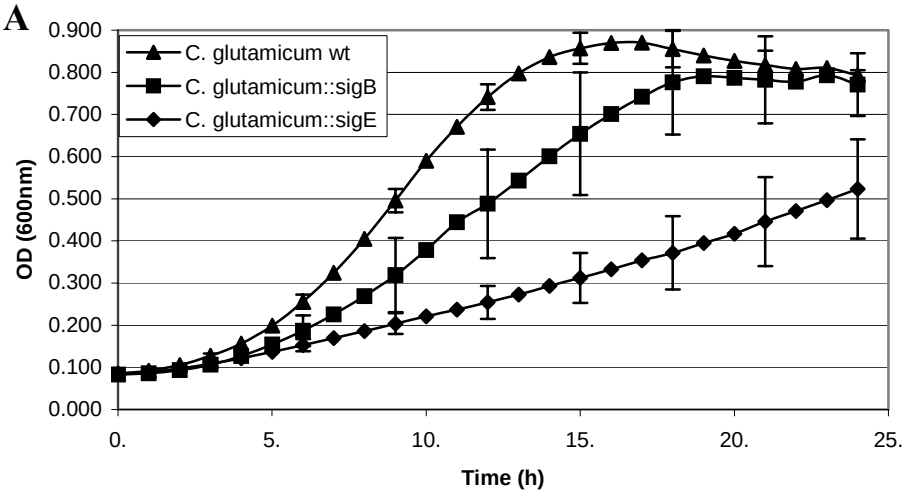
To investigate the function of the up-regulated genes in acid adaptation, single crossover mutants of 26 significantly up-regulated ORFs were generated (see Table 2-6). To test for pH sensitivity of the mutants, growth experiments were performed at pH 7.5 and 5.7 in a honeycomb microplate reader Bioscreen C. The control strain is a wild type containing the plasmid pWLQ2 to exclude the effect of the kanamycin resistance genes used in creating the disruption mutants. Only two mutants, *sigB* and *sigE,* showed a decreased growth rate when grown at pH 5.7 compared to the wild type strain (Figure 3-4 A, B). As shown in figure 3-4 A, the *sigB* mutant was more susceptible to pH 5.7 compared to the *sigE* mutant, whereas at pH 7.5 *sigE's* (Figure 3-4 C) doubling time was about three fold higher compared to the other two strains. This result suggests that *sigE* effects growth under normal condition but is not essential for growth under stressed conditions, whereas *sigB* is primarily responsible for the acid tolerance response in the acid adapted *C. glutamicum* cells.

To further analyze the response of the mutants to acidic pH, acid shock experiments were performed. Exponentially growing cells were harvested, and shocked for 30 minutes in TSB pH 4.0. Samples were taken before and after acid shock and survival were determined. The *sigE* mutant exhibited a 10fold and the *sigB* mutant 1000 fold reduction in survival compared to the control strain (Figure 3-5). These results strongly confirm the hypothesis that *sigB* plays a major role in the regulation of the pH adaptation response.

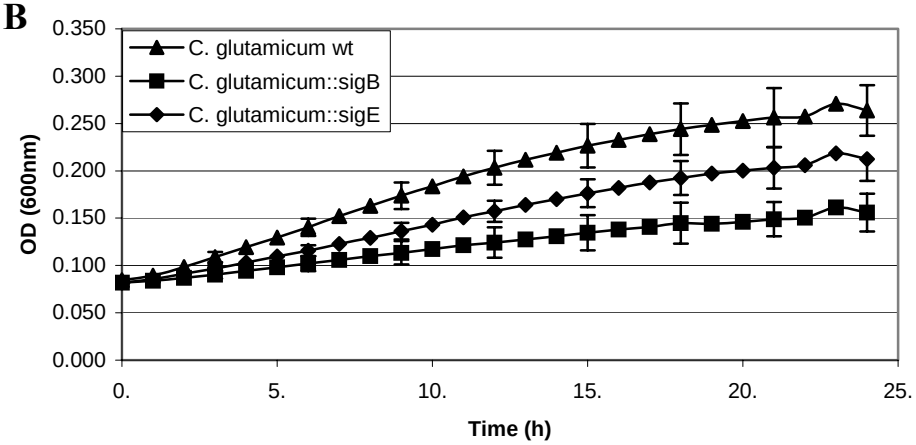
There were no significant differences observed in the acid shock response and pH sensitivity of the other disruption mutants.

Fig. 3-4 (overleaf): **A**) growth curves and doubling times of *C. glutamicum* ATCC 13032 wildtype, *ΔsigB,* and *ΔsigE* mutants. Cells were precultured overnight in TSB pH 7.5, 50 fold diluted and inoculated in pH 5.7 TSB. **B**) As panel A) but cells are inoculated in pH 7.5 TSB. **C**) Doubling times calculated from panels A) and B) at pH 5.7 and 7.5.

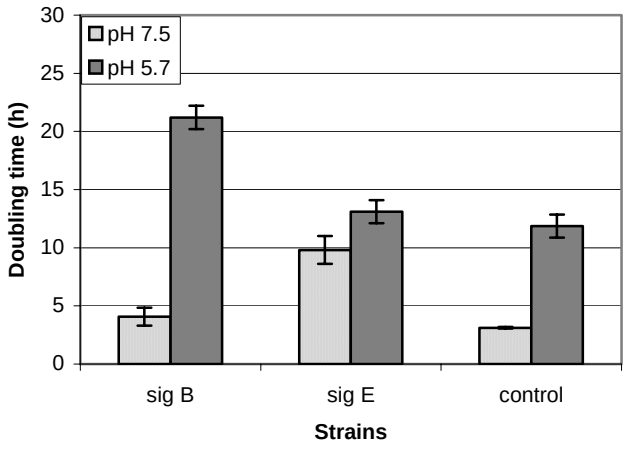
A


C. glutamicum wt
C. glutamicum::sigB
C. glutamicum::sigE
OD (600nm)
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0.
5.
10.
15.
20.
25.
Time (h)

B


C. glutamicum wt
C. glutamicum::sigB
C. glutamicum::sigE
OD (600nm)
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0.
5.
10.
15.
20.
25.
Time (h)

C


pH 7.5
pH 5.7
Doubling time (h)
30
25
20
15
10
5
0
sig B
sig E
control
Strains

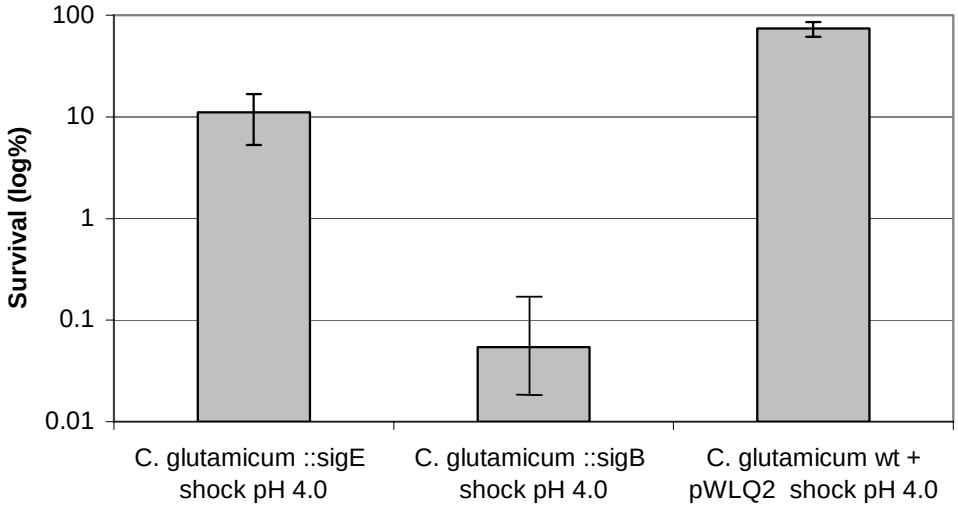


Fig. 3-5: The acid shock of *C. glutamicum* ATCC 13032, *sigB* and *sigE* mutants. An overnight preculture of the three strains was inoculated in TSB pH 7.5 in which the strains were allowed to grow until an OD_{600} of 0.5.

3.1.8 Iron bioavailability in undefined complex and defined minimal medium

To further investigate the up regulation of ABC type cobalamin/Fe^{3+} siderophore tranport operons an experiment was performed using three different complex media (TSB, BHI, and TSB+Fe) and one defined minimal medium (CGXII). ORF 855, one of the genes in the iron siderophore operons, was analyzed by real time PCR for expression level changes in cells grown at neutral and acidic pH in all of the media. First, we calculated expression level changes of ORF 855 in each media separately by comparing mRNA level at pH 5.7 to pH 7.5. The mRNA level increased in TSB, TSB+Fe and BHI whereas it decreased in CGXII (Figure 3-6). Next, we compared expression level of ORF 855 at pH 5.7 in TSB+Fe, CGXII and BHI with TSB at pH 5.7. At acidic pH the mRNA level decreased in all three media compared to TSB. In TSB+Fe and BHI decrease in mRNA expression was modest, but at drastic decrease was observed in CGXII compared to TSB. The same comparison was performed at neutral pH. At pH 7.5 we also observed a decrease in mRNA level in all three media with a considerable decrease in mRNA level in CGXII and TSB+Fe, but only a slight decrease in BHI compared to the expression level detected in TSB at neutral pH (Figure 3-7).

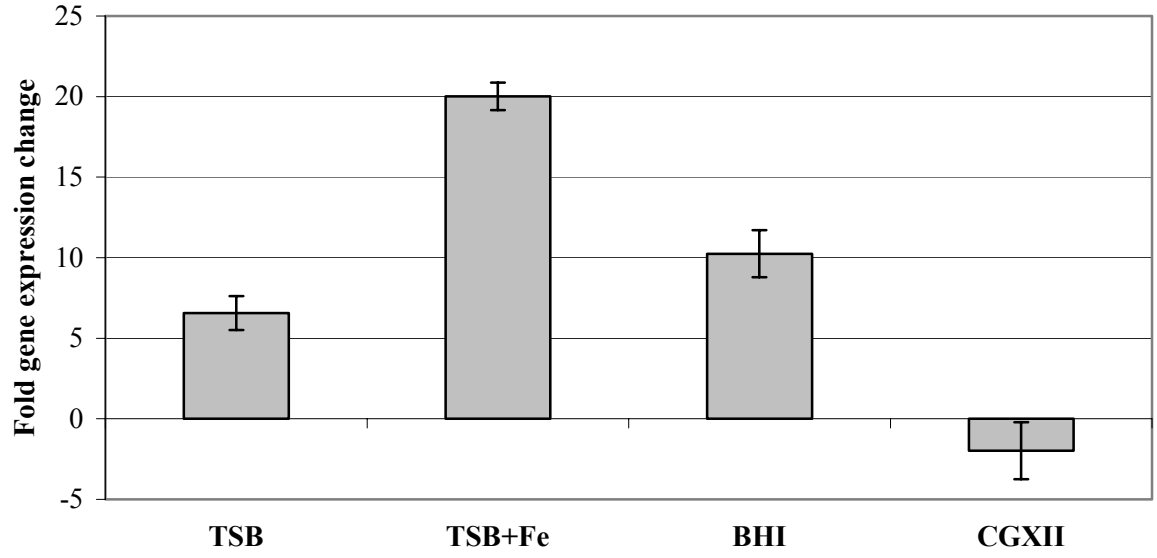


Fig. 3-6: Expression of iron siderophore transporter in *C. glutamicum* in complex and minimal media. Expression level of ORF 855 was determined at acidic pH by real time PCR in three complex (TSB, TSB+Fe, BHI) media and one minimal medium (CGXII) compared to the same media separately at neutral pH. Expression level of ORF 855 at acidic pH showed a increase in TSB, TSB+Fe, BHI and decreased in CGXII. Error bars represent standard deviations obtained from two independent biological experiments.

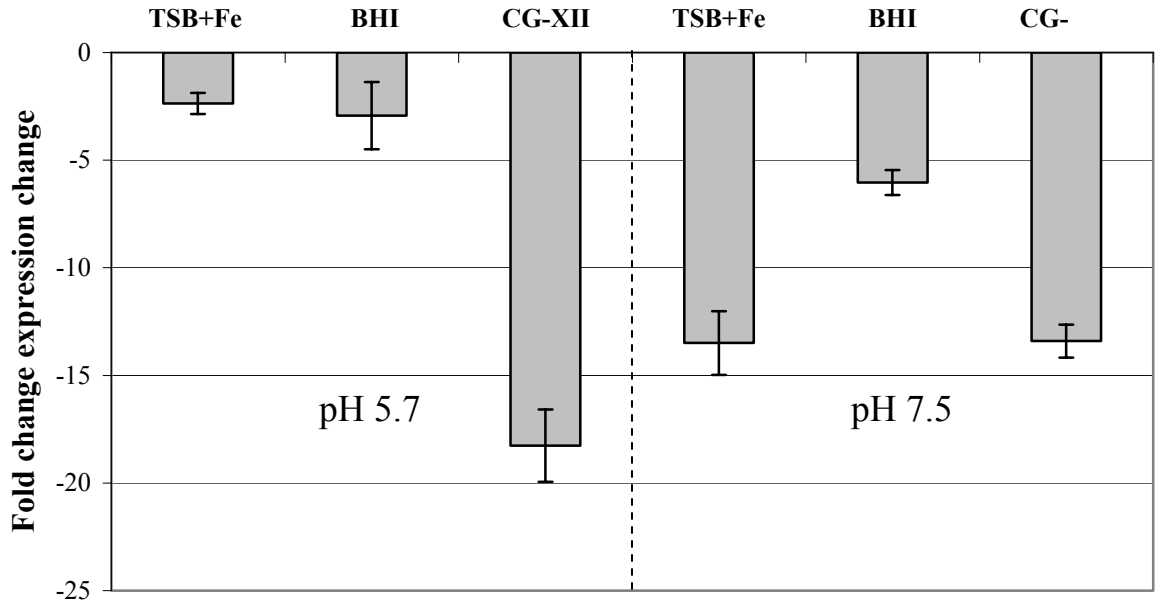


Fig. 3-7: Bioavailability of iron in complex and minimal medium. mRNA level of ORF 855 in three media (TSB+Fe, BHI, CGXII at pH 5.7 and 7.5) were compared to mRNA levels in TSB pH 5.7 as well as 7.5. At pH 5.7 the expression level dramatically decreased in CGXII, however in TSB+Fe and BHI it was only slightly lower compared to the expression in acidic TSB. At pH 7.5 a considerable decrease in mRNA level in CGXII and TSB+Fe was noticed, but only a slight decrease was detected in BHI compared to the mRNA level in TSB at neutral pH.

3.2 Results of the acid shock and adaptation experiments of *Listeria monocytogenes*

3.2.1 Growth characteristic of the acid shocked cells of *Listeria monocytogenes*

Cells were exposed to sublethal acidic pH at 37°C in the middle exponentially growth phase (Figure 3-8 B). Immediately after the acid challenges the cells showed no growth, after 30 min adaptation phase, diminished growth was detected. Similar results were obtained when the cells were cultured and shocked at 25°C (Figure 3-8 A).

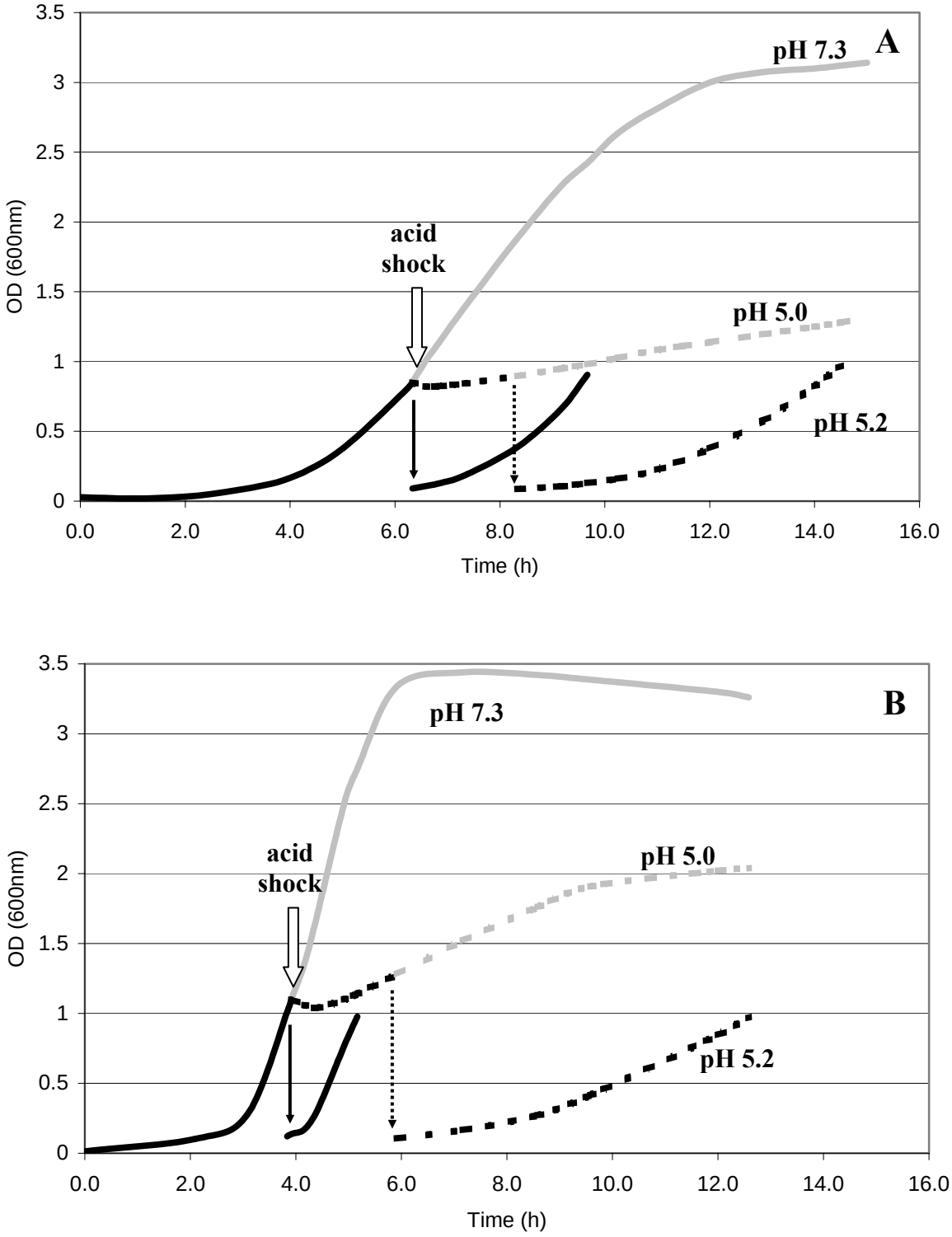


Fig. 3-8 A and **B**: Growth characteristic of *Listeria monocytogenes* EGDe at neutral and acidic pH in BHI broth at (A) 25°C and (B) 37°C. In the mid log phase the cells were challenged to sublethal acidic pH (pH 5.0) by addition of 5M HCl (white arrow shows the time point of the acid shock). Samples were harvested before and 15, 30, 60 and 120 minutes after addition of the acid. For analysis of the acid adaptation response fresh BHI broth at pH 5,2 was inoculated with cells already shocked for 120 minutes (dashed black

arrow). The acid adaptation sample was taken in the mid log phase. To obtain a correct control for the acid adaptation experiment, neutral BHI was inoculated with non-stressed cells (solid black arrow) and sample was harvested in the mid log phase.

3.2.2 Microarray analysis of *Listeria monocytogenes* after acidic treatment

It is well established, that the ability of *L. monocytogenes* to withstand acidic pH, like HCl in the stomach, has an impact on the virulence of this microorganism. There is a lot of information available concerning acid response systems used by this pathogen to survive acid stress. But relatively little is known about global transcriptomic changes, and about regulatory networks that coordinate listerial responses to environmental acidic pH.

After acid treatment, samples were taken at six time points (0, 15, 30, 60, 120 min and adaptation after 12 hours), total RNA was purified and cDNA was fluorescently labeled using a reverse transcription reaction. The two populations of labeled cDNAs, treatment and control were simultaneously hybridized with the cDNA microarray. Competitive microarray hybridization were performed in duplicate from two independent biologically experiments, with both dye arrangements (i.e., Cy3-labeled treated plus Cy5-labeled control cDNAs and Cy5-labeled treated plus Cy3-labeled control cDNAs). Data were normalized based on total intensity for each channel, and ratios of acid shocked to control cDNA were calculated from the normalized data. Similarly to most studies published (21) two-fold up- or down-regulation was used for a post-normalization cutoff to define differential expression ratio. Of the 2853 sequences present on the slide, all spots were detected by both the fluorophores, indicating that the microarray slides were well printed and recognized. No significant variability in signal intensities was observed when dye swap was performed between the experiments.

Tables 3-1 shows the number of the genes that were up- or down-regulated ≥2-fold, ≥3-fold and ≥5-fold respectively.

3.2.3 Acid shock and adaptation at 25°C

In our 25°C study the number of the induced genes was slowly increased (Table 3-1), the maximum was achieved 120 min after the acidification. In the adaptation phase, only a very small amount of significantly induced genes were detected compared to the acid stress experiment at 37°C.

Tab. 3-1: Quantitative summary of *L. monocytogenes* up- and down-regulated genes in acidic medium at 25°C.

No. of up-regulated genes	15 min	30 min	60 min	120 min	adaptation
ratio≥2	68	217	338	466	29
ratio≥3	27	120	239	299	10
ratio≥5	8	62	146	175	2

No. of down-regulated genes	15 min	30 min	60 min	120 min	adaptation
ratio≤1/2	123	350	739	875	80
ratio≤1/3	42	146	402	595	40
ratio≤1/5	12	51	152	304	14

3.2.4 Acid shock and adaptation of *Listeria monocytogenes* at 37°C

There was a progressive increase in the number of the genes changing expression in the first 30 minutes after the medium pH was lowered; the maximum number was reached after 60 min. This number of the significantly regulated genes decreased with long time adaptation towards acidic pH. More genes were up-regulated than were down-regulated by low pH at all time points in this experiment. The Table 3-2 shows the amount of the up and down-regulated genes after acidic treatment.

Tab. 3-2: Quantitative summary of *L. monocytogenes* up- and down-regulated genes in acidic medium at 37°C

No. of up-regulated genes	15 min	30 min	60 min	120 min	adaptation
ratio≥2	158	326	383	376	137
ratio≥3	95	192	192	180	26
ratio≥5	62	101	77	64	13

No. of down-regulated genes	15 min	30 min	60 min	120 min	adaptation
ratio≤1/2	110	323	314	257	39
ratio≤1/3	22	129	146	127	11
ratio≤1/5	7	30	70	56	7

The scatter plot of normalized gene expression data gives the best overview about the dynamics of the gene induction level under the acid adaptation experiment. The plots show, that already 15 minutes after the acid induction many genes were both induced and repressed greater than 2-fold or even 5-fold. Than, 60 minutes after the pH drop, the maximum number the transcriptional changes were found. In response to acid adaptation, many genes returned to the same level as before, which can be seen on the scatter plot by fewer points remaining outside the threshold lines (Figure 3-9).

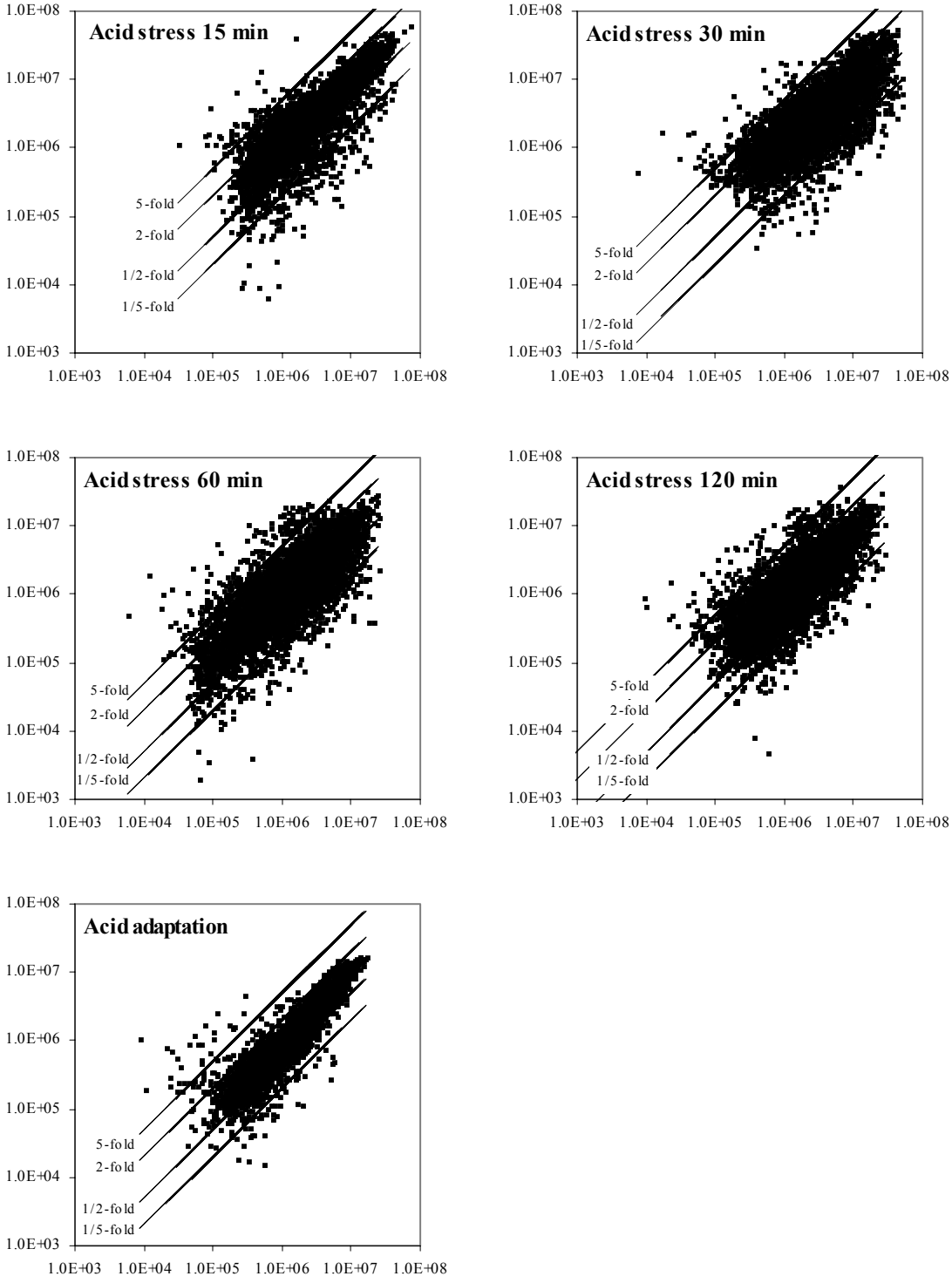


Fig. 3-9: Microarray scatter plot of gene expression 15, 30, 60 and 120 min after acid treatment and of acid adapted *L. monocytogenes* at 37°C. The mean of the log signal intensities from acid-treated samples (y-axis) was plotted over control samples (x-axis). Thresholds are provided in the plots showing when signal ratios are 5-fold, 2-fold, 0.5-fold and 0.2-fold.

3.2.5 General observations about the acid shock experiments

Exposure to low pH results in a progressive increase in the number of genes with altered gene expression. About 40% of the acid induced genes show no homology to other genes with known functions. The time point of the maximum number of the expressional changes was temperature dependent in our experiments. The complete adaptation to the mild acidic pH requires less expressional gene changes. There was a striking difference in the relation of the up- and down-regulated genes between the acid adaptation experiments conducted at 25°C and 37°C.

3.2.6 Temperature shift from 25 to 37°C

Only 5.7% of the *Listeria* genes displayed ratios >2-fold in comparison of normal (25°C) to elevated temperature (37°C). Of these 52.1% were induced less than threefold, and 17.8% showed considerably higher levels of induction (>5-fold). Table 3-3 represent the distribution of the induction level of the up-regulated genes.

Tab. 3-3: Quantitative summary of *L. monocytogenes* up-regulated genes in temperature shift experiments, when temperature was increased from 25°C to 37°C.

No. of up-regulated genes	
ratio≥2	163
ratio≥3	85
ratio≥5	29

The Table 3-4 summarizes selected genes based on their putative function in the general stress response or in the listerial virulence at elevated temperature.

Tab. 3-4: Selected genes of *L. monocytogenes*, which showed induced expression (≥ 2-fold) in response to temperature shift from 25 to 37°C.

Group	**Avg. ratio**	**Gene information**	
		***Lmo* No.**	**Fuction**
Transport/ binding proteins	2.89	lmo0021	similar to PTS system, fructose-specific IIA component
	3.75	lmo0400	similar to fructose-specific phosphotransferase enzyme IIC
	2.08	lmo0401	highly similar to *E. coli* YbgG protein, a putative sugar hydrolase
	4.51	lmo0427	similar to PTS fructose-specific enzyme IIB component
	5.51	lmo0428	similar to PTS fructose-specific enzyme IIC component
	2.65	lmo0782	similar to mannose-specific phosphotransferase system (PTS) component IIC
	2.54	lmo0783	similar to mannose-specific phosphotransferase system (PTS) component IIB
	6.45	lmo0915	similar to phosphotransferase system enzyme IIC
	5.14	lmo1255	similar to PTS system trehalose specific enzyme IIBC
	18.28	lmo1997	similar to PTS mannose-specific enzyme IIA component
	13.35	lmo2000	similar to PTS mannose-specific enzyme IID component

	10.22	lmo2001	similar to PTS mannose-specific enzyme IIC component
	8.57	lmo2650	similar to hypothetical PTS enzyme IIB component
	11.73	lmo2651	similar to mannitol-specific PTS enzyme IIA component
	2.58	lmo2665	similar to PTS system galactitol-specific enzyme IIC component
	2.01	lmo2667	similar to PTS system galactitol-specific enzyme IIA component
	5.87	lmo2797	similar to phosphotransferase system mannitol-specific enzyme IIA
	30.45	lmo2799	similar to phosphotransferase system mannitol-specific enzyme IIBC
Cell surface proteins	2.09	lmo0160	putative peptidoglycan bound protein (LPXTG motif)
	3.14	lmo0320	similar to surface protein (peptidoglycan bound, LPXTG motif)
Regulation	4.92	lmo0051	similar to 2-components response regulator protein (AgrA from *Staphylococcus*)
	2.01	lmo0297	lmo0297similar to transcriptional antiterminator (BglG family)
	9.14	lmo0425	similar to transcription antiterminator (BglG family)
	2.03	lmo2668	similar to transcriptional antiterminator (BglG family)
Adaptation to atypical conditions	2.44	lmo0211	*ctc* similar to *B. subtilis* general stress protein
	2.41	lmo1138	similar to ATP-dependent Clp protease proteolytic component

Many up-regulated genes belonging to the transport/binding group are involved in the PEP dependent uptake and phosphorylation of sugars. Three genes in the group of regulational genes (lmo0297, lmo0425, lmo2668) are coding for the transcriptional regulation of these PTS systems. Additionally, AgrA, the response regulator of a listerial two component regulatory system, was induced by the temperature shift (Table 3-4). Furthermore two genes *ctc* (lmo0211), encoding a general stress protein and *clpP,* codes for the proteolytic component of ATP-dependent Clp protease, exhibited increased expression. The up-regulated lmo0160 and lmo0320 belong presumably to the cell surface proteins.

In response to the temperature shift 146 genes *L. monocytogenes* appeared to be repressed more than two-fold. Table 3-5 represents the distribution of the fold changes of the genes with repressed expression.

Tab. 3-5: Quantitative summary of *L. monocytogenes* down-regulated genes in the temperature shift experiment, when temperature was increased from 25°C to 37°C.

Nr. of down-regulated genes	
ratio<1/2	146
ratio<1/3	79
ratio<1/5	56

Most of the down-regulated genes coding for putative proteins involved in the motility and flagellar biosynthesis (lmo675 to lmo724). Additionally, two genes coding for a peptidoglycan bound protein (lmo0130 and lmo0835) were also down-regulated after the temperature shift (Table 3-6).

Tab.3-6: Selected genes of *L. monocytogenes*, which showed induced expression (≤ 2-fold) in response to temperature shift from 25 to 37°C. Additionally, genes without function, but that formed an putative operon with other selected genes, were also listed.

Group	Avg. ratio	Gene information	
		Lmo Nr.	Function
Cell surface proteins	0.11	lmo0130	similar to 5 -nucleotidase, putative peptidoglycan bound protein (LPXTG motif)
	0.04	lmo0835	putative peptidoglycan bound protein (LPXTG motif)
Mobility and chemotaxis	0.27	lmo0675	Unknown
	0.33	lmo0676	similar to flagellar biosynthetic protein FliP
	0.03	lmo0680	similar to flagella-associated protein FlhA
	0.07	lmo0681	similar to flagellar biosynthesis protein FlhF
	0.17	lmo0682	similar to flagellar hook-basal body protein FlgG
	0.39	lmo0683	similar to chemotactic methyltransferase CheR
	0.06	lmo0684	Unknown
	0.10	lmo0685	similar to motility protein (flagellar motor rotation) MotA
	0.40	lmo0686	similar to motility protein (flagellar motor rotation) MotB
	0.13	lmo0687	Unknown
	0.12	lmo0688	similar to unknown protein
	0.06	lmo0689	similar to CheA activity-modulating chemotaxis protein CheV
	0.05	lmo0690	flagellin protein
	0.38	lmo0691	Chemotaxis response regulator CheY
	0.03	lmo0692	two-component sensor histidine kinase CheA
	0.18	lmo0695	Unknown
	0.07	lmo0696	similar to flagellar hook assembly protein
	0.11	lmo0697	similar to flagellar hook protein FlgE
	0.08	lmo0698	weakly similar to flagellar switch protein
	0.07	lmo0699	similar to flagellar switch protein FliM
	0.08	lmo0700	similar to flagellar motor switch protein FliY
	0.07	lmo0701	Unknown
	0.12	lmo0702	Unknown
	0.04	lmo0703	Unknown
	0.08	lmo0704	Unknown
	0.25	lmo0705	similar to flagellar hook-associated protein FlgK
	0.18	lmo0706	similar to flagellar hook-associated protein 3 FlgL
	0.10	lmo0707	similar to flagellar hook-associated protein 2 FliD
	0.05	lmo0708	similar to hypothetical flagellar protein
	0.05	lmo0709	Unknown
	0.09	lmo0710	similar to flagellar basal-body rod protein FlgB
	0.14	lmo0711	similar to flagellar basal-body rod protein FlgC
	0.12	lmo0712	similar to flagellar hook-basal body complex protein FliE
	0.05	lmo0713	similar to flagellar basal-body M-ring protein FliF

	0.14	lmo0714	similar to flagellar motor switch protein FliG
	0.20	lmo0715	Unknown
	0.19	lmo0716	similar to H+-transporting ATP synthase alpha chain FliI, flagellar-specific, -
	0.18	lmo0717	similar to transglycosylase
	0.29	lmo0718	Unknown
	0.17	lmo0723	similar to metyl-accepting chemotaxis protein
	0.12	lmo0724	similar to B. subtilis YvpB protein

3.2.7 Confirmation of *Listeria monocytogenes* DNA-microarray data with real-time quantitative RT-PCR

Although cDNA arrays are highly sensitive and produce robust data, the reliability of the arrays data is prevalently concerned. Several factors, such as contamination with other genes, dust or scratches on the cDNA spots, and high background can lead to false profiling. In the present study we have utilized repeated biological experiments, multiple arrays and technical replicates on each array to avoid the potential problems, and subsequently used real-time qRT-PCR to validate the results (109). Twelve genes were chosen for analysis by real-time qRT-PCR based on the level of changes seen in the microarray experiment or because of suspected involvement in pH shock response or virulence of *L. monocytogenes*. Suggested by previous studies, the hybridization intensity of the genes in the prfA operon (*prfA*, *plcA*, *hly*, *mpl*, *actA* and *plcB*) on the DNA-array are not reliable (T. Hain, B. Joseph, personal communication). For this reason, all genes belonging to the *prfA* operon, used in this study, validation by real-time quantitative RT-PCR was necessary.

The real-time RT-PCR analysis confirmed the up-regulation of the genes for Lmo0109, Lmo1389, Lmo1740, Lmo2434 and Lmo2748, the results are shown in figure 3-7 to 3-9 and confirmed down regulation of the genes; Lmo0889, Lmo0679, Lmo0847, Lmo0679 and Lmo0692. Both results according to the microarray experiments (Figure 3-10 to 3-12). In general, the trends of differential expression were maintained in both, the microarray and quantitative RT-PCR analyses (with the exception of *prfA* and *hly*), but we often found a greater dynamic range of expression in microarray analyses relative to the corresponding quantitative RT-PCR expression data.

Comparison of the DNA-microarray gene expression ratios and quantitative real time RT-PCR data of two genes *prfA* (lmo0200) and *hly* (lmo0202), belonging to the *prfA* operon, showed a large discrepancy. Presumably due to the unsuitable design of the

oligonucleotides-probes spotted on the slides (T. Hain, B. Joseph, personal communication).

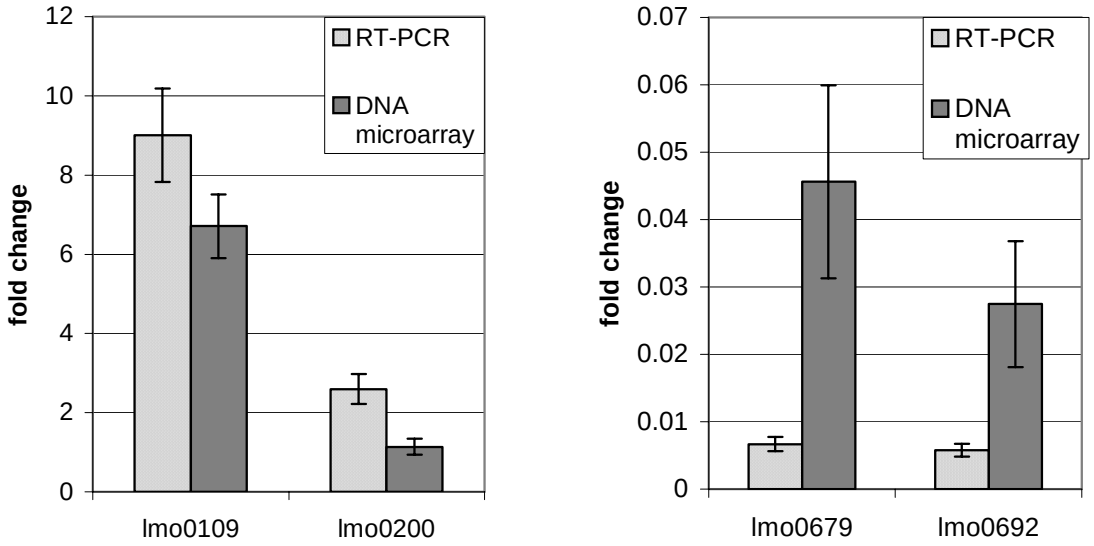
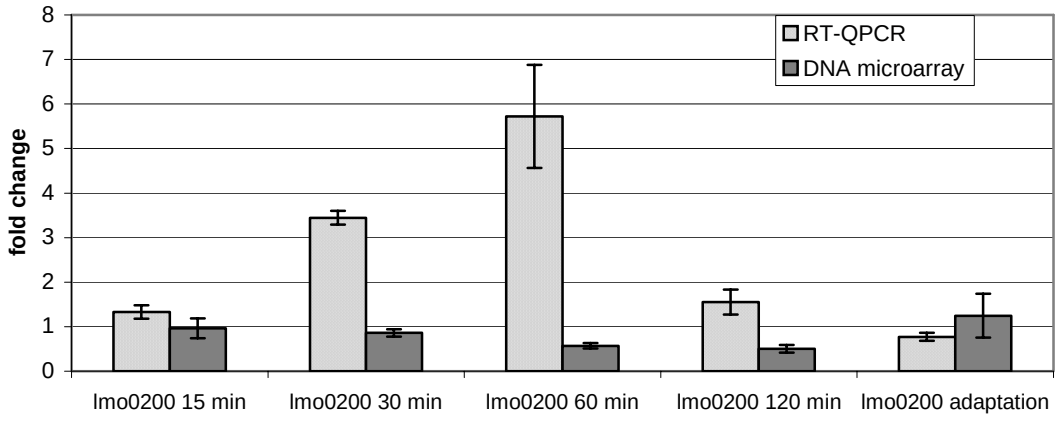


Fig. 3-10: Comparison of the DNA microarray gene expression ratios and quantitative real time RT-PCR data for selected differentially expressed genes in the temperature shift experiment. The genes were identified by microarray analysis of the total RNA obtained from *L. monocytogenes* cells growing in the exponential phase at 25°C and 37°C respectively, at neutral pH in BHI broth. The fold induction of the genes after acid challenge was plotted relative to untreated control. The error bars indicate the standard deviation.


RT-QPCR
DNA microarray
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lmo0200 15 min
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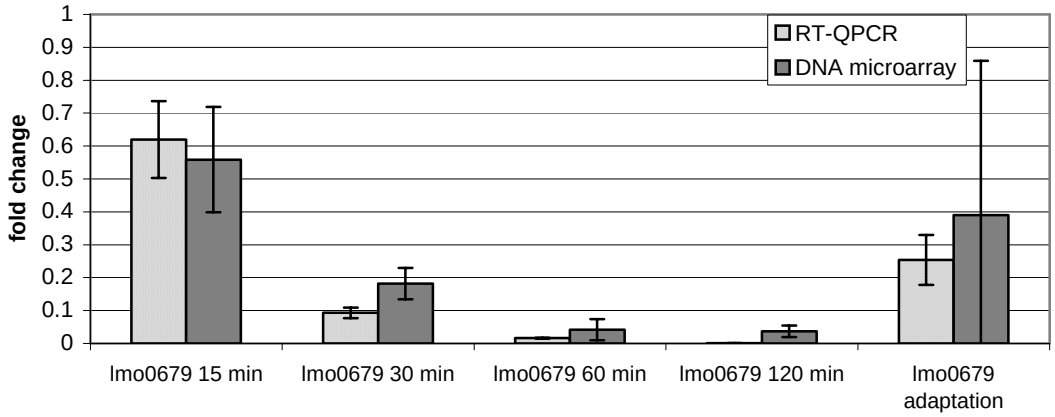
lmo0200 60 min
lmo0200 120 min
lmo0200 adaptation


RT-QPCR
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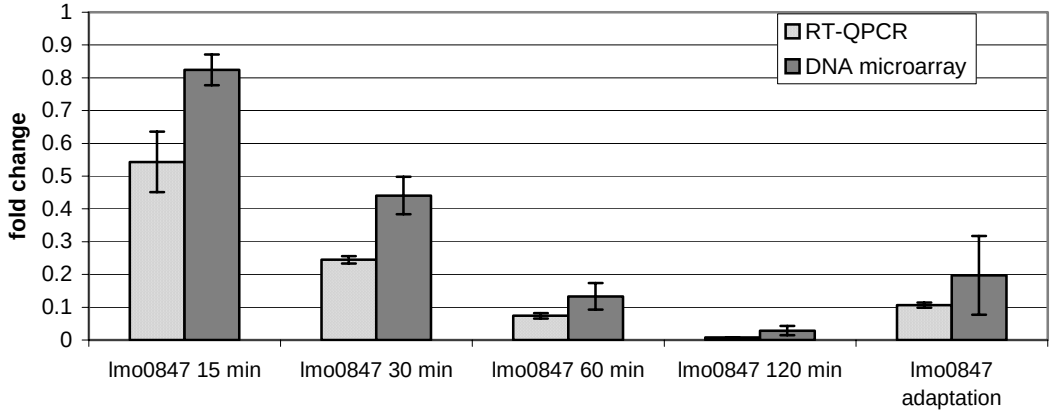
lmo0679 adaptation


RT-QPCR
DNA microarray
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lmo0847 120 min
lmo0847 adaptation

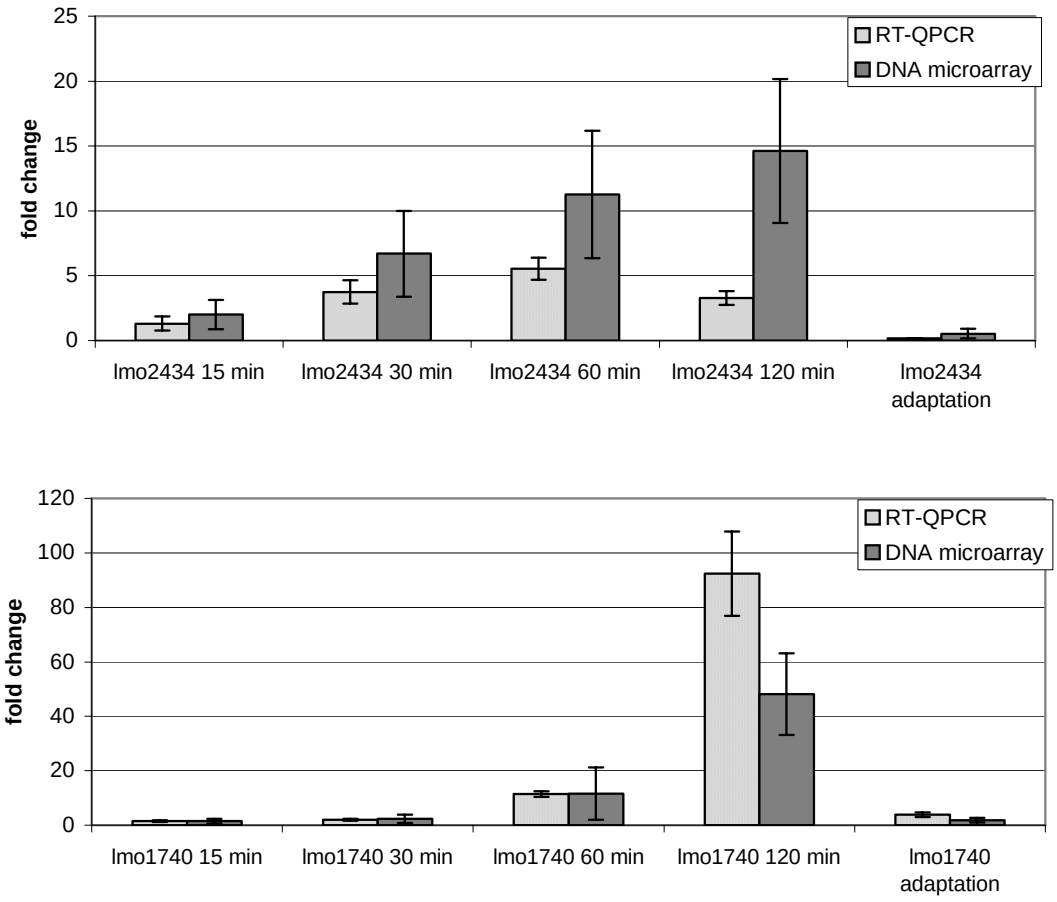
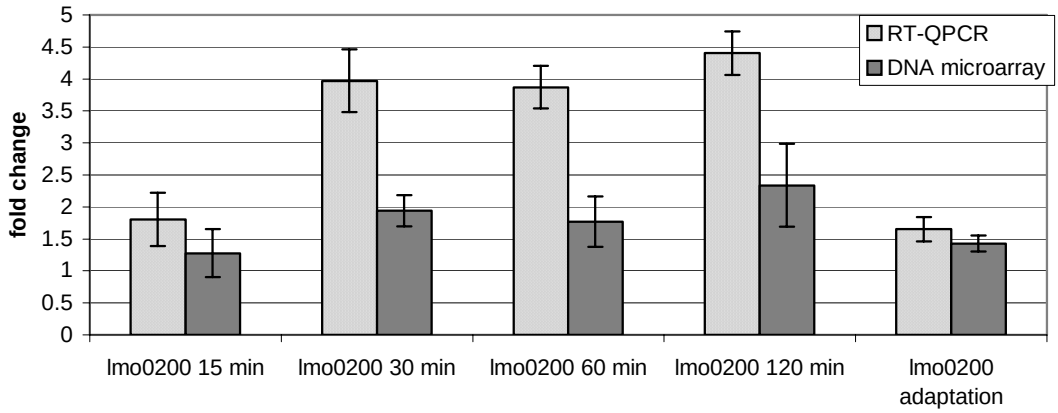


Fig. 3-11: Validation of the results of the DNA microarray hybridization. The relative gene expression levels of a selected set of genes (see text) from *L. monocytogenes* measured by real time quantitative RT-PCR was compared with the results derived from DNA microarray hybridizations. The data were determined with total RNA from cells grown at neutral and at acidic pH in BHI bouillon at 25°C. The values are means of two independent biologically experiment, standard deviations are indicated.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DNA microarray
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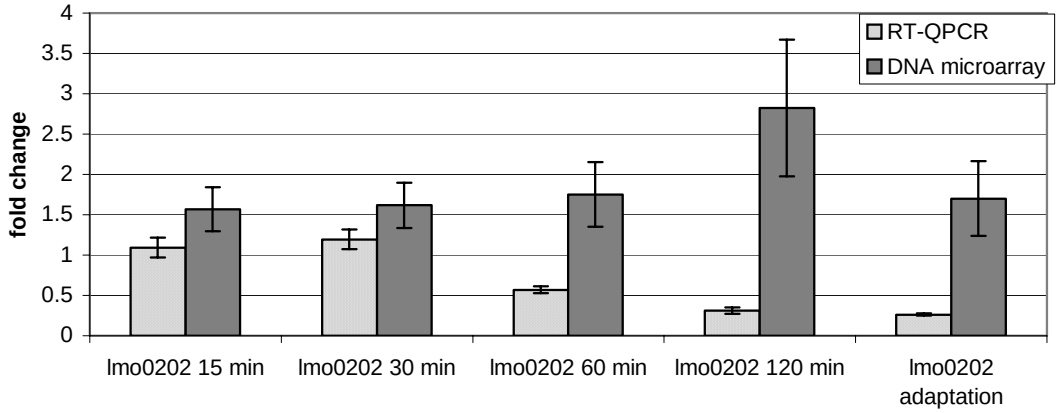
lmo0200 120 min
lmo0200 adaptation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RT-QPCR
DNA microarray
lmo0202 15 min
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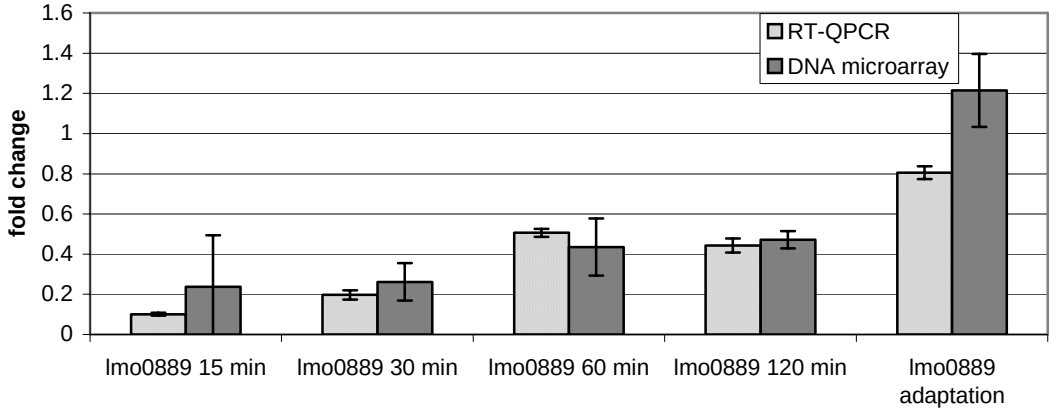
lmo0202 60 min
lmo0202 120 min
lmo0202 adaptation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RT-QPCR
DNA microarray
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lmo0889 30 min
lmo0889 60 min
lmo0889 120 min
lmo0889 adaptation

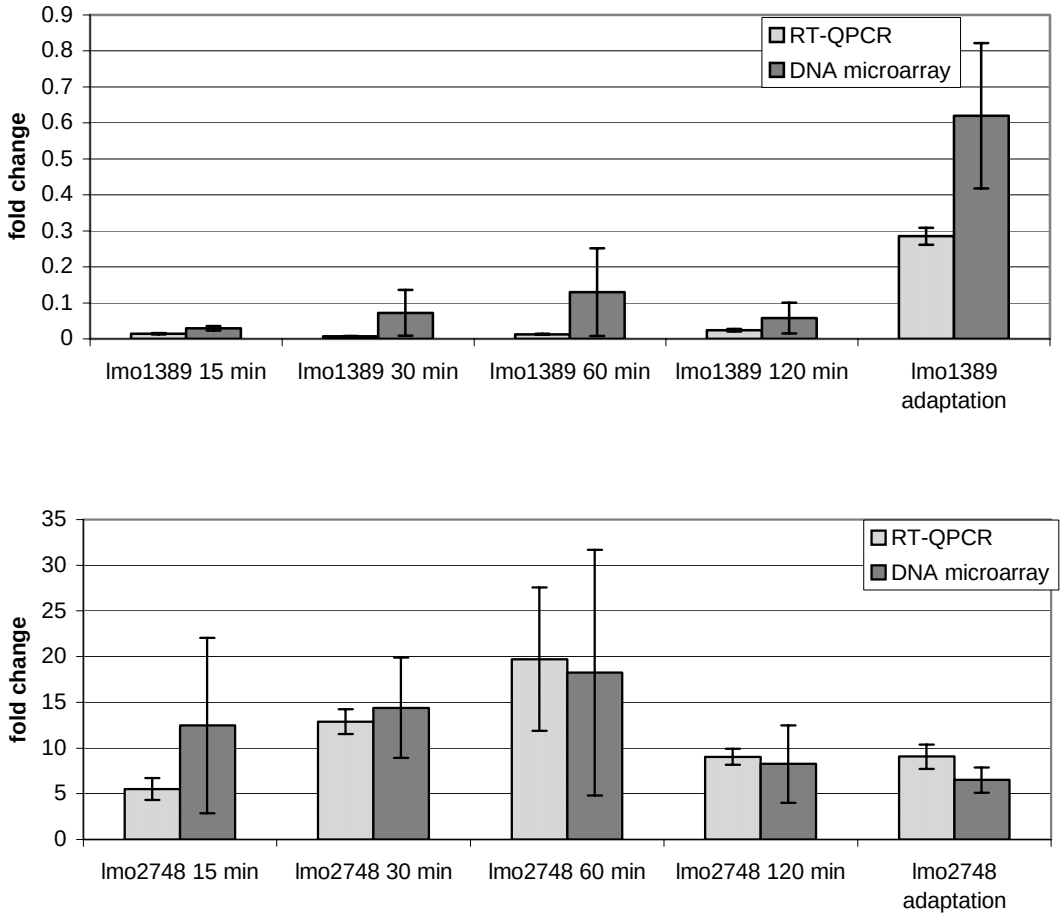


Fig. 3-12: Validation of DNA microarray measurements by real-time RT-PCR. Exponentially phase cells of *L. monocytogenes* EGD-e, grown in BHI media at 37°C were exposed to sublethal acid stress. Probes were taken after acid shock at 0 (control), 15, 30, 60, 120 minutes and after adaptation. Five selected genes predicted by microarray analysis to be differentially expressed were chosen for confirmation of the DNA microarray analysis. Each gene tested by real time RT-PCR was measured in duplicate for each of two independent biologically experiments. Data are presented as the mean change of expression ± standard deviation for each gene between acid and normal condition.

3.2.8 Analysis of the DNA-microarray experiments of *Listeria monocytogenes*

The aim of this work was to identify the specific acid stress response of *L. monocytogenes*. The results of the DNA microarray analyses after acid shock and adaptation suggested the importance of the acid induction of the listerial virulence. The acidification of the medium causes the changes of many of the known virulence genes of *L. monocytogenes*.

I wanted to distinguish clearly between the acid adaptation response and the virulence gene expression. For that reason I compared the effect of a well-documented signal for the virulence gene induction (temperature) with the effect of the acid challenge.

The Venn diagram, shown in Figure 3-13, gives an overview of the induced genes in the array experiments after acid treatment at 25°C and 37°C, and after temperature shift. Analysis of overlapping genes on a Venn diagram can help to determine the cluster of genes which are involved primarily in the listerial stress response. The induced genes are classified into seven groups based on their expression pattern: (**1**) genes induced only after acid treatment at 37°C; (**2**) genes induced only after acid treatment at 25°C; (**3**) genes induced only in the temperature shift experiment; (**4**) genes induced in both, the temperature shift experiment and after acid treatment at 37°C; (**5**) genes induced only after acid treatment at 25°C or 37°C; (**6**) genes induced in both, the temperature shift and after acid treatment at 25°C; and (**7**) genes induced in all experiments (Figure 3-13).

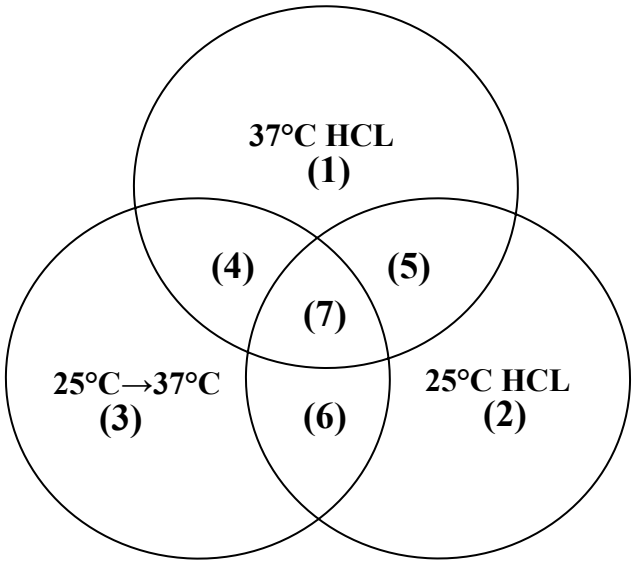


Fig. 3-13: Venn diagram analysis of genes with substantial altered expression rate in the DNA microarray experiments of *L. monocytogenes*. Compare to text to grouping details.

A time course of acid and/or temperature-inducible gene expression was constructed in Supplement Table S-5 to S-11. Only genes for which exhibited a substantial change at transcriptional level as a result of acid treatment and/or temperature shift are listed. Genes with substantial expressional changes are the genes, which exhibited a signal intensities more than 3-fold above local background level, plus their expression ratios were higher than 2 at least at one time point in the time series, and their time series possessed more than 3 valid ratio values.

The Venn diagram analysis shows that acid shock and adaptation response share some of the cellular response with of the temperature shift expression. 164 genes (group 1) were induced only at 37°C after acid treatment, indicating that the expression of these genes is

acid dependent but only at higher temperature. The induction of 143 genes takes place only at 25°C following acidification (group 2). Onehundred twentyfive genes were induced only after a temperature shift, mimicking the internalization of this pathogen (group 3).
In the other cases (group 4 and 6), genes were induced under both stress conditions (acid stress and temperature shift), suggesting that the expressions of these acid induced genes are also temperature-dependent. The genes in the group 5 were induced only by acid stress; the expression of these genes in our experiment seems to be temperature-independent. The genes belonging to group 7 were induced in response to all stressors used in these experiments. The quantitative tabulation of the significant differences in the gene expression under acid stress and temperature shift conditions is presented in Table 3-7.

Tab.3-7: Characterization of the groups based on the Venn diagram.

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**
Sum of the regulated genes	255	767	165	4	371	120	20
Up-regulated genes	164	143	125	4	187	20	15
Down-regulated genes	91	624	40	0	184	100	5
Genes without homology	45%	45%	34%	0%	50%	36%	50%

3.2.9 Kinetics of gene expression changes after a pH stress and adaptation

In contrast to the set of experiments described in other studies, in which gene expression was analyzed in cells grown at neutral pH and shocked to strong acidic pH, our experiments were performed to detect both the short time acid shock response and the smooth transition into the long term acid adaptation response. Cells growing exponentially under neutral pH condition (pH 7.3) were harvested, and RNA was isolated from one aliquot. Other parallel cultures were shocked to pH 5.0; from these cultures RNA was prepared 15, 30, 60, 120 min after pH drop. After 120 min fresh culture medium at pH 5.2 was inoculated using a 10% volume of the acid-shocked cells (see Figure 3-8 A and B). By using *L. monocytogenes* DNA-microarrays the global gene expression was compared to cultures before the acid shock. Gene expression differences of genes that were i) not less than in three time points reliably detected ii) showed at least two time point expression significantly at least factor 2, were analyzed using a k-means cluster analysis method.
Clustering is a useful exploratory technique for analysis different groups of genes based on their expressions pattern in the investigated time interval (139). This method allows the rapid visualization of the clusters, and differentiation between early phase acid shock genes transiently expressed and gene with putative function in the SOS response, or with late phase expressed pH adaptation genes.

Eisen *et al.* (38) found, that genes of similar function cluster together. However, other studies query this principle. Clare *et al.* (22) reported, that the clusters found in microarray data do not in general agree with functional annotation classes. Although many statistically significant relationships could be found, the majority of clusters were not related to known biology function.

3.2.10 Kinetics of gene expression changes after a pH stress and adaptation at 25°C

To identify the patterns of the gene transcription in response to the acid shock and adaptation at 25°C, k-means cluster analysis was performed on those genes that were regulated more than twofold (up or down), (Figure 3-14 A and B).

Cluster 25UP-1 (Figure 3-14 A) contains 104 genes, which showed slowly increased expression and exhibited high expression 60 or 120 min after acidic induction. After adaptation, the RNA levels decreased to the same levels as before the pH drop. The table 3-8 represent the genes belonging to the cluster 25UP-1. The most highly induced genes after acid shock, as determined by microarray analysis, are coding for the putative glutamine ABC transporter (lmo1738 and lmo1740). Interestingly, the expression part of a chromosomal locus encoding a glutamate synthase increased in a similar manner. In addition, cluster 25UP-1 comprises other induced transport proteins presumably involved in drug-, cation-, and heavy metal-efflux system (lmo2741, lmo2575, lmo0641). Furthermore three other genes thioredoxin (lmo2830), glutathione peroxidase (lmo0983), and glutathione reductase (lmo0906), putatively related to the oxidative stress response, showed increased RNA levels after the pH drop. Two of these genes are regulators and are putatively involved in the regulation of the iron transport (lmo1683) and in the SOS stress response regulation (lmo1302). Besides, these genes of a predicted general stress response (lmo0211) and toxic ion resistance gene (lmo1967) belong to the cluster 25UP-1 (Table 3-8).

Tab 3-8: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of up-regulated genes in the cluster 25UP-1 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Transport/binding proteins and lipoproteins		1.89	5.38	19.73	1.84	lmo1738	similar to amino acid ABC transporter (binding protein)
	1.61	2.20	10.75	46.01	1.85	lmo1740	similar to amino acid (glutamine) ABC transporter, permease protein
	1.76	2.71	4.54	4.99		lmo2741	similar to drug-efflux transporters
	5.70	11.06	12.57	10.63	4.89	lmo2575	similar to cation transport protein (efflux)
	2.50	5.87	6.25	4.86	2.35	lmo0641	similar to heavy metal-transporting ATPase

Membrane bioenergetics	1.65	2.61	3.60	5.10		lmo2830	similar to thioredoxin
Metabolism of amino acids and related molecules	1.66	1.97	5.26			lmo1734	similar to glutamate synthase (large subunit)
Regulation		2.65	3.49	5.41		lmo1683	similar to transcription regulators (Fur family), PerR in *B. subtilis*
	1.82	2.84	3.53	4.96		lmo1302	highly similar to SOS response regulator *lexA*, transcription repressor protein
Adaptation to atypical conditions		1.55	2.51	6.41	1.95	lmo0983	similar to glutathione peroxidase
	1.74	2.24	2.63	2.41	1.60	lmo0906	similar to glutathione Reductase
	2.71	5.26	7.05	9.09	1.98	lmo0211	similar to *B. subtilis* general stress protein
Detoxification	1.81	2.75	5.10	10.35	1.47	lmo1967	similar to toxic ion resistance proteins

Cluster 25UP-2 (Figure 3-14 A) includes 123 genes whose expression was transiently increased after the acid shock, but after the acidic pH adaptation their expression showed either the same level or a decreased level as before the acid shock (Table 3-9). Five of these genes belong to the cell surface proteins, *inlE* (lmo0264) and *inlA* (lmo0433) with well-documented function in the listerial virulence, also other three cell surface protein (lmo0610, lmo0880, lmo2085) showed the same expression pattern in the acid adaptation experiment. The up-regulation of the predicted glutamate decarboxylase in response to the acid induction suggests its involvement in a hypothetical glutamate decarboxylase system. In addition, cluster 25UP-2 included genes presumably involved DNA repair (lmo0233), and in the stress response of *L. monocytogenes*, including ClpC endopeptidase (lmo0232), a putative heat shock protein (lmo0963), glutathione reductase (lmo1433), and one stress protein with similarity to YdaG (lmo2748). Furthermore, three genes with putative function in the listerial detoxification belong to this group; the bile acid dehydratase (lmo0754), the conjugated bile acid hydrolase (lmo2067) and the arsenate reductase (lmo2230). One of these genes (lmo2607) is recently recognized as virulence factor too (37).

Tab. 3-9: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of up-regulated genes in the cluster 25UP-2 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene.	Function
Cell surface proteins	1.90	5.71	14.41	9.54	0.52	lmo0264	*InlE,* internalin E
		4.59	6.55	4.45		lmo0433	*InlA,* Internalin A
	1.50	3.71	6.35	5.68	0.39	lmo0610	similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)
	2.41	6.88	9.09	8.78		lmo0880	similar to wall associated protein precursor (LPXTG motif)
	1.70	6.32	9.41	7.31	0.66	lmo2085	putative peptidoglycan bound

							protein (LPXTG motif)
Metabolism of amino acids and related molecules		5.74	10.09	11.12	0.39	lmo2434	highly similar to glutamate decarboxylases
DNA restriction/ modification and repair		2.09	2.02	1.71	0.46	lmo0233	similar to DNA repair protein Sms
Adaptation to atypical conditions	1.81	2.94	5.91	5.83	0.77	lmo0232	*clpC* endopeptidase Clp ATP-binding chain C
	2.07	2.98	3.47	3.08		lmo0963	similar to putative heat shock protein HtpX, *Listeria* epitope LemB
	2.68	7.16	9.87	7.89		lmo1433	similar to glutathione reductase
	2.48	7.90	11.88	8.17	0.69	lmo2748	similar to *B. subtilis* stress protein YdaG
Detoxification	1.85	2.01	1.87	1.52		lmo0754	weakly similar to a bile acid 7-alpha dehydratase
	3.08	7.05	9.10	6.98	0.44	lmo2067	similar to conjugated bile acid hydrolase
	3.47	5.74	7.20	8.95		lmo2230	similar to arsenate reductase
		4.00	4.69	3.46	0.77	Lmo2231	similar to cation efflux system

Cluster 25UP-3 (Figure 3-14 A) contained 57 genes, which showed 15 min after the acid shock the same (or slightly decreased) expression as preinduction levels, but increase rapidly afterwards. Most of these genes were down-regulated or not significantly changed with acid adaptation. Two of these genes (lmo1852 and lmo1853) are a part of an operon with putative function in heavy metal transport. In addition, cluster 25UP-3 comprises two genes *inlB* (lmo0434) and lmo1653 coding for cell surface proteins. Furthermore genes encoding Clp protease (lmo1138) and DNA repair protein (lmo1975) belong this cluster (Table 3-10).

Tab. 3-10: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of up-regulated genes in the cluster 25UP-3 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Transport/binding proteins and lipoproteins		1.89	9.09	4.45		lmo1852	similar to putative mercuric ion binding proteins
	0.87	2.89	8.06	5.33		lmo1853	similar to heavy metal-transporting ATPases
Cell surface proteins		3.26	10.94		0.67	lmo0434	*inlB* Internalin B
		2.41	5.78	6.20		lmo1653	putative cellsurface protein
Adaptation to atypical conditions		1.83	3.35	4.48		lmo1138	similar to ATP-dependent Clp protease proteolytic component
DNA restriction/ modification and repair	0.71	1.07	1.82	2.09		lmo1975	similar to *E. coli* DNA-damage-inducible protein *dinP*

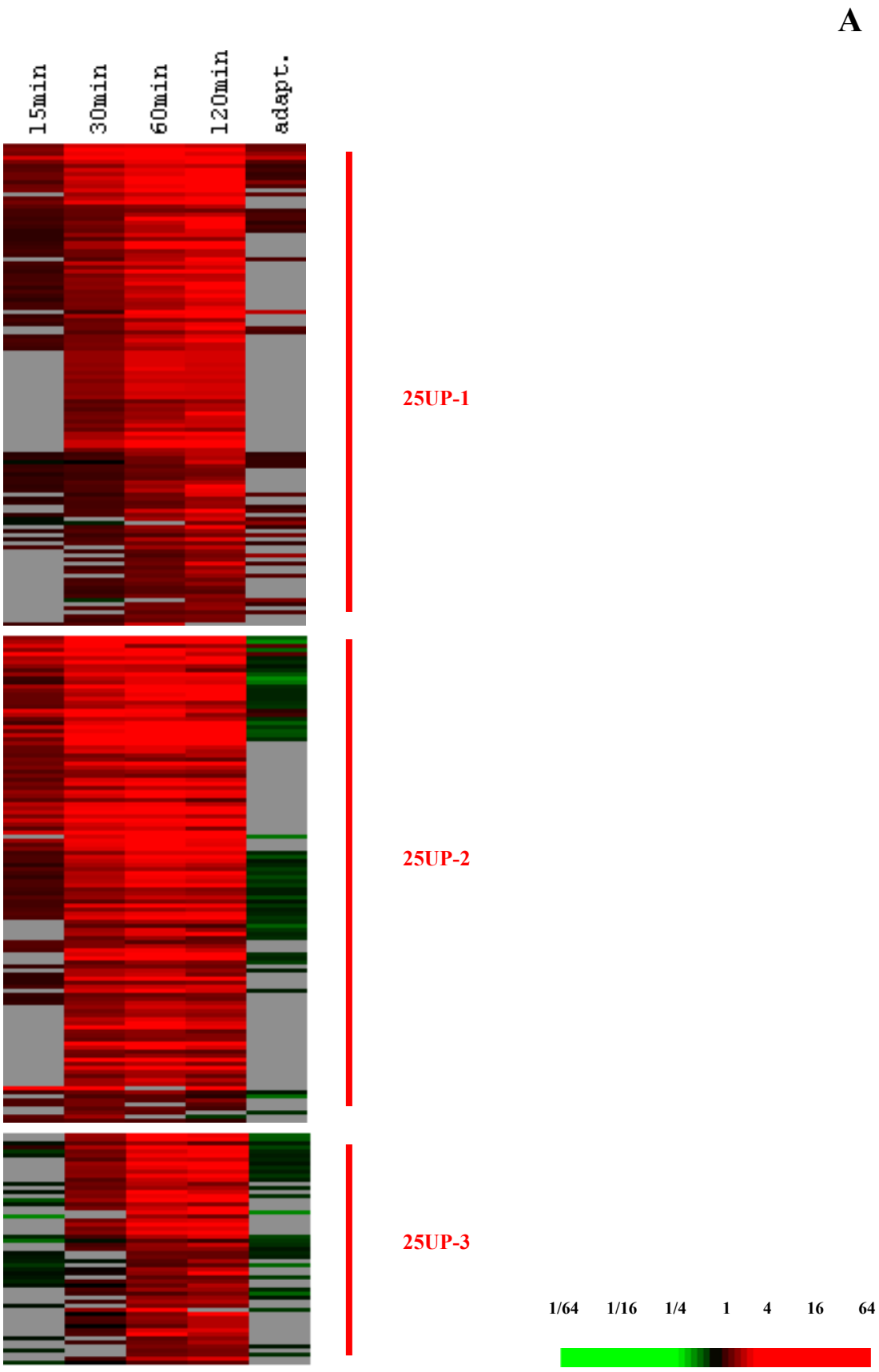
Cluster 25DOWN-4 (Fig. 3-14 B) contained 139 genes whose expression rapidly decreased after the onset of acidification and continued low levels up to the acid adaptation. The gene expression in this group after acid adaptation showed only a weak repression. The cluster

25DOWN-4 contained *fliP* (lmo0676) coding for the flagellar biosynthesis and CheR (lmo 0683), a chemotactic methyltransferase. CheY is a response regulator involved in the chemotaxis of *L. monocytogenes*. Besides of these genes, AgrC (lmo0050), the histidine kinase part of a two component system, and RsbR (lmo0889), a positive regulator of sigma-B activity, belong to cluster 25DOWN-4 (Table 3-11).

Tab. 3-11: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 25DOWN-4 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Sensors (signal transduction)	0.19		0.06		0.44	lmo0050	similar to sensor histidine kinase (AgrC from *Staphylococcus*)
Mobility and chemotaxis	0.35	0.22	0.13	0.21		lmo0676	similar to flagellar biosynthesic protein FliP
	0.33	0.21	0.18	0.21	0.74	lmo0683	similar to chemotactic methyltransferase CheR
Regulation	0.75	0.48	0.42	0.37	0.71	lmo0691	Chemotaxis response regulator CheY
Adaptation to atypical conditions	0.57	0.28	0.17	0.25		lmo0889	RsbR, similar to positive regulator of sigma-B activity

Fig. 3-14 A and **B**: (Overleaf) Cluster analysis of gene expression patterns after acidic treatment at 25°C. K-means clustering was applied for 987 genes after acidification of the medium. A) K-means cluster analysis up-regulated genes after acid treatment, cluster 25UP-1 to 3. B) K-means cluster analysis down-regulated genes after acid treatment cluster 25DOWN-4 to 8.The microarray experiments included comparing gene expression of *L. monocytogenes* before and 15, 30, 60, 120 min after pH drop, the last sample represent the acid adapted cells. The red (up-regulated) and green (down-regulated) bars with labels (1 to 8) refer to the identified clusters of genes. The scale bar indicates the color-coding of the relative RNA levels. The gray color shows the not determinable gene expression ratios.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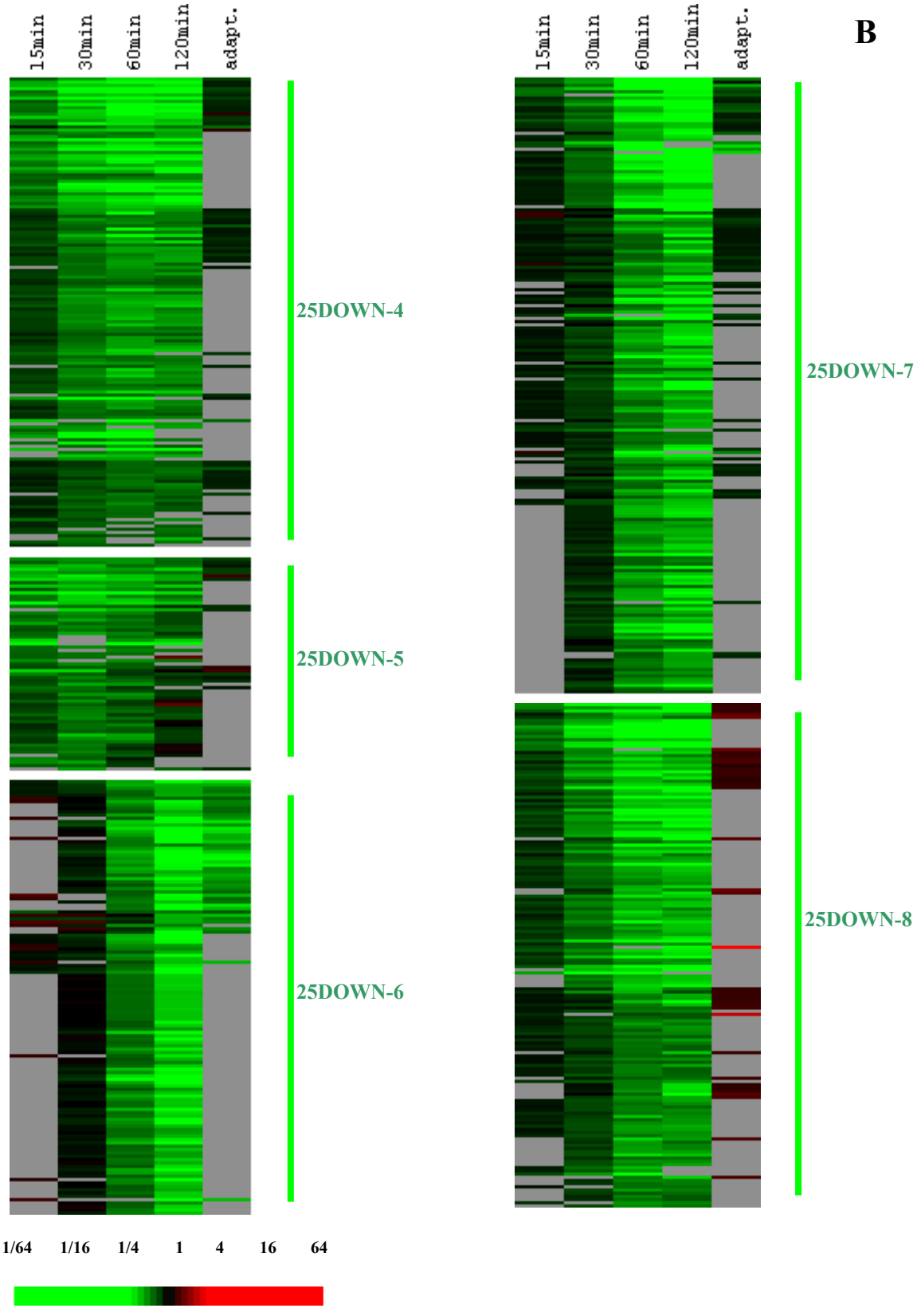
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64

Cluster 25DOWN-5 (Figure 3-14 B) comprised 62 genes that showed a decreased expression at early stage of adaptation, but 120 min after the acid induction the expression achieved the same level as before the pH drop. Two of these genes (lmo0841 and lmo2062) are presumably involved in the cation transport. In addition AgrB (lmo0048), the histidine kinase of a putative two-component system belong to this cluster (Table 3-12).

Tab. 3-12: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 25DOWN-5 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Sensors (signal transduction)	0.31	0.29	0.30	0.41	0.56	lmo0048	similar to *Staphylococcus* two-component sensor histidine kinase AgrB
Transport/binding proteins and lipoproteins	0.48	0.42	0.61			lmo0841	similar to cation (calcium) transporting ATPase
	0.49	0.32	0.47			lmo2062	similar to copper export proteins

Cluster 25DOWN-6 (Figure 3-14 B) included 118 genes that showed weak expression in early stage, than rapid decrease in the expression rate and exhibited further low expression levels until the pH adaptation of the cells. Most of these genes code for proteins involved in the mobility and chemotaxis regulons (lmo0685-lmo0688, lmo0685, lmo0689, lmo0692, lmo0693, lmo0695, lmo0699, lmo0700-lmo0713, lmo0715-lmo0718, lmo0723). Furthermore two genes, encoding a putative glutamine transporter (lmo0847 and lmo0848), and lmo1057, encoding a putative teichoic acid translocation involved in the cell wall biosynthesis, showed a similar expressions pattern (Table 3-13).

Tab. 3-13: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 25DOWN-6 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Cell wall		0.63	0.09	0.02		lmo1075	similar to teichoic acid translocation ATP-binding protein TagH (ABC transporter)
Transport/binding proteins and lipoproteins	0.82	0.45		0.02	0.17	lmo0847	similar to Glutamine ABC transporter (binding and transport protein)
		0.62	0.22	0.06	0.27	lmo0848	similar to amino acid ABC transporter, ATP-binding protein
Mobility and chemotaxis		0.90	0.28	0.14		lmo0685	similar to motility protein (flagellar motor rotation) MotA
		0.96	0.35	0.23		lmo0686	similar to motility protein (flagellar motor rotation) MotB
	1.45		0.48	0.12	0.29	lmo0687	Unknown
		1.61	0.71	0.20	0.31	lmo0688	similar to unknown protein
	1.51	1.58	0.99	0.23	0.21	lmo0689	similar to CheA activity-modulating chemotaxis protein CheV

	0.80	0.48	0.26	0.12	0.15	lmo0692	*cheA,* two-component sensor histidine kinase CheA
		0.59	0.19	0.08	0.15	lmo0693	similar to flagellar motor switch protein FliY C-terminal part
		0.52	0.21	0.21	0.30	lmo0695	Unknown
		0.90	0.16	0.07	0.15	lmo0699	similar to flagellar switch protein FliM
		1.06	0.23	0.12	0.17	lmo0700	similar to flagellar motor switch protein FliY
		1.15	0.27	0.06	0.24	lmo0701	Unknown
	1.55		0.35	0.12	0.24	lmo0702	Unknown
			0.41	0.07	0.15	lmo0703	Unknown
		1.13	0.39	0.12	0.19	lmo0704	Unknown
		0.81	0.38	0.37	0.34	lmo0705	similar to flagellar hook-associated protein FlgK
		0.74	0.30	0.14	0.29	lmo0706	similar to flagellar hook-associated protein 3 FlgL
		0.58	0.22	0.09	0.18	lmo0707	similar to flagellar hook-associated protein 2 FliD
		0.71	0.25	0.05	0.13	lmo0708	similar to hypothetical flagellar protein
		0.93	0.29	0.06	0.12	lmo0709	Unknown
		0.85	0.25	0.08	0.18	lmo0710	similar to flagellar basal-body rod protein FligB
	1.53		0.47	0.15		lmo0711	similar to flagellar basal-body rod protein FlgC
			0.38	0.12	0.21	lmo0712	similar to flagellar hook-basal body complex protein FliE
		0.87	0.32	0.14	0.13	lmo0713	similar to flagellar basal-body M-ring protein FliF
		0.79	0.25	0.13	0.32	lmo0715	Unknown
		0.77	0.27	0.24	0.29	lmo0716	similar to H+-transporting ATP synthase alpha chain FliI, flagellar-specific, -
	0.84	0.61	0.24	0.24	0.25	lmo0717	similar to transglycosylase
	0.78	0.66	0.31	0.26	0.35	lmo0718	Unknown
	0.66	0.60	0.64	0.24	0.37	lmo0723	similar to metyl-accepting chemotaxis protein

Cluster 25DOWN-7 (Figure 3-14 B) comprised 197 genes, which showed transiently decreased expression after the acidification of the medium, but after the acid adaptation the RNA levels reached the same level as before the acidification or they showed slightly decreased expression. The cluster 25DOWN-7 contained 7 genes (lmo0679, lmo0681, lmo0684, lmo0696-lmo0698, lmo0714) encoding proteins with putative function in flagellar biosynthesis or in the motility. Additionally, the genes *inlH* (lmo0263) and lmo0550 encoding a putative cell surface proteins and SigH RNA polymerase sigma-30 factor, respectively, showed short-term expression decreases after the acid challenge (Table 3-14).

Tab. 3-14: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 25DOWN-7 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Mobility and chemotaxis	0.57	0.19	0.03		0.31	lmo0679	similar to flagellar biosynthetic protein FlhB
	0.49	0.27	0.05	0.04	0.35	lmo0681	similar to flagellar biosynthesis protein FlhF
	0.72	0.55	0.12	0.04		lmo0684	Unknown
	0.58	0.33	0.09		0.16	lmo0696	similar to flagellar hook assembly protein
	0.87	0.54	0.15	0.09		lmo0697	similar to flagellar hook protein FlgE
		0.68	0.12	0.04	0.29	lmo0698	weakly similar to flagellar switch protein
		0.70	0.28	0.17		lmo0714	similar to flagellar motor switch protein FliG
Cell surface proteins	1.58	0.72	0.42	0.40	0.83	lmo0263	*inlH* internalin H
		0.71	0.35	0.20		lmo0550	pepdidoglycan bound protein (LPXTG motif)
Initiation		0.83	0.27	0.24		lmo0243	SigH RNA polymerase sigma-30 factor (sigma-H)

Cluster 25DOWN-8 (Figure 3-14 B) contained 158 genes whose expression were slowly decreasing and 60 to 120 min after the acid treatment they showed a minimum value, but after acid adaptation they exhibited a weak increased expression. Four of these genes (lmo0394, lmo1080, lmo1216 and lmo2522) code for cell wall related proteins, one gene (lmo2430) for a putative ferrichrome ABC transporter, and further two genes (lmo0678 and lmo0680) encode flagellar biosynthetic proteins. In addition, cluster 25DOWN-8 comprised genes (lmo0327 and lmo2504) coding for cell surface proteins, *agrA* (lmo0051) which codes for a putative response regulator, and *rsbS* (lmo0890), coding for a protein involved in the regulation of sigma-B activity. The data are summarized in Table 3-15.

Tab. 3-15: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 25DOWN-8 with putative function in the acid stress response, at 25°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Cell wall	0.32	0.10	0.03	0.02	1.57	lmo0394	similar to *L. monocytogenes* extracellular P60 protein
	0.72	0.33	0.22	0.22		lmo1080	similar to *B. subtilis* minor teichoic acids biosynthesis protein GgaB
	0.27	0.11	0.04	0.03		lmo1216	similar to N-acetylmuramoyl-L-alanine amidase (autolysin)
	0.58	0.43		0.12	14.42	lmo2522	similar to hypothetical cell wall binding protein from *B. subtilis*
Transport/binding proteins and lipoproteins	0.57	0.48	0.33	0.22		lmo2430	similar to *B. subtilis* ferrichrome ABC transporter (permease) FhuG
Mobility and chemotaxis	0.60	0.22	0.06	0.06		lmo0678	similar to flagellar biosynthetic protein

							FliR
	0.26	0.10	0.01			lmo0680	similar to flagella-associated protein FlhA
Cell surface proteins	0.62	0.40	0.33	0.28		lmo0327	similar to cell surface proteins (LPXTG motif)
	0.62	0.32	0.18	0.19	1.49	lmo2504	similar to cell wall binding proteins
Regulation	0.71		0.56	0.49	4.99	lmo0051	similar to 2-components response regulator protein (AgrA from *Staphylococcus*)
Adaptation to atypical conditions		0.54	0.28	0.28		lmo0890	*rsbS* highly similar to negative regulation of sigma-B activity

3.2.11 Kinetics of gene expression changes during pH stress and adaptation at 37°C

In the case of acid shock and adaptation experiment at 37°C we used, similarly to the 25°C experiment, k-means clustering to explore the differential expression as a function of time after the acidification of the medium. Five kinetic categories were determined and diagrammed in Figure 3-15.

Cluster 37UP-1 includes 129 genes, which showed increased expression at early stage after acid shock, but after 120 min the RNA levels slowly reached the same levels as before the shock (Figure 3-15 A). Two of these genes lmo2575 and lmo2741 encode transport proteins, involved in cation efflux and in drug efflux, respectively. Seven cell surface proteins belonging to this cluster, *inlE* (lmo0264), *inlA* (lmo0433) and *inlB* (lmo0434) are known function to have in listerial virulence, other three genes (lmo0610, lmo0880, lmo1653, lmo2085) code for cell wall associated proteins with unknown function. In addition, cluster 37UP-1 comprised genes presumably involved in the general stress response, such as putative *clpC* (lmo0232) and *clpP* (lmo1138) genes encoding a Clp endopeptidase ATP-binding chain C and the proteolytic component of an ATP-dependent Clp protease, lmo0963, lmo1601 and lmo2748 encoding predicted stress proteins (Table 3-16).

Tab. 3-16: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of up-regulated genes in the cluster 37UP-1 with putative function in the acid stress response at 37°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Transport/binding proteins and lipoproteins	5.98	4.29	6.68	3.73	2.28	lmo2575	similar to cation transport protein (efflux)
		2.64	2.07		1.71	lmo2741	similar to drug-efflux transporters
Cell surface proteins	5.74	6.54	4.23	2.19		lmo0264	*inlE,* internalin E
	2.73	2.81	2.43		2.30	lmo0433	*inlA,* Internalin A
	3.84	7.11	3.97			lmo0434	*inlB,* Internalin B
	9.06	5.94	5.43			lmo0610	similar to internalin proteins, putative peptidoglycan bound

							protein (LPXTG motif)
	50.91	18.77	12.47	23.92	17.39	lmo0880	similar to wall associated protein precursor (LPXTG motif)
	19.03	8.34	6.06			lmo1653	putative cellsurface protein
	6.11	5.82	3.92	4.17		lmo2085	putative peptidoglycan bound protein (LPXTG motif)
Metabolism of amino acids and related molecules		6.87	6.02		2.41	lmo1734	similar to glutamate synthase (large subunit)
Adaptation to atypical conditions	2.71	9.58	5.94	2.04	1.79	lmo0232	*clpC,* endopeptidase Clp ATP-binding chain C
	0.73	1.13	1.13	1.05	0.92	lmo0906	similar to glutathione reductase
	6.92	4.53	3.84	5.74	2.11	lmo0963	similar to putative heat shock protein HtpX, *Listeria* epitope LemB
	1.96	4.53	2.79	1.40		lmo1138	similar to ATP-dependent Clp protease proteolytic component
		2.23	2.58	1.88	1.57	lmo1601	similar to general stress protein
	9.51	13.45	13.93	7.21		lmo2748	similar to *B. subtilis* stress protein YdaG

Cluster 37UP-2 represents the largest group including 186 acid induced genes (Figure 3-15 A). The genes in this cluster showed a slow increase in expression after acid induction, but acid adapted samples reached acid shock preinduction mRNA ratio. Three of these genes (lmo1738 to lmo1740) are part of an operon encoding a glutamine transporting ABC transporter system. The genes lmo1609, lmo2424 and lmo2830 are presumably coding for a thioredoxin, represented by three products. Additionally, genes encoding cell surface proteins (lmo2178 and lmo2714), a putative glutamate decarboxylase (lmo2434), a putative SOS response regulator (lmo1302), and a transcription regulator (lmo1683) belonging to the Fur family showed the same transcription pattern. Furthermore, the genes encoding a general stress protein (lmo0211), a putative glutathione peroxidase (lmo0983), toxic ion resistance proteins (lmo1967), and arsenate reductase (lmo2230) were induced in response to the stress conditions (Table 3-17).

Tab. 3-17: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of up-regulated genes in the cluster 37UP-2 with putative function in the acid stress response at 37°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Transport/binding proteins and lipoproteins		2.06	10.06	5.70		lmo1738	similar to amino acid ABC transporter (binding protein)
	1.34	1.88	6.82	3.03		lmo1739	similar to amino acid (glutamine) ABC transporter (ATP-binding protein)
		9.19	49.52	16.22		lmo1740	similar to amino acid (glutamine) ABC transporter, permease protein
Membrane bioenergetics			2.77	2.73	1.65	lmo1609	similar to thioredoxin
			2.27	2.97	1.87	lmo2424	similar to thioredoxin

	2.35	6.59	6.87	6.41	1.54	lmo2830	similar to thioredoxin
Cell surface proteins		2.79	10.13	5.78		lmo2178	putative peptidoglycan bound protein (LPXTG motif)
			3.41	4.14	1.74	lmo2714	peptidoglycan anchored protein (LPXTG motif)
Metabolism of amino acids and related molecules	1.78	2.55	2.38		1.45	lmo2434	highly similar to glutamate decarboxylases
DNA restriction/modification and repair		2.38	2.95	2.66		lmo1975	similar to *E. coli* DNA-damage-inducible protein *dinP*
Regulation	2.01	2.41	3.51	2.22		lmo1302	highly similar to SOS response regulator *lexA*, transcription repressor protein
	1.62	4.00	5.06	4.56	2.33	lmo1683	similar to transcription regulators (Fur family), PerR in *B. subtilis*
Adaptation to atypical conditions	1.75	3.51	3.23	3.71	1.64	lmo0211	*ctc,* similar to *B. subtilis* general stress protein
		2.81	4.86	3.12	2.27	lmo0983	similar to glutathione peroxidase
Detoxification	1.58	2.27	2.17	1.57		lmo1967	similar to toxic ion resistance proteins
	8.46	16.91	17.75	14.12		lmo2230	similar to arsenate reductase
	6.63	4.14	4.82	3.46		Lmo2231	similar to cation efflux system

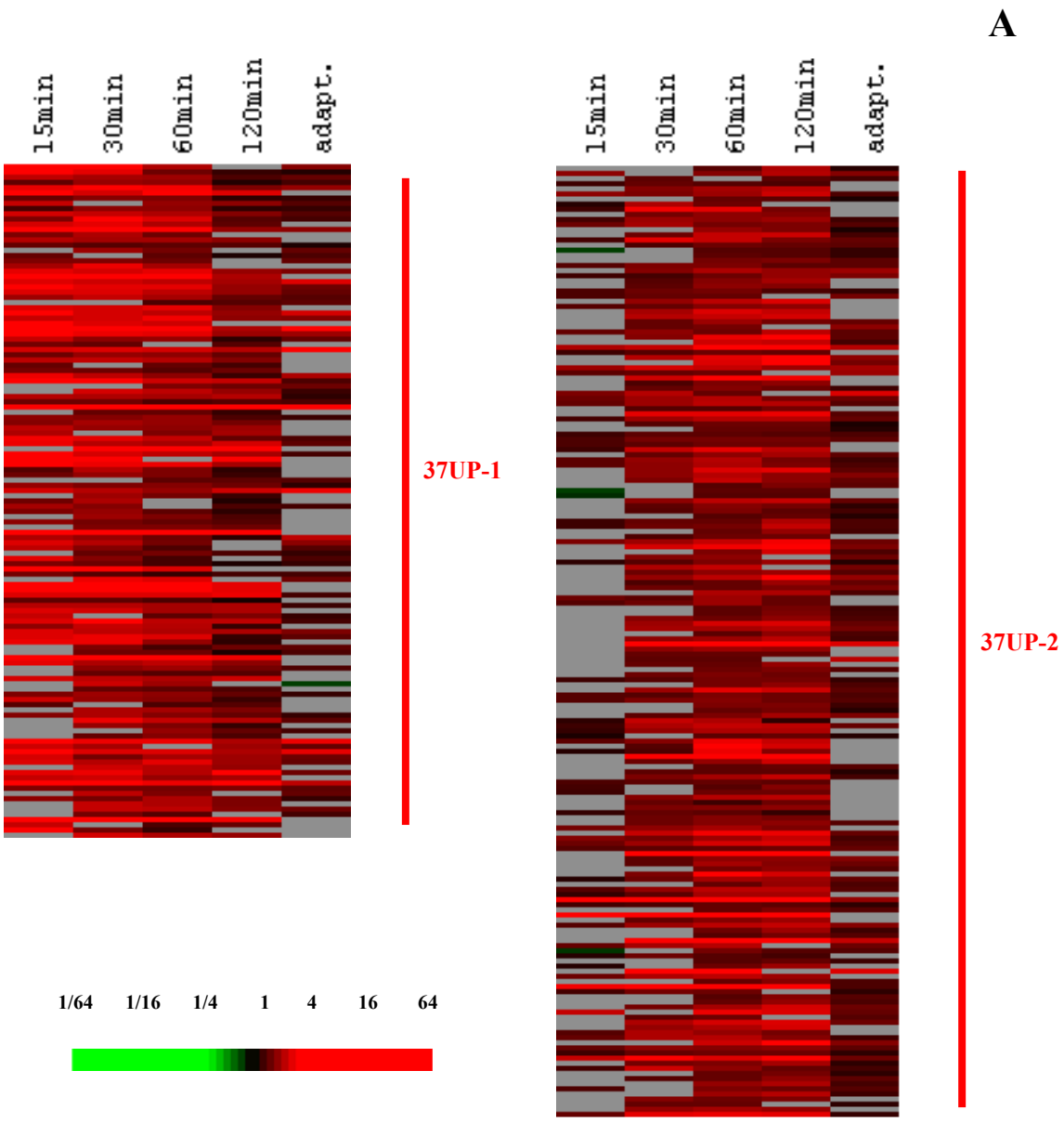
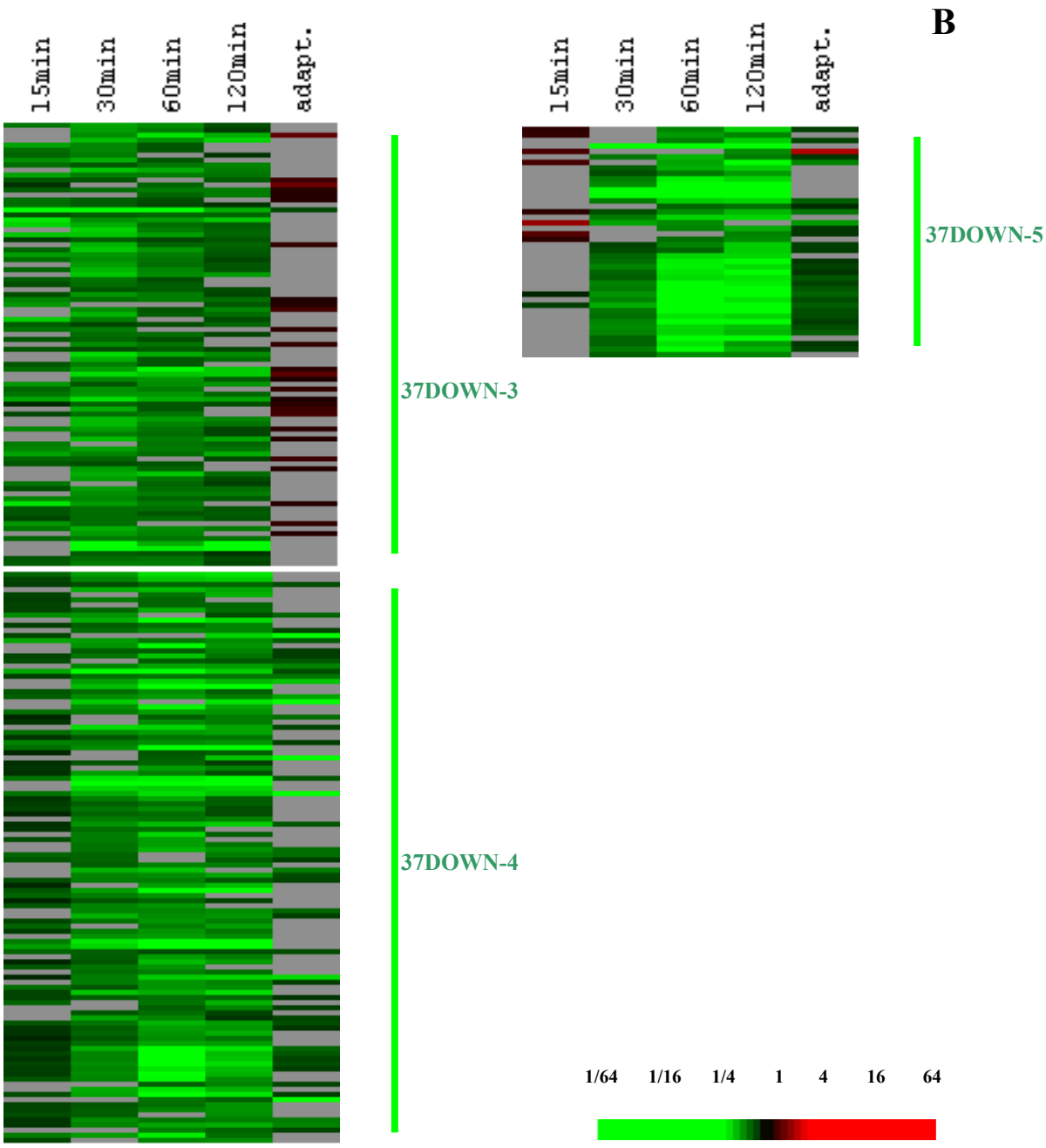


Fig. 3-15 A and **B** (**Fig. 3-15 B** Overleaf): Cluster analysis of gene expression patterns after acidic treatment at 37°C. K-means clustering was applied for 558 genes after acidification of the medium. The microarray experiments included comparing gene expression of *L. monocytogenes* before and 15, 30, 60, 120 min after pH drop, the last sample represent the acid adapted cells.**A**) K-means cluster analysis up-regulated genes after acid treatment, cluster 37UP-1 and 2. **B**) K-means cluster analysis down-regulated genes after acid treatment, cluster 37DOWN-3 to 5. The red (up-regulated) and green (down-regulated) bars with labels (37UP-1 to 37DOWN-5) refer to the identified clusters of genes. The scale bar indicates the color coding of the relative RNA levels. Gray color shows the not determinable gene expression ratios.



Cluster 37DOWN-3 comprises 89 genes showing constantly decreasing RNA level until 120 min after the pH drop, and after acid adaptation these genes demonstrate weak induction or no significant changes in the expression level (Figure 3-15 B). The *iap* gene (lmo0582) encoding P60 extracellular protein involved in the host invasion belongs to this cluster. In addition, the genes encoding a putative cell wall binding protein (lmo2504), and the Sigma-B activity regulators, RsbR (lmo0889), RsbT (lmo0891), RsbU (lmo0892) belong to cluster 37DOWN-3 (Table 3-18).

Tab. 3-18: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 37DOWN-3 with putative function in the acid stress response at 37°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Cell wall	0.45	0.41	0.37	0.36	1.37	lmo0582	*iap,* P60 extracellular protein, invasion associated protein Iap
Cell surface proteins	0.44	0.36	0.38	0.38		lmo2504	similar to cell wall binding proteins
Adaptation to atypical conditions	0.18	0.25	0.42	0.47		lmo0889	RsbR highly similar to positive regulator of sigma-B activity
		0.18	0.37	0.44		lmo0891	RsbT highly similar to positive regulation of sigma-B activity
	0.19	0.19	0.28	0.43		lmo0892	*rsbU,* highly similar to serine phosphatase RsbU

Cluster 37DOWN-4 included 112 genes whose expression was transiently decreased after acid treatment, but the ratio of the mRNAs slowly reached the same levels as before acid shock (Figure 3-15 B). This cluster contains *flgE* (lmo0697), encoding flagellar hook protein, the putative glutamine ABC transporter (lmolmo0847) and lmo2689 presumably codes an Mg2+ transport ATPase (Table 3-19).

Tab. 3-19: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 37DOWN-4 with putative function in the acid stress response at 37°C.

Group	15 min	30 min	60 min	120 min	Adapt.	Gene	Function
Mobility and chemotaxis		0.33	0.25	0.28	0.34	lmo0697	similar to flagellar hook protein FlgE
Transport/binding proteins and lipoproteins		0.18		0.15	0.13	lmo0847	similar to Glutamine ABC transporter (binding and transport protein)
	0.56	0.50	0.40	0.30		lmo2689	highly similar to Mg2+ transport ATPase

Cluster 37DOWN-5 (Figure 3-15 B) included 42 genes that showed after a short initially inductions phase decreased expression, but after the adaptation to the low pH, their RNA ratio were reached the same level as before the acid shock. The genes lmo0394 in this cluster encodes a putative homologue of the extracellular P60 protein (Table 3-20).

T**ab. 3-20**: Average ratio values (mRNA level under acidic conditions/mRNA level under neutral conditions) of down-regulated genes in the cluster 37DOWN-5 with putative function in the acid stress response at 37°C.

Group	15 min	30 min	60 min	120 min	Adapt.	*Lmo* Nr.	Function
Transport/binding proteins and lipoproteins		0.08	0.02	0.02		lmo0394	similar to *L. monocytogenes* extracellular P60 protein

4 Discussion

Adaptation to the changes of the environmental pH is a huge challenge for neutralophile bacteria such as *C. glutamicum* or *L. monocytogenes*. To combat acidic stress, bacteria have complex strategies, which involve different inducible defense systems, called acid tolerance response (ATR), in response to low pH. The acid tolerance response system facilitates bacteria to grow at environmental acidic pH or to transit the human intestinal tract. *C. glutamicum* is able to keep a stable cytoplasmic pH up to a mild acidic outer pH. The ability of *L. monocytogenes* to survive the gastric acid fluid is directly linked to the rapid activation of their acid stress response system. A functional ATR is required for full virulence of *L. monocytogenes* (73).

4.1 Acid adaptation of *Corynebacterium glutamicum*

Acid stress includes the combined effect of low pH caused by inorganic acids and organic acids present in the environment (7). Uncharged organic acids can passively diffuse across the cell membrane and dissociate in the cytoplasm into negatively charged molecules and protons, which can not permeate the cell membrane, thereby lowering the pH of the cytoplasm (128). The lower the external pH the more undissociated organic acid is available to cross the cellular membrane to affect cytoplasmic pH (7). Maintenance of cytoplasmic pH homeostasis under such conditions is crucial for the life of the bacterial cell. Deviations of more than one pH unit from the optimal cytoplasmic pH cause considerable changes in cellular functions, damaging enzymes and DNA integrity (44). Therefore, the cell induces proteins, which aid in the maintenance of pH homeostasis as well as in preventing and repairing damages caused by acid stress. We have shown that *C. glutamicum* can maintain its cytoplasmic pH in a relatively broad range of external pH and now suggest potential mechanisms for this ability.

4.1.1 Induction of cation and multidrug transport systems

Bacteria utilize various mechanisms to maintain pH homeostasis within the cell. One of the well-described mechanisms is based on the F_1F_0 ATPase which either produces ATP using the transmembrane proton gradient through proton influx (acidification of the cytoplasms) or expels protons from the cell using the energy provided by ATP hydrolysis (29). In aerobic organisms such as in *E. coli* and *B. subtilis*, the F_1F_0 ATPase mainly functions in ATP synthesis (89). In organisms without a respiratory chain such as *L. lactis* (70), *E. hirae* (69), *S. mutans*, *S. sanquis* (10) and *L. acidophilus* (76) the F_1F_0 ATPase system plays an important

role in maintaining cytoplasmic pH homeostasis in acid adaptation via proton extrusion (47, 64). However, there are disadvantages in using this system for internal pH homeostasis due to its high energy cost and there is evidence suggesting the presence of other systems in bacterial species lacking a respiratory chain (11). While down-regulation of the F_1F_0 ATPase system in *C. glutamicum* may result in a decrease of acidification of the cytoplasm, it will also decrease ATP synthesis. We therefore believe that its down regulation does not play a major role in cytoplasmic pH homeostasis and may rather be due to a decreased need in ATP synthesis in acid adapted cells which slow down their growth rate.

Among the transport proteins induced through acid adaptation, two cation transport ATPase genes (ORF 851 and 2433) were observed in our study. While it is unknown which cations are transported by these proteins found, cation transport ATPases such as K^+ -or Na^+ -ATPases have been described in various organisms such as in *L. lactis* (66) and *S. mutans* (31) as constituting one of the systems responsible for pH homeostasis by acidifying or alkalinizing cytoplasmic pH through a cation-proton antiport mechanism (12).

Increased expression of a putative multidrug transport system was also observed. Being a soil bacterium *C. glutamicum* encounters a wide variety of cytotoxic compounds in its natural habitat, which lead to the development of various protective mechanisms that can export a wide range of toxic components before these molecules have a chance to damage cellular processes. It is suggested that multidrug efflux system (MDR) are part of the natural defense mechanism of bacteria against toxic compounds, e.g. lipophilic inhibitors, existing in natural environment (100). The broad substrate specificity of these efflux pumps makes them suitable for this defensive role as the bacteria cannot predict the nature of inhibitors it will be confronted with (105). Due to this broad substrate specificity of the MDR efflux pumps we hypothesise that this system could also play a role in pH resistance of *C. glutamicum* by expelling the accumulated anionic form of the dissociated organic acid from the cytoplasm.

4.1.2 Iron transport is induced at low pH in tryptic soy broth

Numerous iron transport genes were induced by acidic pH in our standard growth medium, TSB. These ORFs are organized in 4 putative operons encoding components for different iron Fe^{3+} ABC siderophore transporters. Iron siderophores are extracellular iron chelators secreted by bacteria under iron limiting conditions to help solubilize iron prior to transport (72). According to the literature, at aerobic conditions and neutral pH, iron is present in the insoluble form of Fe^{3+}, whereas low pH and anaerobic conditions increase solubility of iron by reducing it to Fe^{2+} thereby, boosting bioavailability of iron in the medium (57). Our study in complex media (tryptic soy broth, TSB) assessing the iron availability at low pH proved

contradictory to the expected theoretical effect. Expression level of ORF 855 encoding a periplasmic component of an ABC-type cobalamin/Fe^{3+}-siderophores transporter, at pH 5.7 substantially increased in different complex media (BHI, TSB, TSB+Fe) compared to expression levels at neutral pH (Figure 3-6). On the other hand, in defined minimal medium (CGXII) at acidic pH, ORF 855 showed decreased expression level, verifying the theory on pH-dependence of iron bioavailability in the biological experiment. These results suggest an iron sequestering effect of the complex media at acidic pH.

In a second experiment we compared the mRNA level of ORF 855 measured in BHI, CGXII and TSB+Fe to the mRNA level in TSB. In this experiment we observed a decrease in expression level at neutral pH in three media (BHI, CGXII, TSB+Fe) compared to TSB (Figure 3-7). The low mRNA level of ORF 855 in CGXII and TSB+Fe observed in comparison to TSB was possibly due to the relatively high amount of added iron. In BHI, the expression level was moderately lower compared to TSB. BHI could contain more iron resulting from the ingredients present in this medium or does not sequesters iron as well as TSB at low pH. In summary, at neutral pH the iron supplement in TSB media (TSB+Fe) repressed the expression of the iron siderophore transporter gene (ORF 855).

At acidic pH in CGXII the expression level of ORF 855 was substantially lower compared to that in TSB. This result is in accord to the iron excess in CGXII medium at acidic pH. In TSB+Fe medium, despite iron supplement, we observed only a slightly lower mRNA level of ORF 855 compared to TSB medium. In BHI medium we found the same effect as in the iron supplemented TSB medium (TSB+Fe), indicating the possibility, that this effect is presumably coupled generally to the complex components of the rich media. This led us to suggest that the theoretical facts about the pH effect on the bioavailability of iron are true in a defined minimal media, but not in complex media. Therefore, the up regulations of the iron Fe^{3+} ABC siderophore transporters observed in our microarray experiment are surprising and presumably the result of the complex medium which effects iron availability at low pH due to unknown mechanisms.

Regulation of the iron uptake system is an important feature in the survival of bacteria since many enzymatic reactions require iron as a cofactor. Bacteria require a minimum level of 10^{-7} M Fe^{3+} for their survival.

4.1.3 Role of regulatory proteins in acid adaptation

Regulatory proteins play an important role in stress response by directing protein expression in response to environmental fluctuations. We observed induction of six regulatory proteins (ORF 927, 1518, 1642, 1703, 2000 and 3470) during acid adaptation.

SigB (ORF 2000) is strongly induced in *C. glutamicum*. Its role as a general stress protein has been extensively studied in gram-positive organisms under different stress conditions. Null mutations in *B. subtilis* (*sigB)* lead to an increased sensitivity towards low pH, heat and oxidative stress (58). In *L. monocytogenes*, *sigB* has been demonstrated to function in response to several stresses such as oxidative stress, osmotic stress, carbon starvation and growth at low temperatures. E.g., a *sigB* null mutant exhibited a 1,000 to 5,000 fold decrease in survival when exposed to pH 2.5 (138). Studies in *Brevibacterium flavum*, a closely related strain of *C. glutamicum*, showed an effect of *sigB* on growth and viability of cells under acid, salt, alcohol, heat and cold (54). SigB is therefore a general stress response protein and controls transcription of various stress related proteins in Gram-positive bacteria.

ORF 1703 encodes a protein, which belongs to the extracytoplasmic function family (ECF), showing homology to SigE. SigE's role as an alternative sigma factor regulator was demonstrated, e.g., in *Mycobacterium tuberculosis, B. subtilis* and *Pseudomonas aeruginosa* under heat shock and oxidative stress (108). In *M. tuberculosis* a *sigE* disruption mutant exhibited higher sensitivity to various environmental stresses compared to the wild type, but not to acidic pH (78, 86). Our phenotypic studies using the *sigE* disruption mutant also did not support its role in acid stress. We speculate that it is up-regulated by stress factors originating as a consequence of pH stress.

ORF 3470 codes for a protein showing homology to a magnesium dependent transcriptional regulator (MntR). MntR, first characterized in *B. subtilis*, is a member of the DtxR family of metalloregulatory proteins which regulates the expression of metal ion transport systems in response to manganese (82). In vitro studies have shown that DtxR and its relatives respond to various divalent metals such as Fe^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Mn^{2+} which activate the DNA binding function of DtxR and MntR (118-120, 126). DtxR characterized in *Corynebacterium diphtheriae* is a global iron regulator which regulates the expression of iron uptake genes as well as toxin production under iron limited conditions (106). The function of MntR has been studied in various microorganisms demonstrating a wide variety of effects on gene expression. In *B. subtilis* it has been shown that elevated Mn^{2+}levels disturb iron homeostasis leading to an increase in iron availability in the cell. This causes the activation of *sigB* stress response which is depended on the environmental stress sensor RsuB requiring Mn^{2+} as a cofactor (53). In *S. aureus* it has been shown that MntR has an effect on oxidative stress by controlling Mn^{2+} uptake (60). DtxR and MntR differ in their metal ion selectivity as shown by protein sequence comparisons (53). Recently, an MntR homologue has been identified in *C.*

diphtheriae (117). The role of DtxR like proteins in pH stress is unknown but may relate to the regulation of ion transporters of *C. glutamicum*, which are also induced under acid stress
Another regulatory gene that was up-regulated is ORF 1518 encoding the sensory component of the two component histidine kinase system. Two component systems are relatively widespread in bacteria and are reported to assist bacterial cells in the adaptation to various environmental alterations (125). Two component systems are often involved in stress response by directing the synthesis of alternative sigma factors which are required for the synthesis of proteins involved in stress response (92, 94). An increase in the expression of histidine kinase regulators due to stress has been shown in other gram-positive organisms namely in *L. monocytogenes,* but a disruption mutant in the regulatory components of histidine kinase did not reveal differences in growth in presence of stress compared to the wild type strain (65). In *H. pylori* a similar effect was observed in studying the histidine kinase sensory component which demonstrated that deletion of the sensory component has no effect on the cells' function and its role can be overtaken by other genes (136). Similarly, in this study no significant effect was observed at low pH condition on the growth rate of the histidine kinase disruption mutant.

4.1.4 Redundancy of the pH adaptation response of *Corynebacterium glutamicum*

In the majority of the 26 disruption mutants, no significant phenotypical effect at low pH conditions was observed except of the knock-outs of two regulatory proteins SigB and SigE (ORF 2000 and ORF 1703, respectively). This data suggest a considerable redundancy in stress response and, at the same time, demonstrates the complexity of the acid stress response in *C. glutamicum* where the function of the disrupted gene obviously can be overtaken by another gene product. Similar data have been reported for other bacteria, where effects of single insertion mutagenesis on acid stress have been studied. E.g. in studying the acid tolerance response of *S. typhimurium* inactivation of single genes known to contribute to acid stress exhibited only a marginal affect on acid tolerance (111). Other studies of *S. typhimurium* showed that inactivation of at least two or more genes was needed to eliminate acid resistance (26). Similar observations were reported for *B. cereus* where only one acid sensitive single knockout mutant was obtained despite screening 1.7 x 10^8 cells (15). This leads us to conclude that the genes responsible for acid adaptation have widely overlapping functions. Therefore, to obtain acid sensitive mutants, either the disruption of the regulatory protein controlling the expression of multiple genes in the ATR, is needed or more than one gene must be deleted in the genome.

4.2 Acid stress and adaptation of *Listeria monocytogenes*

4.2.1 Use of DNA microarrays to characterize the acidic stress response and the temperature shift in *Listeria monocytogenes*

Most if not all environmental changes act as a signal to modification of the transcriptomics, and thereby changing cellular functions. DNA microarray technology already has been shown to be a useful tool in studying global expression patterns in response to a number of different growth conditions. In this study, we examined the *L. monocytogenes* response to acidic stress. In the experiments we presented, we were able to confirm the induction of several acidic stress genes that were laboriously identified in previous studies. In addition, we identified many new acid-inducible genes. Among the induced putative acid stress response genes, a large set of known and predicted virulence genes shown altered expression after acid shock and adaptation at a temperature of 37°C. As known, in different pathogenic bacteria, including *Salmonella*, *Neisseria*, and *Listeria,* a strong relationship between the synthesis of stressor induced proteins and the manifestation of their virulence exists. The expression of virulence genes greatly depends on environmental conditions, such as high temperature, low pH, high osmolarity, substrate limitation and the amount of reactive oxygen radicals. Many of these factors depend on the extra- or intracellular location of the pathogenic bacteria. In our study we wanted to focus on the acid tolerance response of *L. monocytogenes*, and differentiate the acidic stress response in itself from the virulence gene induction. For this reason we investigated acid shock and adaptation at 25°C and a temperature shift experiment from 25 to 37°C. Furthermore, we analyzed the transcriptional changes both of these experiments using a DNA microarray technology and compared them to acid shock adaptation at 37°C. Thereby, hopefully determining putative acid stress response, general stress response, virulence genes and the overlap of these gene clusters in their regulation.

4.2.2 General observations to the acid shock and adaptation experiments

As can be seen from the results of DNA microarray experiments a large number about 10-20% of the genes have been changed their expression when the cells were exposed to acid. Long time acid adaptation response requires a smaller set of the ATR genes; at this time point the amount of the regulated genes of both of the examined microorganisms (*Corynebacterium* and *Listeria*) are well comparable. At 25°C the listerial acid stress response was delayed about 15-30 minutes compared to the 37°C experiment, presumably due to the slower enzymatic function at lower temperature. About 40% of these genes are without any homology to other genes with known function to date. For the rest of the genes it was possible to deduce or suggest a function, or gene family but in the most cases lacks a rationale reason

for transcriptional changes of these genes in the ATR or in virulence, due to lack of knowledge. The down regulation of many genes involved in metabolic processes was presumably caused by the diminished growth rate following the acid shock.

We do note some limitations of our experiment, for reasons that are unclear; the genes in the *prfA* operon do not give reliable hybridisations results (Torsten Hain, Bijou Joseph, personal communication), therefore for this genes additional RT-PCR analysis was necessary.

4.2.3 Classification of acid-induced gene based on their functions

In the following section, I will try to sort out such induced genes, which perform a possible task in the ATR or in virulence, by analysis of their predicted functions. Such genes can be classified into several groups (according to Pascale Cossart and Philippe Glaser, Institut Pasteur Paris, France): transport/binding proteins and lipoproteins, motility and chemotaxis, cell surface proteins, DNA restriction/modification and repair, regulation, adaptation to atypical conditions and detoxification. However, alternative classifications are also possible.

4.2.3.1 Transport and binding proteins

Three genes (lmo1738, lmo1739 and lmo1740) encoding putative glutamine ABC transporter system showed significant induction after acid shock as determined by microarray analysis. This operon was expressed under acidic condition temperature independent, however, lmo1739 showed only at 37°C significant transcriptional increase. The operon was slowly induced, 60 to 120 minutes after the acid shock the genes lmo1738 and lmo1740 reached approximately 5 and 50-fold induction, respectively. The gene lmo1739 demonstrated a lesser induction level, 3- to 7-fold. After acid adaptation transcription levels of this putative glutamine ABC transporter operon returned to preshock levels.

Interestingly, the genes of another putative glutamine ABC transporter system (lmo0847 and 0848) appeared to be significant repressed. The regulation of those genes was also temperature independent. Real time analysis of the genes lmo0847 and lmo1740 confirmed the expression changes as determined by microarray analysis.

Additionally, two genes involved in the glutamate metabolisms (belong to the functional group of Metabolism of amino acids and related molecules) *gadD* (lmo2434) encoding a glutamate decarboxylase and lmo1734 coding for a putative glutamate synthase are induced.

gadD is part of the listerial glutamate decarboxylase system. This glutamate decarboxylase (GAD) acid resistance system plays a major role in the survival of *L. monocytogenes* under acidic conditions (135). The acid sensitivity of this pathogen was found to be greatly dependent on the free glutamate levels of the media (28). Conte *et al.* (25) identified the following genes belonging to the GAD system: encoding glutamate decarboxylases: *gadA*,

gadB and *gadD,* encoding glutamate/γ-aminobutyrate antiporters: *gadC* and *gadE*. The *gad* genes are located in three loci on the chromosomal DNA, to the first locus belongs to *gadA* (lmo0447*)* and *gadE* (lmo0448), to the second to *gadC* (lmo2362) and *gadB* (lmo2363) and to the third to *gadD* (lmo2434) without any neighbouring antiporter gene.

The significant expression changes of the above mentioned glutamine transporter and several glutamate metabolism genes suggest the existence of an alternative acid tolerance response system in *L. monocytogenes* besides of known GAD system. Mutants of such genes should reveal their roles in the acid resistance of *L. monocytogenes*.

The bioavailability of metal ions for the bacterial cells is linked to the environmental conditions (2). Generally, under anaerobic conditions and acidic pH the solubility of the metals is better compared to the aerobic environment and neutral pH. Many metals are required for growth, but when they are present in excess they can cause toxicity through the formation of reactive radicals or through the unspecific displacement of the metal cofactors in the active centre of enzymes (99). To control the intracellular metal ion level, bacteria require tightly regulated transport systems to acquire sufficient quantities of the metals on the one hand, and prevent deleterious effects of metal overload on the other hand.

The latter might explain thatt four genes (lmo0641, lmo1852, lmo1853, and lmo2875) encoding efflux proteins have increased expression in response to the acid shock. These genes are only induced after the pH drop; but three of them (lmo0641, lmo1852 and lmo1853) were induced in the acid shock experiment only at 25°C after acid shock.

On the level of gene expression, one of the first responses of *L. monocytogenes* to acid stress is the increased constant expression of lmo2575, encoding a putative cation transport (efflux) protein with unknown substrate specificity.

Three other genes code for proteins involved in the heavy metal export, lmo0641 for a putative heavy metal transporting ATPase, lmo1852 and lmo1853 are part of an operon encoding for a predicted mercuric ion transporting ATPase are also induced in response to acid shock.

In addition, the putative drug efflux protein (lmo2741) showed increased expression after acid shock and in the temperature shift experiment too, suggested their role in an undefined general stress response systems.

In the temperature shift experiment the most highly induced genes belong to the family of the sugar phosphotransferase system (PTS). Eighteen genes encoding for parts of a PTS system are up-regulated 2.5 to 30-fold in response to the temperature shift. The bacterial phosphoenolpyruvate : sugar phosphotransferase systems mediate the uptake and

phosphorylation of sugars and hexitols and it regulates metabolism in response to the availability of carbohydrates (71). The ability of Listeria to colonize and grow in a broad range of ecosystems correlates with the large number of their transporter proteins; especially 88 genes are involved in the carbohydrate transport, mediated by phosphoenolpyruvate-dependent phosphotranspherase systems (PTS). Systems for fructose, mannose trehalose, mannitol and galactitol have been found, but two PTS system no substratspecificity could be predicted based on their sequence similarity. The adaptation to the changes in the available sugars sources necessitates the permanent regulation of the carbohydrate uptake gene expression. Presumably, the temperature increase is also a signal for a different ecological niche with changed of the accomplishable carbohydrate composition.

4.2.3.2 Motility and chemotaxis

Our results demonstrated, that the regulation of the motility gene cluster in *L. monocytogenes* by both environmental factor, temperature and acid.

According to the literature (34, 133), motility gene expression is repressed at 37°C, the same behavior was found in the temperature shift experiments. The majority of the motility genes (37 of 43) were down-regulated at physiological temperature compared to 25°C. The same effect was observed after acid shock at 25°C, 41 out of 43 putative genes were significantly repressed as determined by microarray analysis. At 37°C after acid shock no significant gene expression changes of the motility genes was detected. Two genes proved to be an exception, lmo0679 encoding a putative flagellar hook protein and lmo0675 without sequence homology.

L. monocytogenes are highly flagellated and motile at low temperatures, of 30°C and below, and are typically non motile at temperatures of 37°C or above (52). Bacterial flagellins serve as pattern recognition molecules for Toll-like receptor 5-mediated signalling, leading to activation of innate immune responses while infection (34, 133). The transcription of *flaA*, encoding flagellin, is down-regulated at physiological temperature (134). It has been proposed that down regulation of *flaA* expression during *in vivo* infection by *L. monocytogenes* may serve as an adaptive mechanism to avoid host recognition and mobilization of host innate responses. MogR (motility gene repressor) regulates the motility gene cluster in *L. monocytogenes*; the temperature-dependent expression of motility genes in *L. monocytogenes* is independent of PrfA. The mode of action of acid-dependent regulation of the motility gene cluster in *L. monocytogenes* remaines unclear.

4.2.3.3 Cell surface proteins

One hundred and thirty out of the 2853 *L. monocytogenes* genes encode surface proteins. Cell surface proteins interact with the environment or infected hosts and are of primary importance in bacterial adherence, invasion into the mammalian cells and interaction with the host immune system. Cabanes *et al.* (16)classified the cell surface proteins in three major types: i) surface proteins covalently linked to the peptidoglycan by their C-terminal domain, called LPXTG motif (Leu-Pro-X-Thr-Gly, X any amino acid); ii) proteins associated to the surface by way of ionic or hydrophobic interaction mediated by their C-terminal domain (GW proteins, hydrophobic tail proteins and P60-like proteins); iii) proteins attached to the surface by their N-terminal region (lipoproteins). Only for a minority of the cell surface proteins a function is established in the listerial invasion. The abundance of *L. monocytogenes* surface proteins and the variety of anchoring systems are probably related to the ability of this bacterium to survive in diverse environments and to interact with a large variety of cell types during the invasion of the mammalian host. The fact, that about 20% of the *L. monocytogenes* cell surface proteins are absent in the highly related nonpathogenic *L. innocua*, indicates the importance of these proteins in the listerial virulence. Fourteen of these cell surface proteins encoding genes exhibited changed expression after. Other four cell surface genes change after shift to physiological temperature. There is no overlap between these two groups.

Except for three proteins; InlB (lmo0434), Iap (lmo0582), and P60 homologue protein (lmo0394), the other fifteen proteins have a LPXTG motif that mediates their covalent linkage to peptidoglycan (97). Six genes (*inlA*, *inlB*, *inlH*, *inlE*, lmo0327 and lmo0610) with altered gene expression in response to acid shock belong to a family of surface proteins characterized by an N-terminal domain containing leucine rich repeats (LRRs). LRRs are known to be involved in protein-protein interactions and in a variety of functions such as adhesion, ligand-receptor interactions and signalling (63).

The genes *inlA* and *inlB* are up-regulated after acid shock, but they do not showed significant transcriptional changes in the temperature shift experiment. The *inlAB* operon is involved in host cell tropism, InlA is required to invade epithelial cells, InlB is also an invasion protein used for entry into some hepatocyte-like, and some epithelial, endothelial and fibroblast cell lines.

The exact role of the proteins encoded by *inlE* and *inlH* is not known, however, our current knowledge is that these proteins are not involved in listerial invasion processes (16).

Surprisingly, two genes (lmo0394 and lmo0582) encoding P60-like proteins were repressed after acid induction. P60 was originally described as invasion-associated protein (Iap)

required for mouse fibroblast invasion (75). P60 has a murein hydrolase activity that implicated their function also in the cell division. However, recent studies suggested their importance in the binding of intestinal Caco2 cells.

Temperature shift affected the gene expression of four genes (lmo0130, lmo0160, lmo0320 and lmo0835) coding for surface proteins, all of these genes belong to the LPXTG proteins and their function in the listerial virulence or environmental adaptation is unclear. In summary, the induction of a set of known internalin and internalin like cell surface proteins encoding genes underline the importance the acidification of the environment as signal for the induction of listerial virulence.

4.2.3.4 DNA restriction/modification and repair

Two putative DNA repair protein encoded by lmo0233 and lmo1975 were induced after acid shock, lmo0233 shows homology to the *Escherichia coli* gene *sms*. The ubiquity of the *sms* (called also *radA)* gene in bacterial genomes suggests that it has an important role in the promotion of cell growth or survival. Beam *et al.* (5) showed, that *sms* is required for efficient repair of certain forms of DNA damage and for genetic recombination. Kruger *et al.* (74) found an *sms* homolog gene in *B. subtilis*, in which it is a member of *clpC* operon. This is also true for the localization of *sms* in *L. monocytogenes*. Mutation studies in *B. subtilis* established the role of *sms* in the development of a nonspecific stationary phase resistance to UV irradiation. The *clpC* operon in *B. subtilis* is regulated by the general stress σ-factor, sigma-B, suggesting that the induction of the *sms* homolog presumably is a part of a general stress response also in *L. monocytogenes*.

The second gene, lmo1975 shows homology to *dinP,* encoding a putative DNA repair protein. Ohmori *et al.* (102) reported, that similarity searches of DinP against protein sequences suggested their participation in the error-prone repair mechanism. Based on SOS box like consensus sequences in the upstream region, *dinP* gene expression is most likely under the control of the LexA SOS stress response regulator.

4.2.3.5 Regulation

Genes coding for regulatory proteins play vital roles in metabolic processes, including transcription, cell development and in the coordination of the stress responses. It has been observed in this study that the changes in expression profile during acid stress and adaptation were accompanied by the stimulation of a panel of genes encoding regulatory proteins.

PrfA

Real time quantitative RT-PCR revealed that *prfA* (lmo0200) transcription was induced through both, acid shock and temperature shift (Fig. 3-7, 3-8, 3-9). However, after adaptation to acid the expression level of *prfA* dropped to the same level as before acid induction.

PrfA is the master regulator of listerial virulence. Genes in the PrfA regulon are involved in many different steps of the infectious process, e.g. invasion, escape from the phagosome, intracellular multiplication and spreading (73). *Listeria* is a pathogenic facultative intracellular microorganism, living outside of host organisms as saprophytic soil bacterium. During the nonpathogenic life cycle the expression of the virulence factors are energetically disadvantageous. Therefore, *Listeria* must have means to sense its location within or outside a eukaryotic host, and to use these signals for the regulation of the virulence gene expression. During the infection process *Listeria* encounters different conditions as signal for the virulence expression. The first signal for the sensing the warm-blooded host is a rapid temperature shift (62), which results in up-regulation of virulence genes. Our real time RT-PCR data revealed likewise the induction of *prfA* by temperature shift.

Further stimuli, according to literature data, include low iron concentration (23), high osmolarity (95), activated charcoal and oxidative stress, whereas fermentable sugars and low pH repress the PrfA dependent virulence genes (9).

Another important signal for entering a host is a low pH caused by the acidic stomach fluids. Ingested *Listeria* must withstand the strong acidic fluid of the stomach (131). The exposure to acidic conditions is a signal for virulence gene induction of many other pathogens (112), we found a similar reaction in Listeria for the first time. Since the acidic challenge in the stomach does not only switches the acid tolerance response system of this microorganism on, but provides a cross protection against other challenges, such as heat, ethanol, oxidative and osmotic stresses (49, 85). There is recent evidence that acid tolerance response plays a role in infection and intracellular proliferation by *L. monocytogenes*. Marron *et al.* (88)identified a mutant strain in *L. monocytogenes,* which is incapable of ATR, the virulence of this mutant was considerably decreased. Additionally, development of acid tolerance results in increased internalization by Caco-2 cells and higher survival rates in activated macrophages *in vitro* (24). Acid adaptation also results in higher loads of *L. monocytogenes* in the intestine and the mesentheric lymph nodes after oral infection in mice.

Our results demonstrate, in detail for the first time, whole genome gene expression data helping to explain these phenomena (the effect of ATR on the virulence) at the molecular level. The DNA-microarray data after acid shock, revealed the induction of *prfA* and a set of

other virulence genes, thereby establish the relation of the acid tolerance response and virulence gene expression in *Listeria*.

PerR (Fur family)

The acid shock induced the expression of *perR* (lmo1683) coding for PerR transcriptional regulator belongs to the Fur-family. In gram-negative organisms the iron regulator Fur, is known to bind iron as a corepressor, and controls a subset of acid shock proteins in an iron-independent manner, too. Mutations have been identified in *fur* that produces acid-blind/iron-sensing and acid-sensing/iron-blind phenotypes. This suggests that this protein senses iron and acid separately (19). In *L. monocytogenes* a *fur* mutant was not affected in growth at low pH (110). In *S. aureus* PerR was found to control transcription of genes encoding oxidative stress resistance proteins, furthermore PerR regulates the transcription of iron storage genes and repressed transcription of the iron homeostasis regulator Fur (59). Additionally PerR is required for a full virulence of *S. aureus*. The *perR* mutant of *L. monocytogenes* has the same growth characteristic under acidic conditions as the wild type strain. Additionally, Rea *et al.* (110) demonstrated, that the reduction in virulence potential of the *perR* mutant is due to deregulation of iron uptake or storage during intracellular growth. These data suggested that in our case the induction of *perR* belongs rather to the cluster of low pH induced virulence genes, presumably without any function in the acid stress response of *L. monocytogenes*.

Sigma-B

In the low-GC content Gram-positive microorganisms (like *Bacillus*, *Listeria*, *Staphylococcus*) the general stress response is mediated by SigB. Genes of the SigB regulon code for proteins against oxidative, osmotic, heat, acid stress, and are required for survival under other extreme environmental conditions (39). I found in the experiments, that the transcription level of *sigB* (lmo0895) was not significantly affected in response to acid stress or temperature shift. Although three genes (lmo0889, lmo0891 and lmo0892) coding for proteins involved in the *sigma-B* regulation were significant down-regulated after acid shock. According to Ferreira *et al.* (41) survival of *L. monocytogenes* at low pH is sigma-B dependent. However the analysis of the *sigma-B* null mutant showed, that the null mutant still displayed a lesser acid tolerance response, indicating that *sigma-B* is not solely responsible for the acid tolerance response.

Our knowledge about the SigB operon derived from the extensive research in *B. subtilis*, these studies described the model of the post-transcriptional regulation of SigB activity by environmental stress or ATP depletion (Figure 4-1). Eight genes encoding *sigma-B* regulator proteins (Rsb) are involved in the *sigB* regulation, seven of these (RsbRSTUVWX) are

located in the *sigB* operon, RsbP is encoded by a gene located outside the *sigB* operon. The *sigB* operon is cotranscribed from a SigA promoter located upstream of *rsbR* and from a SigB promoter located upstream of *rsbV*.

Under non-stress conditions, RsbV is in a phosphorylated form, thereby binding to the anti-Sig-B protein RsbW, which inactivates SigB. In stressed cells the unphosphorylated form of RsbV competes with SigB for binding RsbW: as the relative concentration of the RsbV-RsbW complex increases, the concentration of the free active form of SigB rises. Environmental stress sensed through up-stream switch module (Figure 4-1). In the case of ATP depletion RsbP (serine protein kinase) dephosphorylates RsbV and thereby activates SigB (39). However one has to keep in mind that our current model is derived from *B. subtilis* and the validity in *L. monocytogenes* has not been yet proved.

The down regulation of RsbRTU suggests that SigB in the acid shock experiment was activated through the energy stress cascade. However it is trivial fact, that low energy stress is one of the major triggers of the general stress response.

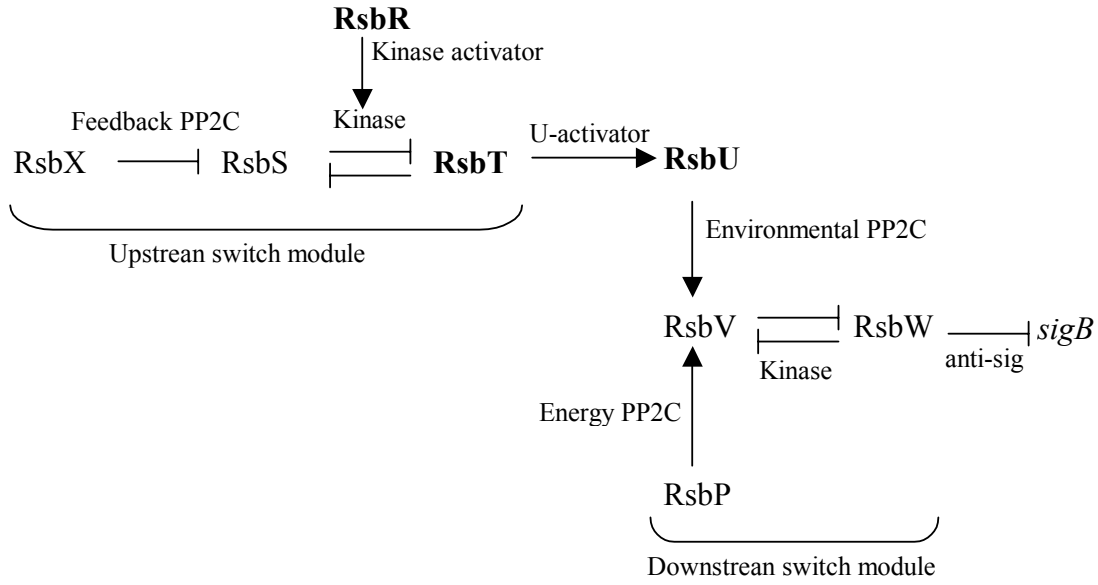


Fig. 4-1: Schematic model for *sigB* regulation by partner-switching in *B. subtilis* (Ferreira *et al.* (41)). sigma-B activity is regulated by a downstream and upstream modules, which response to energy (ATP depletion) or environmental stress. Each partner-switching module consist three components, a serine phosphatase (RsbX, U or P), antagonist proteins (RsbS or V), and serine kinase (RsbT or W). The serine kinase function as a switch protein, binding to either the antagonist or its target depending on the phosphorylation status of the antagonist protein. Protein activation is indicated by arrows, inhibition by T-headed arrows. PP2C designates the protein phosphatase 2C.

LexA repressor type proteins

lmo1302, encoding a putative transcription repressor protein LexA, exhibited an increased expression following acid shock in *L. monocytogenes.*

In *Escherichia coli* LexA is involved in regulation of the adaptive mechanism called the "SOS response", to manage a various types of DNA damage (84). During normal cell growth, in non-stressed cells, LexA is stable and represses a set of SOS response genes, by the binding the SOS-box located upstream to the target genes. The DNA damaging effects activate RecA protein to a form that increases the rate of LexA cleavage. Upon cleavage, LexA is inactivated and the SOS genes are turned on. The physiological effect of the up regulation of LexA in our experiments remaines elusive.

4.2.3.6 Temperature induced regulator genes

Various regulatory genes are up-regulated by temperature shift; three of these (lmo0297, lmo0425, lmo2668) are presumably associated with the altered expression of the carbohydrate transport proteins (PTS systems) at elevated temperature. These genes show a high similarity to transcription antiterminators proteins belong to the BglG family. In *E. coli* such regulators are involved in the β-glucoside utilization. BglG represents a family of transcriptional antiterminators that bind to RNA sequences which partially overlap with rho dependent terminators, and prevent termination by stabilizing an alternative structure of the transcript (48), thereby allowing the transport, phosphorylation and metabolisms of β-glucosides. The activity of BglG is determined by its dimeric state, which is modulated by a reversible phosphorylation through BglF. Only the non-phosphorylated BglG dimer binds RNA and allows read-through of transcription.

The other heat induced regulator gene lmo0051 encodes the putative response regulator AgrA of a two component regulatory system in *L. monocytogenes*. Auret *et al.* (4) investigated the function of this response regulator and found that in a *agr* mutant the production of several secreted proteins was modified, indicating that the *agr* locus influenced protein secretion. On the other hand the inactivation of *agrA* did not affect the ability of the pathogen to invade and multiply in cells in vitro. However, the virulence of the *agrA* mutant was attenuated in the mouse model demonstrating a role for the *agr* locus in virulence of *L. monocytogenes*.

4.2.3.7 Adaptation to atypical conditions

Bacterial cells respond almost immediately to different stress conditions by increasing the production of general stress proteins (GSPs). Nine putative general stress proteins were induced after acid shock and/or elevated temperature. Two of these genes *ctc* and *clpP* were induced by both of these factors, acidic conditions and temperature shift. Sigma-B dependent

transcription was revealed (67, 93) by sequence analysis for the genes *ctc* (lmo0211) encoding a general stress protein, lmo1433 encoding glutathione reductase, lmo2748 coding for a putative stress protein YdaG, and lmo1601 encoding a putative general stress protein.

In *B. subtilis*, *ctc* gene is induced in response to osmotic, heat, oxidative stress, and glucose limitation. The function of Ctc was also in investigated *L. monocytogenes*. Physiological studies indicate, that this protein facilitates growth in minimal medium under high osmolarity in absence of osmoprotectants like glycine betaine.

Chaperones and proteases help for bacteria in the rapid adaptation to sudden changes of the environment. Chaperones assist the proper folding, refolding or assembly of proteins, whereas proteases process those that cannot be refolded or are not needed any longer. Among the chaperones, a large protein complex named Clp (caseinolytic protein) displays both proteolytic and chaperone activities. In *B. subtilis* ClpP is required for a wide variety of functions, including growth at high temperature, motility, competence and sporulation (50). Homologues of ClpP have been identified in a wide range of prokaryotes and eukaryotes. In *L. monocytogenes*, ClpP might play a role in the early fate of intracellular bacteria. Bacterial uptake by macrophages induces a set of proteins, including ClpP, which plays a crucial role in the rapid adaptive response to an intracellular environment during the infectious process(96).

4.2.3.8 Glutathione peroxidase, glutathione reductase and thioredoxins

In our acid shock experiments six genes were up-regulated with putative function in an oxidative stress response. Three of these genes, lmo0983, encoding a predicted glutathione peroxidase and lmo0906 as well as lmo1433, encoding glutathione reductases, are putative members of the glutathione-dependent oxidative stress response system.

In *E. coli* and *Saccharomyces cerevisiae* the glutathione- and thioredoxin-dependent reduction systems are responsible for maintaining the reduced environment of the cytosol (18) and play an important role in survival in the presence of oxidative stress. Cells containing glutathione might employ a glutathione - glutathione peroxidase - glutathione reductase system, to protect themselves against damage from H_2O_2 treatment (79). This system has been identified in yeast also (124), in which the reduction of H_2O_2 to H_2O was catalyzed by glutathione peroxidase. This enzyme uses glutathione as a hydrogen donor, and the oxidized glutathione is reduced in turn by glutathione reductase and NADPH. Glutathione reductase is an enzyme that provides protection from oxidative stress by reducing glutathione disulfide to glutathione. Kazmierczak *et al.* (67) reported, that in *L. monocytogenes* the sigma-B dependent resistance to oxidative damage due, at least in part, to glutathione reductase.

There are three isoform a putative thioredoxin (encoded by lmo1609, lmo2424 and lmo2830) up-regulated after pH drop. Thioredoxin proteins act as disulfide oxidoreductases and are the donors for thioredoxin peroxidases. Therefore an increasing body of evidence suggests that the glutathione- and thioredoxin-dependent reduction system involved in the oxidative stress response in *L. monocytogenes* is induced after acid sock.

4.2.3.9 Detoxification

In this study we identified five genes involved in detoxification processes. All these genes were induced through acid shock, but not by temperature shift. Their expression was strongly increased after acid shock, however after acid adaptation they dropped to the same mRNA level as before. Two of these (lmo0754 and lmo2067) were only induced at 25°C. Three further *bsh* (lmo2067), encoding a conjugated bile acid hydrolase, lmo2230, encoding an arsenate reductase and lmo2231 encoding a putative cation efflux system, are preceded by a predicted PrfA box. Additionally, upstream of these genes putative sigma-B promoter sites were detected. These findings emphasize again, the strong relationship between the acid stress response and virulence gene induction.

Furthermore, lmo1967 coding for a toxic ion resistance protein, involved in detoxification, were up-regulated in the acid shock response experiments. Arsenic is a known toxic metalloid, whose trivalent and pentavalent ions can inhibit many biochemical processes (33). The arsenic resistance efflux system transports arsenite [As(III)], alternatively using a chemiosmotic transporter (putatively lmo2231). The other gene in the arsenic resistance system, presumably in our case lmo2230 encodes an arsenate reductase that converts intracellular arsenate [As(V)] to arsenite [As(III)], the substrate of the efflux system. There is no information about the role of the arsenic resistance system in the acid resistance or in virulence of *L. monocytogenes*.

Furthermore, two more detoxification genes lmo0754, encoding a bile acid 7-alpha dehydrogenase and *bsh* (lmo2067), encoding a conjugated bile acid hydrolase were found up-regulated in after acid shock. Bile is one of many barriers that *Listeria monocytogenes* must overcome in the human gastrointestinal tract in order to infect and cause disease (8). Dussurget *et al.* (36) reported that the *bsh* (lmo2067) gene encodes a functional intracellular enzyme involved in the degradation of bile in all pathogenic *Listeria* species. The *bsh* gene is positively regulated by PrfA, and involved in the intestinal and hepatic phases of listeriosis (36). Comparative sequence analysis revealed, that the putative bile acid 7-alpha hydrolase and conjugated bile acid hydrolase (lmo0754 and lmo2067) were present in *L. monocytogenes*

but not in *L. innocua* probably reflecting the capacity of *L. monocytogenes* to survive in the mammalian gut (51).

4.2.3.10 Shared genes in the acid stress response of *C. glutamicum* and *L. monocytogenes*

The comparative analysis of acid tolerance response of *C. glutamicum* and *L. monocytogenes* revealed only minor overlap. However, as presented below, in both microorganisms sigma-B general stress regulator is mainly responsible for the induction of the stress response genes.

Furthermore cation transporter proteins, involved in the cation efflux with undefined substrate specificity showed induced expression after acidification in both bacteria. This fact emphasize the substantial importance of maintenance the ion homeostasis in response the environmental pH changes.

All bacteria have specific genes for resistances against heavy metal ions, which are toxic. In our DNA-microarray experiments we found any up-regulated genes belonging to putative heavy metal resistance systems in both microorganisms. As mentioned above the bioavailability of many metal ions is dependent on the environmental redox state and pH. At acidic pH the induction of heavy metal resistance genes indicate entity of the danger of metal overload. Parallel with the induction of efflux genes, we found up-regulation of genes involved in DNA-repair systems. Recent studies indicate that free metal ion overload can results in the generation of reactive oxygen species (ROS) and oxidative stress, and that is responsible for the subsequent DNA damage. One has to keep in mind, that many minerals and soils contain heavy metals, which become available for the microorganisms at low pH. The trigger of heavy metal exporter systems during the ATR is likely to increase viability in such environments.

5 Conclusion

These transcriptional profiling experiments support the view that the acid stress response brings about widespread changes in cellular metabolism to achieve a more resistant state. In this study, genome wide DNA-microarray technology was used to describe these changes at transcriptional level in *C. glutamicum* and *L. monocytogenes*. We observed significant shift in the transcriptional patterns of genes encoding transport proteins, motility, cell surface, DNA repair, regulation, stress proteins and proteins in detoxification processes.

In the first part of this work, the acid adaptation response of *Corynebacterium glutamicum* was analyzed. This process include the continuous fermentation of this bacterium at neutral and acidic pH, the transcriptions profiling of the acid adapted cells using DNA-microarray technology and analysis of function by creating disruption mutants of the acid induced genes. Gene disruption of the 26 most highly induced genes showed no significant phenotypic effect at low pH except of the general stress regulator protein sigma-B. The *sigB* disruption strain showed a significant acid sensitive phenotype. These data suggest that i) the regulation of the long-term acid adaptation is at least partially SigB-dependent and ii) the acid stress response of *C. glutamicum* is a highly complex and redundant system.

In *L. monocytogenes* genome-wide transcription profiling was performed in response to hydrochloric acid at 25 and 37°C. The results of the transcriptomic analysis of the acid shock and acid adaptation responses were compared to the effect of a temperature shift (from 25 to 37°C) alone. In the acid stress experiments we discovered a substantial shift in general stress response genes, such as Ctc or ClpC and ClpP. Furthermore, glutamate decarboxylase, involved in the active pH homeostasis, as well as export proteins with function in either detoxification processes or in cation transport, were induced.

The up-regulation of a set of genes, which are transcribed from SigB-dependent promoters, indicates a regulatory function of SigB in the acid shock response of *L. monocytogenes*. Listerial virulence capabilities are linked with environmental stress responses mediated by alternative sigma factors. The pH drop caused not only an ATR, but also up-regulation of virulence genes, such as PrfA, the master regulator of virulence, and InlAB, which are internalins suggesting the involvement of pH in the signaling of virulence gene regulation of *L. monocytogenes*. Additionally, we observed down-regulation of the motility regulon, which may serve to avoid host recognition during the pathogenic cell cycle, and thereby

further promoting the virulence of this pathogen. The analysis of the Listeria DNA-microarray data revealed a strong correlation between the acid stress response and virulence gene expression. Thus I propose that alternative sigma factors as sigma-B, contribute to the ability of *L. monocytogenes* to cause a disease.

In general, we discovered in both bacteria a substantial shift in the transcriptional patterns of known and suspected transporter proteins, revealing the importance of this functional group in the ATR. Transporters contribute to stress resistance by removing toxic compounds and maintaining ion balance. The induction of different metal transporting proteins emphasized the importance of the maintenance of cytoplasmic free metal ion homeostasis in response to the changing environmental pH conditions. The question how could such cation transporters promote the active pH homeostasis, may be addressed by analyzing the role and substrate specificity of individual cation identified in *C. glutamicum* and *L. monocytogenes*.

Besides the genes with known function in the general or acid stress response, many unknown or poorly characterized genes were found to be differentially regulated in cultures grown at acidic conditions. Especially these genes are expected to provide further important information on the response of *L. monocytogenes* to the acidic environmental pH.

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7 Supplement

Tab. S-1: DNA-microarray analysis of acid adapted *C. glutamicum*

	REACTION STEPS	NOTES
DNA-MICROARRAY PREPARATION	**ACID ADAPTATION EXPERIMENT**	two independent fermentation
	EXPERIMENT total RNA / CONTROL total RNA	two replicate RNA isolation
	Cy5-labeled cDNA / Cy3-labeled cDNA	random hexamer primed synthesis of fluorescently labeled cDNA
	competitive hybridization to DNA-microarray	DNA-microarray hybridization and washing
IMAGING	Cy5 signal intensity / Cy3 signal intensity	Fluorescent images at 635nm $I_{Cy5(i=x)}$ and 532nm $I_{Cy3(i=x)}$ using an GenePix 4000 laser scanner
	quantitative analysis / quantitative analysis	raw fluorescent data and analysis using softwareGenePix 3.0
NORMALIZATION	Cy5/Cy3 **RATIO** calculation, $\log_2$ transformation	$\mathbf{R}_{Cy5/Cy3(i=x)} = I_{Cy5(i=x)}/I_{Cy3(i=x)}$ $\mathbf{R}_{\mathbf{SN}Cy5/Cy3(i=x)}$ - Cy5/Cy3 ratio of x-th spot on the slide N signal intensity median value
	total intensity **NORMALIZATION** between slides and dyes (Cy5 and Cy3)	Data were normalized to the average ratio of *C. glutamicum* genomic DNA
FILTER	**FILTER** low signal intensity, bad spot quality	**IF** ($I_{Cy3(i=x)}$ **OR** $I_{Cy5(i=x)} \geq 3*I_{Bkg(i=x)}$) **RIGHT** x-th spot were used in the further analysis **WRONG** x-th spot too weak, omitted $I_{Bkg(i=x)}$ - local background intensity value
	FILTER spots with nonsignificant changes	P values were calculated from replicated ratio values based on Student's t test using log transformed gene ratios and genomic DNAratios, which were normalized to zero **IF** ($P_{(i=x)} \leq 0{,}05$) **RIGHT** x-th spot were used in the further analysis **WRONG** nonsignificant, omitted $P_{(i=x)}$ - P values of the x-th spot on the four slides
	ANALYSIS	Analysis of gene expression data was performed by selecting genes showing at least twofold changed average mRNA level.

Tab. S-2: DNA-microarray analysis of *L. monocytogenes* following acid shock

	REACTION STEPS	NOTES
DNA-MICROARRAY PREPARATION	**ACID SHOCK AND ADAPTATION EXPERIMENT**	two independent biological experiment
	EXPERIMENT total RNA / CONTROL total RNA	two replicate RNA isolations each
	Cy5-labeled cDNA / Cy3-labeled cDNA	random primed cDNA synthesis
	competitive hybridization to DNA-microarray	DNA-microarray hybridization and washing
IMAGING	Cy5 signal intensity / Cy3 signal intensity	Fluorescent images at 633 and 543nm using an Affimetrix GSM 418 scanner.
	quantitative analysis / quantitative analysis	Quantification the spot and local background intensities ($I_{Bkg(i=x)}$), determination the spot quality (flags) using ImaGene.
NORMALIZATION	data conversion and background substraction	using ExpressConverter (TIGR) Intensity = (SM-BM) * SA Signal Median - (SM) Background Median - (BM) Signal Area - (SA)
	total intensity **NORMALIZATION** between slides	Spread sheet analysis $I_{SN(i=x)norm} = I_{SN(i=x)} * (\sum_{(i=1-n)} I_{SNi} / \sum_{(i=1-n)} I_{S1i})$ (n=6912) $I_{S1(i=x)}$ - signal intensity of the x-th spot on the first slide $I_{S1(i=x)norm}$ - normalized intensity value $_{SN}$ - N-th slide
	total intensity **NORMALIZATION** between dyes (Cy5 and Cy3)	Spread sheet analysis $I_{Cy5(i=x)norm} = I_{Cy5(i=x)} * (\sum_{(i=1-n)} I_{Cy5i} / \sum_{(i=1-n)} I_{Cy3i})$ (n=6912) $I_{Cy3(i=x)}$ - signal intensity of the x-th spot on the first slide $I_{Cy5(i=x)norm}$ - Cy5 normalized intensity value
FILTER	**FILTER** low signal intensity, bad spot quality	**IF** ($I_{Cy3(i=x)}$ **OR** $I_{Cy5(i=x)} \geq 3 * I_{Bkg(i=x)}$) **RIGHT** x-th spot were used in the further analysis **WRONG** x-th spot too weak, omitted $I_{Bkg(i=x)}$ - local background intensity value Omit spots with bad spot quality, based on the determination of software ImaGene (flags).

Cy5/Cy3 **RATIO** calculation, $\log_2$ transformation ↓ **FILTER** spots with nonsignificant changes ↓ **ANALYSIS**	$\mathbf{R}_{Cy5/Cy3(i=x)} = I_{Cy5(i=x)}/I_{Cy3(i=x)}$ $\mathbf{R}_{\mathbf{SN}Cy5/Cy3(i=x)}$ - Cy5/Cy3 ratio of x-th spot on the slide N signal intensity value P values were calculated from replicated ratio values based on Student's t test using log transformed gene ratios and standard gene ratios. **IF** ($P_{(i=x)} \leq 0{,}05$) **RIGHT** spot was used in the further analysis **WRONG** nonsignificant, omitted $P_{(i=x)}$ - P values of the x-th spot on the four slides Analysis of gene expression data was performed by selecting genes showing at least twofold changed average mRNA level.

Tab. S-3: List of ORFs of *C. glutamicum* showing a 2 fold decrease in transcription levels in response to acid adaptation at pH 5.7. The ratios listed in the table are the average ratios of 4 micoarray experiments.

Group	ORF	NCBI no.	Annotation	Ratio
Transport protein	1382	NCgl0799	Na^+/proline, Na^+/panthothenate symporter or related permease	0.45
	1011	NCgl0510	ABC-type cobalt transport system, ATPase component	0.32
	1012	NCgl0511	ABC-type cobalt transport system, permease Component, similar to CbiQ and related transporters	0.33
	2042	NCgl1877	glutamate ABC-type transporter, permease component	0.42
	2092	NCgl1915	similar to ABC-type dipeptide/oligopeptide/nickel transport systems	0.31
	2093	NCgl1915	similar to ABC-type dipeptide/oligopeptide/nickel transport systems	0.33
	2094	NCgl1916	similar to ABC-type dipeptide/oligopeptide/nickel transport systems	0.41
	3744	NCgl2562	ABC-type transporter, periplasmic component, similar to ABC-type dipeptide/oligopeptide/nickel transport systems	0.44
	67061	NCgl2562	ABC-type transporter, periplasmic component, similar to ABC-type dipeptide/oligopeptide/nickel transport systems	0.47
	2095		strong similarity to oligopeptide ABC transporter (permease) oppC - Bacillus subtilis	0.40
Regulatory protein	1044	NCgl0536	translation initiation factor IF-1	0.50
	2648	NCgl1324	translation initiation factor IF3	0.45
	1562	NCgl0946	transcription elongation factor	0.42
Metabolic proteins	671	NCgl0251	Catalase	0.30
	670	NCgl0251	Catalase	0.38
	831	NCgl0360	succinate dehydrogenase/fumarate reductase, flavoprotein subunit	0.28
	832	NCgl0361	succinate dehydrogenase/fumarate reductase Fe-S protein	0.29
	976	NCgl0468	ribosomal protein L10	0.49
	978	NCgl0469	ribosomal protein L7/L12	0.50
	988	NCgl0476	ribosomal protein S12	0.46
	989	NCgl0477	ribosomal protein S7	0.40
	3553	NCgl0487	ribosomal protein L3	0.37
	3554	NCgl0488	ribosomal protein L4	0.41
	3555	NCgl0490	ribosomal protein L2	0.36
	3777	NCgl0491	ribosomal protein S19	0.40
	3778	NCgl0492	ribosomal protein L22	0.40
	992	NCgl0493	ribosomal protein S3	0.39
	993	NCgl0494	ribosomal protein L16/L10E	0.40
	994	NCgl0495	ribosomal protein L29	0.42

	999	NCgl0499	ribosomal protein L14	0.48
	1000	NCgl0500	ribosomal protein L24	0.41
	1020	NCgl0517	ribosomal protein L18	0.46
	1022	NCgl0518	ribosomal protein S5	0.41
	1023	NCgl0519	ribosomal protein L30/L7E	0.49
	1044	NCgl0536	translation initiation factor IF-1	0.50
	1046	NCgl0537	ribosomal protein S13	0.48
	1047	NCgl0538	ribosomal protein S11	0.49
	1048	NCgl0539	ribosomal protein S4	0.48
	1050	NCgl0541	ribosomal protein L17	0.48
	1069	NCgl0556	ribosomal protein L13	0.41
	1088	NCgl0572	co-chaperonin GroES, HSP10; chaperonin 10 Kd subunit	0.43
	1092	NCgl0573	chaperonin GroEL, member of the HSP60 family	0.47
	1377	NCgl0795	citrate synthase	0.48
	1421	NCgl0834	ribosomal protein L28	0.48
	1508	NCgl0902	general stress protein Ctc	0.47
	1807	NCgl1159	F0F1-type ATP synthase a subunit	0.25
	1808	NCgl1160	F0F1-type ATP synthase c subunit	0.25
	1809	NCgl1161	F0F1-type ATP synthase b subunit	0.33
	1810	NCgl1162	F0F1-type ATP synthase delta subunit	0.27
	1811	NCgl1163	F_0F_1-type ATP synthase alpha subunit	0.27
	1812	NCgl1164	F_0F_1-type ATP synthase gamma subunit	0.31
	1813	NCgl1165	F_0F_1-type ATP synthase beta subunit	0.31
	2768	NCgl1241	2-keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase	0.41
	2675	NCgl1304	ribosomal protein S1	0.48
	2647	NCgl1325	ribosomal protein L35	0.46
	2425	NCgl1482	aconitase A	0.20
	2511	NCgl1546	orotidine-5'-phosphate decarboxylase	0.44
	2512	NCgl1547	carbamoylphosphate synthase large subunit	0.40
	2513	NCgl1548	carbamoylphosphate synthase small subunit	0.30
	2515	NCgl1549	similar to related cyclic amidohydrolases	0.37
	2516	NCgl1550	aspartate carbamoyltransferase, catalytic chain	0.46
	3792	NCgl2112	similar to heme/copper-type cytochrome/quinol oxidase, subunit 3	0.48
	3478	NCgl2436	phosphoserine phosphatase, contains ACT domain	0.44
	65297	NCgl2555	glucosamine-6-phosphate isomerase	0.41
	3737	NCgl2555	glucosamine-6-phosphate isomerase	0.45
	2095		strong similarity to oligopeptide ABC transporter (permease) oppC - Bacillus subtilis	0.40
Hypothetical proteins	787	NCgl0334	hypothetical protein	0.41
	830	NCgl0359	hypothetical membrane protein	0.29
	1013	NCgl0512	hypothetical membrane protein, similar to dihydrolipoamide dehydrogenase/glutathione oxidoreductase and related enzymes	0.28
	1381	NCgl0798	uncharacterized membrane protein	0.21
	1477	NCgl0878	hypothetical protein	0.46
	1545	NCgl0932	hypothetical protein	0.38
	1751	NCgl1114	hypothetical membrane protein	0.40
	2690	NCgl1294	hypothetical protein	0.45
	2402	NCgl1465	hypothetical membrane protein	0.41

	2546	NCgl1576	predicted membrane protein	0.48
	2547	NCgl1577	hypothetical protein	0.49
	3392	NCgl1676	hypothetical protein	0.42
	2111	NCgl1929	hypothetical membrane protein	0.46
	2112	NCgl1930	hypothetical membrane protein	0.42
	3191	NCgl2170	hypotheical protein	0.37
	3479	NCgl2435	hypothetical protein	0.39
	2850	NCgl2504	hypothetical protein	0.36
	219	NCgl2734	hypothetical protein, similar to ABC-type transporter permease component	0.46
	2844		hypothetical protein	0.33
	1380		hypothetical protein	0.47
	1332		hypothetical protein	0.49
	2406		hypothetical protein	0.49

Tab. S-4:. List of ORFs of *C. glutamicum* showing at least a two-fold increase in transcription levels in response to acid adaptation at pH 5.7. The ratios are the average ratios of 4 DNA-microarray experiments.

Group	ORF	NCBI no.	Annotation	Ratio
Transport proteins	851	NCgl0375	cation transport ATPase	2.38
	855	NCgl0378	ABC-type transporter, periplasmic component ABC-type cobalamin/Fe^{3+}-siderophores transport systems, periplasmic components	3.57
	856	NCgl0379	ABC-type transporter, permease component, similar to FecCD transport family proteins and ABC-type cobalamin/Fe^{3+}-siderophores transport systems, permease components	3.22
	857	NCgl0380	ABC-type transporter, ATPase component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems, ATPase components	4.43
	3549	NCgl0482	ABC-type transporter, ATPase component, similar to ABC-type cobalamin/Fe^{3+}-siderophore transport system	5.06
	3550	NCgl0483	ABC-type transporter, permease component, FecCD transport family; similar to ABC-type cobalamin/Fe^{3+}-siderophore transport system	4.36
	3551	NCgl0484	ABC-type transporter, permease component, FecCD transport family; similar to ABC-type cobalamin/Fe^{3+}-siderophore transport system	4.32
	1168	NCgl0635	hypothetical protein, similar to siderophore-interacting proteins	3.78
	1169	NCgl0636	ABC-type transporter, ATPase component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	4.99
	1170	NCgl0637	ABC-type transporter, permease component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	5.58
	1171	NCgl0638	ABC-type transporter, permease component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	2.09
	1173	NCgl0639	ABC-type transporter, periplasmic component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	6.84
	1346	NCgl0773	siderophore-interacting protein	7.40
	1347	NCgl0774	ABC-type cobalamin/Fe^{3+}-siderophore transport system, periplasmic component	10.53
	1349	NCgl0776	ABC-type cobalamin/Fe^{3+}-siderophore transport system, periplasmic component	3.82
	1351	NCgl0778	ABC-type cobalamin/Fe^{3+}-siderophore transport system, permease component	2.79
	1352	NCgl0779	ABC-type cobalamin/Fe^{3+}-siderophore transport system, ATPase component	3.58
	1516	NCgl0909	ABC-type transporter, ATPase component, similar to ABC-type multidrug transport system	3.18
	2779	NCgl1232	Co/Zn/Cd efflux system component	6.92
	2583	NCgl1379	predicted divalent heavy-metal cations transporter	2.91
	2433	NCgl1488	cation transport ATPase	3.31
	2534	NCgl1565	ABC-type transporter, periplasmic component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	2.12
	2146	NCgl1959	ABC-type transport systems, periplasmic component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	8.43
	2969	NCgl2352	ABC-type transporter, permease component, similar to ABC-type dipeptide/oligopeptide/nickel transport systems	2.11

	2905	NCgl2463	Na+/H+-dicarboxylate symporter	2.62
	3458	NCgl2970	ABC-type transport systems, periplasmic component, similar to ABC-type cobalamin/Fe^{3+}-siderophores transport systems	2.57
Regulatory proteins	927	NCgl0430	bacterial regulatory protein, predicted arsR family transcriptional regulator	4.87
	1518	NCgl0911	two-component system sensory transduction histidine kinase	2.47
	1642	NCgl1019	transcriptional regulator	2.26
	1703	NCgl1075	DNA-directed RNA polymerase specialized sigma Subunits, similar to sigma-70 factor (ECF subfamily); and sigma24	2.03
	2000	NCgl1844	DNA-directed RNA polymerase sigma subunit SigB, similar to sigma70/sigma32 factors	2.17
	3470	NCgl2441	Mn-dependent transcriptional regulator	2.40
Metabolic proteins	422	NCgl0049	NAD-dependent aldehyde dehydrogenase	2.31
	498	NCgl0106	lactoylglutathione lyase or related lyase	2.28
	727	NCgl0294	phosphoserine phosphatase	2.75
	777	NCgl0328	nitroreductase	3.23
	1432	NCgl0842	molybdopterin biosynthesis enzyme	2.17
	1491	NCgl0888	demethylmenaquinone methyltransferase	2.15
	1504	NCgl0899	Dioxygenase, similar to 2-nitropropane dioxygenase	2.14
	1558	NCgl0943 [a]	AraC-type DNA-binding domain-containing protein	7.69
	2578	NCgl1383	hydrolase of the alpha/beta superfamily	2.20
	2428	NCgl1485	predicted nucleoside-diphosphate-sugar epimerase	2.66
	2480	NCgl1521	ammonia permease	2.90
	2006	NCgl1848	hypothetical protein, similar to archaeal enzymes of ATP-grasp superfamily	2.47
	2149	NCgl1961	thiamine monophosphate synthase	2.09
	3056	NCgl2277	aldo/keto reductase, related to diketogulonate reductase	2.03
	3700	NCgl2297	malate/lactate dehydrogenase	2.67
	64895	NCgl2297	malate/lactate dehydrogenase	2.97
	3467	NCgl2443	ribonucleotide reductase alpha subunit	5.23
	2930	NCgl2444	ribonucleotide reduction protein	4.35
	2929	NCgl2445	glutaredoxin-like protein	3.48
	2828	NCgl2521	thiamine pyrophosphate-requiring enzyme, similar to acetolactate synthase, pyruvate dehydrogenase (cytochrome), glyoxylate carboligase,phosphonopyruvate decarboxylase	3.25
	279	NCgl2785	membrane-associated phospholipid phosphatase	2.54
	309	NCgl2813	predicted flavoprotein	2.09
	1894	NCgl2908	putative mercuric reductase, similar to dihydrolipoamide dehydrogenase/glutathione oxidoreductase and related enzymes	2.94
	1922	NCgl2934	hypothetical protein, similar to phosphotransferase system mannitol/fructose-specific IIA domain (Ntr-type)	2.21
	3438	NCgl2956	hypothetical protein, similar to sugar phosphate isomerases/epimerases	2.40
	3439	NCgl2957	myo-inositol dehydrogenase, similar to predicted dehydrogenases and related proteins	2.12
	3452	NCgl2965 [a]	hypothetical membrane protein, similar to permeases of the major facilitator superfamily	5.42
Hypothetical proteins	518	NCgl0123 [a]	hypothetical protein	6.99
	534	NCgl0138	hypothetical protein	2.31
	546	NCgl0148	hypothetical protein	2.32
	588	NCgl0191	hypothetical protein	3.25

	748	NCgl0308	uncharacterized phage-associated protein	3.47
	854	NCgl0377 [a, b]	hypothetical membrane protein	7.05
	3543	NCgl0381 [a]	hypothetical membrane protein	17.53
	3544	NCgl0382	hypothetical membrane protein	6.23
	1059	NCgl0549	hypothetical membrane protein	2.04
	1080	NCgl0565	putative membrane protein	2.46
	1106	NCgl0584	hypothetical membrane protein, similar to putative stress-responsive transcriptional regulator	2.42
	1152	NCgl0623 [a]	hypothetical protein	5.45
	1168	NCgl0635 [b]	hypothetical protein, similar to siderophore-interacting proteins	3.78
	1239	NCgl0691	hypothetical protein, similar to dihydrofolate reductase	2.17
	1293	NCgl0734 [a]	hypothetical protein, similar to transcription factor WhiB	2.82
	1495	NCgl0891	hypothetical protein	2.26
	1540	NCgl0927	hypothetical protein	2.62
	1541	NCgl0928	hypothetical membrane protein, similar to esterases	2.46
	1631	NCgl1011	hypothetical protein	3.28
	1704	NCgl1076	hypothetical protein	2.06
	1718	NCgl1090	hypothetical protein	2.07
	1851	NCgl1197	hypothetical protein	2.14
	2706	NCgl1287	hypothetical protein	2.26
	2705	NCgl1288	hypothetical protein	3.23
	2703	NCgl1289 [a]	hypothetical protein	4.88
	2645	NCgl1327	hypothetical protein	2.24
	2579	NCgl1382	hypothetical protein	2.16
	2431	NCgl1487	hypothetical protein	2.07
	2489	NCgl1528	hypothetical protein	2.45
	3347	NCgl1646 [a]	hypothetical protein	4.29
	3348	NCgl1647	hypothetical protein	2.32
	2006	NCgl1848	hypothetical protein, similar to archaeal enzymes of ATP-grasp superfamily	2.47
	2974	NCgl2356	hypothetical protein	2.21
	2920	NCgl2450 [a]	hypothetical protein, involved in propionate catabolism	35.16
	2919	NCgl2451 [a]	hypothetical protein	13.75
	21	NCgl2584 [a]	hypothetical protein, involved in biosynthesis of extracellular polysaccharides	5.21
	242	NCgl2753	hypotheical protein, similar to vancomycin resistance protein	2.04
	1884	NCgl2900	hypothetical protein	2.28
	1898	NCgl2912	hypothetical membrane protein	2.42
	1922	NCgl2934	hypothetical protein, similar to phosphotransferase system mannitol/fructose-specific IIA domain (Ntr-type)	2.21
	1938	NCgl2944	hypothetical protein	2.63
	1941	NCgl2946 [a]	hypothetical protein	3.89
	535		hypothetical protein	2.08
	963		hypothetical protein	2.08
	1940		hypothetical protein	2.09
	810		hypothetical protein, similar to transposases	2.12
	749		hypothetical protein	2.15
	1939		hypothetical protein	2.36
	325		hypothetical protein	2.40
	3190		hypothetical protein	2.45
	853		hypothetical protein	2.71
	2147		hypothetical protein	3.57
	517	[a]	hypothetical protein	9.96
	2754		hypothetical protein	25.11

Tab. S-5: *L. monocytogenes* genes, which showed significant changed in transcription level only after acid treatment at 37°C.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	adapt.	temp. shift	Id	LmoNr.	Function
		1.02	2.07	0.48		B	lmo0011	unknown, similar to mevalonate diphosphate decarboxylase
1.64		1.54	2.16	1.55		B	lmo0013	QoxA, AA3-600 quinol oxidase subunit II
	1.07		1.08	0.84		B	lmo0015	QoxC, AA3-600 quinol oxidase subunit III
1.18		0.64	0.70			B	lmo0025	Unknown, similar to phosphoheptose isomerase
		1.04	0.59	0.42		B	lmo0067	Unknown, similar to dinitrogenase reductase ADP-ribosylation system
	-1.66	-2.73	-2.04	1.15		B	lmo0099	unknown
	1.57	1.96	2.19			B	lmo0101	Unknwon, similar to transcription regulator
-0.73	-0.93	-1.15	-1.18	-0.87		B	lmo0145	hypothetical protein
		0.89	1.19	0.46		B	lmo0153	Unknown, similar to a probable high-affinity zinc ABC transporter (Zn(II)-binding lipoprotein)
1.66	1.49	1.82	2.46	0.89		B	lmo0189	Unknwon, highly similar to *B. subtilis* Veg protein
		1.20	1.11	0.32		B	lmo0198	GcaD, highly similar to UDP-N-acetylglucosamine pyrophosphorylase
		1.77	1.46	0.30		B	lmo0224	Sul, highly similar to dihydropteroate synthases
1.44	3.26	2.57	1.03	0.84		B	lmo0232	ClpC, endopeptidase Clp ATP-binding chain C
		0.85	1.07	0.61		B	lmo0246	NusG, transcription antitermination factor
		-1.49	-2.40	-0.79		B	lmo0251	RplL, ribosomal protein L12
-0.83		-1.41	-2.12			B	lmo0254	Unknown
-0.69	-1.45	-1.17				B	lmo0256	Unknown, conserved hypothetical protein
-0.79		-1.24	-1.23			B	lmo0257	Unknown, similar to unknown protein
-0.76		0.99	2.29			B	lmo0261	Unknown, similar to phospho-beta-glucosidase
		-0.90	-1.92	-0.74		B	lmo0273	Unknown
-1.62	-1.79		-0.93	-1.22		B	lmo0280	Unknown, highly similar to anaerobic ribonucleotide reductase activator protein
	1.32	2.33	2.40	0.80		B	lmo0304	Unknown
	0.91	1.28	1.23	1.07		B	lmo0379	Unknown
		0.88	0.47	1.03		B	lmo0387	Unknown, similar to *B. subtilis* YhdG protein
-0.72	-1.10	-1.25	-1.58			B	lmo0391	Unknown
-1.30	-1.04	-1.39	-1.56			B	lmo0392	Unknown, highly similar to *B. subtilis* YqfA protein
	-1.29	-1.62	-1.61	-0.78		B	lmo0393	Unknown
-0.73		1.05	1.00	0.59		B	lmo0397	Unknown, similar to unknown proteins
-0.94		-1.11	-0.99			B	lmo0401	Unknown, highly similar to *E. c*ol YbgG protein, a putative sugar hydrolase
0.70			-1.22	0.97		B	lmo0406	Unknown, similar to *B. subtilis* YyaH protein
-0.82			-2.53	-3.11		B	lmo0412	Unknown
-1.99	-1.86	-1.39	-1.64	-1.13		B	lmo0414	Unknown, conserved membrane protein
	-1.73	-2.98	-1.79			B	lmo0428	Unknown, similar to PTS fructose-specific enzyme IIC component
		1.07	1.03	0.66		B	lmo0453	Unknown, conserved hypothetical proteins
		1.42	1.49	1.10		B	lmo0454	Unknown, conserved hypothetical protein similar to *B. subtilis* YeaC
0.96	1.43	1.52	1.06	1.13		B	lmo0479	Unknown, putative secreted protein
	1.53	2.07	1.79	1.89		B	lmo0481	Unknown, similar to unknown proteins
0.74	0.85	1.27	1.72	1.51		B	lmo0486	RpmF, ribosomal protein L32
	-1.12	-1.14	-1.54	-0.70		B	lmo0488	Unknown, similar to transcriptional regulator (LysR family)
	-2.33	-2.02	-1.48			B	lmo0534	Unknown, similar to unknown proteins
-1.95	-1.45	-1.50	-1.38			B	lmo0536	Unknown, similar to 6-phospho-beta-glucosidase
-0.92		-1.08	-1.04	-0.96		B	lmo0552	Unknown, similar to unknown protein

0.86			-1.62	2.08		B	lmo0554	Unknown, similar to NADH-dependent butanol dehydrogenase
0.94	1.23	1.16		1.41		B	lmo0555	Unknown, similar to di-tripeptide transporter
	-1.32	-0.94	-0.63			B	lmo0557	Unknown, similar to phosphoglycerate mutase
-0.99	-1.03		-1.07	0.76		B	lmo0558	Unknown, conserved hypothetical protein
1.02	1.21	1.60	1.70			B	lmo0575	Unknown, similar to transcription regulator GntR family
	1.98	2.58	2.37			B	lmo0589	Unknown
	2.10	2.33	2.55			B	lmo0590	Unknown, similar to a fusion of two types of conserved hypothetical proteinconserved hypothetical
	1.16	1.84	1.40	1.23		B	lmo0591	Unknown, similar to unknown membrane proteins
		0.91	1.04	1.11		B	lmo0592	Unknown
	1.17	1.37		1.69		B	lmo0606	Unknown, similar to transcription regulator MarR family
		0.49	1.26	0.44		B	lmo0607	Unknown, similar to ABC transporter, ATP-binding protein
1.19	1.67	1.27	2.25	1.07		B	lmo0608	Unknown, similar to ABC transporter, ATP-binding protein
		2.44	2.32	0.78		B	lmo0612	Unknown, similar to transcription regulator MarR family
		-1.16	-1.46	0.41		B	lmo0642	Unknown
0.73	0.99	1.33	1.24			B	lmo0653	Unknown
	2.16	2.74	3.29	1.62		B	lmo0667	Unknown, similar to ABC transporter (ATP-binding protein)
		2.61	3.06	1.36		B	lmo0668	Unknown, similar to putative ABC transporter, permease protein
-1.30	-1.08	-0.83	-0.60			B	lmo0675	
	-1.58	-1.98	-1.85	-1.56		B	lmo0697	Unknown, similar to flagellar hook protein FlgE
2.55	2.63	2.55	2.10	3.41		B	lmo0722	Unknown, similar to pyruvate oxidase
1.10	1.21	1.32		1.82		B	lmo0723	Unknown, similar to metyl-accepting chemotaxis protein
-0.86	-0.96	-1.17		0.40		B	lmo0745	Unknown
1.37	1.31	0.97	1.25			B	lmo0763	Unknown, similar to unknown proteins
	0.91	1.43	1.69			B	lmo0778	Unknown
-0.69	-1.29	-1.40	-1.39	-1.35		B	lmo0789	Unknown, similar to conserved hypothetical proteins
4.89	3.67	2.43	2.34	0.93		B	lmo0793	Unknown, similar to conserved hypothetical protein
	0.99	1.01	1.41			B	lmo0804	Unknown
		1.49	1.25	1.31		B	lmo0812	unknown
	-2.26	-3.08	-3.12			B	lmo0814	unknown, similar to oxidoreductases
-0.93	-1.08	-1.15	-1.20			B	lmo0838	UhpT, highly similar to hexose phosphate transport protein
		0.95	1.24	0.90		B	lmo0857	unknown, similar to carboxylesterase
0.53		0.40	1.36			B	lmo0865	Unknown, similar to phosphomannomutase
1.29	1.25	0.94	1.01	0.90		B	lmo0878	unknown, similar to oxidoreductases
	1.17	1.18	0.60			B	lmo0899	unknown, similar to *B. subtilis* YdcK protein
		1.19	1.24	0.44		B	lmo0901	unknown, similar to PTS system, cellobiose-specific IIC component
-0.91	-1.33	-0.97				B	lmo0916	Unknown, similar to phosphotransferase system enzyme IIA
1.07	1.65	2.08	1.45	0.91		B	lmo0958	unknown, similar to transcription regulator (GntR family)
	-1.66	-2.92	-1.98			B	lmo0959	unknown, similar to undacaprenyl-phosphate N-acetylglucosaminyltransferase
2.93	2.74	2.34	1.21	0.88		B	lmo0960	unknown, similar to proteases
-0.71	-1.11	-0.85	-1.01			B	lmo0961	similar to proteases

	1.67	2.60	2.72	1.10		B	lmo0977	unknown, similar to *B. subtilis* YjcH protein
		0.97	1.48	1.07		B	lmo0984	unknown, weakly similar to two-component response regulator
-0.76	-0.94		-1.10	-0.81		B	lmo0985	unknown
2.95	2.50	2.27	1.92	0.86		B	lmo0998	unknown, similar to hypothetical protein
3.79	3.19		3.01			B	lmo1002	PtsH, PTS phosphocarrier protein Hpr (histidine containing protein)
1.77			0.47	0.40		B	lmo1012	unknown, similar to N-acyl-L-amino acid amidohydrolases
-0.51		1.01	1.02			B	lmo1027	unknown, similar to conserved hypothetical proteins (in particular *B. subtilis* YkqC)
-0.47		-1.09	-1.48	-1.32		B	lmo1036	unknown
-0.66		-1.66	-1.52			B	lmo1041	unknown, similar to molybdate ABC transporter binding protein
0.74	1.29	0.99	0.86	0.55		B	lmo1051	unknown, similar to formylmethionine deformylase and to *B. subtilis* YkrB protein
	1.09	1.58	1.35	0.57		B	lmo1053	PdhB, highly similar to pyruvate dehydrogenase (E1 beta subunit)
	0.96	1.47	1.08	0.78		B	lmo1054	PdhC, highly similar to pyruvate dehydrogenase (dihydrolipoamide acetyltransferase E2 subunit)
		1.04	1.22	0.39		B	lmo1056	unknown
0.70	1.16	1.15	2.01	0.92		B	lmo1057	unknown, similar to L-lactate dehydrogenase
0.76	1.31	1.40	2.37	0.90		B	lmo1058	unknown, similar to *B. subtilis* YktA protein
		1.23	1.74	0.77		B	lmo1060	unknown, similar to transcription response regulator
	0.90	1.26	1.27			B	lmo1125	unknown
	-1.39	-2.12	-1.51	-0.65		B	lmo1149	unknown, similar to alpha-ribazole-5 -phosphatase
-1.31	-1.70	-1.83	-1.92			B	lmo1170	unknown, similar to *Salmonella enterica* PduX protein
	1.08	0.74	0.84			B	lmo1181	unknown, similar to cobalamin adenosyl transferase
1.37		0.75	0.48			B	lmo1229	unknown, similar to *B. subtilis* YshA protein
1.43	1.87	2.32	2.90	1.25		B	lmo1233	
	2.54	2.87	3.77	1.44		B	lmo1246	Unknown, similar to ATP-dependent RNA helicase (DEAD motif)
		1.46	2.50	0.94		B	lmo1247	unknown
-1.31	-1.65	-3.29	-3.73			B	lmo1254	Unknown, similar to alpha,alpha-phosphotrehalase
-0.41		-1.05	-1.11			B	lmo1255	Unknown, similar to PTS system trehalose specific enzyme IIBC
		-1.07	-2.28	-4.63		B	lmo1257	unknown
1.80	1.51	1.55	1.31	0.52		B	lmo1265	unknown, weakly similar to oligopeptide ABC transporter AppA (binding protein)
0.55	0.96	1.46	1.83	0.46		B	lmo1268	
	1.27	1.71	1.84	0.98		B	lmo1270	Unknown, similar to signal peptidase I
		0.66	1.31	0.50		B	lmo1272	unknown, conserved hypothetical protein similar to B. subtilis YlqF protein
	0.95	1.21	1.88	1.16		B	lmo1283	Unknown, similar to *Lactococcus lactis* LacX protein
	1.30	1.20	1.48	1.43		B	lmo1288	Unknown, similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)
		2.00	2.03	0.94		B	lmo1292	
-0.63		-1.68	-0.97			B	lmo1351	unknown
		1.06	1.43	0.77		B	lmo1365	
		1.08	1.71	0.91		B	lmo1392	Unknown, similar to putative proteases
-1.79			-1.04	0.43		B	lmo1400	Unknown, similar to N-acetyltransferase
	1.21	1.24	1.22			B	lmo1424	Unknown, similar to manganese transport proteins NRAMP
	0.91	0.97	0.97	1.01		B	lmo1425	

	1.09	1.08		2.23		B	lmo1428	
	1.23	1.15	1.80			B	lmo1438	unknown, similar to penicillin-binding protein
		1.33	1.45	1.00		B	lmo1439	
	1.81	1.44	0.94			B	lmo1474	
1.68	1.38	1.16	1.05			B	lmo1475	
	1.29	1.44	1.28			B	lmo1501	Unknown, similar to unknown proteins
	1.52	1.50	1.30			B	lmo1507	Unknown, similar to two-component response regulators
		1.38	1.68	0.93		B	lmo1513	Unknown, similar to iron-sulfur cofactor synthesis protein
-0.46	-1.22	-1.33	-0.87			B	lmo1541	Unknown, similar to unknown protein
	-1.30	-1.28	-0.86			B	lmo1542	
		1.10	0.69	0.49		B	lmo1565	
	1.84	2.73	2.44	1.14		B	lmo1569	Unknown, similar to unknown proteins
0.48			1.13	0.86		B	lmo1577	Unknown, similar to unknown proteins
0.96	1.03	1.45	1.27	0.88		B	lmo1595	Unknown, similar to unknown protein
0.68		1.37	0.68	0.69		B	lmo1599	
	1.16	1.37	0.91	0.65		B	lmo1601	Unknown, similar to general stress protein
		1.47	1.45	0.72		B	lmo1609	Unknown, similar to thioredoxin
		1.19	1.35	0.40		B	lmo1613	Unknown, similar to unknown proteins
2.31			-0.87	0.60		B	lmo1626	
-1.15	-1.15		-1.47	-1.28		B	lmo1629	
0.81		-1.95	-3.10	-1.60		B	lmo1634	Unknown, similar to Alcohol-acetaldehyde dehydrogenase
1.51	1.32	0.81	0.36	0.59		B	lmo1646	unknown, similar to putative exonucleases SbcD
	1.12	1.49	1.91	1.50		B	lmo1676	
0.53	2.37	2.06	0.49			B	lmo1681	unknown, similar to cobalamin-independent methionine synthase
0.36		1.13	1.16	0.48		B	lmo1691	unknown, similar to deoxyuridine triphosphate nucleotidohydrolases
	0.87		1.06	0.55		B	lmo1693	unknown, similar to hypothetical proteins
1.35	1.02	0.65	0.79	0.80		B	lmo1706	unknown, similar to transport proteins
1.12	1.56	2.84	2.28			B	lmo1736	Unknown, similar to unknown proteins
0.42	0.91	2.77	1.60			B	lmo1739	Unknown, similar to amino acid (glutamine) ABC transporter (ATP-binding protein)
-0.93		0.67	1.07	0.76		B	lmo1753	Unknown, similar to unknown protein
-1.09			0.40	0.34		B	lmo1760	Unknown, similar to unknown protein
	1.31	1.75	2.38			B	lmo1763	Unknown, similar to unknown protein
-0.47		-1.83	-2.20			B	lmo1784	
	0.99	0.88	1.17	0.42		B	lmo1829	unknown, similar to fibronectin binding proteins
	-1.59	-1.60	-1.70	-1.18		B	lmo1831	
	-2.10	-1.65	-1.79	-0.70		B	lmo1832	
		0.79	1.02	0.74		B	lmo1840	
-1.45		-1.38	-1.22			B	lmo1846	unknown, similar to conserved hypothetical proteins
		0.94	1.16	0.68		B	lmo1862	unknown, similar to hypothetical proteins
-1.17		-1.50	-1.90			B	lmo1868	unknown, similar to conserved hypothetical proteins
	-0.91	-1.38	-1.83			B	lmo1879	
	1.14	0.98	0.42	0.97		B	lmo1887	unknown, similar to conserved hypothetical proteins
	1.07	1.10	1.14	0.48		B	lmo1888	unknown, similar to hypothetical proteins
		0.99	1.22	0.43		B	lmo1889	unknown, similar to conserved hypothetical proteins
	1.43	1.92	2.28	0.79		B	lmo1890	unknown, similar to conserved hypothetical proteins
0.88	1.14	1.18	1.08			B	lmo1895	

0.77		1.02	1.54			B	lmo1901	
0.73		1.29	1.29			B	lmo1902	
1.26	0.94		0.31			B	lmo1912	Unknown, similar to unknown proteins (hypothetical sensory transduction histidine kinase)
		0.77	1.12	0.63		B	lmo1922	Unknown, similar to unknown proteins
0.59		0.82	1.19			B	lmo1946	Unknown, similar to similar to acyl-CoA hydrolase
-0.52	-0.78	-1.49	-0.86			B	lmo1955	Unknown, similar to integrase/recombinase
0.65		0.84	1.17			B	lmo1961	Unknown, similar to oxidoreductases
	1.31	1.57	1.65	1.00		B	lmo1978	Unknown, similar to glucose-6-phosphate 1-dehydrogenase
	-1.64	-4.56	-4.41			B	lmo2000	Unknown, similar to PTS mannose-specific enzyme IID component
-1.89	-2.55	-5.55	-4.60			B	lmo2001	Unknown, similar to PTS mannose-specific enzyme IIC component
	-3.61	-5.42	-5.52			B	lmo2003	Unknown, similar to transcription regulator GntR family
-1.23	-1.09	-0.88	-0.89	-0.88		B	lmo2004	Unknown, similar to transcription regulator GntR family
2.10	2.10	1.94	2.09	0.70		B	lmo2028	Unknown, similar to unknown proteins
	-1.29	-1.45	-1.28			B	lmo2049	Unknown, similar to unknown proteins
1.26	1.86	2.56	2.65	0.98		B	lmo2057	
1.01	1.13	1.19	1.27	1.24		B	lmo2064	Unknown, similar to large conductance mechanosensitive channel protein
		0.76	1.51	0.77		B	lmo2078	Unknown, similar to unknown proteins
	3.01	3.22	3.42			B	lmo2090	
		0.66	1.02	0.40		B	lmo2107	Unknown, similar to transcriptional regulator (DeoR family)
-0.47	-1.43	-2.42	-2.55	-2.60		B	lmo2124	Unknown, similar to maltodextrin ABC-transport system (permease)
1.25			0.80	0.46		B	lmo2154	Unknown, similar to ribonucleoside-diphosphate reductase, subunit beta
	-1.01	-0.82	0.38			B	lmo2163	Unknown, similar to oxidoreductase
		1.37	1.67	1.05		B	lmo2167	Unknown, similar to unknown proteins
-0.84	-1.28	-1.27	-0.97			B	lmo2170	Unknown, similar to unknown proteins
	1.48	3.34	2.53			B	lmo2178	Unknown, putative peptidoglycan bound protein (LPXTG motif)
0.48	1.28	1.94	1.81	1.15		B	lmo2188	Unknown, similar to oligoendopeptidase
		1.15	1.73	0.90		B	lmo2189	Unknown, similar to a putative competence protein from streptococcus pneumoniae
0.85	0.91	1.18	1.09	0.57		B	lmo2204	Unknown, similar to unknown protein
	1.43	0.84	1.27	1.12		B	lmo2209	Unknown
		1.18	1.46	1.03		B	lmo2224	Unknown, similar to unknown proteins
	1.70	1.88	1.41	0.89		B	lmo2232	Unknown, similar to unknown proteins
	1.16	1.57	1.90			B	lmo2238	Unknown, similar to transport system permease protein
	1.22	1.03	1.03			B	lmo2250	
		1.32	1.86	0.72		B	lmo2298	Protein gp4 [Bacteriophage A118]
		1.02	1.37	1.00		B	lmo2308	unknown, similar to single-stranded DNA-binding protein
	1.15	1.13	0.91			B	lmo2321	
	-1.48	-2.54	-2.56			B	lmo2336	
-0.79	-2.28	-2.05	-2.62			B	lmo2341	Unknown, similar to carbohydrate kinases
-0.85	-1.14	-1.30	-1.18	-0.61		B	lmo2364	Hypothetical protein
-1.23		-1.35	-2.12			B	lmo2367	
		1.03	1.24	0.76		B	lmo2376	Unknown, similar to peptidyl-prolyl cis-trans isomerase
0.54		-0.91	-1.13			B	lmo2405	Unknown

		1.18	1.57	0.90		B	lmo2424	Unknown, similar to thioredoxin
		0.84	1.62	0.57		B	lmo2450	Unknown, similar to carboxylesterase
		-1.12	-1.59	-0.76		B	lmo2457	
1.00			-1.54	-0.58		B	lmo2459	
0.51		-1.26	-1.71			B	lmo2460	Unknown, similar to *B. subtilis* CggR hypothetical transcriptional regulator
		0.98	1.61	1.00		B	lmo2471	Unknwon, similar to NADH oxidase
		1.19	1.75	0.89		B	lmo2472	Unknown, conserved hypothetical protein
2.36		2.77	2.61	1.15		B	lmo2478	
		1.24	0.95	0.35		B	lmo2489	
		1.80	1.22	0.83		B	lmo2491	Unknown
		0.90	1.40	0.78		B	lmo2526	
	2.03	2.53	2.63	1.30		B	lmo2527	Unknown, similar to *B. subtilis* YwzB protein
	-0.92	-1.10		-0.58		B	lmo2531	
	1.73	2.09	2.49			B	lmo2557	Unknown, conserved hypothetical protein
1.21	2.05	2.17	1.56	0.72		B	lmo2580	Unknwon, similar to ABC transporter, ATP-binding protein
-0.46		-1.05	-0.92			B	lmo2589	Unknwon, similar to transcription regulator TetR/AcrR family
		2.42	3.08	1.54		B	lmo2593	Unknown, similar to transcription regulators (MerR family)
-0.45	-0.98	-1.62	-1.37			B	lmo2609	
	-0.87	-1.22	-2.46	-0.71		B	lmo2612	
	-1.22	-2.54	-2.49			B	lmo2613	
	-1.32	-2.82	-3.03	-0.60		B	lmo2614	
	-1.55	-2.87	-2.57	-0.78		B	lmo2615	
	-1.18	-2.66	-3.00	-0.66		B	lmo2616	
	-1.12	-2.61	-2.72	-1.03		B	lmo2617	
	-1.56	-3.51	-2.84	-0.83		B	lmo2618	
	-1.47	-3.38	-3.03	-1.02		B	lmo2619	
	-1.58	-3.25	-3.38	-0.76		B	lmo2625	
		-1.07	-1.59	-0.84		B	lmo2638	Unknown, similar to NADH dehydrogenase
0.73	1.16	1.27	1.28	0.54		B	lmo2643	RpmF, Unknown
	-2.01	-2.67	-2.13			B	lmo2651	Unknwon, similar to mannitol-specific PTS enzyme IIA component
	-1.19	-2.72	-1.83	-0.66		B	lmo2654	
-0.83	-1.00	-1.31	-1.75			B	lmo2689	Unknown, highly similar to Mg2+ transport ATPase
	1.35	1.45	1.97	0.52		B	lmo2692	Unknown
-0.94	-1.10		-1.63			B	lmo2700	Unknown, similar to aldo/keto reductase
		1.21	1.36	0.56		B	lmo2701	unknown, similar to *B. subtilis* YaaL protein
		1.77	2.05	0.80		B	lmo2714	Unknown, peptidoglycan anchored protein (LPXTG motif)
		-1.29	-0.83	-0.58		B	lmo2716	
		-0.84	-0.70	-1.81		B	lmo2720	Unknown, similar to acetate-CoA ligase
	2.33	2.05	1.50			B	lmo2739	unknown, similar to regulatory proteins of the SIR2 family
		1.82	2.12	0.73		B	lmo2769	Unknown, similar to ABC transporter, ATP-binding protein
	1.55	1.32	1.48			B	lmo2784	Unknown, similar to lichenan operon transcription antiterminator *licR*
-0.80		0.83	1.15			B	lmo2790	
2.37		0.88	1.17			B	lmo2791	
3.33	1.42	0.67				B	lmo2813	Unknown

Tab. S-6: *L. monocytogenes* genes, which showed significant change in transcription level only after acid treatment at 25°C.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	adapt.	temp. shift	Id*	LmoNr.	Function
	-0.04	-0.46	-1.45			A	lmo0007	GyrA, DNA gyrase subunit A
-0.40	-1.14	-1.29				A	lmo0009	Unknown, similar to spermidine N1-acetyltransferase
-0.50	-0.97	-1.09				A	lmo0011	unknown, similar to mevalonate diphosphate decarboxylase
	-1.22	-1.07	-0.25			A	lmo0012	Unknown, similar to mevalonate kinases
-0.51		1.17	0.31	-0.95		A	lmo0014	QoxB, AA3-600 quinol oxidase subunit I
	0.04	-1.01	-2.89			A	lmo0015	QoxC, AA3-600 quinol oxidase subunit III
	0.83	1.73	2.43	-0.52		A	lmo0019	Unknown
-1.12	-1.57	-1.40	-1.83			A	lmo0020	Unknown, similar to transcriptional regulator (GntR family)
	-0.52	-0.87	-1.33			A	lmo0027	Unknown, similar to PTS system, beta-glucosides specific enzyme IIABC
-0.23	-0.89	-1.35	-1.43			A	lmo0029	Unknown
	-0.41	-1.01	-1.29			A	lmo0033	Unknown, similar to endoglucanase
	-0.08	-1.00	-2.45			A	lmo0035	Unknown, similar to Glucosamine--fructose-6-phosphate aminotransferase (C-terminal domain)
-1.08	-1.90	-2.67	-2.58	-0.85		A	lmo0046	RpsR, ribosomal protein S18
0.48	0.69	1.73	2.21	0.55		A	lmo0047	unknown
-1.71	-1.79	-1.75	-1.29	-0.83		A	lmo0048	Unknown, similar to *Staphylococcus* two-component sensor histidine kinase AgrB
-2.39		-4.06		-1.20		A	lmo0050	Unknown, similar to sensor histidine kinase (AgrC from *Staphylococcus*)
-0.46	-0.94	-2.13	-3.37			A	lmo0055	PurA, highly similar to adenylosuccinate synthetase
-0.34	-0.73	-1.19	-1.04			A	lmo0070	unknown
-0.33	-0.01	-0.07	-0.48	2.10		A	lmo0071	unknown
-1.14	-1.60	-1.76	-1.16	-0.45		A	lmo0074	Unknown
	-0.16	-1.60	-2.72	-1.48		A	lmo0090	Unknwon, similar to ATP synthase alpha chain
0.60	1.98	3.96	4.47			A	lmo0095	unknown
	0.10	-0.64	-1.08			A	lmo0097	Unknown, similar to PTS system mannose-specific, factor IIC
-1.33		-1.32	-1.09			A	lmo0111	Unknwon
	-0.28	-2.00	-4.03	-1.07		A	lmo0112	Unknown, weakly similar to transcription regulators, Fnr/Crp family
-0.75	-1.02	-0.23	0.28			A	lmo0123	Unknown, similar to protein gp18 from Bacteriophage A118
	-0.03	-1.33	-2.48	-1.21		A	lmo0136	Unknwon, similar to oligopeptide ABC transporter, permease protein
	-0.16	-0.81	-1.44			A	lmo0138	Unknwon
-1.94	-1.27	-1.53	-1.99			A	lmo0141	unknown
-0.23	-1.10	-2.13	-2.30			A	lmo0152	Unknown, similar to oligopeptide ABC transporter-binding protein
-1.20	-1.91	-2.46	-1.88			A	lmo0164	Unknwon, similar to B. subtilis YabA protein
-2.53		-1.78	-2.36			A	lmo0165	unknwon, conserved hypothetical protein
	0.14	-0.56	-1.20	0.52		A	lmo0166	unknwon, similar to B. subtilis YazA protein
	1.42	2.04	1.85			A	lmo0170	Unknwon

		-0.59	-0.97	-1.31		A	lmo0175	Unknown, putative peptidoglycan bound protein (LPXTG motif)
	-0.37	-0.88	-1.38			A	lmo0176	Unknwon, similar to glucose uptake protein
-0.88	-1.99	-1.74	-0.84			A	lmo0186	Unknwon, similar to *B. subtilis* YabE protein
	0.25	-0.40	-1.04			A	lmo0187	Unknown, similar to *B. subtilis* YabF protein
-0.82	-1.40	-0.93				A	lmo0188	KsgA, dimethyladenosine transferase (16S rRNA dimethylase)
	-0.46	-2.42	-4.09			A	lmo0190	Unknwon, similar to B. subtilis YabH protein
-2.00	-2.24	-1.33	-1.35	-0.25		A	lmo0194	ABC transporter, ATP-binding protein
	0.16	-1.22	-2.48			A	lmo0195	Unknown, similar to membrane protein (putative ABC transporter component)
	-0.44	-1.79	-1.31	-0.44		A	lmo0196	Unknwon, similar to *B. subtilis* SpoVG protein
-0.26	-0.92	-1.49	-1.66			A	lmo0199	Prs, phosphoribosyl pyrophosphate synthetase
-0.78	-1.67	-1.89	-1.44	0.61		A	lmo0213	Pth, similar to peptidyl-tRNA hydrolase
-1.44	-1.64	-1.55	-0.82			A	lmo0214	Mfd, transcription-repair coupling factor
-0.98	-1.41	-0.47				A	lmo0215	Unknown, conserved membrane-spanning protein
-0.24	-0.05	-1.21	-3.30	-0.33		A	lmo0216	Unknwon, highly similar to B. subtilis YabO protein
-1.43	-2.59	-2.78	-2.51			A	lmo0220	FtsH, highly similar to cell division protein ftsH
-1.19	-1.92	-2.65	-3.04			A	lmo0221	Unknwon, conserved hypothetical protein
-0.79	-1.34	-1.83	-1.21			A	lmo0222	Unknwon, conserved hypothetical protein
	0.01	-0.55	-1.15			A	lmo0226	Folk, similar to 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase
-0.35	-0.77		-2.01			A	lmo0228	LysS, lysyl-tRNA synthetase
	0.70	1.99	2.10			A	lmo0230	Unknown, similar to B. subtilis YacH protein
	1.06	1.01	0.77	-1.12		A	lmo0233	unknown, similar to DNA repair protein Sms
	0.00	-0.84	-1.79			A	lmo0237	GltX, highly similar to glutamyl-tRNA synthetase
	-1.23	-1.80	-1.80	-0.26		A	lmo0239	CysS, cysteinyl-tRNA synthetase
	-0.40	-1.84	-1.64			A	lmo0241	Unknown, similar to conserved hypothetical proteins like to *B. subtilis* YacO protein
0.86	1.33	1.77	2.19			A	lmo0242	Unknown, similar to *B. subtilis* Yacp protein
	-0.27	-1.87	-2.06			A	lmo0243	SigH, RNA polymerase sigma-30 factor (sigma-H)
	-0.42	-1.08	-0.80			A	lmo0247	Unknown
-0.53	-0.53	-0.70	-2.25	-0.31		A	lmo0249	ribosomal protein L1
1.41	2.57	3.41	3.60			A	lmo0250	RplJ, ribosomal protein L10
	0.16	0.73	1.18			A	lmo0257	Unknown, similar to unknown protein
	0.20	-0.85	-1.91			A	lmo0260	Unknown, similar to unknown proteins
-0.83	-1.83	-2.03	-1.81	-0.27		A	lmo0261	Unknown, similar to phospho-beta-glucosidase
0.66	-0.47	-1.24	-1.33	-0.27		A	lmo0263	InlH, internalin H
0.63	-0.05	-2.71	-3.26	-0.33		A	lmo0265	Unknown, similar to succinyldiaminopimelate desuccinylase
-1.00	-1.69	-3.50	-3.65			A	lmo0267	Unknown, similar to other proteins
0.69	1.28	2.02	2.25			A	lmo0268	Unknown, similar to phosphoglycerate mutase
-2.88		-3.29	-2.94			A	lmo0270	Unknown
-0.31	-0.43	-0.75	-1.97			A	lmo0271	unknown, highly similar to phospho-beta-glucosidase
	-0.78	-0.93	-1.55			A	lmo0274	Unknown
-0.75	-0.75	-1.05	-0.94			A	lmo0275	Unknown, C-terminal part similar to B. subtilis ComEC protein

	-0.40	-1.23	-0.84			A	lmo0276	Unknown, conserved hypothetical protein
-0.46	-1.03	-1.96	-2.59			A	lmo0281	Unknown
	0.06	-1.35	-2.40	-0.29		A	lmo0284	Unknown, similar to ABC transporter (ATP-binding protein)
	-0.77	-0.89	-1.20			A	lmo0285	Unknown, putative lipoprotein
	-0.65	-1.16	-0.94			A	lmo0286	Unknown, similar to aminotransferase
	-0.56	-1.84	-1.23			A	lmo0289	Unknown, similar to B. subtilis YycH protein
-0.27	-0.67	-1.88	-1.41			A	lmo0303	Unknown, putaive secreted, lysin rich protein
	0.15	-0.99	-1.17			A	lmo0313	Unknown, conserved hypothetical protein
	0.93	1.05	0.45	-1.28		A	lmo0322	Unknown, similar to unknown proteins
-0.22	-0.91	-1.07	-0.78			A	lmo0325	Unknown, similar to transcriptional regulators
1.01	2.37	2.21	1.18	-0.53		A	lmo0326	Unknown, similar to transcriptional regulators
-0.68	-1.32	-1.58	-1.85			A	lmo0327	Unknown, similar to cell surface proteins (LPXTG motif)
-0.69	-1.14	-0.79	-0.80			A	lmo0335	Unknown
	0.00	0.66	1.24			A	lmo0336	Unknown
-0.15		1.19	1.23	-0.43		A	lmo0339	Unknown, weakly similar to inorganic pyrophosphatase
	1.04	2.17	3.63	0.87		A	lmo0341	Unknown
	0.59		1.61	-0.43		A	lmo0363	Unknown, similar to *Salmonella typhimurium* peptidase E
	-0.23	-1.42	-0.88	-0.36		A	lmo0364	Unknown, similar to transcription regulator
	-0.36	-0.98	-1.06			A	lmo0367	Unknown, conserved hypothetical protein similar to B. subtilis YwbN protein
-0.68	-1.67	-1.48	-1.22			A	lmo0372	Unknown, similar to beta-glucosidase
	0.74	1.06	1.57			A	lmo0375	unknown
1.00	1.57	2.45	2.23	0.64		A	lmo0376	Unknown, similar to putative transcription regulator
	1.88	2.35	2.63	1.06		A	lmo0378	Unknown
	-2.12	-2.48	-3.10			A	lmo0382	Unknown, similar to B. subtilis transcription repressor of myo-inositol catabolism operon IolR
	0.19	0.72	1.41			A	lmo0389	LtrA, low temperature requirement protein A
-1.69	-1.95	-0.67				A	lmo0390	Unknown, similar to uracil-DNA glycosylase
-0.41	-0.83	-1.51	-1.80			A	lmo0402	Unknown, similar to transcriptional antiterminator (BglG family)
-1.12	-1.24	-0.89	-0.60			A	lmo0404	Unknown
-0.49		1.22	0.16			A	lmo0406	Unknown, similar to B. subtilis YyaH protein
-0.32	-0.81	-2.31	-1.87	-0.71		A	lmo0410	Unknown, similar to phosphoenolpyruvate synthase, C-terminal part
-0.48	-0.88	-1.81	-0.82	-0.83		A	lmo0411	Unknown, similar to phosphoenolpyruvate synthase (N-terminal part)
	0.59	0.81	1.04			A	lmo0431	Unknown, similar to acetyltransferase
-0.66	-1.77	-3.04	-1.43	-0.43		A	lmo0436	Unknown, similar to unknown proteins
		2.81	3.46	-1.58		A	lmo0439	Unknown, weakly similar to a module of peptide synthetase
		0.87	1.41	1.77		A	lmo0443	Unknown, similar to B. subtilis transcription regulator LytR
-0.15	-0.54	-2.54	-2.08			A	lmo0452	Unknown, similar to unknown proteins
-0.46	-1.09	-2.16	-2.57	-0.40		A	lmo0455	Unknown, similar to unknown proteins
-1.28	-0.77		-1.67			A	lmo0468	Unknown
-1.22	-1.87	-1.87	-1.19			A	lmo0472	Unknown

-1.42	-1.68	-1.99	-1.77			A	lmo0473	Unknown
-1.90	-2.52	-2.69	-2.77			A	lmo0474	Unknown
-2.05	-2.52	-3.76	-3.31			A	lmo0475	Unknown
	-0.42	-2.16	-2.17			A	lmo0484	Unknown
-0.35	-0.71	-1.12	-0.98			A	lmo0494	Unknown, weakly similar to esterase
	1.98	3.15	2.59			A	lmo0496	Unknown, similar *to B. subtilis* YnzC protein
-0.16	-0.79	-2.19				A	lmo0497	Unknown, similar to sugar transferase
	-0.23	-1.02	-0.95			A	lmo0499	Unknown, similar to ribulose-5-phosphate 3 epimerase
	-0.72	-1.00	-0.99			A	lmo0509	Prs, similar to phosphoribosyl pyrophosphate synthetase
-2.02	-2.35	-2.37	-2.73	-0.35		A	lmo0510	Unknown
	-0.32	-1.65	-1.92			A	lmo0517	Unknown, similar to phosphoglycerate mutase
	-0.63	-1.53	-0.79			A	lmo0522	Unknown, similar to transcription regulator
-0.88			-1.82	-0.43		A	lmo0525	Unknown
-0.86	-1.53	-2.39	-2.56			A	lmo0527	Unknown, transmembrane protein
-0.24	-1.02	-1.85	-2.28			A	lmo0532	Unknown
-0.40	-1.04	-1.03	-1.03			A	lmo0533	Unknown, similar to unknown proteins
0.50	0.82	1.13	0.82			A	lmo0535	Unknown, similar to transcription regulator (LacI family)
-0.72	-1.79	-1.78	-0.76			A	lmo0537	Unknown, similar to N-carbamyl-L-amino acid amidohydrolase
-0.25	-0.64	-2.28	-1.48	0.58		A	lmo0538	Unknown, similar to N-acyl-L-amino acid amidohydrolase
-0.56	-0.82	-1.07	-1.66			A	lmo0540	Unknown, similar to penicillin-binding protein
	-0.49	-1.51	-2.33			A	lmo0550	Unknown, pepdidoglycan bound protein (LPXTG motif)
	0.82	1.03	0.57			A	lmo0552	Unknown, similar to unknown protein
-1.01	-0.96	-0.15	-0.51	-0.64		A	lmo0555	Unknown, similar to di-tripeptide transporter
-2.73	-4.50	-3.54	-4.01	-0.86		A	lmo0559	Unknown, putative membrane protein
-0.33	-0.18	1.03	1.97			A	lmo0568	HisG, similar to ATP phosphoribosyltransferase
-0.77	-1.14	-1.14	-0.63	0.52		A	lmo0571	Unknown, similar to methyltransferase
-0.46	-0.72	-0.40	1.09			A	lmo0588	Unknown, similar to DNA photolyase
-0.76	-0.79	-1.21	-1.25	-0.99		A	lmo0589	Unknown
-0.73	-0.77	-1.50	-1.58			A	lmo0590	Unknown, similar to a fusion of two types of conserved hypothetical proteinconserved hypothetical
-0.57	-0.50	-1.18	-1.52	-0.67		A	lmo0591	Unknown, similar to unknown membrane proteins
-1.33	-2.86	-3.98	-4.75	-0.61		A	lmo0598	Unknown, similar to proteins involved in biotin metabolism (BioY)
-0.65	-1.47	-3.36	-4.02	-0.24		A	lmo0599	Unknown, conserved hypothetical protein
	-0.38	-2.54	-3.72	-0.35		A	lmo0600	Unknown
2.64	3.22	1.55	1.90	1.01		A	lmo0604	Unknown, similar to B. subtilis YvlA protein
-1.10	-1.83	-2.70				A	lmo0605	Unknown, conserved hypothetical membrane protein
	0.06	1.47	3.77			A	lmo0613	Unknown, similar to oxidoreductase
	-0.16	-1.19	-2.04			A	lmo0616	Unknown, C-terminal domain similar to glycerophosphoryl diester phosphodiesterase
-0.47	-0.61	-0.95	-1.06			A	lmo0618	Unknown, similar to protein kinase
-1.00	-0.86	-0.71	0.23			A	lmo0622	Unknown, hypothetical
	1.04	1.63	2.07			A	lmo0624	Unknown, similar to unknown proteins

	-0.21	-1.03		-0.50		A	lmo0637	Unknown, weakly similar to methyltransferase
-0.39	-1.02	-2.10	-2.37			A	lmo0645	Unknown, similar to amino acid transporter
-0.99	-0.15	-0.97	-1.37	-1.04		A	lmo0648	Unknown, similar to membrane proteins
	-0.10	-0.61	-1.78			A	lmo0650	Unknown, conserved membrane protein
-1.24	-1.62	-1.33				A	lmo0656	Unknown, conserved hypothetical protein
	3.10	3.70	2.80			A	lmo0660	unknown, similar to transposases
-0.93	-2.07	-2.72	-2.12	0.77		A	lmo0664	Unknown, similar to acetyl transferase
	1.44	1.64	1.24			A	lmo0670	unknown
-0.73	-2.19	-3.97	-4.04			A	lmo0678	Unknown, similar to flagellar biosynthetic protein FliR
-0.80	-2.43	-4.90		-1.71		A	lmo0679	Unknown, similar to flagellar biosynthetic protein flhB
	-0.75	-2.42	-3.65	-2.72		A	lmo0693	Unknown, similar to flagellar motor switch protein fliY C-terminal part
	-0.49	-1.37		-0.37		A	lmo0694	Unknown
0.78	1.63	2.41	2.33			A	lmo0720	Unknown
-0.64	-0.20	0.79	2.20	-0.26		A	lmo0729	Unknown
	0.20	-0.73	-3.29	-1.42		A	lmo0731	Unknown
-0.41	-0.87	-0.49	1.12			A	lmo0734	Unknown, similar to transcriptional regulator (LacI family)
0.89	1.01	0.90	0.60			A	lmo0754	Unknown, weakly similar to a bile acid 7-alpha dehydratase
	0.99	1.33	2.11			A	lmo0758	Unknown
-0.68	-1.12	-1.48	-0.45			A	lmo0764	Unknown, similar to lipoate-protein ligase
	-0.03	-0.86	-1.39			A	lmo0765	Unknown
-1.13	-1.74	-0.73	0.32			A	lmo0778	Unknown
	-0.56	-1.05		-0.52		A	lmo0785	Unknown, similar to transcriptional regulator (NifA/NtrC family)
-1.55	-3.02	-3.29				A	lmo0787	Unknown, similar to amino acid transporter
-1.03	-2.06	-2.54	-2.01	-0.42		A	lmo0790	Unknown, similar to transcription regulator (EbsC from *Enterococcus faecalis*)
0.66	2.02	2.86	2.70	-0.86		A	lmo0794	Unknown, similar to *B. subtilis* YwnB protein
	1.30	1.87	3.46			A	lmo0800	Unknown, similar to *B. subtilis* YqkB protein
-0.94	-0.83		-0.98	1.39		A	lmo0802	Unknown, weakly similar to GTP-pyrophosphokinase
-0.96	-1.60	-0.75		0.79		A	lmo0812	unknown
-0.18	-0.52	-1.06	-1.12			A	lmo0813	unknown, similar to fructokinases
	-0.62	-0.96	-1.09			A	lmo0816	Unknown, similar to *B. subtilis* regulatory protein PaiA
	-0.81	-1.78	-1.60			A	lmo0817	Unknown, similar to *E. coli* PhnB protein
-0.72	-0.33	0.85	1.17	-0.24		A	lmo0819	unknown
		0.91	1.37	0.94		A	lmo0824	unknown
-0.24	-0.59	-1.51	-2.69			A	lmo0825	unknown, similar to 3-hydroxy-3-methylglutaryl-coenzyme a reductase
	-0.40	-0.77	-1.10			A	lmo0826	Unknown, similar to transport protein
-0.25	-0.59	-1.66	-1.63	-0.39		A	lmo0830	Fbp, highly similar to fructose-1,6-bisphosphatase
-1.20		-1.56	-1.34			A	lmo0831	unknwon
-0.62	-1.07	-0.72	1.10			A	lmo0836	unknown, similar to *B. subtilis* YrkR protein
-1.06	-1.24	-0.72				A	lmo0841	unknown, similar to cation (calcium) transporting ATPase
	-0.78	-1.07	-1.00			A	lmo0842	Unknown, putative peptidoglycan bound protein (LPXTG motif)

	-0.69	-2.19	-4.04	-1.90		A	lmo0848	unknown, similar to amino acid ABC transporter, ATP-binding protein
	0.16	-1.09	-1.59			A	lmo0852	Unknown, similar to transcription regulator TetR/AcrR family
	-0.57	-1.86	-1.53			A	lmo0854	Unknown, similar to *E. coli* SugE protein (transmembrane chaperone)
	-0.25	-1.20	-2.39			A	lmo0855	D-alanine--D-alanine ligase
-0.48	-1.12	-1.31				A	lmo0856	MurF, UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diamino pimelate-D-alanyl-D-alanyl ligase
-0.41	-1.07		-1.10			A	lmo0867	unknown
-0.26	-0.04	-0.07	-1.11			A	lmo0877	unknown, similar to *B. subtilis* NagB protein (glucosamine-6-phosphate isomerase)
-0.65	-1.89	-2.61	-2.61			A	lmo0882	unknown, similar to *B. subtilis* YdbS protein
-1.40	-2.83	-3.24	-3.25	-0.39		A	lmo0883	Unknown, similar to *B. subtilis* YbtB protein
	-0.43	-1.42		0.55		A	lmo0886	Dal, similar to alanine racemase
	-0.90	-1.83	-1.84			A	lmo0890	RsbS, highly similar to negative regulation of sigma-B activity
	-0.79	-1.27	-0.94			A	lmo0898	unknown, conserved hypothetical protein
	1.16	1.86	1.11	-0.57		A	lmo0903	unknown, conserved hypothetical protein
0.97	1.49	1.01	1.16			A	lmo0910	Unknown
1.50	2.16	2.42	2.27			A	lmo0914	Unknown, similar to PTS system, IIB component
	-0.30	-0.86	-0.89	1.06		A	lmo0919	unknown, similar to ABC transporter ATP-binding protein (antibiotic resistance)
-0.70	-1.51	-1.51	-1.18			A	lmo0927	unknown, hypothetical transmembrane protein
		0.97	1.99	0.68		A	lmo0933	unknown, similar to sugar transferase
0.90	1.38	2.19	2.67			A	lmo0937	unknown
-0.35	-0.77		1.47			A	lmo0949	unknown, conserved hypothetical membrane protein
-0.24	-0.56	-1.15				A	lmo0951	unknown
1.20	2.00	2.83	2.98	-0.40		A	lmo0954	Unknown
0.66	0.93	2.15	2.81			A	lmo0956	Unknown, similar to N-acetylglucosamine-6P-phosphate deacetylase (EC 3.5.1.25)
-0.20	0.22	1.67	3.19			A	lmo0957	Unknown, similar to glucosamine-6-Phoasphate isomerase (EC 5.3.1.10)
0.96	1.49	2.10	1.79			A	lmo0962	unknown, similar to proteases
	-0.40	-2.22	-3.37			A	lmo0967	unknown, similar to *B. subtilis* YjbM protein
	-0.44	-1.60	-2.06			A	lmo0968	unknown, similar to conserved hypothetical proteins like to *B. subtilis* YjbN protein
	-0.19	-0.07	1.87			A	lmo0976	unknown, similar to B. subtilis YjcF protein
-0.73	-1.35	-2.69	-3.11	-0.34		A	lmo0981	unknown, similar to efflux transporter
-0.47	-0.64	-1.24				A	lmo0984	unknown, weakly similar to two-component response regulator
-0.46	-1.05	-0.68	-1.22			A	lmo0988	unknown, similar to peptide chain release factor 3 (RF-3)
	0.15		0.32	-1.26		A	lmo0990	unknown, conserved hypothetical protein
	-0.15	-0.50	-1.00			A	lmo0992	unknown, conserved hypothetical protein
	-0.36	-0.80	-1.29			A	lmo0993	unknown, similar to Na+-transporting ATP synthase subunit J
	1.83	3.01	2.79	-1.09		A	lmo0994	unknown
-2.36	-2.34	-1.94	-2.41			A	lmo0997	ClpE, ATP-dependent protease
-0.69		-1.01	-1.42			A	lmo1000	unknown, similar to phytoene dehydrogenase

-0.96	-1.95	-1.84	-1.95			A	lmo1003	phosphotransferase system enzyme I
	0.09	-0.36	-1.62			A	lmo1004	unknown, conserved hypothetical protein
-0.52	-1.65	-1.91	-1.94			A	lmo1006	unknown, similar to aminotransferases (to B. subtilis PatA protein)
-0.65	-0.84	-1.50	-1.86			A	lmo1007	unknown
-0.68	-1.34	-1.66	-1.17	-0.24		A	lmo1008	unknown, similar to *B. subtilis* YkuJ protein
	-0.35	-1.08	-1.33			A	lmo1011	unknown, similar to tetrahydrodipicolinate succinylase
-0.54	-1.13	-0.95	-0.70			A	lmo1012	unknown, similar to N-acyl-L-amino acid amidohydrolases
	-0.33	-1.07	-1.55			A	lmo1014	GbuA, highly similar to glycine betaine ABC transporter (ATP-binding protein)
	0.81	1.42	1.31			A	lmo1017	unknown, similar to phosphotransferase system glucose-specific enzyme IIA
-1.96	-3.02	-4.15				A	lmo1024	unknown
-0.62	-1.49	-1.78	-2.77			A	lmo1025	unknown
	-0.99	-1.49	-1.20	-0.68		A	lmo1027	unknown, similar to conserved hypothetical proteins (in particular B. subtilis YkqC)
-0.81	-1.24	-1.23	-1.38	-0.99		A	lmo1028	unknown, similar to B. subtilis YkzG protein
	-0.18	-1.07	-1.18			A	lmo1035	unknown, similar to phosphotransferase system (PTS) beta-glucoside-specific enzyme IIABC
-0.69	-1.34	-1.13	-0.75			A	lmo1037	unknown, highly similar to B. subtilis YoaT protein
-0.47	-1.05	-0.95	0.34	-0.39		A	lmo1049	Unknown, similar to molybdopterin biosynthesis protein MoeB
	0.15		1.09	-0.58		A	lmo1065	unknown, similar to *B. subtilis* YktB protein
-0.56	-1.69	-2.16	-0.93	0.60		A	lmo1066	unknown, similar to extragenic suppressor protein SuhB and to myo-inositol-1(or 4)-monophosphatase
-0.19	-0.85	-1.25	-1.52			A	lmo1071	unknown, similar to cell-division protein RodA and FtsW
-0.18	-1.10	-4.27	-6.14			A	lmo1074	unknown, highly similar to teichoic acid translocation permease protein TagG
	-0.67	-3.46	-5.41			A	lmo1075	unknown, similar to teichoic acid translocation ATP-binding protein TagH (ABC transporter)
-0.24	-1.19	-2.56	-3.25			A	lmo1077	unknown, similar to teichoic acid biosynthesis protein B
-0.78	-1.61		-1.28			A	lmo1078	unknown, similar to putative UDP-glucose pyrophosphorylases
-0.48	-1.62	-2.18	-2.20			A	lmo1080	unknown, similar to *B. subtilis* minor teichoic acids biosynthesis protein GgaB
-0.34	-0.90	-1.09	-0.79			A	lmo1085	unknown, similar to teichoic acid biosynthesis protein B
-0.78	-1.48	-1.29	-0.76			A	lmo1086	unknown, similar to CDP-ribitol pyrophosphorylase
-0.57	-1.30	-1.28	-0.89			A	lmo1087	unknown, similar to glucitol dehydrogenase
	-1.22	-1.62	-1.38			A	lmo1088	
-0.85	-2.06	-2.72	-2.43	0.46		A	lmo1089	
0.83	1.34	1.80	1.55	-0.34		A	lmo1099	unknown, similar to a protein encoded by Tn916
	3.58	4.63	4.85			A	lmo1113	unknown, highly similar to TN916 ORF22
	2.56	3.83	3.90	-0.55		A	lmo1114	unknown, highly similar to TN916 ORF23

-1.22	-1.57	-0.73	-0.97			A	lmo1118	unknown
-1.95	-1.19	-1.15	-0.83			A	lmo1119	unknown, similar to methylases
-0.64	-1.12	-0.95		-0.25		A	lmo1121	unknown
-0.39	-0.84	-1.41	-1.08			A	lmo1122	unknown
-0.28	-0.35	-0.72	-1.06	-0.46		A	lmo1124	unknown
-0.34	-0.31	-0.82	-1.08	-0.28		A	lmo1126	unknown, similar to E. coli YjaB protein
	-0.13	0.90	1.01			A	lmo1135	unknown
-0.14	-0.35	-1.18	-1.37			A	lmo1144	Unknown, similar to *Salmonelle enterica* PduU protein
-0.46	-0.37	-0.71	-1.95			A	lmo1188	unknown
1.42	1.50	1.26	1.16			A	lmo1189	Unknown, similar to transcriptional regulator
-0.21	-0.54	-1.27	-1.14			A	lmo1191	
-0.38	-0.56	-1.02	-0.69			A	lmo1207	Unknown, similar to cobalt transport ATP-binding protein CbiO
	0.81	1.58	2.11			A	lmo1220	unknown, similar to unknown protein
	-0.55	-1.22	-1.33			A	lmo1221	
	0.12	-0.95	-1.16			A	lmo1222	
-0.22	-0.02	-0.15	-1.29			A	lmo1226	unknown, similar to transporter, (to B. subtilis YdgH protein)
-0.68	-1.67	-1.32	-0.93			A	lmo1237	
	-0.88	-1.72	-1.71			A	lmo1238	Unknown, similar to ribonuclease PH
0.46	-0.32	-1.69	-1.81			A	lmo1239	unknown, conserved hypothetical protein, similar to B. subtilis YsnA protein
0.58	0.07	-1.49	-1.64			A	lmo1240	unknown, conserved hypothetical protein, similar to B. subtilis YsnB protein
	3.68	4.84	3.73	-0.46		A	lmo1241	unknown
-1.05	-1.21	-0.91	0.15			A	lmo1243	unknown
	-0.65	-1.72	-3.11	1.05		A	lmo1250	unknown, similar to antibiotic resistance protein
	-0.15	-1.32	-2.58	-1.53		A	lmo1254	Unknown, similar to alpha,alpha-phosphotrehalase
-0.44	-1.32	-1.56	0.05			A	lmo1260	
-1.54	-1.10	-0.65	-0.84	-0.75		A	lmo1261	unknown
-0.32	-1.03	-1.29	-1.64			A	lmo1262	unknown, similar to transcriptional regulator (phage-related)
-2.57	-2.06	-1.90	-2.47	-0.72		A	lmo1266	unknown
	-0.45	-0.97	-1.26	-0.44		A	lmo1267	
-0.52	-0.90	-1.06	-0.74			A	lmo1272	unknown, conserved hypothetical protein similar to *B. subtilis* YlqF protein
	-0.42	-1.09	-1.13			A	lmo1273	
	-0.26	-1.93	-2.20			A	lmo1275	
	-0.45	-2.15	-2.92			A	lmo1276	
-0.20	-0.21	-1.05	-2.04			A	lmo1279	
	-0.06		1.50	0.52		A	lmo1281	Unknown, similar to *B. subtilis* YneP protein
	-0.52	-1.72	-2.46			A	lmo1286	unknown, conserved hypothetical protein
	-0.03	-1.01	-2.33	-0.21		A	lmo1287	Unknown, similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)
-1.06	-1.36	-1.32				A	lmo1292	
	-0.80	-1.64	-2.74			A	lmo1294	

	-0.15	-1.57	-2.28			A	lmo1297	unknown, similar to aluminum resistance protein and to *B. subtilis* YnbB protein (hypothetical)
	0.87	1.17	1.72			A	lmo1303	unknown, similar to *B. subtilis* YneA protein
	-0.34	-1.30	-1.22			A	lmo1308	unknown, weakly similar to arginine N-methyltransferases
-0.65	-1.23	-2.77	-3.19			A	lmo1313	
-0.18	-0.83	-1.55	-2.40			A	lmo1314	
-0.54	-1.44	-2.12	-1.98	0.65		A	lmo1315	Unknown, similar to undecaprenyl diphosphate synthase
-0.43	-1.56	-3.04	-2.93			A	lmo1316	
-0.21	-0.60	-1.98	-2.95			A	lmo1318	unknown, conserved hypothetical protein similar to *B. subtilis* YluC protein
-0.31	-0.75	-1.75	-2.11	-0.26		A	lmo1321	unknown, conserved hypothetical protein, similar to *B. subtilis* YlxS protein
-0.44	-0.98	-2.21	-2.66			A	lmo1322	
	-0.25	-1.56	-2.34			A	lmo1323	unknown, similar to B. subtilis YlxR protein
	-0.08	-1.30	-2.39			A	lmo1324	unknown, conserved hypothetical protein, similar to *B. subtilis* YlxQ protein
	0.03	-1.25	-2.31			A	lmo1325	
	0.06	-0.62	-2.09	-0.41		A	lmo1326	unknown, conserved hypothetical protein similar to *B. subtilis* YlxP protein
-0.53	-1.22	-1.95	-1.91			A	lmo1328	
-0.38	-0.73	-1.84	-2.75			A	lmo1329	
	-0.41	-0.62	-1.35			A	lmo1330	
-0.86	-1.73	-2.26	-2.20			A	lmo1333	unknown, similar to *B. subtilis* YqzC protein
-0.85	-1.98	-2.59	-2.65			A	lmo1334	unknown, similar to *B. subtilis* YqzD protein
-0.15	-0.02	-0.48	-2.16			A	lmo1335	
-0.49	-1.31	-1.09	-0.50			A	lmo1337	unknown similar to *B. subtilis* yqgP
-0.20	-1.13	-2.25	-1.41			A	lmo1338	unknown similar to *B. subtilis* yqgQ
-1.61	-1.71	-1.84	-2.90	0.67		A	lmo1348	Unknown, similar to aminomethyltransferase
	-0.22	-1.28	-2.32	0.65		A	lmo1349	Unknown, similar to glycine dehydrogenase (decarboxylating) subunit 1
-0.16	-0.70	-1.19	-1.12			A	lmo1352	Unknown
	-0.17	-0.95	-2.03			A	lmo1354	Unknown, similar to aminopeptidase P
	-0.40	-1.97	-2.76			A	lmo1357	acetyl-CoA carboxylase subunit (biotin carboxylase subunit)
	-0.71	-2.06	-3.15			A	lmo1359	Unknown, similar to transcription termination protein (NusB)
-0.23	-0.99	-3.54	-4.19			A	lmo1361	Unknown, similar to exodeoxyribonuclease VII (large subunit)
	-0.35	-1.46	-0.76			A	lmo1362	Unknown, similar to exodeoxyribonuclease small subunit
	0.24		1.28	-0.25		A	lmo1367	Unknown, similar to arginine repressor
-0.53	-1.14	-3.76	-2.16	-0.25		A	lmo1369	Unknown, similar to phosphotransbutyrylase
-0.97	-1.61	-2.08	-2.38			A	lmo1370	Unknown, similar to branched-chain fatty-acid kinase
	-0.88	-2.07	-2.71			A	lmo1371	Unknown, similar to branched-chain alpha-keto acid dehydrogenase E3 subunit

	-0.36	-1.28	-2.62			A	lmo1372	Unknown, similar to branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha subunit)
	-0.11	-1.34	-2.38			A	lmo1374	Unknown, similar to branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase)
	-1.29	-1.97	-2.28			A	lmo1384	Unknown, similar to unknown protein
-0.48	-0.92	-1.27	-1.26			A	lmo1385	Unknown, similar to unknown protein
-0.76	-1.05	-0.96				A	lmo1386	Unknown, similar to DNA translocase
	-0.17	-0.01	2.07	0.80		A	lmo1387	Unknown, similar to pyrroline-5-carboxylate reductase
	0.18	-0.82	-1.05			A	lmo1391	Unknown, similar to sugar ABC transporter, permease protein
-0.65	-1.06	-1.65	-1.86			A	lmo1394	Unknown, similar to 3-ketoacyl-acyl carrier protein reductase
-0.79	-1.25	-1.92	-2.09			A	lmo1395	Unknown, similar to unknown protein
-1.54	-2.65	-4.36	-4.06			A	lmo1396	Unknown, similar to phosphatidylglycerophosphate synthase
-1.26	-1.88	-3.06	-2.80	-0.30		A	lmo1410	Unknown
-0.88	-1.36	-1.37	-1.52	-0.24		A	lmo1411	Unknown
-0.72	-1.02	-0.11	0.23			A	lmo1415	Unknown, similar to hydroxy-3-methylglutaryl coenzyme A synthase
-0.84	-1.84	-3.25	-3.63			A	lmo1419	Unknown, conserved hypothetical protein
-0.37	-1.01	-1.99	-2.85			A	lmo1420	Unknown, weakly similar to UDP-N-acetylglucosaminyl-3-enolpyruvate reductase
-1.10	-1.06	-1.08	-1.56			A	lmo1425	
-0.75	-1.07	-1.01	-0.68			A	lmo1426	
-1.16	-1.23	-1.45	-1.56			A	lmo1427	
-1.92	-1.65	-1.53	-1.41	-0.70		A	lmo1428	
-1.19	-2.49	-3.79	-3.67	-0.34		A	lmo1431	Unknown, similar to ABC transporter (ATP-binding protein)
1.42	2.84	3.30	2.98			A	lmo1433	Unknown, similar to glutathione reductase
-0.78	-1.48	-1.97	-1.70			A	lmo1440	unknown, similar to unknown proteins
-0.71	-0.54	0.88	1.56	-0.30		A	lmo1441	Unknown, similar to putative peptidoglycan acetylation protein
-0.59	-1.09			-0.64		A	lmo1442	Unknown, similar to transport proteins
-0.47	-1.02	-0.96		-0.63		A	lmo1443	Unknown
	-1.01		-3.12	-0.52		A	lmo1445	
	-0.34	-1.31	-1.80			A	lmo1448	Unknown, conserved hypothetical protein
	-0.50	-1.87	-2.79			A	lmo1449	unknown, similar to endonuclease IV
-0.82	-1.71	-2.06	-1.82			A	lmo1450	Unknown, similar to ATP-dependent RNA helicase, DEAD-box family (deaD)
-1.29	-0.36			-0.38		A	lmo1453	Unknown, conserved hypothetical protein
	-0.48	-1.82	-3.22			A	lmo1455	
	-0.47	-1.64	-3.22			A	lmo1456	Unknown, similar to unknown proteins
	0.10	-0.58	-1.35			A	lmo1457	Unknown, similar to unknown protein
	0.20	-0.67	-1.48			A	lmo1458	
-0.59	-0.99	-1.07	-1.38	-0.32		A	lmo1461	Unknown
-0.29	-0.82	-1.58	-2.32			A	lmo1462	Unknown, similar to GTP binding proteins
	-0.89	-1.61	-1.94			A	lmo1464	Unknown, similar to diacylglycerol kinase
-0.20	-0.97	-1.53	-1.69			A	lmo1465	Unknown, similar to unknown proteins

-0.13	-0.61	-1.11	-1.10			A	lmo1466	Unknown, similar to unknown proteins
	-0.04	-1.52	-2.68	-0.28		A	lmo1470	Unknown, similar to unknown proteins
	-0.12	-1.49	-2.20			A	lmo1471	Unknown, similar to ribosomal protein L11 methyltransferase
-1.04	-1.91	-2.09	-1.99			A	lmo1479	
	-0.31	-0.87	-2.66	-0.31		A	lmo1480	
-0.76	-1.55	-2.31	-1.76			A	lmo1483	
	-0.58	-1.78	-2.09			A	lmo1488	Unknown, similar to unknown proteins
	-0.45	-1.06	-1.57			A	lmo1489	Unknown, similar to unknown proteins
	-0.82	-1.95	-2.38			A	lmo1490	Unknown, similar to shikimate 5-dehydrogenase (AroD)
-0.28	-0.97	-1.98	-2.30			A	lmo1491	Unknown, similar to unknown proteins
-0.32	-0.91	-0.76	-1.04			A	lmo1492	Unknown, similar to unknown proteins
	-0.01	-0.63	-1.47	0.50		A	lmo1493	Unknown, similar to oligopeptidase
-0.34	-1.31	-2.72	-3.22			A	lmo1497	
-1.19	-3.05	-3.36	-2.97			A	lmo1498	Unknown, similar to O-methyltransferase
-0.74	-1.72	-1.67	-1.78			A	lmo1499	Unknown, similar to unknown proteins
-2.66	-3.26	-3.15	-2.66			A	lmo1500	Unknown, similar to unknown proteins
	-0.17	-1.24	-1.76			A	lmo1504	
	-0.10	-0.73	-1.39			A	lmo1509	Unknown, similar to exodeoxyribonuclease V
	-0.50	-0.95	-1.16			A	lmo1510	Unknown, similar to unknown proteins
-0.16	-0.44	-1.20	-1.21			A	lmo1512	Unknown, similar to putative tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
	-0.33	-1.18	-0.77			A	lmo1513	Unknown, similar to iron-sulfur cofactor synthesis protein
-0.66	-1.25	-1.95	-2.31			A	lmo1515	Unknown, similar to unknown protein
	0.84	2.47	2.64			A	lmo1518	Unknown
	0.09	-1.25	-1.89			A	lmo1519	
	-0.45	-1.85	-1.92			A	lmo1520	
-0.62	-0.92	-1.52	-1.96			A	lmo1524	
-0.44	-1.24	-1.77	-2.18			A	lmo1530	Unknown, similar to tRNA-guanine transglycosylase Tgt
-0.21	-0.61	-1.46	-1.14			A	lmo1532	
-0.34	-0.65	-1.47	-1.07			A	lmo1533	
	0.18	0.64	1.37			A	lmo1535	Unknown, similar to unknown proteins
-0.32	-0.96	-1.35	-1.22			A	lmo1536	Unknown, similar to prephenate dehydratase PheA
-0.71	-1.47	-1.50	-1.16			A	lmo1537	Unknown, conserved GTP binding protein
-0.65	-1.51	-4.09	-4.61			A	lmo1546	
	-0.11	-0.83	-1.49			A	lmo1552	
	-0.30	-2.15	-3.35			A	lmo1553	
-1.22	-2.55	-3.16	-3.30			A	lmo1555	Unknown, similar to uroporphyrinogen III cosynthase (HemD)
-2.16	-2.82	-2.33	-1.96	0.72		A	lmo1557	
-0.74	-1.33	-1.84	-1.98			A	lmo1558	Unknown, similar to hypothetical GTP binding protein
	0.01	-0.59	-1.13			A	lmo1560	
	-0.58	-1.56	-1.49	-0.36		A	lmo1566	

-0.72	-1.64	-1.85	-1.11	-0.57		A	lmo1567	
-2.61	-2.50	-2.38	-2.03	-0.56		A	lmo1568	Unknown, similar to unknown proteins
	-0.33	-1.09	-1.33	0.68		A	lmo1571	
-0.15	-0.20	-1.14	-3.19	-0.24		A	lmo1572	
-0.60	-1.00	-1.48	-1.79			A	lmo1574	
0.64	0.86	1.12	1.59	0.67		A	lmo1579	Unknown, similar to alanine dehydrogenase
	0.07	0.12	1.24	0.72		A	lmo1583	Unknown, similar to thiol peroxidases
-0.31	-1.28	-2.22				A	lmo1585	Unknown, similar to proteases
	-1.17	-2.23	-2.53			A	lmo1594	Unknown, similar to *B. subtilis* negative regulator of FtsZ ring formation (EzrA)
	0.02	-0.11	-1.20	-0.30		A	lmo1596	
	-0.40	0.82	2.09			A	lmo1600	
-0.41	-0.98	-0.84	-2.03			A	lmo1603	Unknown, similar to aminopeptidase
-0.56	-0.70	-0.53	-1.76			A	lmo1605	
-0.30	-0.16	-0.04	-1.15			A	lmo1606	Unknown, similar to DNA translocase
-0.17	-0.62	-1.66	-1.60			A	lmo1622	Unknown, similar to unknown proteins
-0.78	-1.52	-2.27	-2.57	-0.48		A	lmo1625	unknown
-0.19	-0.37		-1.79	-0.36		A	lmo1627	
-0.51	-1.06	-0.72	-0.53	0.55		A	lmo1636	Unknown, similar to similar to ABC transporter (ATP-binding protein)
-0.45	-1.02	-1.35	-1.68			A	lmo1638	Unknown, similar to unknown proteins
-2.49	-2.36	-2.65	-2.68	-0.59		A	lmo1639	Unknown, similar to dna-3-methyladenine glycosidase
-1.00	-0.88	-1.42	-1.22	-0.31		A	lmo1640	Unknown
	-0.19	-1.23	-2.30			A	lmo1642	Unknown, similar to putative sigma factor regulator
-0.12	-0.33	-1.48	-2.32			A	lmo1643	Unknown
	-0.44	-1.54		-0.31		A	lmo1644	Unknown, similar to SNF2-type helicase
-0.40	-0.96	-1.34	-1.17	-0.44		A	lmo1657	
-0.45	-0.78	-1.22	-0.76			A	lmo1658	
	-0.11	-1.34	-1.81			A	lmo1660	
-0.50	-1.23	-2.04	-2.56			A	lmo1661	unknown, similar to conserved hypothetical proteins
-0.29	-0.74	-1.61	-1.97			A	lmo1664	
-0.42	-0.55		1.37			A	lmo1665	unknown
-1.36	-1.16	-1.71	-2.90	-0.68		A	lmo1666	unknown, peptidoglycan linked protein (LPxTG)
	0.07	0.91	1.71			A	lmo1670	unknown, similar to conserved hypothetical proteins
	-0.34	-1.13	-1.86			A	lmo1673	
-0.16	-0.55	-1.48	-2.06	0.67		A	lmo1674	unknown, similar to prolyl aminopetidases
-0.23	-0.39	-1.38	-1.98	0.67		A	lmo1675	
-1.41	-2.44	-1.56	-1.17			A	lmo1677	unknown, similar to menaquinone biosynthesis proteins
	0.82	1.41	0.92	0.73		A	lmo1679	unknown, similar to cystathionine beta-lyase
-0.18	-0.61	-1.59	-2.28			A	lmo1688	unknown, similar to glucose 1-dehydrogenase
-0.72	-1.16	-1.90	-1.65			A	lmo1689	unknown, similar to A/G-specific adenine glycosylase
	1.39	1.15	1.08			A	lmo1690	unknown, similar to hypothetical proteins
0.99	1.76	2.38	2.34	-0.82		A	lmo1694	unknown, similar to CDP-abequose synthase

	-0.06	-0.99	-1.79			A	lmo1695	unknown, similar to putative membrane proteins
-1.03	-1.50	-2.35	-2.31			A	lmo1696	unknown, similar to unknown proteins
	-0.31	-1.07	-1.77	-1.20		A	lmo1700	unknown
-0.18	-0.62	-1.09	-0.95			A	lmo1702	unknown, similar to glutathione transferase - fosfomycin resistance protein
	0.08	-1.40	-2.39			A	lmo1712	unknown, similar to multidrug resistance protein, integral membrane protein
	-0.97	-0.90	-1.10			A	lmo1714	unknown
	-0.09	-0.81	-1.05			A	lmo1718	unknown, similar to putative outer surface protein
	0.16	-0.56	-1.00			A	lmo1720	unknown, similar to phosphotransferase system (PTS) lichenan-specific enzyme IIB component
-0.74	-1.53	-1.92	-1.78	-0.26		A	lmo1723	unknown
-0.87	-1.89	-3.06				A	lmo1724	unknown, similar to ABC transporter, ATP-binding protein
	0.20	0.85	1.96			A	lmo1726	unknown, similar to hypothetical proteins
-0.16	-0.58	-1.09	-1.28	-0.28		A	lmo1735	
	-0.31	-1.71	-0.76			A	lmo1742	
-0.92	-2.33	-1.96	-0.70			A	lmo1744	Unknown, similar to unknown proteins
-0.98	-1.60	-0.19	0.65			A	lmo1749	Unknown, similar to shikimate kinase
-0.57	-1.13	-1.93	-2.24	-0.34		A	lmo1751	Unknown, similar to hypothetical RNA methyltransferase
	-1.22	-1.74	-1.12			A	lmo1752	Unknown
	0.18	-1.26	-2.03			A	lmo1754	
	-0.58	-1.71	-1.99			A	lmo1761	Unknown, similar to putative sodium-dependent transporter
		-0.05	-1.45	0.54		A	lmo1764	
	0.05	-0.56	-1.49	0.72		A	lmo1765	
	-0.05	-1.17	-3.09	0.97		A	lmo1766	
		-1.37	-2.51	0.86		A	lmo1768	
	-0.72	-1.74	-1.91	0.87		A	lmo1769	
	-0.75	-1.12	-1.00	0.78		A	lmo1771	Unknown, similar to unknown protein
	-0.53	-2.24	-1.47	1.31		A	lmo1772	
	-0.05	-0.24	1.24			A	lmo1773	
	-0.22		1.15	0.80		A	lmo1774	
-0.40	-1.17	-1.90	-1.05			A	lmo1778	Unknown, similar to ABC transporter (ATP-binding protein)
	-0.20	-0.69	-1.90	-0.40		A	lmo1787	
1.06	1.88	2.51	2.18	-0.67		A	lmo1788	Unknown, similar to transcription regulator
	1.15	1.87	2.02	-0.47		A	lmo1789	Unknown, weakly similar to Nad(P)h Oxidoreductase chain B
	1.22	2.01	2.32			A	lmo1791	Unknown
	1.27	1.72	1.53			A	lmo1792	
0.52	0.89	1.57	2.23			A	lmo1795	Unknown, similar to unknown proteins
-0.21	-0.16	-0.16	-1.01	-0.54		A	lmo1797	
-0.42	-0.66	-1.18	-1.75			A	lmo1801	
-0.29	-0.51	-1.12	-1.82			A	lmo1802	Unknown, similar to unknown proteins

-0.16	-0.29	-2.24	-3.07	0.60		A	lmo1804	
	0.02	-1.68		0.74		A	lmo1805	
-0.32	-0.63	-1.87	-1.98			A	lmo1812	Unknown, similar to L-serine dehydratase
-0.28	-0.23	-1.22	-2.71			A	lmo1816	
	-0.19	-1.86	-2.43			A	lmo1817	Unknown, weakly similar to thiamin pyrophosphokinase
	-0.46	-1.08	-0.99			A	lmo1819	Unknown, similar to unknown proteins
	-0.01	-1.30	-1.10			A	lmo1822	Unknown, similar to RNA-binding Sun protein
	0.03	-1.42	-1.41			A	lmo1823	
-0.29	-0.87	-1.59	-1.96			A	lmo1826	unknown
-0.95	-1.49	-1.88	-2.01			A	lmo1827	unknown, similar to guanylate kinases
-1.04	-1.62	-1.62	-2.06			A	lmo1828	unknown, similar to conserved hypothetical protein
	0.86	1.37	1.67			A	lmo1830	unknown, similar to conserved hypotheticl proteins
	0.22	-0.83	-2.17			A	lmo1836	
-0.14		-1.33	-1.25			A	lmo1839	
-0.87	-1.70	-2.54	-2.31			A	lmo1840	
	-0.45	-1.13	-1.54			A	lmo1845	unknown, similar to conserved hypothetical proteins
	0.92	3.18	2.16			A	lmo1852	unknown, similar to putative mercuric ion binding proteins
-0.20	1.53	3.01	2.41			A	lmo1853	unknown, similar to heavy metal-transporting ATPases
	-0.08		1.02	0.69		A	lmo1863	unknown, similar to hypothetical proteins
-0.33	-1.06	-1.68	-0.83			A	lmo1865	unknown, similar to conserved hypothetical proteins
	-0.13	-1.18	-2.22	-0.47		A	lmo1869	unknown, similar to conserved hypothetical proteins, putative integral membrane protein
-1.65		-1.75	-1.42			A	lmo1870	unknown, similar to alkaline phosphatase
-0.83	-1.66	-2.09	-2.80	0.55		A	lmo1871	unknown, similar to phosphoglucomutases
	-0.36	-2.09	-2.90			A	lmo1874	unknown, similar to thymidylate synthase
	0.05	-0.71	-1.36			A	lmo1876	Unknown, similar to formyl-tetrahydrofolate synthetase C-terminal part
-0.85	-1.57	-2.10	-2.34			A	lmo1878	unknown, similar o transcriptional regulators
-0.36	-1.00	-1.22	-0.70			A	lmo1881	unknown, similar to 5 -3 exonuclease
-0.22	-0.83	-1.53	-1.91			A	lmo1886	unknown, similar to probable thermostable carboxypeptidases
-0.99	-1.59	-1.36	-1.14			A	lmo1891	
-0.80	-1.25	-1.26	-0.99			A	lmo1892	
	-0.30	-0.92	-1.56			A	lmo1897	
	0.04	-0.31	-1.63			A	lmo1899	
	-0.16	-0.61	-1.20	-0.32		A	lmo1903	unknown, similar to thioredoxin
	0.09	-0.48	-1.52			A	lmo1905	
-0.24	-0.91	-1.25	-1.00			A	lmo1908	Unknown, similar to unknown proteins
-2.29		-3.99	-4.32			A	lmo1911	Unknown, similar to unknown proteins (hypothetical sensory transduction histidine kinase)
	-1.49	-1.74	-1.40			A	lmo1915	Unknown, similar to malolactic enzyme (malate dehydrogenase)

	-0.21	-1.12	-2.07			A	lmo1916	Unknown, similar to peptidase
-0.65	-1.50	-2.01	-2.42			A	lmo1920	Unknown, similar to unknown proteins
	-0.23	-0.64	1.16			A	lmo1925	
-0.58	-0.85	-1.47				A	lmo1926	Unknown, similar to chorismate mutase
	-0.07	-0.75	1.33			A	lmo1927	
-0.29	-0.72	-0.75	-2.26			A	lmo1930	Unknown, similar to heptaprenyl diphosphate synthase component II (menaquinone biosynthesis)
-0.60	-0.55	-0.62	-1.46			A	lmo1931	
-0.93	-1.39	-1.02	-1.15			A	lmo1939	
-0.19	-0.40	-0.49	-1.27			A	lmo1942	
-0.62	-0.48		-1.15			A	lmo1943	Unknown, similar to unknown proteins
-0.55	-0.85	-1.01	-1.87	-0.39		A	lmo1945	Unknown, similar to unknown protein
		-1.04	-1.81	-0.55		A	lmo1949	Unknown, similar to unknown proteins
-0.22	-0.61	-1.17	-1.02	-0.31		A	lmo1954	
-0.39	-0.97	-1.18	-0.54	-0.41		A	lmo1956	
-1.15	-1.85	-2.33		-0.63		A	lmo1957	
-0.36	-1.13	-1.22		-0.34		A	lmo1958	
0.60	1.35	1.37	1.29			A	lmo1963	Unknown, similar to unknown proteins
-0.94	-1.17	-1.20	-1.43			A	lmo1965	Unknown, similar to unknown proteins
-0.29	-0.86	-1.32	-1.67			A	lmo1976	Unknown, similar to oxidoreductase
	-2.43	-2.20	-2.02			A	lmo1977	Unknown, similar to unknown proteins
-0.86	-1.42	-0.98				A	lmo1981	Unknown, similar to unknown proteins
-0.93	-1.57	-1.03	-0.45	-0.24		A	lmo1982	Unknown, similar to unknown proteins
-0.44	-1.06	-1.36	-0.63	-0.38		A	lmo2004	Unknown, similar to transcription regulator GntR family
	-0.24	-1.34	-1.86			A	lmo2019	
	-0.47	-1.00	-1.02			A	lmo2021	Unknown, similar to unknown protein
0.58	1.12	0.74	0.46			A	lmo2031	Unknown, similar to unknown proteins
-0.80	-1.53	-1.28	-1.33			A	lmo2033	
	-0.13	-0.85	-1.52			A	lmo2034	
	-0.04	-1.59	-2.30			A	lmo2035	
	-0.59	-1.43	-1.96			A	lmo2037	
	-0.85	-1.37	-1.93			A	lmo2038	
-0.18	-0.82	-1.50	-1.21			A	lmo2044	Unknown, similar to transporter binding proteins
-1.25	-1.88	-2.51	-2.14	-0.61		A	lmo2046	Unknown, weakly similar to ketopantoate reductase involved in thiamin biosynthesis
-0.75	-1.30	-2.46		-0.55		A	lmo2050	Unknown, similar to excinuclease ABC (subunit A)
-0.62	-1.57	-2.48	-1.82			A	lmo2051	Unknown, weakly similar to proteases
-0.79	-1.74	-2.42	-1.72			A	lmo2053	Unknown, similar to unknown proteins
	-0.68	-1.04	-1.25			A	lmo2060	Unknown, similar to unknown protein
-0.26	-1.25	-2.23	-1.73			A	lmo2061	Unknown, similar to unknown protein
-1.04	-1.63	-1.08				A	lmo2062	Unknown, similar to copper export proteins
1.62	2.82	3.19	2.80	-1.18		A	lmo2067	Unknown, similar to conjugated bile acid hydrolase
0.99	1.13	0.92	0.48	-0.59		A	lmo2069	
-0.17	-0.45	-1.20	-1.48			A	lmo2070	Unknown, similar to unknown proteins

	-0.48	-1.53	-1.02			A	lmo2074	Unknown, similar to unknown proteins
-0.15	-0.25	-1.41	-1.38			A	lmo2075	Unknown, similar to glycoprotein endopeptidase
-0.20	-0.62	-1.39	-1.58			A	lmo2076	Unknown, similar to ribosomal protein alanine acetyltransferase
-0.29	-0.84	-1.52	-1.68			A	lmo2077	Unknown, similar to glycoprotease
-0.66	-1.28	-1.75	-1.63			A	lmo2078	Unknown, similar to unknown proteins
-0.42	-1.26	-1.49	-1.86	-0.66		A	lmo2079	unknown
-0.53	-0.56	-1.39	-1.46			A	lmo2080	Unknown
	0.07	-0.70	-1.82			A	lmo2081	Unknown, similar to unknown protein
	-0.23	-0.99	-1.07			A	lmo2083	unknown
	-1.35		-2.26	-0.42		A	lmo2086	Unknown, weakly similar to transcription regulators
-0.32	-0.55	-1.23	-0.97	-0.47		A	lmo2100	Unknown, similar to transcriptional regulator (GntR family) and to aminotransferase (MocR-like)
-1.36	-3.73	-2.72	-2.42	-0.39		A	lmo2104	Unknown
0.95	0.78	1.06	0.18			A	lmo2106	Unknown, similar to unknown proteins
-0.41	-1.33	-1.99	-2.12			A	lmo2118	Unknown, similar to phosphoglucomutase
		1.46	1.57	-0.28		A	lmo2122	Unknown, similar to maltodextrose utilization protein MalA
		0.70	0.51	1.26		A	lmo2127	Unknown
	2.69	3.91	3.51			A	lmo2132	Unknown
-0.30		1.15	1.41			A	lmo2136	Unknown, similar to PTS system, fructose-specific enzyme IIB component
-0.37	-0.75	-0.98	-1.07			A	lmo2139	Unknown, similar to ABC transporter (ATP-binding protein)
-0.38	-0.66	-0.98	-2.27			A	lmo2142	Unknown
	-0.06	-1.01	-2.14			A	lmo2146	Unknown, similar to transcription regulator LysR family
	0.20	-0.86	-1.27			A	lmo2148	Unknown, similar to unknown proteins
1.06	2.27	2.81	3.24			A	lmo2158	unknown, similar to B. subtilis YwmG protein
-1.00	-1.31		-1.38			A	lmo2164	Unknown, similar to transcriptional regulator (AraC/XylS family)
-0.33	-0.72	-2.33	-1.62			A	lmo2165	Unknown, similar to transcription regulator CRP/FNR family
-0.63	-1.02	-2.16	-1.60	0.52		A	lmo2166	Unknown
-0.63	-1.39	-1.39	-0.58			A	lmo2167	Unknown, similar to unknown proteins
	1.36	1.06		-0.49		A	lmo2174	Unknown, similar to unknown proteins
-0.16	0.08	1.18	2.18			A	lmo2176	Unknown, similar to transcriptional regulator (tetR family)
1.29	2.64	3.12	3.28	0.70		A	lmo2177	Unknown, similar to unknown protein
		-0.22	-1.73	-0.45		A	lmo2192	Unknown, similar to oligopeptide ABC transporter (ATP-binding protein)
	-0.17	-0.86	-1.87			A	lmo2194	Unknown, similar to oligopeptide ABC transporter (permease)
	0.07	-0.67	-1.44			A	lmo2195	Unknown, similar to oligopeptide ABC transporter (permease)
-0.29	-0.99	-1.40	-1.59	-0.35		A	lmo2197	Unknown
	-0.12	-0.96	-1.01			A	lmo2198	
	-0.45	-1.06	-1.91			A	lmo2201	Unknown, similar to 3-oxoacyl-acyl-carrier protein synthase

	0.04	-0.42	-1.08			A	lmo2203	Unknown, similar to N-acetylmuramoyl-L-alanine amidase and to internalin B
-0.19	0.06		1.01	0.55		A	lmo2207	Unknown, similar to unknown protein
	-0.12	-1.29	-2.40			A	lmo2211	
-0.83	-1.42	-1.71	-2.36			A	lmo2212	
-0.42	-0.59	0.07	1.03			A	lmo2217	Unknown, similar to unknown protein
-0.57	-1.14	-1.81	-2.29	-0.29		A	lmo2218	Unknown
	-0.01	0.62	1.07			A	lmo2222	Unknown, similar to unknown proteins
-0.77	-1.47	-1.51	-1.54			A	lmo2229	Unknown, similar to penicillin-binding protein
-0.30	-0.51	-1.00	-0.98			A	lmo2237	Unknown, similar to transport system permease protein
-0.36	-0.52	-0.99	-1.33			A	lmo2239	Unknown
-1.15	-2.04	-2.49	-2.74			A	lmo2240	Unknown, similar to ABC transporter (ATP-binding protein)
-0.87	-2.00	-2.64	-2.38			A	lmo2241	Unknown, similar to transcriptional regulators (GntR family)
0.90	1.42	0.96	1.59			A	lmo2242	Unknown, similar to O6-methylguanine-DNA methyltransferase
	-0.44	-1.21	-0.81			A	lmo2244	Unknown, similar to putative ribosomal large subunit pseudouridine synthase
	-0.50	-1.02	-2.40			A	lmo2249	Unknown, similar to low-affinity inorganic phosphate transporter
		-0.10	-1.65	-0.48		A	lmo2250	
	0.68	0.94	1.41	-1.21		A	lmo2257	Unknown, hypothetical CDS
-1.36	-1.50	-0.68	0.17			A	lmo2259	unknown, similar to phosphotransferase system (PTS) beta-glucoside-specific enzyme IIA
-0.51	-0.90	-1.12		-0.27		A	lmo2261	Unknown, similar to unknown proteins
	0.00	-0.65	-1.59	-0.31		A	lmo2264	Unknown, similar to unknown proteins
	0.12	-0.51	-1.05			A	lmo2266	Unknown, similar to unknown proteins
0.52	0.74	0.98	1.68	0.57		A	lmo2338	
		-0.51	-0.92	-1.05		A	lmo2347	Unknown, similar to amino acid ABC transporter (permease)
	0.07	-0.66	-1.34	-0.32		A	lmo2351	Unknown, similar to NADH-dependent FMN reductase
-0.22	-0.66	-1.42		-0.38		A	lmo2352	Unknown, similar to LysR family transcription regulator
	1.16	1.82	1.67			A	lmo2357	Unknown, similar to unknown protein
	1.74	1.92	1.93			A	lmo2358	Unknown, similar to N-acetylglucosamine-6-phosphate isomerase
	-0.16	-0.48	-1.27			A	lmo2362	Unknown, similar to amino acid antiporter (acid resistance)
0.61	1.08	0.80	0.50	0.52		A	lmo2370	Unknown, similar to aminotransferase
-0.35	-0.90	-2.62	-2.83			A	lmo2371	Unknown, similar to putative ABC-transporter transmembrane subunit
	-0.13	-0.99	-1.31			A	lmo2372	unknown, similar to ABC-transporter ATP binding proteins
-0.30	-0.33	-1.74	-3.10	-0.31		A	lmo2379	unknown, similar to proteins involved in resistance to cholate and to NA(+) and in pH homeostasis
-0.18	-0.12	-1.14	-1.93	-0.36		A	lmo2380	unknown, similar to proteins involved in resistance to cholate and to NA(+) and in pH

								homeostasis
-0.38	-0.17	-0.90	-1.45	-0.32		A	lmo2382	unknown, similar to proteins involved in resistance to cholate and to NA(+) and in pH homeostasis
0.87	1.57	1.83	2.10	-0.30		A	lmo2386	Unknown, similar to *B. subtilis* YuiD protein
0.57	2.09	2.41	1.38			A	lmo2387	Unknown, conserved hypothetical protein
	0.60	1.31	0.58	0.50		A	lmo2389	Unknown, similar to NADH dehydrogenase
1.40	2.81	3.99	3.82			A	lmo2391	Unknown, conserved hypothetical protein similar to *B. subtilis* YhfK protein
	0.08		2.20	0.63		A	lmo2397	Unknown, similar to NifU protein
1.11	2.28	2.48	1.51			A	lmo2399	Unknown, similar to conserved hypothetical proteins
	-1.28	-1.15	-1.64	-0.51		A	lmo2416	Unknown
	-0.15	-0.89	-1.01			A	lmo2417	Unknown, conserved lipoprotein (putative ABC transporter binding protein)
-0.62	-1.50	-1.87	-1.15	1.34		A	lmo2420	unknown
-1.00	-1.27	-0.44	0.37			A	lmo2422	Unknown, similar to two-component response regulator
-1.89	-3.52	-5.74		-0.28		A	lmo2423	Unknown, conserved hypothetical protein
-1.44	-2.64	-4.01	-4.09			A	lmo2427	Unknown, similar to cell division proteins RodA, FtsW
-1.30	-2.72	-3.85	-3.73			A	lmo2428	Unknown, similar to cell division proteins RodA, FtsW
-0.44	-0.79	-1.17	-1.79			A	lmo2429	Unknown, similar to *B. subtilis* ferrichrome ABC transporter (ATP-binding protein) FhuC
-0.80	-1.07	-1.59	-2.20			A	lmo2430	Unknown, similar to *B. subtilis* ferrichrome ABC transporter (permease) FhuG
-0.40	-0.28	-0.72	-1.72			A	lmo2431	Unknown, similar to *B. subtilis* ferrichrome ABC transporter fhuD precursor (ferrichrome-binding protein)
0.51	2.76	3.74	2.97			A	lmo2436	Unknown, similar to transcription antiterminator
-0.29	-0.36	-0.20	1.29	-0.35		A	lmo2437	Unknown
	-0.25	-1.01	-1.34			A	lmo2446	Unknown, similar to glycosidase
-0.79	-1.54	-1.94	-1.56			A	lmo2450	Unknown, similar to carboxylesterase
-0.21		0.95	1.24	0.73		A	lmo2452	Unknown, similar to carboxylesterase
2.34	3.17	4.03	4.56	-0.58		A	lmo2454	Unknown
	-0.22	-0.43	-1.22			A	lmo2458	
0.62	0.96	1.13	0.73			A	lmo2460	Unknown, similar to *B. subtilis* CggR hypothetical transcriptional regulator
	-0.06	-1.06	-1.63			A	lmo2464	Unknown, similar to transcription regulator
-0.34	-1.04	-1.60	-2.26			A	lmo2465	Unknown
-0.70	-1.42	-1.73	-1.68			A	lmo2476	Unknown, similar to aldose 1-epimerase (mutarotase)
-0.49	-1.04	-0.98	-0.69			A	lmo2477	
0.56	1.23	2.07	2.58	0.62		A	lmo2486	unknown
0.92	1.42	2.63	3.40			A	lmo2487	Unknown, similar to B. subtilis YvlB protein
	-0.04	-0.88	-1.82			A	lmo2488	
-0.18	-0.68	-1.35				A	lmo2491	Unknown
-0.49	-1.10	-1.05				A	lmo2492	unknown
-0.51	-1.18	-1.84	-1.96			A	lmo2499	Unknown, similar to phosphate ABC transporter (binding protein)

	-0.30	-1.67	-2.37	-0.38		A	lmo2502	Unknown
-0.17	-0.45	-0.78	-1.14			A	lmo2503	Unknown, similar to cardiolipin synthase
-0.66	-1.33	-2.24	-2.75			A	lmo2505	
	-0.13	-1.54	-1.73			A	lmo2508	Unknown, similar to conserved hypothetical proteins
	-0.42	-1.23	-1.36			A	lmo2509	
0.98	2.38	2.82	2.94	-0.41		A	lmo2511	Unknown, similar to conserved hypothetical proteins like to B. subtilis YvyD protein
-0.26	-0.68	-1.62	-1.79			A	lmo2512	
-0.39	-1.07	-1.74	-1.75			A	lmo2516	Unknown, similar to conserved hypothetical proteins
-0.24	-0.93	-1.64	-1.80			A	lmo2517	unknwon
-1.21	-1.55	-0.93	0.17			A	lmo2518	Unknown, similar to *B. subtilis* putative transcriptional regulator LytR
-0.30	-0.66	-1.13	-1.53	-0.61		A	lmo2519	Unknown, similar to *B. subtilis* TagO teichoic acid linkage unit synthesis protein
	-0.63	-1.11	-1.31	-0.33		A	lmo2520	Unknwon, similar to *B. subtilis* O-succinylbenzoate-CoA synthase (MenC)
-0.77	-1.22	-1.63	-1.97	-0.37		A	lmo2521	Unknown, similar to *B. subtilis* TagA protein involved in polyglycerol phosphate biosynthesis
-0.78	-1.21		-3.03	3.85		A	lmo2522	Unknwon, similar to hypothetical cell wall binding protein from *B. subtilis*
	-0.22	-1.31	-1.94	-0.54		A	lmo2524	Unknwon, similar to hydroxymyristoyl-(acyl carrier protein) dehydratase
-0.21	-0.52	-1.26	-1.49	-0.30		A	lmo2525	
	-0.04	-0.12	-1.41			A	lmo2529	
0.49		-0.41	-1.67			A	lmo2530	
	0.13	-0.99	-2.29			A	lmo2533	
	0.17	-0.95	-2.08			A	lmo2536	
-0.30	-1.08	-2.66	-2.86			A	lmo2537	Unknwon, similar to UDP-N-acetylglucosamine 2-epimerase
-0.96	-1.65	-2.49	-2.77			A	lmo2538	
-1.16	-2.00	-1.55	-0.60			A	lmo2539	
	-0.19	-1.53	-1.94			A	lmo2542	Unknown, similar to protoporphyrinogen oxidase
	-0.49	-1.80	-2.72	-0.35		A	lmo2543	
-0.21	-1.10	-2.40	-2.71			A	lmo2544	Unknwon, similar to thymidine kinase
	0.14	-1.50	-2.30	-0.44		A	lmo2545	
-0.36	-1.33	-1.91	-1.29			A	lmo2547	
	-0.09	-0.44	-1.42			A	lmo2548	
-0.46	-1.27	-2.12	-2.64			A	lmo2549	
-0.65	-1.10	-1.76	-1.69			A	lmo2551	
-0.51	-1.04	-1.45	-1.82			A	lmo2552	
-0.39	-0.96	-2.52	-2.87			A	lmo2553	Unknown, conserved hypothetical protein
-0.62	-0.87	-1.89	-2.01			A	lmo2554	Unknown, similar to galactosyltransferase
-0.20	-0.92	-1.89	-1.93			A	lmo2558	
-0.38	-1.38	-3.34	-5.06			A	lmo2559	

-0.25	-0.75	-1.76	-1.64			A	lmo2560	Unknown, similar to *B. subtilis* RNA polymerase delta subunit
-0.96	-2.28	-3.34	-3.24			A	lmo2562	Unknwon
-0.74	-1.34	-1.06	-1.27			A	lmo2563	Unknwon, conserved hypothetical protein
-0.12	-0.38	-1.35	-1.10			A	lmo2565	Unknown, conserved hypothetical protein
	-0.22	-1.52	-2.29	-1.37		A	lmo2569	Unknwon, similar to dipeptide ABC transporter (dipeptide-binding protein)
	-0.56	-1.32	-1.01			A	lmo2590	Unknown, similar to ATP binding proteins
0.49	0.78	0.84	1.48			A	lmo2592	Unknown, similar to oxidoreductase, aldo/keto reductase family
-0.27	-0.54	-1.17	-2.17			A	lmo2596	
-0.46	-0.95	-1.32	-1.74			A	lmo2597	
	-0.27	-0.70	-1.21			A	lmo2598	
0.77	2.19	2.42	2.60			A	lmo2602	Unknown, conserved hypothetical protein
-0.39	-0.69	-1.09	-1.83			A	lmo2605	
	-0.31	-1.52	-2.27			A	lmo2634	Unknown, similar to *B. subtilis* YbaF protein
	-0.47	-0.87	-1.47	-0.42		A	lmo2641	Unknown, similar to heptaprenyl diphosphate synthase component II
	0.97	1.33	1.41	0.54		A	lmo2652	Unknown, similar to transcriptional antiterminator
0.64	0.89	1.03	0.93			A	lmo2679	Unknown, similar to the two components sensor protein kdpD
	0.97	1.74	0.58			A	lmo2680	
1.07	1.86		-0.60			A	lmo2681	
-0.23	1.45	2.85	3.70	-0.33		A	lmo2697	unknown
0.57	1.42	1.58	1.27			A	lmo2708	Unknown, similar to PTS system, cellobiose-specific enzyme IIC
1.08	1.31	1.64	2.16			A	lmo2710	Unknown
-0.36	-1.01	-0.51	0.23	0.77		A	lmo2711	unknown, similar to hypothetical proteins
	-0.23	-1.02	0.66	0.60		A	lmo2719	Unknown, conserved hypothetical proteins
-0.55		-1.79	-1.56			A	lmo2735	Unknown, similar to Sucrose phosphorylase
	-0.18	-0.69	-1.11			A	lmo2737	Unknown, similar to transcriptional regulator (LacI family)
	0.94	1.38	1.47			A	lmo2743	Unknown, similar to transaldolase
	-0.25	-1.78	-2.12	-0.55		A	lmo2751	Unknown, similar to ABC transporter, ATP-binding protein
	0.10	-1.08	-1.76	-0.34		A	lmo2752	Unknown, similar to ABC transporter, ATP-binding protein
-1.28	-1.56		-2.14	1.26		A	lmo2753	Unknown
-0.34	-0.58	-0.52	-1.39			A	lmo2754	Unknown, similar to D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 5)
	-0.08	-0.09	0.24	1.21		A	lmo2756	
	-0.73	-2.52	-1.85	0.90		A	lmo2757	Unknown, similar to ATP-dependent DNA helicases
	-1.85		-1.16	-0.52		A	lmo2762	Unknown, similar to PTS cellobiose-specific enzyme IIB
	-0.12	-0.42	-1.85			A	lmo2765	Unknown, similar to PTS cellobiose-specific enzyme IIA
-1.74	-2.05	-2.28	-2.16			A	lmo2770	Unknown, similar to gamma-glutamylcysteine synthetase (for the N_terminal part) and to cyanophycin synthetase (C-terminal part)

	-0.53		-1.50	-0.79		A	lmo2774	Unknwon, similar to ABC transporter, ATP-binding protein
-1.17	-1.73	-1.88				A	lmo2779	Unknown, similar to probable GTP-binding protein
-0.25	-0.92	-1.96	-2.22			A	lmo2783	Unknown, similar to cellobiose phosphotransferase system enzyme IIC
-0.19	-0.78	-1.44	-0.84	-0.32		A	lmo2793	Unknown
	-0.79	-1.69	-1.40			A	lmo2794	Unknown, highly similar to B. subtilis DNA-binding protein Spo0J-like homolog YyaA
-0.48	-0.83	-1.02	-0.59			A	lmo2801	Unknown, similar to a putative N-acetylmannosamine-6-phosphate epimerase
	-0.08	-0.67	-1.11			A	lmo2809	Unknown, hypothetical secreted protein
-0.37	-0.89	-0.99	-1.39			A	lmo2812	unknown, similar to D-alanyl-D-alanine carboxypeptidase
	1.87	2.24	1.46			A	lmo2817	unknown, similar to peptidases
	2.45	3.82	3.91			A	lmo2818	Unknown, similar to transmembrane efflux protein
5.48	6.14		4.71			A	lmo2820	Unknown, amino-terminal domain similar to transcription regulators
-0.53	-1.22	-1.21	-1.02			A	lmo2824	unknown, similar to D-3-phosphoglycerate dehydrogenase
-0.26	-0.57		-1.29	-0.30		A	lmo2843	unknown
-0.51	-1.29	-1.44	-1.57			A	lmo2844	Unknown, similar to unknown proteins
	0.20		0.89	1.03		A	lmo2845	unknown, similar to transmembrane efflux proteins
	-0.26	-1.02	-1.02			A	lmo2848	unknown, highly similar to L-rhamnose isomerase
-0.18	-0.19	-1.26	-2.11	-0.34		A	lmo2854	Unknown, highly similar to *B. subtilis* SpoIIIJ protein
-0.53	-0.61	-1.72	-3.52			A	lmo2855	
-0.65	-0.85	-1.24	-1.37	-0.39		A	lmo2856	
-1.58	-2.23	-2.41	-2.12	0.54		A	lmo2857	hypothetical protein

Tab. S-7: *L. monocytogenes* genes, which showed significant change in transcription level only after temperature shift.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	Adapt.	temp. shift	Id*	LmoNr.	Function
					2.03	C	lmo0002	DnaN, DNA polymerase III, beta chain
					1.53	C	lmo0021	Unknown, similar to PTS system, fructose-specific IIA component
					-1.34	C	lmo0032	Unknown, similar to xylose repressor
					1.13	C	lmo0043	Unknown, similar to arginine deiminase
					-2.39	C	lmo0094	unknown
					-3.40	C	lmo0098	Unknown, similar to PTS system mannose-specific, factor IID
					2.71	C	lmo0099	unknown
					-1.38	C	lmo0100	Unknwon
					-2.72	C	lmo0102	Unknwon
					-2.46	C	lmo0104	Unknwon
					2.16	C	lmo0106	Unknown, similar to transcription regulator
					1.13	C	lmo0113	Unknwon, similar to protein gp35 from Bacteriophage A118
					-3.07	C	lmo0118	LmaA, antigen A
					1.93	C	lmo0119	Unknwon
					-2.97	C	lmo0120	unknown
					2.09	C	lmo0121	unknown, similar to bacteriophage minor tail proteins
					-2.69	C	lmo0122	Unknown, similar to phage proteins
					-3.80	C	lmo0124	Unknown
					-3.19	C	lmo0126	Unknwon
					-2.92	C	lmo0128	Unknwon, similar to a protein from Bacteriophage phi-105 (ORF 45)
					-3.20	C	lmo0130	Unknwon, similar to 5 -nucleotidase, putative peptidoglycan bound protein (LPXTG motif)
					2.15	C	lmo0133	Unknown, similar to *E. coli* YjdI protein
					1.59	C	lmo0156	Unknwon
					1.07	C	lmo0160	Unknown, putative peptidoglycan bound protein (LPXTG motif)
					1.84	C	lmo0180	Unknwon, similar to sugar ABC transporter, permease protein
					2.05	C	lmo0182	Unknwon, similar to alpha-xylosidase and alpha-glucosidase
					2.00	C	lmo0184	Unknwon, similar to oligo-1,6-glucosidase
					2.06	C	lmo0227	Unknwon, conserved hypothetical protein
					2.55	C	lmo0254	Unknown
					1.36	C	lmo0256	Unknown, conserved hypothetical protein
					1.53	C	lmo0280	Unknown, highly similar to anaerobic ribonucleotide reductase activator protein
					1.01	C	lmo0297	Unknown, similar to transcriptional antiterminator (BglG family)
					1.65	C	lmo0320	Unknown, similar to surface protein (peptidoglycan bound, LPXTG motif)
					1.26	C	lmo0324	Unknown

					1.46	C	lmo0352	unknown, highly similar to regulatory proteins (DeoR family)
					1.19	C	lmo0355	Unknown, similar to Flavocytochrome C Fumarate Reductase chain A
					1.91	C	lmo0400	Unknown, similar to fructose-specific phosphotransferase enzyme IIC
					1.05	C	lmo0401	Unknown, highly similar to *E. coli* YbgG protein, a putative sugar hydrolase
					-1.19	C	lmo0404	Unknown
					1.47	C	lmo0421	Unknown, similar to rod shape-determining protein RodA
					3.19	C	lmo0425	Unknown, similar to transcription antiterminator BglG family
					2.17	C	lmo0427	Unknown, similar to PTS fructose-specific enzyme IIB component
					2.46	C	lmo0428	Unknown, similar to PTS fructose-specific enzyme IIC component
					2.51	C	lmo0429	Unknown, similar to sugar hydrolase
					1.60	C	lmo0450	Unknown, similar to unknown proteins
					1.77	C	lmo0477	Unknown, putative secreted protein
					2.10	C	lmo0478	Unknown, putative secreted protein
					1.25	C	lmo0483	Unknown
					1.05	C	lmo0536	Unknown, similar to 6-phospho-beta-glucosidase
					-1.18	C	lmo0596	Unknown, similar to unknown proteins
					2.04	C	lmo0643	Unknown, similar to putative transaldolase
					1.25	C	lmo0788	Unknown
					2.63	C	lmo0814	unknown, similar to oxidoreductases
					-1.05	C	lmo0822	unknown, similar to transcriptional regulators
					1.24	C	lmo0839	unknown, similar to Tetracycline resistance protein
					1.33	C	lmo0863	unknown
					1.85	C	lmo0913	Unknown, similar to succinate semialdehyde dehydrogenase
					2.69	C	lmo0915	Unknown, similar to phosphotransferase system enzyme IIC
					1.18	C	lmo0940	Unknown
					1.01	C	lmo0948	unknown, similar to transcription regulator
					-1.14	C	lmo0977	unknown, similar to *B. subtilis* YjcH protein
					-1.22	C	lmo0995	unknown, similar to *B. subtilis* YkrP protein
					1.54	C	lmo1042	unknown, similar to molybdopterin biosynthesis protein moeA
					1.09	C	lmo1043	unknown, similar to molybdopterin-guanine dinucleotide biosynthesis MobB
					1.87	C	lmo1044	Unknown, similar to molybdopterin converting factor, subunit 2
					1.78	C	lmo1045	unknown, similar to molybdopterin converting factor (subunit 1).
					1.99	C	lmo1046	unknown, similar to molybdenum cofactor biosynthesis protein C
					1.69	C	lmo1048	unknown, similar to molybdenum cofactor biosynthesis protein B

					1.34	C	lmo1050	unknown, similar to *B. subtilis* YdfE protein
					1.45	C	lmo1098	unknown, highly similar to TN916 ORF8
					1.64	C	lmo1170	unknown, similar to *Salmonella enterica* PduX protein
					3.37	C	lmo1254	Unknown, similar to alpha,alpha-phosphotrehalase
					2.36	C	lmo1255	Unknown, similar to PTS system trehalose specific enzyme IIBC
					1.52	C	lmo1257	unknown
					-1.21	C	lmo1269	unknown, similar to type-I signal peptidase
					1.21	C	lmo1278	
					1.66	C	lmo1348	Unknown, similar to aminomethyltransferase
					1.77	C	lmo1349	Unknown, similar to glycine dehydrogenase (decarboxylating) subunit 1
					1.91	C	lmo1350	Unknown, similar to glycine dehydrogenase (decarboxylating) subunit 2
					1.96	C	lmo1406	
					-1.01	C	lmo1619	Unknown, similar to Xaa-His dipeptidase
					1.44	C	lmo1623	Unknown, similar to putative transporters
					1.11	C	lmo1627	
					1.06	C	lmo1629	
					1.70	C	lmo1631	
					1.86	C	lmo1632	
					2.56	C	lmo1634	Unknown, similar to Alcohol-acetaldehyde dehydrogenase
					-1.00	C	lmo1665	unknown
					1.17	C	lmo1728	unknown, some similarities to cellobiose-phosphorylase
					1.23	C	lmo1732	Unknown, similar to sugar ABC transporter, permease protein
					1.56	C	lmo1786	
					2.24	C	lmo1867	unknown, similar to pyruvate phosphate dikinase
					1.03	C	lmo1876	Unknown, similar to formyl-tetrahydrofolate synthetase C-terminal part
					1.54	C	lmo1879	
					2.50	C	lmo1883	unknown, similar to chitinases
					-1.02	C	lmo1933	Unknown, similar to GTP cyclohydrolase I
					1.04	C	lmo1955	Unknown, similar to integrase/recombinase
					1.70	C	lmo1993	
					4.19	C	lmo1997	Unknown, similar to PTS mannose-specific enzyme IIA component
					3.82	C	lmo1998	Unknown, similar to opine catabolism protein
					4.22	C	lmo1999	Unknown, weakly similar to glucosamine-fructose-6-phosphate aminotransferase
					3.74	C	lmo2000	Unknown, similar to PTS mannose-specific enzyme IID component
					3.35	C	lmo2001	Unknown, similar to PTS mannose-specific enzyme IIC component

					1.14	C	lmo2068	
					2.07	C	lmo2084	Unknown
					1.51	C	lmo2124	Unknown, similar to maltodextrin ABC-transport system (permease)
					-1.70	C	lmo2127	Unknown
					-1.17	C	lmo2130	Unknown, similar to unknown protein
					1.53	C	lmo2159	Unknown, similar to oxidoreductase
					1.65	C	lmo2160	Unknown, similar to unknown proteins
					2.78	C	lmo2161	Unknown
					1.34	C	lmo2162	Unknown, similar to unknown proteins
					1.63	C	lmo2163	Unknown, similar to oxidoreductase
					-1.16	C	lmo2269	Unknown
					-1.85	C	lmo2278	
					-3.02	C	lmo2279	holin [Bacteriophage A118]
					-2.02	C	lmo2281	
					-2.76	C	lmo2284	Protein gp19 [Bacteriophage A118]
					-1.89	C	lmo2290	Portein gp13 [Bacteriophage A118]
					-2.36	C	lmo2291	major tail shaft protein [Bacteriophage A118]
					-1.37	C	lmo2293	Protein gp10 [Bacteriophage A118]
					-3.91	C	lmo2294	Protein gp9 [Bacteriophage A118]
					-3.82	C	lmo2296	Unknown, similar to coat protein [Bacteriophage SPP1]
					-1.64	C	lmo2297	Unknown, putative scaffolding protein [Bacteriophage A118]
					-1.29	C	lmo2300	putative terminase large subunit from Bacteriophage A118
					1.75	C	lmo2332	
					1.50	C	lmo2340	Unknown, similar to *Erwinia chrysanthemi* IndA protein
					2.25	C	lmo2341	Unknown, similar to carbohydrate kinases
					-1.12	C	lmo2360	Unknown, transmembrane protein
					-1.65	C	lmo2361	Unknown, conserved hypothetical protein
					-1.00	C	lmo2409	Unknown
					1.83	C	lmo2584	Unknwon, similar to formate dehydrogenase associated protein
					1.06	C	lmo2585	Unknown, similar to *B. subtilis* YrhD protein
					2.54	C	lmo2586	Unknwon, similar to formate dehydrogenase alpha chain
					-1.02	C	lmo2587	Unknwon, conserved hypothetical proteins
					1.00	C	lmo2590	Unknown, similar to ATP binding proteins
					1.01	C	lmo2637	Unknown, conserved lipoprotein
					1.38	C	lmo2645	Unknown
					3.52	C	lmo2646	Unknown
					1.58	C	lmo2648	Unknown, similar to Phosphotriesterase
					3.10	C	lmo2650	Unknwon, similar to hypothetical PTS enzyme IIB component
					3.55	C	lmo2651	Unknwon, similar to mannitol-specific PTS enzyme IIA component

					1.37	C	lmo2665	Unknwon, similar to PTS system galactitol-specific enzyme IIC component
					1.02	C	lmo2668	unknown, similar to transcriptional antiterminator (BglG family)
					1.38	C	lmo2670	Unknown, conserved hypothetical protein
					1.60	C	lmo2687	Unknown, similar to cell division protein FtsW
					1.19	C	lmo2715	
					1.00	C	lmo2718	
					1.15	C	lmo2721	Unknown, similar to glucosamine-6-phosphate isomerase
					1.48	C	lmo2730	unknown, similar to phosphatase
					1.45	C	lmo2731	unknown, similar to transcription regulator (RpiR family)
					1.62	C	lmo2732	unknown
					1.13	C	lmo2735	Unknown, similar to Sucrose phosphorylase
					1.15	C	lmo2759	Unknown, similar to unknown protein
					1.83	C	lmo2774	Unknwon, similar to ABC transporter, ATP-binding protein
					1.01	C	lmo2788	
					1.61	C	lmo2795	Unknown, similar to E. coli RpiR transcription regulator
					2.55	C	lmo2797	Unknown, similar to phosphotransferase system mannitol-specific enzyme IIA
					1.43	C	lmo2798	Unknown, similar to phosphatase
					4.93	C	lmo2799	Unknown, similar to phosphotransferase system mannitol-specific enzyme IIBC
					4.58	C	lmo2800	Unknown, similar to dehydrogenase
					1.81	C	lmo2852	unknown

Tab. S-8: *L. monocytogenes* genes, which showed significant change in transcription levels after acid shock at 37°C and in the temperature shift experiment.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	adapt.	temp. shift	Id*	LmoNr.	Function
					1.09	C	lmo0520	Unknown, similar to transcription regulator
	1.07	1.65	1.56			B	lmo0520	Unknown, similar to transcription regulator
					1.06	C	lmo1227	Unknown, similar to uracil-DNA glycosylase
		1.12	1.09	0.99		B	lmo1227	Unknown, similar to uracil-DNA glycosylase
					2.24	C	lmo1538	Unknown, similar to glycerol kinase
2.09	1.80	1.30	0.56	2.21		B	lmo1538	Unknown, similar to glycerol kinase
					2.31	C	lmo2796	Unknown, similar to transcription regulator
		0.78	1.08	0.39		B	lmo2796	Unknown, similar to transcription regulator

Tab. S-9: *L. monocytogenes* genes, which showed significant change in transcription levels after acid shock at 25 and 37°C.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	adapt.	temp. shift	Id*	LmoNr.	Function
-0.63	-1.30	-2.42	-3.04			A	lmo0001	DnaA, Chromosomal replication initiation protein DnaA
-1.40	-1.93	-1.62	-0.95			B	lmo0001	DnaA, Chromosomal replication initiation protein DnaA
-0.18	1.10	1.81	1.14	-0.33		A	lmo0016	QoxD, highly similar to quinol oxidase aa3-600 chain IV
1.08		0.66		0.97		B	lmo0016	QoxD, highly similar to quinol oxidase aa3-600 chain IV
-0.27	-0.75	-1.89	-2.80			A	lmo0023	Unknown, similar to PTS system, fructose-specific IIC component
	-1.82	-1.47	-0.73			B	lmo0023	Unknown, similar to PTS system, fructose-specific IIC component
1.69	3.19	3.65	3.39	-1.01		A	lmo0044	RpsF, ribosomal protein S6
3.90	3.11	2.05		1.55		B	lmo0044	RpsF, ribosomal protein S6
	1.00	1.30	1.67			A	lmo0054	DnaC, highly similar to replicative DNA helicases
0.70	1.15	1.03	0.87			B	lmo0054	DnaC, highly similar to replicative DNA helicases
	0.15	-0.27	-1.47			A	lmo0063	Unknwon
0.55		-1.53	-2.39	-0.72		B	lmo0063	Unknwon
0.57	1.03	1.70	1.63			A	lmo0091	unknwon, similar to ATP synthase gamma chain
2.24	1.97	1.84	0.87	1.17		B	lmo0091	unknwon, similar to ATP synthase gamma chain
	-2.57	-2.18	-2.01	-0.51		A	lmo0106	Unknown, similar to transcription regulator
	-2.26	-1.64	-2.49			B	lmo0106	Unknown, similar to transcription regulator
0.68	1.94	3.25	3.71			A	lmo0110	Unknown, similar to lipase
1.08	1.79	1.62	0.41	0.81		B	lmo0110	Unknown, similar to lipase
	0.12	-0.97	-2.25			A	lmo0119	Unknwon
-1.15	-1.81	-2.60	-2.71			B	lmo0119	Unknwon
-0.15	-0.06	-0.90	-2.51			A	lmo0121	unknown, similar to bacteriophage minor tail proteins
-0.79	-1.53	-2.33	-2.38			B	lmo0121	unknown, similar to bacteriophage minor tail proteins
0.68	0.79	1.31	1.34			A	lmo0132	Unknown, similar to inosine monophosphate dehydrogenase
	1.03	0.62	0.56			B	lmo0132	Unknown, similar to inosine monophosphate dehydrogenase
1.78	3.36	4.12	4.16			A	lmo0134	Unknwon, similar to E. coli YjdJ protein
2.67	3.59	2.89	1.47	1.79		B	lmo0134	Unknwon, similar to E. coli YjdJ protein
1.99	2.31	2.54	3.25	-0.30		A	lmo0137	Unknwon, similar to oligopeptide ABC transporter, permease protein
4.26	2.50	2.99	2.65			B	lmo0137	Unknwon, similar to oligopeptide ABC transporter, permease protein
	1.45	1.93	2.02	-0.49		A	lmo0146	Unknwon, hypothetical protein
1.18	1.58	1.49	0.57	0.65		B	lmo0146	Unknwon, hypothetical protein
0.86		1.86	2.34			A	lmo0148	unknown

2.28		1.79	1.64	0.96		B	lmo0148	unknown
	-0.12	-1.29	-1.79			A	lmo0156	Unknwon
	-2.09	-2.27	-1.91			B	lmo0156	Unknwon
		1.66	1.83	-0.24		A	lmo0158	Unknown, conserved hypothetical protein
0.80	1.68	1.68	0.56	0.61		B	lmo0158	Unknown, conserved hypothetical protein
1.21	2.31	2.69	2.39			A	lmo0174	Unknown, similar to transposase
2.51	2.29	2.04	1.57	0.96		B	lmo0174	Unknown, similar to transposase
-0.33	-1.51	-2.54	-2.55	0.70		A	lmo0178	Unknwon, similar to xylose repressor
-1.25	-1.53	-1.02				B	lmo0178	Unknwon, similar to xylose repressor
	-0.36	-1.62	-1.98			A	lmo0191	Unknwon, similar to a putative phospho-beta-glucosidase
	-1.18		0.52	0.54		B	lmo0191	Unknwon, similar to a putative phospho-beta-glucosidase
	0.97	1.66	1.62	-0.22		A	lmo0206	Unknwon
0.47	2.05	1.25				B	lmo0206	Unknwon
	1.05	2.38	2.32	-0.41		A	lmo0208	Unknown, conserved hypothetical protein
0.70	2.85	2.66	1.39			B	lmo0208	Unknown, conserved hypothetical protein
	1.23	1.81	2.15			A	lmo0209	Unknown
	1.34	1.68	1.61			B	lmo0209	Unknown
-1.53	-1.08	-0.26				A	lmo0218	Unknown, polyribonucleotide nucleotidyltransferase domain present
-1.25		-0.81	-0.90			B	lmo0218	Unknown, polyribonucleotide nucleotidyltransferase domain present
	0.12	-0.87	-1.63			A	lmo0236	Unknown, similar to B. subtilis YacN protein
-1.20	-1.44		-0.61			B	lmo0236	Unknown, similar to B. subtilis YacN protein
-1.11	-1.92	-4.16	-4.31	-0.21		A	lmo0245	SecE, highly similar to preprotein translocase subunit
-1.69	-2.04	-0.97				B	lmo0245	SecE, highly similar to preprotein translocase subunit
	-0.28	-0.97	-1.45	-0.27		A	lmo0258	RpoB, RNA polymerase (beta subunit)
		-1.47	-0.84	-0.69		B	lmo0258	RpoB, RNA polymerase (beta subunit)
	0.20	-1.33	-3.31			A	lmo0259	RpoC, RNA polymerase (beta' subunit)
-0.86	-1.24	-2.09	-1.31			B	lmo0259	RpoC, RNA polymerase (beta' subunit)
0.93	2.51	3.85	3.25	-0.93		A	lmo0264	InlE, internalin E
2.52	2.71	2.08	1.13			B	lmo0264	InlE, internalin E
2.25	3.78	4.33	4.05	-0.57		A	lmo0266	Unknown, similar to transcriptional regulators
2.96	3.01	2.99	1.73	1.71		B	lmo0266	Unknown, similar to transcriptional regulators
1.66	2.20	2.13	1.90	-0.21		A	lmo0291	Unknown, conserved hypothetical protein similar to B. subtilis YycJ protein
1.48	2.07	1.33				B	lmo0291	Unknown, conserved hypothetical protein similar to B. subtilis YycJ protein
-0.63	-1.33	-1.60	-1.32			A	lmo0311	Unknown
	-1.12		0.40	0.60		B	lmo0311	Unknown
	1.55	1.89	1.64			A	lmo0315	Unknown, similar to thiamin biosynthesis protein
0.82	2.73	2.35	1.51	1.18		B	lmo0315	Unknown, similar to thiamin biosynthesis protein
1.14	1.40	1.79	2.10			A	lmo0318	Unknown, similar to thiamin-phosphate pyrophosphorylase (ThiE)
2.18	1.94	1.93	1.71			B	lmo0318	Unknown, similar to thiamin-phosphate pyrophosphorylase (ThiE)

0.45	0.72	1.18	1.32			A	lmo0365	Unknown, similar to conserved hypothetical protein
1.17	0.96	1.11	1.03	0.36		B	lmo0365	Unknown, similar to conserved hypothetical protein
-0.56	-1.11	-1.29	-1.02			A	lmo0377	Unknown
	-2.08	-3.58	-2.57			B	lmo0377	Unknown
-1.64	-3.32	-4.97	-5.51	0.65		A	lmo0394	Unknown, similar to L. monocytogenes extracellular P60 protein
	-3.73	-5.35	-5.38			B	lmo0394	Unknown, similar to L. monocytogenes extracellular P60 protein
	1.90	2.68	1.74	-0.58		A	lmo0405	Unknown, similar to phosphate transport protein
	1.85	1.38		1.21		B	lmo0405	Unknown, similar to phosphate transport protein
-0.16		1.44	1.44			A	lmo0407	Unknown
1.17		1.13	0.30	0.88		B	lmo0407	Unknown
-0.23		1.31	1.20	-0.37		A	lmo0408	Unknown
0.94	1.14			0.81		B	lmo0408	Unknown
	2.20	2.71	2.15			A	lmo0433	InlA, Internalin A
1.45	1.49	1.28		1.20		B	lmo0433	InlA, Internalin A
	1.71	3.45		-0.59		A	lmo0434	InlB, Internalin B
1.94	2.83	1.99				B	lmo0434	InlB, Internalin B
1.72	2.79	3.34	3.12	-0.98		A	lmo0445	Unknown, similar to transcription regulator
2.72	2.47	2.71	1.72	1.45		B	lmo0445	Unknown, similar to transcription regulator
		-0.96	-2.24	-0.35		A	lmo0489	Unknown, similar to NADH:flavin oxidoreductase
-0.85	-1.38	-2.35	-1.37	-0.76		B	lmo0489	Unknown, similar to NADH:flavin oxidoreductase
-0.23	-0.29		1.13			A	lmo0513	Unknown, weakly similar to transcription regulator
	1.05	1.58	1.98			B	lmo0513	Unknown, weakly similar to transcription regulator
2.00	3.50	4.26	4.22	-1.62		A	lmo0515	Unknown, conserved hypothetical protein
3.22	3.38	3.37	2.03			B	lmo0515	Unknown, conserved hypothetical protein
0.78	2.31	2.61	2.84			A	lmo0539	Unknown, similar to tagatose-1,6-diphosphate aldolase
2.64	2.41	2.25	1.92	2.65		B	lmo0539	Unknown, similar to tagatose-1,6-diphosphate aldolase
0.76	1.36	1.85	2.42			A	lmo0553	Unknown
0.89	1.26	1.35	0.90	0.59		B	lmo0553	Unknown
	0.81	1.19	1.59			A	lmo0574	Unknown, similar to beta-glucosidase
	1.73	2.22	2.59			B	lmo0574	Unknown, similar to beta-glucosidase
0.93	2.23	2.82	2.67	-0.28		A	lmo0579	Unknown, similar to unknown protein
3.10	2.70	2.72	1.76	1.29		B	lmo0579	Unknown, similar to unknown protein
0.49	1.94	2.50	2.15	-0.39		A	lmo0580	Unknown, weakly similar to carboxylesterase
2.64	2.64	2.19	1.80	1.20		B	lmo0580	Unknown, weakly similar to carboxylesterase
	-0.27	-1.48	-1.84			A	lmo0581	Unknown, conserved hypothetical protein
-0.57		-1.17		1.28		B	lmo0581	Unknown, conserved hypothetical protein
0.66	-0.36	-1.63	-1.47			A	lmo0582	Iap, P60 extracellular protein, invasion associated protein Iap
-1.14	-1.30	-1.44	-1.48	0.45		B	lmo0582	Iap, P60 extracellular protein, invasion associated protein Iap

	1.86	3.21	3.37	-0.29		A	lmo0584	Unknown, conserved hypothetical membrane protein
1.86	2.28	1.93	1.21	0.96		B	lmo0584	Unknown, conserved hypothetical membrane protein
-0.66		0.93	1.14	-1.19		A	lmo0593	Unknown, similar to transport proteins (formate?)
2.62	2.50	2.73	1.53			B	lmo0593	Unknown, similar to transport proteins (formate?)
-0.27	0.76	1.02	0.76	-0.96		A	lmo0596	Unknown, similar to unknown proteins
3.33	2.56	2.21	1.73	2.51		B	lmo0596	Unknown, similar to unknown proteins
	1.62	2.74	3.25	-0.55		A	lmo0602	Unknown, weakly similar to transcription regulator
2.29	2.48	2.85	1.70	1.59		B	lmo0602	Unknown, weakly similar to transcription regulator
		1.29	2.80	0.82		A	lmo0609	Unknown, similar to E. coli phage shock protein E
	1.56	2.83	2.97	1.09		B	lmo0609	Unknown, similar to E. coli phage shock protein E
0.59	1.89	2.67	2.50	-1.37		A	lmo0610	Unknown, similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)
3.18	2.57	2.44				B	lmo0610	Unknown, similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)
1.93	3.19	4.27	4.20	-0.49		A	lmo0628	Unknown
4.84	4.28	3.81	2.03	3.14		B	lmo0628	Unknown
0.65	2.17	3.71	3.78			A	lmo0629	Unknown
4.09	2.79	2.73	1.84	1.82		B	lmo0629	Unknown
-0.19	-0.33	-1.45	-1.77			A	lmo0644	Unknown, similar to conserved hypothetical proteins
-1.32	-1.13	-0.87		0.46		B	lmo0644	Unknown, similar to conserved hypothetical proteins
0.60	2.09	2.99	3.86	-0.77		A	lmo0647	Unknown
2.07	2.36	3.08	3.33	2.10		B	lmo0647	Unknown
0.97	1.69	1.96	1.74	-0.68		A	lmo0654	unknown
2.35	1.63	1.75	0.55	1.23		B	lmo0654	unknown
0.83	2.14	3.06	2.95	-0.56		A	lmo0669	Unknown, similar to oxidoreductase
1.83	2.52	2.33	1.89	1.94		B	lmo0669	Unknown, similar to oxidoreductase
0.79	1.42	2.10	2.24			A	lmo0719	Unknown, similar to unknown protein
1.35	1.15		1.04			B	lmo0719	Unknown, similar to unknown protein
-2.45	-3.92	-4.92	-4.50	-0.73		A	lmo0726	Hypothetical CDS
-4.72	-4.45	-3.38	-2.09	-0.81		B	lmo0726	Hypothetical CDS
-0.87	-1.94	-3.61	-4.15	-0.83		A	lmo0727	Unknown, similar to L-glutamine-D-fructose-6-phosphate amidotransferase
-1.96	-3.48	-3.65	-2.38	-0.92		B	lmo0727	Unknown, similar to L-glutamine-D-fructose-6-phosphate amidotransferase
	1.56	2.10	2.50	-0.35		A	lmo0736	Unknown, similar to ribose 5-phosphate isomerase
1.31	1.73	1.64	1.15			B	lmo0736	Unknown, similar to ribose 5-phosphate isomerase
1.40	2.29	3.03	3.46	-0.40		A	lmo0759	Unknown
2.26	2.28	2.16	1.55			B	lmo0759	Unknown

	2.03	2.72	2.95	-0.43		A	lmo0760	Unknown
1.15		1.24	0.83			B	lmo0760	Unknown
0.51	0.88	1.73	2.97			A	lmo0769	Unknown, similar to alpha-1,6-mannanase
	2.45	3.51	2.90			B	lmo0769	Unknown, similar to alpha-1,6-mannanase
-0.63	-0.20	0.90	1.13			A	lmo0781	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IID
	1.18	1.53	1.28	0.73		B	lmo0781	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IID
	1.38	1.64	1.59			A	lmo0784	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIA
2.77	2.25	1.67	0.68	2.02		B	lmo0784	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIA
-0.51	-1.34	-3.83	-3.56	-1.98		A	lmo0791	Unknown
	-1.84	-2.56	-2.17	-2.31		B	lmo0791	Unknown
	1.28	2.51	2.59			A	lmo0796	Unknown, conserved hypothetical protein
1.32	1.85	2.18	1.35	1.33		B	lmo0796	Unknown, conserved hypothetical protein
		1.17	1.72	1.00		A	lmo0799	Unknown
1.18	1.88	2.19	2.16	1.00		B	lmo0799	Unknown
	-0.02	-1.21	-1.72	-0.47		A	lmo0808	unknown, similar to spermidine/putrescine ABC transporter, permease protein
-1.41	-0.94	-0.88	-0.93			B	lmo0808	unknown, similar to spermidine/putrescine ABC transporter, permease protein
	1.69	2.94	3.15	-0.65		A	lmo0820	unknown, some similarity to acatyltransferases
	2.55	2.24	1.72	0.68		B	lmo0820	unknown, some similarity to acatyltransferases
0.88	1.53	2.80	4.14			A	lmo0821	unknown
	2.70	2.71	2.79	1.02		B	lmo0821	unknown
	-0.13	-0.74	-1.37			A	lmo0823	unknown, similar to oxydoreductases
-0.98	-1.27	-1.64	-1.13			B	lmo0823	unknown, similar to oxydoreductases
1.39	1.64	1.42	1.23			A	lmo0829	NifJ, highly similar to pyruvate-flavodoxin oxidoreductase
2.11	2.05	1.94	2.17	0.50		B	lmo0829	NifJ, highly similar to pyruvate-flavodoxin oxidoreductase
-0.26	-0.91	-2.19	-2.46	-0.27		A	lmo0837	Unknown, similar to ABC transporter (ATP binding protein)
-1.10	-1.68	-1.88	-1.51	-1.95		B	lmo0837	Unknown, similar to ABC transporter (ATP binding protein)
-0.28	-1.16		-5.42	-2.56		A	lmo0847	unknown, similar to Glutamine ABC transporter (binding and transport protein)
	-2.45		-2.75	-2.95		B	lmo0847	unknown, similar to Glutamine ABC transporter (binding and transport protein)
		0.72	1.37	0.64		A	lmo0851	Unknown
0.86	1.82	2.17	2.11	1.16		B	lmo0851	Unknown
0.64	1.64	2.02	2.95			A	lmo0870	unknown
	0.98	1.31	1.29	0.33		B	lmo0870	unknown
-0.84	-1.26	-1.63	-1.35			A	lmo0879	unknown
-0.57	-1.12			0.44		B	lmo0879	unknown

1.27	2.78	3.18	3.13			A	lmo0880	Unknown, similar to wall associated protein precursor (LPXTG motif)
5.67	4.23	3.64	4.58	4.12		B	lmo0880	Unknown, similar to wall associated protein precursor (LPXTG motif)
-1.35	-1.95	-1.49	-0.77			A	lmo0888	Unknown, similar to B. subtilis YdcE protein
-2.75	-1.67	-1.88	-2.37			B	lmo0888	Unknown, similar to B. subtilis YdcE protein
-0.81	-1.82	-2.56	-2.01			A	lmo0889	highly similar to positive regulator of sigma-B activity
-2.51	-2.01	-1.26	-1.09			B	lmo0889	highly similar to positive regulator of sigma-B activity
-0.15	-0.83	-2.37	-2.24			A	lmo0891	RsbT, highly similar to positive regulation of sigma-B activity
	-2.50	-1.45	-1.17			B	lmo0891	RsbT, highly similar to positive regulation of sigma-B activity
	-0.66	-2.26	-2.49			A	lmo0892	RsbU, highly similar to serine phosphatase RsbU
-2.36	-2.37	-1.82	-1.21			B	lmo0892	RsbU, highly similar to serine phosphatase RsbU
0.80	1.16	1.40	1.27	0.68		A	lmo0906	Unknown, similar to glutathione Reductase
0.73	1.13	1.13	1.05	0.92		B	lmo0906	Unknown, similar to glutathione Reductase
0.45	0.94	1.13	0.73			A	lmo0909	Unknown, similar to transcription regulator, GntR family
1.06		0.94	0.94			B	lmo0909	Unknown, similar to transcription regulator, GntR family
0.89	2.15	2.75	2.40	-0.55		A	lmo0912	unknown, similar to transporters (formate)
1.69	1.61	1.75	0.90	0.69		B	lmo0912	unknown, similar to transporters (formate)
	1.22	1.48	1.57			A	lmo0923	Unknown, similar to ABC transporter, ATP-binding protein (N-terminal part)
1.53	1.96	1.80	1.36			B	lmo0923	Unknown, similar to ABC transporter, ATP-binding protein (N-terminal part)
0.81	1.16	1.96	2.21	0.87		A	lmo0930	unknown, conserved hypothetical protein, similar to B. subtilis YhfI protein
0.88	1.38	1.39	1.39	1.10		B	lmo0930	unknown, conserved hypothetical protein, similar to B. subtilis YhfI protein
		0.72	1.22	0.60		A	lmo0931	unknown, similar to lipoate protein ligase A
0.89	1.10	1.28	1.32			B	lmo0931	unknown, similar to lipoate protein ligase A
	1.94	3.85	3.98			A	lmo0932	unknown, conserved hypothetical protein
2.19	1.73	1.63	1.88			B	lmo0932	unknown, conserved hypothetical protein
-0.37	0.13	1.83	2.27			A	lmo0944	unknown, similar to B. subtilis YneR protein
0.71	2.32	2.73	2.25			B	lmo0944	unknown, similar to B. subtilis YneR protein
-0.52	-0.73		1.84			A	lmo0952	Unknown
	1.18	1.69	1.92	1.10		B	lmo0952	Unknown
0.76	1.46	2.48	3.20			A	lmo0953	Unknown
2.08		1.49	1.98			B	lmo0953	Unknown
	1.04	2.31	2.65	-0.30		A	lmo0955	unknown
1.11	1.76	2.18	1.37	0.75		B	lmo0955	unknown
1.05	1.57	1.79	1.62			A	lmo0963	unknown, similar to putative heat shock protein HtpX, Listeria epitope LemB
2.79	2.18	1.94	2.52	1.08		B	lmo0963	unknown, similar to putative heat shock protein HtpX, Listeria epitope LemB
-0.55	-1.42	-3.52	-4.39			A	lmo0969	unknown, similar to ribosomal large subunit pseudouridine synthetase

-1.30	-1.50	-1.83	-1.74			B	lmo0969	unknown, similar to ribosomal large subunit pseudouridine synthetase
	-0.78	-2.72	-4.16			A	lmo0970	unknown, similar to enoyl- acyl-carrier protein reductase
	-2.15	-1.24	-1.17	0.60		B	lmo0970	unknown, similar to enoyl- acyl-carrier protein reductase
	0.22	0.80	1.98	0.97		A	lmo0982	unknown, similar to glucanase and peptidase
	1.64	2.12	1.71	1.54		B	lmo0982	unknown, similar to glucanase and peptidase
	0.63	1.33	2.68	0.96		A	lmo0983	unknown, similar to glutathione peroxidase
	1.49	2.28	1.64	1.18		B	lmo0983	unknown, similar to glutathione peroxidase
	0.00		1.14	0.52		A	lmo0986	unknown, similar to antibiotic ABC transporter, ATP-binding protein,
-0.75		1.09	1.13			B	lmo0986	unknown, similar to antibiotic ABC transporter, ATP-binding protein,
-0.44	0.83	2.08	2.59	-0.82		A	lmo0995	unknown, similar to B. subtilis YkrP protein
	4.24	4.07	3.54			B	lmo0995	unknown, similar to B. subtilis YkrP protein
-0.17	-0.41	-2.17	-3.60			A	lmo1033	unknown, similar to transketolase
-1.16	-2.34	-1.78	-1.10			B	lmo1033	unknown, similar to transketolase
0.44	0.91	1.36	1.70			A	lmo1052	PdhA, highly similar to pyruvate dehydrogenase (E1 alpha subunit)
	1.70	2.19	2.30	1.15		B	lmo1052	pdhA ,highly similar to pyruvate dehydrogenase (E1 alpha subunit)
0.90	2.39	3.14	3.23	-0.75		A	lmo1068	unknown
3.49	2.83	2.82	1.98			B	lmo1068	unknown
	-0.89	-2.63	-2.34			A	lmo1072	PycA, highly similar to pyruvate carboxylase
-1.89	-1.80	-1.49	-1.02			B	lmo1072	PycA, highly similar to pyruvate carboxylase
	-0.99	-3.41	-3.69	-0.50		A	lmo1079	unknown, similar to B. subtilis YfhO protein
	-2.64	-2.56	-1.97	-0.82		B	lmo1079	unknown, similar to B. subtilis YfhO protein
	-0.26	-1.98	-3.03			A	lmo1081	Unknown, similar to glucose-1-phosphate thymidyl transferase
-1.31	-1.68	-1.34	-0.82			B	lmo1081	Unknown, similar to glucose-1-phosphate thymidyl transferase
	-0.40	-2.23	-3.05			A	lmo1082	Unknown, similar to dTDP-sugar epimerase
	-1.82	-1.27	-0.99			B	lmo1082	Unknown, similar to dTDP-sugar epimerase
-0.13	-0.31	-1.91	-2.89			A	lmo1083	Unknown, similar to dTDP-D-glucose 4,6-dehydratase
-1.19	-2.15	-1.59	-0.96			B	lmo1083	Unknown, similar to dTDP-D-glucose 4,6-dehydratase
	0.03	-1.44	-2.73			A	lmo1084	unknown, similar to DTDP-L-rhamnose synthetase
-0.84	-1.07		-0.90			B	lmo1084	unknown, similar to DTDP-L-rhamnose synthetase
-0.66	-1.79	-2.94	-2.78			A	lmo1090	unknown, similar to glycosyltransferases
	-2.29	-1.67	-1.88			B	lmo1090	unknown, similar to glycosyltransferases
1.19	2.65	3.99	4.26	-0.48		A	lmo1140	unknown
1.30	1.77	1.50	0.69			B	lmo1140	unknown
-0.18	-0.49	-0.94	-1.02			A	lmo1192	Unknown, similar to cobalamine synthesis protein CbiB
-0.63	-1.05	-1.36	-1.40			B	lmo1192	Unknown, similar to cobalamine synthesis protein CbiB
	-0.82	-1.07	-1.06			A	lmo1228	
-1.54	-1.51	-1.71	-1.78	-0.73		B	lmo1228	

0.53	0.83	1.13	1.87			A	lmo1258	unknown
	1.61	1.65		1.27		B	lmo1258	unknown
-0.16	-0.01		1.41	0.70		A	lmo1269	unknown, similar to type-I signal peptidase
	2.02	2.31		1.34		B	lmo1269	unknown, similar to type-I signal peptidase
	-0.29	-1.05	-1.86			A	lmo1277	
-0.57	-1.03	-1.16	-0.74			B	lmo1277	
	-0.31	0.04	1.22			A	lmo1282	Unknown, similar to B. subtilis YneQ protein
	1.72	2.19	3.01	1.72		B	lmo1282	Unknown, similar to B. subtilis YneQ protein
	1.09	1.74	1.48	-0.72		A	lmo1295	Unknown, similar to host factor-1 protein
	1.87	1.36	0.72			B	lmo1295	Unknown, similar to host factor-1 protein
-0.33	-0.87	-2.02	-2.46			A	lmo1296	unknown, conserved hypothetical protein similar to B. subtilis YnbA protein
-0.84	-1.33	-0.97	-0.85			B	lmo1296	unknown, conserved hypothetical protein similar to B. subtilis YnbA protein
0.57	1.35	2.07	2.62			A	lmo1301	unknown, conserved hypothetical protein
1.28	1.13	1.90	1.18			B	lmo1301	unknown, conserved hypothetical protein
0.86	1.51	1.82	2.31			A	lmo1302	unknown, highly similar to SOS response regulator lexA, transcription repressor protein
1.01	1.27	1.81	1.15			B	lmo1302	unknown, highly similar to SOS response regulator lexA, transcription repressor protein
-0.23	-0.61	-1.67	-3.79			A	lmo1306	unknown, highly similar to B. subtilis YneF protein
-0.59		-1.89	-1.57			B	lmo1306	unknown, highly similar to B. subtilis YneF protein
-0.16	-0.74	-1.98	-2.48			A	lmo1317	Unknown, similar to deoxyxylulose 5-phosphate reductoisomerase
-1.06	-1.37	-0.94				B	lmo1317	Unknown, similar to deoxyxylulose 5-phosphate reductoisomerase
	-0.28	-1.93	-2.93			A	lmo1319	
-0.55	-1.87	-1.45	-0.90			B	lmo1319	
-0.14	-0.44	-1.64	-1.45			A	lmo1331	
	-1.21	-0.91	-0.62			B	lmo1331	
0.53	0.86	1.32	2.00	0.64		A	lmo1332	unknown, similar to conserved hypothetical proteins
		1.07	1.38	0.73		B	lmo1332	unknown, similar to conserved hypothetical proteins
1.21	2.05	2.01	1.71	-0.54		A	lmo1340	unknown, similar to B. subtilis YqgU protein
1.27	2.00		0.41			B	lmo1340	unknown, similar to B. subtilis YqgU protein
	0.20	-0.96	-2.23	0.66		A	lmo1350	Unknown, similar to glycine dehydrogenase (decarboxylating) subunit 2
-0.79			-1.04	0.74		B	lmo1350	Unknown, similar to glycine dehydrogenase (decarboxylating) subunit 2
-0.13	-0.73	-2.16	-2.16			A	lmo1356	Unknown, similar to acetyl-CoA carboxylase subunit (biotin carboxyl carrier subunit)
	-1.04	-1.07	-0.90			B	lmo1356	Unknown, similar to acetyl-CoA carboxylase subunit (biotin carboxyl carrier subunit)
	-0.36	-2.18	-3.42			A	lmo1358	unknown, similar to B. subtilis YqhY protein
-0.89	-1.80	-1.45	-0.83			B	lmo1358	unknown, similar to B. subtilis YqhY protein
-1.47	-2.58	-4.23	-4.79			A	lmo1360	
-1.53	-1.28	-1.44	-1.30	0.30		B	lmo1360	

-0.26		1.08	1.20	-0.70		A	lmo1375	Unknown, similar to aminotripeptidase
0.89	1.11	0.93				B	lmo1375	Unknown, similar to aminotripeptidase
	-0.09	0.75	1.52			A	lmo1377	
		0.97	1.01	0.41		B	lmo1377	
	0.73	1.05	1.20			A	lmo1379	Unknown, similar to B. subtilis SpoIIIJ protein
	0.91	0.97	1.58	0.90		B	lmo1379	Unknown, similar to B. subtilis SpoIIIJ protein
	0.83	1.20	1.74	0.58		A	lmo1380	Unknown
		0.79	1.28	0.76		B	lmo1380	Unknown
	-0.08	1.12	2.13			A	lmo1382	Unknown
	1.81	2.48	2.58	1.19		B	lmo1382	Unknown
-0.16	-0.08	1.27	2.54	0.59		A	lmo1383	Unknown, similar to unknown protein
	1.95	1.99	2.18	1.40		B	lmo1383	Unknown, similar to unknown protein
-0.42	-1.04	-2.89	-3.30	-0.35		A	lmo1388	
-1.42	-2.72	-2.79	-2.98	-0.99		B	lmo1388	
-1.10	-2.78	-4.53	-5.35			A	lmo1389	Unknown, similar to sugar ABC transporter, ATP-binding protein
	-4.31	-3.99	-4.64			B	lmo1389	Unknown, similar to sugar ABC transporter, ATP-binding protein
-0.19	-1.24	-3.29	-4.22			A	lmo1390	Unknown, similar to ABC transporter (permease proteins)
	-2.48	-2.38	-2.51			B	lmo1390	Unknown, similar to ABC transporter (permease proteins)
	0.95	0.99	0.25	-2.19		A	lmo1406	
-1.39	-2.01	-2.62	-2.33	-3.48		B	lmo1406	
-1.10	-1.25		1.19			A	lmo1414	Unknown, similar to Acetyl-CoA:acetyltransferase
	1.69	1.87	2.24	0.82		B	lmo1414	Unknown, similar to Acetyl-CoA:acetyltransferase
	0.73	2.97	3.52	2.24		A	lmo1416	Unknown
	3.16	2.95	3.24	2.57		B	lmo1416	Unknown
	0.69	0.95	1.47			A	lmo1423	Unknown
	1.63	1.81	1.74			B	lmo1423	Unknown
-0.44	0.78	1.59	1.44			A	lmo1432	Unknown
1.94	1.61		0.91	0.82		B	lmo1432	Unknown
	-0.34	-2.16	-1.87			A	lmo1436	Unknown, similar to aspartokinase I (alpha and beta subunits)
-0.89	-1.56		-0.72	0.47		B	lmo1436	Unknown, similar to aspartokinase I (alpha and beta subunits)
-0.56	-1.53	-1.89	-1.38			A	lmo1437	Unknown, similar to aspartate-semialdehyde dehydrogenase
	-1.66	-0.98	-1.56	0.79		B	lmo1437	Unknown, similar to aspartate-semialdehyde dehydrogenase
0.70	1.71	1.99	1.78	-0.36		A	lmo1454	
1.44	1.80	1.11	0.50			B	lmo1454	
-0.41	-1.15	-1.92	-2.35			A	lmo1463	Unknown, similar to cytidine deaminase
	-2.12	-1.12	-1.30			B	lmo1463	Unknown, similar to cytidine deaminase
-0.77	-2.13	-3.09	-2.60	-0.35		A	lmo1496	Unknown, similar to transcription elongation factor GreA
-2.42	-1.50		-0.89			B	lmo1496	Unknown, similar to transcription elongation factor GreA

	0.65	1.00	1.04			A	lmo1503	Unknown
0.70	1.05	1.35	1.35	0.59		B	lmo1503	Unknown
		0.79	1.81	0.56		A	lmo1514	Unknown, similar to unknown protein
	0.94	1.03	0.57			B	lmo1514	Unknown, similar to unknown protein
0.59	2.05	2.83	3.01			A	lmo1526	Unknown, similar to unknown proteins
4.80	4.01	4.26	3.14			B	lmo1526	Unknown, similar to unknown proteins
	0.19	-0.93	-3.09			A	lmo1527	Unknown, similar to protein-export membrane protein SecDF
-0.59	-1.51	-1.94	-1.08			B	lmo1527	Unknown, similar to protein-export membrane protein SecDF
-0.73	-1.51	-2.29	-1.97			A	lmo1528	Unknown, similar to unknown proteins
-1.09	-0.80	-0.88	-1.23			B	lmo1528	Unknown, similar to unknown proteins
	-0.23	-1.25	-2.14			A	lmo1529	Unknown, similar to unknown proteins
-0.73	-1.06	-1.31	-1.13			B	lmo1529	Unknown, similar to unknown proteins
-0.31	-0.97	-2.59	-2.46			A	lmo1531	Unknown, similar to S-adenosylmethionine:tRNA ribosyltransferase-isomerase
-1.39	-1.47			0.52		B	lmo1531	Unknown, similar to S-adenosylmethionine:tRNA ribosyltransferase-isomerase
-0.26	-0.30	-0.66	-1.73	-0.27		A	lmo1540	
-0.81	-1.41	-1.67	-0.92			B	lmo1540	
-1.57	-2.45	-3.13	-2.70	0.54		A	lmo1544	
-1.05	-1.16	-0.89				B	lmo1544	
-0.31	-1.27	-2.59	-2.99			A	lmo1547	
-1.01	-0.95	-1.08	-0.85			B	lmo1547	
-0.86	-1.94	-2.82	-3.26	0.70		A	lmo1548	
-1.77			-0.86	0.63		B	lmo1548	
	-0.18	-1.79	-2.72			A	lmo1551	
	-1.45	-1.38	-1.55			B	lmo1551	
	-1.54	-2.17	-2.36			A	lmo1554	
-0.56	-1.74	-2.19	-2.45	-1.02		B	lmo1554	
-1.05	-2.67	-3.35	-3.34	0.64		A	lmo1556	
	-2.59	-2.05	-1.69			B	lmo1556	
-0.60	-1.10	-1.59	-1.94			A	lmo1561	
	-2.09	-0.96	-1.29			B	lmo1561	
-0.33	-0.81	-2.02	-3.57			A	lmo1573	
-0.64	-1.28	-1.29				B	lmo1573	
1.22	1.48	2.19	2.11			A	lmo1578	Unknown, similar to X-Pro dipeptidase
2.62	2.17	1.34		1.68		B	lmo1578	Unknown, similar to X-Pro dipeptidase
1.79	2.82	2.95	2.93			A	lmo1580	Unknown, similar to unknown protein
2.25	1.56	0.92		1.00		B	lmo1580	Unknown, similar to unknown protein
-0.28	-0.82	-2.07	-3.63			A	lmo1581	
	-1.50	-2.49	-1.08			B	lmo1581	
-0.40	-0.99	-2.56	-4.05			A	lmo1582	Unknown, weakly similar to site specific DNA-methyltransferase

-0.88	-1.46	-1.88				B	lmo1582	Unknown, weakly similar to site specific DNA-methyltransferase
	-0.95	-2.80	-3.52			A	lmo1584	Unknown, similar to unknown proteins
	-1.38	-1.86	-1.38			B	lmo1584	Unknown, similar to unknown proteins
		0.73	1.04	-0.34		A	lmo1602	Unknown, similar to unknown proteins
0.98	1.44	1.56	1.29	1.00		B	lmo1602	Unknown, similar to unknown proteins
-0.17	-0.13	-0.26	-1.07	-0.56		A	lmo1604	Unknown, similar to 2-cys peroxiredoxin
	-1.34	-2.12	-1.80	-1.33		B	lmo1604	Unknown, similar to 2-cys peroxiredoxin
-0.35	-1.03	-1.79				A	lmo1616	Unknown, similar to unknown proteins
-0.97	-1.44	-1.03				B	lmo1616	Unknown, similar to unknown proteins
	-0.11	-1.11	-1.76			A	lmo1623	Unknown, similar to putative transporters
-1.27	-1.84	-2.92	-2.42	0.55		B	lmo1623	Unknown, similar to putative transporters
1.60	2.56	3.07	2.81			A	lmo1628	
2.69	1.54	0.77		0.94		B	lmo1628	
	0.05	-0.58	-1.47	-0.37		A	lmo1631	
-1.03	-1.19		-1.10	-0.75		B	lmo1631	
-0.96	-2.32	-3.05	-2.22	0.70		A	lmo1635	Unknown, similar to unknown proteins
	-2.61	-2.35	-2.45	1.08		B	lmo1635	Unknown, similar to unknown proteins
	0.03	-0.42	-1.50			A	lmo1649	unknown
	-1.07	-1.54	-1.44			B	lmo1649	unknown
0.49		-0.88	-2.23	-2.15		A	lmo1651	unknown, similar to ABC transporter (ATP-binding protein)
	-2.11	-2.37		-1.43		B	lmo1651	unknown, similar to ABC transporter (ATP-binding protein)
	0.04	-1.01	-2.02	-1.72		A	lmo1652	unknown, similar to ABC transporter (ATP-binding protein)
	-1.54	-1.66	-1.97	-0.94		B	lmo1652	unknown, similar to ABC transporter (ATP-binding protein)
	1.27	2.53	2.63			A	lmo1653	unknown, putative cellsurface protein
4.25	3.06	2.60				B	lmo1653	unknown, putative cellsurface protein
	-0.61	-2.37	-3.47			A	lmo1663	
	-1.59	-1.79	-1.10	0.38		B	lmo1663	
-0.67	-1.23	-1.48	-1.44			A	lmo1667	unknown, similar to L-lactate dehydrogenases
-1.79	-1.01	-1.68	-1.80			B	lmo1667	unknown, similar to L-lactate dehydrogenases
-0.34	-1.01	-1.05	-1.01			A	lmo1682	unknown, similar to transmembrane transport proteins
-1.23	-1.68	-1.43		0.66		B	lmo1682	unknown, similar to transmembrane transport proteins
	1.40	1.80	2.44			A	lmo1683	unknown, similar to transcription regulators (Fur family), PerR in B. subtilis
0.70	2.00	2.34	2.19	1.22		B	lmo1683	unknown, similar to transcription regulators (Fur family), PerR in B. subtilis
1.11	2.52	2.83	3.29	0.80		A	lmo1684	unknown, similar to glycerate dehydrogenases
0.61	1.80	2.04	2.00			B	lmo1684	unknown, similar to glycerate dehydrogenases
-0.51	-1.50	-1.95	-1.91	-0.50		A	lmo1710	unknown, similar to putative flavodoxin
-0.89	-1.52	-1.44	-1.02	-0.77		B	lmo1710	unknown, similar to putative flavodoxin
0.73	0.98	2.39				A	lmo1734	Unknown, similar to glutamate synthase (large subunit)
	2.78	2.59		1.27		B	lmo1734	Unknown, similar to glutamate synthase (large subunit)

	0.92	2.43	4.30	0.88		A	lmo1738	Unknown, similar to amino acid ABC transporter (binding protein)
	1.04	3.33	2.51			B	lmo1738	Unknown, similar to amino acid ABC transporter (binding protein)
0.69	1.14	3.43	5.52	0.89		A	lmo1740	Unknown, similar to amino acid (glutamine) ABC transporter, permease protein
	3.20	5.63	4.02			B	lmo1740	Unknown, similar to amino acid (glutamine) ABC transporter, permease protein
	-0.63	-2.88	-3.36			A	lmo1755	
-1.34	-1.79	-1.72	-1.21			B	lmo1755	
	-1.02	-3.34	-3.44			A	lmo1756	
-1.98	-2.64	-2.05	-1.94	0.36		B	lmo1756	
	-0.20	-1.43	-2.59	0.54		A	lmo1757	Unknown, similar to unknown protein
-0.48	-1.15	-1.03	-0.87	0.51		B	lmo1757	Unknown, similar to unknown protein
	-0.14	-1.46	-2.57	0.54		A	lmo1758	Unknown, similar to DNA ligase
	-1.44	-0.91	-0.97	0.42		B	lmo1758	Unknown, similar to DNA ligase
	-0.37	-1.79	-2.50	0.66		A	lmo1759	
	-1.70	-0.85		0.57		B	lmo1759	
-0.24	-1.32	-2.37	-1.99	1.07		A	lmo1770	
	-2.01	-1.02		0.69		B	lmo1770	
	-0.68	-1.99	-2.70	1.02		A	lmo1775	
-0.86	-1.26	-1.34		0.78		B	lmo1775	
-0.23	-0.19	-0.28	-1.43	-0.34		A	lmo1783	
	-1.13	-2.02	-2.42			B	lmo1783	
	0.19	0.78	1.47	0.57		A	lmo1794	Unknown, similar to unknown proteins
		0.61	1.02	0.53		B	lmo1794	Unknown, similar to unknown proteins
	-0.27	-1.02	-2.59			A	lmo1806	
-0.96	-1.90	-3.30	-4.29			B	lmo1806	
	-0.37	-1.99	-3.08			A	lmo1807	
	-2.20	-1.88	-1.57			B	lmo1807	
	-0.48	-2.27	-3.06			A	lmo1808	
	-2.22	-1.61	-1.40			B	lmo1808	
	-0.57	-1.59	-1.46			A	lmo1809	
-1.17	-1.50	-1.76				B	lmo1809	
-0.92	-1.70	-2.72	-2.40			A	lmo1810	Unknown, similar to unknown proteins
-1.54	-1.83	-1.41	-0.86	0.56		B	lmo1810	Unknown, similar to unknown proteins
	-0.60	-1.30	-1.47			A	lmo1813	Unknown, similar to phosphoglycerate dehydrogenase
-0.44	-1.01	-1.19	-0.81			B	lmo1813	Unknown, similar to phosphoglycerate dehydrogenase
-0.44	-1.20	-1.68	-1.87			A	lmo1814	Unknown, similar to unknown proteins
	-1.44	-1.26	-0.77			B	lmo1814	Unknown, similar to unknown proteins
-0.60	-1.34	-1.90	-1.63			A	lmo1815	Unknown, similar to unknown protein
-1.05	-1.55	-1.80				B	lmo1815	Unknown, similar to unknown protein
-0.28	-0.73	-2.15	-2.90			A	lmo1818	Unknown, similar to ribulose-5-phosphate 3-epimerase

	-2.19	-1.52	-1.94	0.45		B	lmo1818	Unknown, similar to ribulose-5-phosphate 3-epimerase
2.70	4.92	4.50	2.71	0.49		A	lmo1854	unknown, similar to conserved hypothetical proteins
	1.04	0.98	0.74	0.44		B	lmo1854	unknown, similar to conserved hypothetical proteins
	-0.50	-0.85	-1.30			A	lmo1855	unknown, similar to similar to D-alanyl-D-alanine carboxypeptidases
-1.60	-1.91	-1.52	-1.39			B	lmo1855	unknown, similar to similar to D-alanyl-D-alanine carboxypeptidases
-0.27	-0.64	-1.18	-1.99			A	lmo1856	
-0.80	-1.34	-1.78	-1.47			B	lmo1856	
-0.27	-0.74	-1.22	-1.85			A	lmo1857	unknown, similar to hypoyhetical protein
-1.97	-1.55	-1.27	-2.00			B	lmo1857	unknown, similar to hypoyhetical protein
	0.02	-0.96	-1.11	0.67		A	lmo1858	unknown, similar to dehydogenases and hypothetical proteins
-0.63	-1.42	-0.91	-0.93			B	lmo1858	unknown, similar to dehydogenases and hypothetical proteins
		0.68	1.30	0.63		A	lmo1860	unknown, similar to peptidyl methionine sulfoxide reductases
		1.08	1.21	0.97		B	lmo1860	unknown, similar to peptidyl methionine sulfoxide reductases
-0.45	-0.69	-1.42	-2.21	0.69		A	lmo1877	Unknown, similar to formyl-tetrahydrofolate synthetase N-terminal part
-1.18	-1.22		-0.83	0.70		B	lmo1877	Unknown, similar to formyl-tetrahydrofolate synthetase N-terminal part
-0.14		1.12	1.66			A	lmo1880	unknown, similar to similar to RNase HI
		0.95	1.17	0.58		B	lmo1880	unknown, similar to similar to RNase HI
	-0.43	-0.61	-1.94			A	lmo1882	unknown, similar to ribosomal protein S14
	-1.44	-2.81	-2.48			B	lmo1882	unknown, similar to ribosomal protein S14
-0.60	-0.70	-1.24	-2.05			A	lmo1898	unknown, similar to hypothetical proteins
-1.01	-0.92	-1.03				B	lmo1898	unknown, similar to hypothetical proteins
-0.14		1.94	2.58	0.89		A	lmo1919	Unknown, similar to unknown proteins
	1.53	2.12	2.19			B	lmo1919	Unknown, similar to unknown proteins
-0.71	-1.65	-1.81	-0.72			A	lmo1935	Unknown, similar to protein-tyrosine/serine phosphatase
	-1.81	-1.19		0.41		B	lmo1935	Unknown, similar to protein-tyrosine/serine phosphatase
0.48		-1.54	-3.10			A	lmo1936	
	-2.08	-2.33	-1.29			B	lmo1936	
	-1.50	-2.55	-2.94			A	lmo1937	Unknown, similar to unknown protein
	-1.88	-1.35	-0.89			B	lmo1937	Unknown, similar to unknown protein
-0.25	-1.00	-1.75	-2.27			A	lmo1951	Unknown, similar to unknown proteins
-1.34		-1.15	-0.73			B	lmo1951	Unknown, similar to unknown proteins
-1.43	-2.31	-2.57	-3.24			A	lmo1952	
-0.73	-1.46	-1.75	-1.41			B	lmo1952	
1.33	2.04	2.67	3.52	0.66		A	lmo1966	Unknown, similar to unknown proteins
	1.48	1.56	1.67			B	lmo1966	Unknown, similar to unknown proteins
0.86	1.46	2.35	3.37	0.56		A	lmo1967	Unknown, similar to toxic ion resistance proteins

0.66	1.18	1.12	0.65			B	lmo1967	Unknown, similar to toxic ion resistance proteins
-0.49	0.10	0.87	1.06			A	lmo1975	Unknown, similar to E. coli DNA-damage-inducible protein dinP
	1.25	1.56	1.41			B	lmo1975	Unknown, similar to E. coli DNA-damage-inducible protein dinP
	-0.19	-0.87	-1.23			A	lmo1986	
-0.82		-1.17	-0.76	-0.54		B	lmo1986	
1.85	2.89	3.37	3.50			A	lmo1990	
3.63	3.10	3.68	2.66			B	lmo1990	
0.80	1.06	1.55	1.65	-0.21		A	lmo1991	
1.42	1.71	1.93	1.52			B	lmo1991	
2.30	3.09	3.41	3.41			A	lmo1992	Unknown, similar to alpha-acetolactate decarboxylase
4.70	3.91	3.54	2.68			B	lmo1992	Unknown, similar to alpha-acetolactate decarboxylase
0.59	-0.09	-1.46	-1.28	-1.07		A	lmo1993	
	-1.74	-1.85	-1.71			B	lmo1993	
	-0.53	-1.56	-0.84	-0.44		A	lmo1995	
-0.94	-1.85	-1.30	-1.30			B	lmo1995	
		-1.08	-1.87	-0.42		A	lmo1999	Unknown, weakly similar to glucosamine-fructose-6-phosphate aminotransferase
-1.50	-2.73	-4.71	-4.51			B	lmo1999	Unknown, weakly similar to glucosamine-fructose-6-phosphate aminotransferase
-0.45	-1.07	-1.92	-1.52			A	lmo2020	
	-1.66	-1.49	-1.50			B	lmo2020	
0.79	1.25	1.77	0.73	-0.25		A	lmo2024	
1.09	1.12	0.93	0.28			B	lmo2024	
	-0.82	-1.88	-1.43			A	lmo2032	
-1.60	-1.61	-1.24	-1.07			B	lmo2032	
-0.74	-1.54	-2.59	-2.03			A	lmo2039	
-2.69	-1.87	-1.45		0.40		B	lmo2039	
-1.35	-2.54	-2.34	-1.63			A	lmo2041	Unknown, similar to unknown proteins
-0.76	-1.34			0.42		B	lmo2041	Unknown, similar to unknown proteins
-0.88	-1.90	-1.80	-1.45			A	lmo2042	Unknown, similar to unknown proteins
	-1.41	-0.86	-0.80	0.59		B	lmo2042	Unknown, similar to unknown proteins
	-1.10	-1.05	-1.66	-0.48		A	lmo2045	Unknown
	-1.09	-1.81	-1.45			B	lmo2045	Unknown
-0.53	-0.52	-0.76	-2.01			A	lmo2047	
-0.46	-1.04	-2.22	-1.84			B	lmo2047	
-1.70	-2.42	-2.77	-3.35			A	lmo2048	Unknown, similar to unknown proteins
-0.96	-1.37	-1.80				B	lmo2048	Unknown, similar to unknown proteins
-0.59	-1.51	-2.07	-1.47			A	lmo2052	Unknown, similar to phosphopantetheine adenylyltransferase
-1.30		-0.85	-0.95			B	lmo2052	Unknown, similar to phosphopantetheine adenylyltransferase
	0.73	1.33	2.00			A	lmo2054	Unknown, similar to unknown proteins

	2.30	2.87	2.81	1.19		B	lmo2054	Unknown, similar to unknown proteins
0.83	1.15	1.66	1.84			A	lmo2055	Unknown, similar to unknown proteins
1.56	1.34	1.82	2.29	0.78		B	lmo2055	Unknown, similar to unknown proteins
-0.81	-1.35	-1.56	-1.55			A	lmo2063	unknown
-0.97	-1.26	-1.32	-1.05			B	lmo2063	unknown
0.77	2.66	3.23	2.87	-0.59		A	lmo2085	Unknown, putative peptidoglycan bound protein (LPXTG motif)
2.61	2.54	1.97	2.06			B	lmo2085	Unknown, putative peptidoglycan bound protein (LPXTG motif)
		0.80	1.55	0.80		A	lmo2089	Unknown, similar to lipases
		0.88	1.22	0.85		B	lmo2089	Unknown, similar to lipases
-0.27	-0.56	-1.04	-1.94	-0.27		A	lmo2092	
-1.04	-1.30	-0.99	-1.08			B	lmo2092	
2.18	2.95	2.90	1.68	0.81		A	lmo2108	Unknown, similar to N-acetylglucosamine-6-phosphate deacetylase
2.30		1.11	1.50	0.62		B	lmo2108	Unknown, similar to N-acetylglucosamine-6-phosphate deacetylase
	-0.50		1.37	1.49		A	lmo2119	Unknown, similar to unknown proteins
	1.06	1.70	1.62	1.55		B	lmo2119	Unknown, similar to unknown proteins
-0.14	-0.36		1.41	1.64		A	lmo2120	Unknown, similar to unknown proteins
	1.09	1.91	1.56	1.25		B	lmo2120	Unknown, similar to unknown proteins
	-0.92		-2.19	-0.28		A	lmo2129	unknown
	-3.26	-3.84	-4.92			B	lmo2129	unknown
3.11	4.17	3.47	2.20	1.02		A	lmo2156	Unknown
2.63	3.31	2.21	2.21	0.77		B	lmo2156	Unknown
	1.46	2.57	2.42			A	lmo2157	
1.69	2.33	2.10	0.65			B	lmo2157	
0.58	1.27	2.50	2.77			A	lmo2190	
1.00	1.79	2.25	2.23	0.99		B	lmo2190	
	0.91	1.86	1.83	-0.40		A	lmo2191	Unknown, similar to unknown proteins
2.62	2.23	2.03	2.05	1.49		B	lmo2191	Unknown, similar to unknown proteins
	-0.16	-0.15	-1.66	-0.36		A	lmo2196	Unknown, similar to pheromone ABC transporter (binding protein)
	-1.51	-2.45	-2.51	-1.14		B	lmo2196	Unknown, similar to pheromone ABC transporter (binding protein)
	0.80	1.76	2.89	0.61		A	lmo2199	Unknown, similar to unknown protein
0.61	1.08	1.93	1.95			B	lmo2199	Unknown, similar to unknown protein
	1.31	2.38	3.35	0.97		A	lmo2200	Unknown, similar to transcription regulator
3.29	3.48	3.49	3.81	1.77		B	lmo2200	Unknown, similar to transcription regulator
-0.74	-1.62	-3.02	-2.58			A	lmo2202	Unknown, similar to 3-oxoacyl- acyl-carrier protein synthase
-1.76	-1.33			0.59		B	lmo2202	Unknown, similar to 3-oxoacyl- acyl-carrier protein synthase
1.19	2.22	2.28	2.13	-0.49		A	lmo2205	Unknown, similar to phosphoglyceromutase 1
2.65	2.83	2.57	0.66	1.07		B	lmo2205	Unknown, similar to phosphoglyceromutase 1
0.91	2.35	3.31	3.19	1.59		A	lmo2210	Unknown
	1.27	1.33	0.34	0.90		B	lmo2210	Unknown
2.41	3.83	4.64	4.42			A	lmo2213	Unknown, similar to unknown protein
4.57	3.23	2.99	2.81			B	lmo2213	Unknown, similar to unknown protein
1.79	2.52	2.85	3.16			A	lmo2230	Unknown, similar to arsenate reductase

3.08	4.08	4.15	3.82			B	lmo2230	Unknown, similar to arsenate reductase
	2.00	2.23	1.79	-0.36		A	lmo2231	Unknown, similar to unknown proteins
2.73	2.05	2.27	1.63			B	lmo2231	Unknown, similar to unknown proteins
-0.13	-0.42	-1.09	-1.44			A	lmo2247	Unknown, similar to oxidoreductase
		-1.24	-1.30	-0.54		B	lmo2247	Unknown, similar to oxidoreductase
-0.33	-0.67	-1.61	-2.70			A	lmo2248	Unknown, similar to unknown proteins
	-1.50	-1.75	-1.54	-0.76		B	lmo2248	Unknown, similar to unknown proteins
-0.54	-1.03	-2.07	-2.29			A	lmo2254	Unknown, similar to unknown proteins
-1.33	-1.05	-1.78	-1.62			B	lmo2254	Unknown, similar to unknown proteins
	1.02	1.52	2.07	0.85		A	lmo2256	Unknown, similar to unknown proteins
1.00	1.86	2.24	2.29	1.56		B	lmo2256	Unknown, similar to unknown proteins
		1.25	1.70	0.66		A	lmo2263	unknown, similar to unkown proteins
2.51	2.27	2.05	2.43			B	lmo2263	unknown, similar to unkown proteins
0.79	2.75	3.85	4.09	-1.14		A	lmo2269	Unknown
	2.46	1.82		-0.74		B	lmo2269	Unknown
0.54	0.97	1.47	1.86			A	lmo2323	gp43 [Bacteriophage A118]
1.49	1.83	1.69	1.19	0.62		B	lmo2323	gp43 [Bacteriophage A118]
	0.68	0.92	1.42	1.29		A	lmo2360	Unknown, transmembrane protein
	3.17	3.13	3.07	2.35		B	lmo2360	Unknown, transmembrane protein
		0.76	1.22	0.98		A	lmo2361	Unknown, conserved hypothetical protein
1.23	1.99	2.18		1.25		B	lmo2361	Unknown, conserved hypothetical protein
	0.85	0.76	1.12			A	lmo2366	Unknown, similar to transcription regulator DeoR family
1.10	0.95		0.93			B	lmo2366	Unknown, similar to transcription regulator DeoR family
	-0.28	-0.37	1.12	0.67		A	lmo2369	Unknown, similar to B. subtilis general stress protein 13 containing a ribosomal S1 protein domain
-0.63		1.40	1.05	0.98		B	lmo2369	Unknown, similar to B. subtilis general stress protein 13 containing a ribosomal S1 protein domain
0.75	1.37	2.14	2.68			A	lmo2373	Unknown, similar to phosphotransferase system (PTS) beta-glucoside-specific enzyme IIB component
0.46	1.31	1.00	0.82			B	lmo2373	Unknown, similar to phosphotransferase system (PTS) beta-glucoside-specific enzyme IIB component
	0.92	1.35	1.77			A	lmo2393	Unknown, similar to B. subtilis YuzD protein
0.41		1.28	1.85			B	lmo2393	Unknown, similar to B. subtilis YuzD protein
	1.53	2.87	3.52			A	lmo2398	
	3.22	3.52		2.62		B	lmo2398	
0.70	1.33	2.06	2.98	0.82		A	lmo2406	unknown, similar to B. subtilis YunF protein
1.27	2.05	2.21	1.82			B	lmo2406	unknown, similar to B. subtilis YunF protein
-0.71	-0.83	-0.74	-1.26			A	lmo2409	Unknown
		-1.53	-2.08	-0.67		B	lmo2409	Unknown
	2.52	3.34	3.47	-1.34		A	lmo2434	unknown, highly similar to glutamate decarboxylases
0.83	1.35	1.25		0.54		B	lmo2434	unknown, highly similar to glutamate decarboxylases
		1.06	1.21	0.91		A	lmo2442	Unknown
2.42	1.78	1.59	0.72			B	lmo2442	Unknown

0.57	1.28	1.34	1.45			A	lmo2453	Unknown, similar to lipolytic enzyme
1.02		0.88	1.05			B	lmo2453	Unknown, similar to lipolytic enzyme
-1.19	-0.16	1.09	0.74	-0.67		A	lmo2463	unknown, similar to transport protein
	2.09	1.98	1.44			B	lmo2463	unknown, similar to transport protein
0.44	0.94	2.04	2.67			A	lmo2468	
1.54	2.05	1.54	1.21	0.61		B	lmo2468	
	0.00	0.59	1.46	0.62		A	lmo2474	unknown, conserved hypothetical protein
	1.36	1.93	2.50	1.25		B	lmo2474	unknown, conserved hypothetical protein
-0.38	-1.06	-1.97	-2.91			A	lmo2481	Unknown, similar to B. subtilis P-Ser-HPr phosphatase
-1.41	-2.03	-1.71	-1.48			B	lmo2481	Unknown, similar to B. subtilis P-Ser-HPr phosphatase
-0.30	-1.03	-2.04	-2.89			A	lmo2482	
	-1.87	-1.55		0.45		B	lmo2482	
	1.51	2.79	3.34	-0.57		A	lmo2484	Unknown, similar to B. subtilis YvlD protein
	2.77	2.37	2.18	1.06		B	lmo2484	Unknown, similar to B. subtilis YvlD protein
	1.75	3.07	3.50	-1.04		A	lmo2485	Unknown, similar to B. subtilis yvlC protein
	1.74	1.47	0.61			B	lmo2485	Unknown, similar to B. subtilis yvlC protein
		1.25	1.47	-0.53		A	lmo2494	Unknown, similar to negative regulator of phosphate regulon
	1.12	1.25		1.02		B	lmo2494	Unknown, similar to negative regulator of phosphate regulon
-0.69	-1.66	-2.47	-2.38	0.58		A	lmo2504	Unknown, similar to cell wall binding proteins
-1.17	-1.49	-1.39	-1.40			B	lmo2504	Unknown, similar to cell wall binding proteins
-0.96	-2.60	-3.92	-4.19			A	lmo2506	
	-3.12	-2.06	-3.58			B	lmo2506	
-0.93	-2.77	-4.83	-4.74	0.66		A	lmo2507	
	-3.96	-2.83	-3.59			B	lmo2507	
-0.24	-0.13	0.99	1.60			A	lmo2515	Unknown, similar to B. subtilis two-component response regulator DegU
	1.22	1.53	0.91			B	lmo2515	Unknown, similar to B. subtilis two-component response regulator DegU
	0.23	-1.12	-2.48			A	lmo2532	
	-1.03	-1.25	-0.80			B	lmo2532	
	0.12	-1.39	-2.70			A	lmo2534	
	-1.11	-1.01	-0.62			B	lmo2534	
0.50	-0.22	-2.16	-2.38	-0.32		A	lmo2546	
	-1.91	-1.59	-0.64	-0.78		B	lmo2546	
-0.82	-2.11	-3.11	-3.58			A	lmo2550	Unknwon, similar to glycosyl transferases
-1.02	-1.31	-1.40	-0.94			B	lmo2550	Unknwon, similar to glycosyl transferases
	-0.76	-1.70	-2.13			A	lmo2556	
-0.75		-1.28		-0.93		B	lmo2556	
	-0.87	-2.54	-3.25			A	lmo2561	
-1.09	-1.45	-1.45	-0.78			B	lmo2561	
-0.52	-0.72	-0.21	1.56			A	lmo2564	Unknwon, similar to 4-oxalocrotonate isomerase
		1.07	0.58	0.60		B	lmo2564	Unknwon, similar to 4-oxalocrotonate isomerase

-0.60	1.33	2.54	2.93	-0.41		A	lmo2570	Unknwon
1.01	2.05	1.77	1.42			B	lmo2570	Unknwon
-0.94	1.84	3.03	3.34			A	lmo2571	Unknwon, similar to nicotinamidase
3.65	2.82	2.84	2.05	2.77		B	lmo2571	Unknwon, similar to nicotinamidase
	1.97	3.10	3.19			A	lmo2572	Unknwon, similar to Chain A, Dihydrofolate Reductase
2.45	1.96		1.89	1.22		B	lmo2572	Unknwon, similar to Chain A, Dihydrofolate Reductase
1.22	2.77	3.41	3.32	-1.00		A	lmo2573	Unknown, similar to zinc-binding dehydrogenase
3.84	2.61	2.63	2.09	2.69		B	lmo2573	Unknown, similar to zinc-binding dehydrogenase
1.39	2.76	3.04	2.63	1.76		A	lmo2574	Unknwon
2.51	1.42	1.95	1.87	1.27		B	lmo2574	Unknwon
2.51	3.47	3.65	3.41	2.29		A	lmo2575	Unknwon, similar to cation transport protein (efflux)
2.58	2.10	2.74	1.90	1.19		B	lmo2575	Unknwon, similar to cation transport protein (efflux)
	1.98	2.40	2.68			A	lmo2603	Unknown
1.21	1.69	1.56	1.65	1.06		B	lmo2603	Unknown
-0.14	-0.41	-0.89	-1.55			A	lmo2606	
-1.04	-1.38	-2.10	-1.89	-0.95		B	lmo2606	
-0.27	-0.46	-0.78	-1.65			A	lmo2607	
-0.60	-1.11	-1.83	-1.74	-0.59		B	lmo2607	
-0.18	-0.40	-0.58	-1.55			A	lmo2608	
-0.65	-1.19	-1.80	-2.05			B	lmo2608	
	-0.40	-0.90	-1.65			A	lmo2610	
-0.72	-1.29	-1.95	-1.87			B	lmo2610	
	-0.16	-0.21	-1.01			A	lmo2611	
	-0.80	-1.59	-2.44	-0.76		B	lmo2611	
	-0.01	-0.10	-1.87	-0.30		A	lmo2620	
-0.42	-1.76	-3.50	-3.57	-1.15		B	lmo2620	
-0.11	-0.23	-0.13	-1.31			A	lmo2621	
	-1.61	-3.25	-2.71	-0.93		B	lmo2621	
	-0.31	-0.32	-1.61	-0.29		A	lmo2622	
-0.68	-2.04	-3.87	-3.68	-1.08		B	lmo2622	
	-0.04	0.02	-1.08	-0.32		A	lmo2623	
	-1.38	-3.14	-3.02	-0.90		B	lmo2623	
	-0.18	-0.16	-1.68			A	lmo2624	
	-1.33	-2.67	-2.50	-0.90		B	lmo2624	
	-0.15	-0.46	-2.02			A	lmo2626	
	-1.63	-2.49	-2.25	-0.92		B	lmo2626	
	0.06	-0.15	-1.80			A	lmo2627	
-0.58	-2.02	-3.51	-2.75	-1.16		B	lmo2627	
	0.15	-0.26	-2.29			A	lmo2628	
-0.85	-1.58	-3.65	-2.25	-1.05		B	lmo2628	

	-0.16	-0.18	-1.82	-0.30		A	lmo2629	
	-1.57	-2.54	-2.17	-1.08		B	lmo2629	
	-0.20	-0.39	-1.84			A	lmo2630	
-0.58	-1.56	-3.22	-2.30	-0.78		B	lmo2630	
	-0.21	-0.21	-1.16			A	lmo2631	
-0.84	-1.67	-3.30	-2.57	-0.91		B	lmo2631	
	-0.22	-0.48	-2.57			A	lmo2632	
-0.70	-1.55	-2.68	-2.23	-0.70		B	lmo2632	
-0.23	-0.35	-0.82	-3.37			A	lmo2633	
-0.73	-1.62	-3.01	-2.10			B	lmo2633	
	-0.40	-2.10	-3.22	-0.29		A	lmo2635	Unknown, weakly similar to E. coli MenA protein
-1.04	-1.56	-2.94	-1.67			B	lmo2635	Unknown, weakly similar to E. coli MenA protein
	-0.56	-1.22	-1.72			A	lmo2636	Unknown, conserved hypothetical lipoprotein
	-0.91	-1.44	-0.93			B	lmo2636	Unknown, conserved hypothetical lipoprotein
-0.25	-0.01	-0.06	-1.55	-0.88		A	lmo2637	Unknown, conserved lipoprotein
	-1.15	-3.39	-3.86			B	lmo2637	Unknown, conserved lipoprotein
	-0.20	-0.26	-1.95			A	lmo2655	
	-1.46	-3.16	-2.17	-0.81		B	lmo2655	
-0.16	-0.42	-1.27	-3.56			A	lmo2656	
-0.92	-1.93	-2.97	-2.67	-0.73		B	lmo2656	
	1.86	2.59	2.10			A	lmo2669	Unknown
	2.42	2.16	1.75			B	lmo2669	Unknown
1.88	2.93	3.35	2.77			A	lmo2671	Unknown
2.31	3.18	2.57	3.23	1.28		B	lmo2671	Unknown
1.08	2.83	3.06	2.71			A	lmo2672	Unknown, weakly similar to transcription regulator
2.74	2.81	2.16	3.04	1.31		B	lmo2672	Unknown, weakly similar to transcription regulator
0.90	2.75	3.79	3.65	-0.67		A	lmo2673	Unknown, conserved hypothetical protein
2.31	2.39	1.75	1.99			B	lmo2673	Unknown, conserved hypothetical protein
2.28	3.30	3.25	3.03	-0.95		A	lmo2674	Unknown, similar to ribose 5-phosphate epimerase
3.56	3.11	2.83	3.10	2.25		B	lmo2674	Unknown, similar to ribose 5-phosphate epimerase
-0.62	-0.21			-1.88		A	lmo2686	unknown
		-1.40	-1.13	-5.99		B	lmo2686	unknown
0.53		-0.55	-1.22			A	lmo2691	Unknown, similar to autolysin, N-acetylmuramidase
-0.85	-1.13	-1.57	-1.73			B	lmo2691	Unknown, similar to autolysin, N-acetylmuramidase
	2.47	3.03	2.55	-0.31		A	lmo2695	Unknown, similar to dihydroxyacetone kinase
1.47	1.40	1.40	1.50	1.09		B	lmo2695	Unknown, similar to dihydroxyacetone kinase
0.53	1.06		0.90	0.59		A	lmo2705	Unknown
	0.98	1.21	1.62	1.09		B	lmo2705	Unknown
0.61	1.16	1.19	1.41			A	lmo2706	Unknown
	2.23	1.50		1.19		B	lmo2706	Unknown

0.62	1.60	2.33	1.98			A	lmo2713	Unknown, secreted protein with 1 GW repeat
1.62	1.35	2.01	1.50	0.61		B	lmo2713	Unknown, secreted protein with 1 GW repeat
		-0.58	-2.50	-0.79		A	lmo2717	
-0.64	-1.65	-1.36	-1.50	-1.52		B	lmo2717	
	0.68	-0.23	-2.55	-0.97		A	lmo2718	
-0.62	-1.54	-1.98	-2.01	-1.54		B	lmo2718	
	0.84	2.23	2.65			A	lmo2723	Unknown, similar to unknown proteins
0.94		1.44	1.25	0.98		B	lmo2723	Unknown, similar to unknown proteins
-0.22	1.14	2.06	1.59			A	lmo2740	Unknown
	2.35	2.24	1.53	1.29		B	lmo2740	Unknown
1.31	2.98	3.57	3.03	-0.54		A	lmo2748	Unknown, similar to B. subtilis stress protein YdaG
3.25	3.75	3.80	2.85			B	lmo2748	Unknown, similar to B. subtilis stress protein YdaG
-0.23	-1.04	-2.21	-2.87	-0.33		A	lmo2767	Unknwon
		-1.46	-1.19	-0.78		B	lmo2767	Unknwon
-0.34	-0.60	-0.93	-1.49	-0.37		A	lmo2788	
-1.50	-1.88	-2.83	-1.32			B	lmo2788	
	0.74	1.19	1.41			A	lmo2789	
	1.18	1.19		0.54		B	lmo2789	
-0.16	-0.47		1.30	0.53		A	lmo2825	
		0.89	1.58	0.91		B	lmo2825	
	1.39	1.93	2.23			A	lmo2828	Unknown
	2.47	2.07	1.76			B	lmo2828	Unknown
0.72	1.39	1.85	2.35			A	lmo2830	Unknown, similar to thioredoxin
1.23	2.72	2.78	2.68	0.62		B	lmo2830	Unknown, similar to thioredoxin
-0.42	-0.85	-1.78	-2.34			A	lmo2831	Unknown, similar to phosphoglucomutase
-0.78		-1.54	-1.69			B	lmo2831	Unknown, similar to phosphoglucomutase
-0.58	-0.83	-1.51	-1.92	-0.54		A	lmo2853	unknown, highly similar to B. subtilis Jag protein
	-1.14	-1.67	-1.60			B	lmo2853	unknown, highly similar to B. subtilis Jag protein

Tab. S-10: *L. monocytogenes* genes, which showed significant change in transcription levels after acid shock at 25 and in the temperature shift experiment.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	adapt.	temp. shift	Id*	LmoNr.	Function
					2.30	C	lmo0051	Unknwon, similar to 2-components response regulator protein (AgrA from Staphylococcus)
-0.50		-0.83	-1.04	2.32		A	lmo0051	Unknwon, similar to 2-components response regulator protein (AgrA from Staphylococcus)
					-1.07	C	lmo0069	unknown
-0.20	-0.03	-0.19	-1.39			A	lmo0069	unknown
					2.05	C	lmo0073	unknown
-0.95	-0.54	-0.71		2.34		A	lmo0073	unknown
					-1.34	C	lmo0193	unknwon
	-1.42	-2.15	-1.89			A	lmo0193	unknwon
					4.06	C	lmo0225	FolA, highly similar to dihydroneopterin aldolase
	1.48	2.46	3.52			A	lmo0225	FolA, highly similar to dihydroneopterin aldolase
					-1.51	C	lmo0269	Unknown, similar to transporter
-2.03	-2.82	-3.48	-3.74	-0.30		A	lmo0269	Unknown, similar to transporter
					-1.16	C	lmo0309	Unknown, similar to unknown protein
-0.29	-1.13	-2.22	-2.17			A	lmo0309	Unknown, similar to unknown protein
					-1.25	C	lmo0403	Unknown
-1.31	-1.26	-0.60		-0.27		A	lmo0403	Unknown
					-1.12	C	lmo0416	Unknown, similar to putative transcription regulator
-1.51	-1.32	-1.17	-0.55	-0.52		A	lmo0416	Unknown, similar to putative transcription regulator
					-1.13	C	lmo0449	Unknown
-1.07	-1.46	-1.11	-0.80			A	lmo0449	Unknown
					-1.69	C	lmo0470	Unknown, weakly similar to site-specific DNA-methyltransferase
-1.91	-3.02	-4.46	-3.79			A	lmo0470	Unknown, weakly similar to site-specific DNA-methyltransferase
					1.64	C	lmo0471	Unknown
0.68	2.19	2.67	2.66	-1.68		A	lmo0471	Unknown
					-1.39	C	lmo0485	Unknown
	-0.21	-1.01	-2.07	-1.18		A	lmo0485	Unknown
					-1.94	C	lmo0511	Unknown, conserved hypothetical protein
	-0.62	-1.96	-2.35	-1.56		A	lmo0511	Unknown, conserved hypothetical protein
					-1.09	C	lmo0519	Unknown, similar to multidrug resistance protein
	-0.18	-1.13	-2.48			A	lmo0519	Unknown, similar to multidrug resistance protein
					1.66	C	lmo0521	Unknown, similar to 6-phospho-beta-glucosidase
0.65	1.00	1.92	2.07			A	lmo0521	Unknown, similar to 6-phospho-beta-glucosidase
					-1.67	C	lmo0597	Unknown, similar to transcription regulator CRP/FNR family
-1.70	-1.73		-1.98			A	lmo0597	Unknown, similar to transcription regulator CRP/FNR family

					-2.02	C	lmo0611	Unknown, similar to acyl-carrier protein phosphodiesterase and NAD(P)H dehydrogenase
-2.48	-2.55	-0.75	0.19	0.72		A	lmo0611	Unknown, similar to acyl-carrier protein phosphodiesterase and NAD(P)H dehydrogenase
					1.32	C	lmo0640	Unknown, similar to oxidoreductase
	0.73	1.06	1.67			A	lmo0640	Unknown, similar to oxidoreductase
					1.93	C	lmo0641	Unknown, similar to heavy metal-transporting ATPase
1.32	2.55	2.64	2.28	1.23		A	lmo0641	Unknown, similar to heavy metal-transporting ATPase
					-1.05	C	lmo0665	Unknown
-0.35	-1.22	-2.09	-1.89	0.89		A	lmo0665	Unknown
					-1.34	C	lmo0667	Unknown, similar to ABC transporter (ATP-binding protein)
	0.04	0.70	1.64	0.64		A	lmo0667	Unknown, similar to ABC transporter (ATP-binding protein)
					-1.41	C	lmo0672	Unknown, similar to unknown protein
-1.28		-3.77	-4.76	-1.04		A	lmo0672	Unknown, similar to unknown protein
					-1.91	C	lmo0675	
-1.11	-2.18	-2.96	-3.12			A	lmo0675	
					-1.60	C	lmo0676	Unknown, similar to flagellar biosynthesic protein FliP
-1.51	-2.16	-2.94	-2.23			A	lmo0676	Unknown, similar to flagellar biosynthesic protein FliP
					-5.13	C	lmo0680	Unknown, similar to flagella-associated protein flhA
-1.92	-3.29	-6.23				A	lmo0680	Unknown, similar to flagella-associated protein flhA
					-3.90	C	lmo0681	Unknown, similar to flagellar biosynthesis protein FlhF
-1.02	-1.87	-4.21	-4.62	-1.50		A	lmo0681	Unknown, similar to flagellar biosynthesis protein FlhF
					-2.54	C	lmo0682	Unknown, similar to flagellar hook-basal body protein FlgG
-0.23	-0.96	-2.95		-0.98		A	lmo0682	Unknown, similar to flagellar hook-basal body protein FlgG
					-1.36	C	lmo0683	Unknown, similar to chemotactic methyltransferase CheR
-1.60	-2.22	-2.44	-2.23	-0.44		A	lmo0683	Unknown, similar to chemotactic methyltransferase CheR
					-4.08	C	lmo0684	Unknown
-0.47	-0.87	-3.03	-4.81			A	lmo0684	Unknown
					-3.30	C	lmo0685	Unknown, similar to motility protein (flagellar motor rotation) MotA
	-0.15	-1.84	-2.80			A	lmo0685	Unknown, similar to motility protein (flagellar motor rotation) MotA
					-1.32	C	lmo0686	MotB, similar to motility protein (flagellar motor rotation) MotB
	-0.06	-1.52	-2.12			A	lmo0686	MotB, similar to motility protein (flagellar motor rotation) MotB
					-2.95	C	lmo0687	Unknown
0.54		-1.06	-3.06	-1.80		A	lmo0687	Unknown
					-3.00	C	lmo0688	Unknown, similar to unknown protein

	0.69	-0.50	-2.35	-1.67		A	lmo0688	Unknown, similar to unknown protein
					-4.08	C	lmo0689	Unknown, similar to CheA activity-modulating chemotaxis protein CheV
0.59	0.66	-0.02	-2.12	-2.22		A	lmo0689	Unknown, similar to CheA activity-modulating chemotaxis protein CheV
					-4.35	C	lmo0690	FlaA, flagellin protein
	-0.04	-0.23		-3.57		A	lmo0690	FlaA, flagellin protein
					-1.39	C	lmo0691	CheY, Chemotaxis response regulator CheY
-0.42	-1.06	-1.26	-1.45	-0.49		A	lmo0691	CheY, Chemotaxis response regulator CheY
					-5.29	C	lmo0692	CheA, two-component sensor histidine kinase CheA
-0.32	-1.05	-1.95	-3.05	-2.72		A	lmo0692	CheA, two-component sensor histidine kinase CheA
					-2.47	C	lmo0695	Unknown
	-0.94	-2.27	-2.24	-1.74		A	lmo0695	Unknown
					-3.88	C	lmo0696	unknown, similar to flagellar hook assembly protein
-0.78	-1.61	-3.41		-2.64		A	lmo0696	unknown, similar to flagellar hook assembly protein
					-3.19	C	lmo0697	Unknown, similar to flagellar hook protein FlgE
-0.20	-0.89	-2.69	-3.47			A	lmo0697	Unknown, similar to flagellar hook protein FlgE
					-3.60	C	lmo0698	Unknown, weakly similar to flagellar switch protein
	-0.55	-3.08	-4.71	-1.79		A	lmo0698	Unknown, weakly similar to flagellar switch protein
					-3.81	C	lmo0699	Unknown, similar to flagellar switch protein FliM
	-0.16	-2.61	-3.91	-2.78		A	lmo0699	Unknown, similar to flagellar switch protein FliM
					-3.73	C	lmo0700	UNknown, similar to flagellar motor switch protein fliY
	0.09	-2.11	-3.08	-2.58		A	lmo0700	UNknown, similar to flagellar motor switch protein fliY
					-3.81	C	lmo0701	Unknown
	0.20	-1.90	-4.03	-2.03		A	lmo0701	Unknown
					-3.11	C	lmo0702	Unknown
0.63		-1.51	-3.02	-2.08		A	lmo0702	Unknown
					-4.59	C	lmo0703	Unknown
		-1.30	-3.79	-2.70		A	lmo0703	Unknown
					-3.60	C	lmo0704	Unknown
	0.17	-1.37	-3.08	-2.41		A	lmo0704	Unknown
					-2.01	C	lmo0705	Unknown, similar to flagellar hook-associated protein FlgK
	-0.31	-1.38	-1.45	-1.55		A	lmo0705	Unknown, similar to flagellar hook-associated protein FlgK
					-2.47	C	lmo0706	Unknown, similar to flagellar hook-associated protein 3 FlgL
	-0.44	-1.75	-2.79	-1.79		A	lmo0706	Unknown, similar to flagellar hook-associated protein 3 FlgL
					-3.36	C	lmo0707	Unknown, similar to flagellar hook-associated protein 2 FliD
	-0.79	-2.21	-3.43	-2.44		A	lmo0707	Unknown, similar to flagellar hook-associated protein 2 FliD
					-4.22	C	lmo0708	Unknown, similar to hypothetical flagellar protein

	-0.50	-1.99	-4.25	-2.91		A	lmo0708	Unknown, similar to hypothetical flagellar protein
					-4.24	C	lmo0709	Unknown
	-0.11	-1.77	-4.04	-3.01		A	lmo0709	Unknown
					-3.52	C	lmo0710	Unknown, similar to flagellar basal-body rod protein flgB
	-0.24	-1.98	-3.69	-2.49		A	lmo0710	Unknown, similar to flagellar basal-body rod protein flgB
					-2.81	C	lmo0711	Unknown, similar to flagellar basal-body rod protein flgC
0.61		-1.08	-2.72			A	lmo0711	Unknown, similar to flagellar basal-body rod protein flgC
					-3.06	C	lmo0712	Unknown, similar to flagellar hook-basal body complex protein FliE
		-1.40	-3.10	-2.27		A	lmo0712	Unknown, similar to flagellar hook-basal body complex protein FliE
					-4.28	C	lmo0713	Unknown, similar to flagellar basal-body M-ring protein fliF
	-0.20	-1.66	-2.88	-2.91		A	lmo0713	Unknown, similar to flagellar basal-body M-ring protein fliF
					-2.88	C	lmo0714	Unknown, similar to flagellar motor switch protein fliG
	-0.51	-1.85	-2.52			A	lmo0714	Unknown, similar to flagellar motor switch protein fliG
					-2.31	C	lmo0715	Unknown
	-0.34	-2.00	-2.91	-1.66		A	lmo0715	Unknown
					-2.43	C	lmo0716	Unknwon, similar to H+-transporting ATP synthase alpha chain FliI, flagellar-specific, -
	-0.38	-1.87	-2.07	-1.79		A	lmo0716	Unknwon, similar to H+-transporting ATP synthase alpha chain FliI, flagellar-specific, -
					-2.44	C	lmo0717	Unknown, similar to transglycosylase
-0.26	-0.72	-2.08	-2.04	-2.02		A	lmo0717	Unknown, similar to transglycosylase
					-1.80	C	lmo0718	Unknown
-0.35	-0.61	-1.70	-1.94	-1.50		A	lmo0718	Unknown
					-3.03	C	lmo0724	Unknown, similar to B. subtilis YvpB protein
	-0.40	-1.21	-2.46	-2.05		A	lmo0724	Unknown, similar to B. subtilis YvpB protein
					1.34	C	lmo0783	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIB
-0.51		1.13	0.97	-0.33		A	lmo0783	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIB
					-1.66	C	lmo0795	Unknown, conserved hypothetical protein
	-0.01	-1.03	-2.15			A	lmo0795	Unknown, conserved hypothetical protein
					1.39	C	lmo0797	Unknown
0.87	1.39		1.01			A	lmo0797	Unknown
					-1.08	C	lmo0805	unknown
-0.27	-0.71	-2.03	-3.89			A	lmo0805	unknown
					1.37	C	lmo0806	unknown, similar to transcription regulator
	0.98	1.34	0.83			A	lmo0806	unknown, similar to transcription regulator
					-4.43	C	lmo0834	unknown
-0.33	-1.12	-2.22	-3.53	-0.92		A	lmo0834	unknown
					-4.63	C	lmo0835	Unknown, putative peptidoglycan bound protein (LPXTG motif)

-0.32	-0.91	-2.00	-2.83	-1.06		A	lmo0835	Unknown, putative peptidoglycan bound protein (LPXTG motif)
					-1.47	C	lmo0868	unknown
-1.00	-1.30	-3.36	-3.11			A	lmo0868	unknown
					-1.13	C	lmo0904	Unknown
	-0.72	-2.19	-0.66	-0.87		A	lmo0904	Unknown
					-1.04	C	lmo0907	unknown, similar to phosphoglycerate mutase
-0.57	-1.75	-1.52	-1.49	-0.37		A	lmo0907	unknown, similar to phosphoglycerate mutase
					1.67	C	lmo0998	unknown, similar to hypothetical protein
0.88	2.81	2.91	2.38			A	lmo0998	unknown, similar to hypothetical protein
					-1.53	C	lmo1013	unknown, similar to conserved hypothetical proteins like to B. subtilis YkuT protein
-0.78	-1.88	-3.53	-4.13			A	lmo1013	unknown, similar to conserved hypothetical proteins like to B. subtilis YkuT protein
					-1.45	C	lmo1015	GbuB, highly similar to glycine betaine ABC transporters (permease)
	-0.23	-1.64	-2.83			A	lmo1015	GbuB, highly similar to glycine betaine ABC transporters (permease)
					-1.41	C	lmo1016	GbuC, highly similar to glycine betaine ABC transporters (glycine betaine-binding protein)
	-0.55	-2.63	-4.24			A	lmo1016	GbuC, highly similar to glycine betaine ABC transporters (glycine betaine-binding protein)
					-1.99	C	lmo1067	unknown, similar to GTP-binding elongation factor
-0.91	-1.64	-2.52	-3.00			A	lmo1067	unknown, similar to GTP-binding elongation factor
					-1.06	C	lmo1073	unknown, similar to metal binding protein (ABC transporter)
-0.51	-2.22		-5.05	-0.68		A	lmo1073	unknown, similar to metal binding protein (ABC transporter)
					-1.02	C	lmo1095	unknown, similar to PTS system, cellobiose-specific IIB component (cel A)
-0.73	-1.95	-3.46	-3.41			A	lmo1095	unknown, similar to PTS system, cellobiose-specific IIB component (cel A)
					-1.24	C	lmo1210	unknown, similar to unknown proteins
-1.30	-2.94	-3.41				A	lmo1210	unknown, similar to unknown proteins
					-2.34	C	lmo1216	unknown, similar to N-acetylmuramoyl-L-alanine amidase (autolysin)
-1.90	-3.13	-4.73	-5.01			A	lmo1216	unknown, similar to N-acetylmuramoyl-L-alanine amidase (autolysin)
					-1.11	C	lmo1248	unknown, weakly similar to 8-oxo-dGTPase (mutT)
-2.08	-2.84	-2.67	-3.27			A	lmo1248	unknown, weakly similar to 8-oxo-dGTPase (mutT)
					-1.42	C	lmo1298	
-1.32	-2.42	-4.58	-5.83	-0.99		A	lmo1298	
					-2.00	C	lmo1299	
-0.23	-0.06	-0.54	-1.80	-0.73		A	lmo1299	
					-1.26	C	lmo1353	Unknown, similar to B. subtilis YqhQ protein
-1.02	-1.83	-1.68	-1.24			A	lmo1353	Unknown, similar to B. subtilis YqhQ protein
					2.35	C	lmo1407	

1.05	2.51	2.83	2.55			A	lmo1407	
					-1.76	C	lmo1421	Unknown, similar to glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)
-1.21	-0.82	-0.30	-0.88			A	lmo1421	Unknown, similar to glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)
					-1.37	C	lmo1422	Unknown, similar to glycine betaine/carnitine/choline ABC transporter (membrane protein)
-1.57	-2.05	-2.02	-3.01			A	lmo1422	Unknown, similar to glycine betaine/carnitine/choline ABC transporter (membrane protein)
					-1.06	C	lmo1446	
-0.96	-1.58	-3.89	-4.32	-0.59		A	lmo1446	
					-1.26	C	lmo1516	Unknown, similar to ammonium transporter NrgA
-0.26	-0.67	-1.18	-1.97	-1.40		A	lmo1516	Unknown, similar to ammonium transporter NrgA
					-1.34	C	lmo1517	Unknown, similar to nitrogen regulatory PII protein
-0.37	-0.37	-1.13	-2.01	-1.74		A	lmo1517	Unknown, similar to nitrogen regulatory PII protein
					-1.01	C	lmo1525	Unknown, similar to single-stranded-DNA-specific exonuclease (RecJ)
-0.41	-0.63	-1.57	-2.16			A	lmo1525	Unknown, similar to single-stranded-DNA-specific exonuclease (RecJ)
					2.12	C	lmo1539	Unknown, similar to glycerol uptake facilitator
-0.28	1.33	2.14	1.36			A	lmo1539	Unknown, similar to glycerol uptake facilitator
					-2.63	C	lmo1699	unknown, some similarities to methyl-accepting chemotaxis proteins
	-0.36	-1.53	-2.69	-1.59		A	lmo1699	unknown, some similarities to methyl-accepting chemotaxis proteins
					-1.01	C	lmo1843	unknown, similar to conserved hypothetical proteins
-0.24	-0.41	-1.20	-2.78	-0.28		A	lmo1843	unknown, similar to conserved hypothetical proteins
					2.04	C	lmo1917	
	3.07	3.55	2.86			A	lmo1917	
					1.17	C	lmo1964	Unknown, similar to ABC transporter, ATP-binding protein
2.21	2.77	2.94	2.25			A	lmo1964	Unknown, similar to ABC transporter, ATP-binding protein
					-1.12	C	lmo2114	Unknown, similar to ABC transporter (ATP-binding protein)
-0.21	-0.21	-1.31	-1.21	0.84		A	lmo2114	Unknown, similar to ABC transporter (ATP-binding protein)
					-1.55	C	lmo2208	Unknown, similar to unknown protein
-0.84	-1.31	-0.07				A	lmo2208	Unknown, similar to unknown protein
					-1.09	C	lmo2223	Unknown, similar to unknown proteins
-0.87	-1.15	-1.28	-1.09			A	lmo2223	Unknown, similar to unknown proteins
					-1.25	C	lmo2277	Unknown
-2.88	-2.37	-2.65	-2.31			A	lmo2277	Unknown
					-1.43	C	lmo2288	Protein gp15 [Bacteriophage A118]

-0.26	-0.45	-1.04	-0.93			A	lmo2288	Protein gp15 [Bacteriophage A118]
					-1.80	C	lmo2343	Unknown, similar to nitrilotriacetate monooxygenase
0.94	0.94	-0.38	-2.02	-1.70		A	lmo2343	Unknown, similar to nitrilotriacetate monooxygenase
					-2.12	C	lmo2344	Unknown, similar to B. subtilis YtnI protein
0.90		-1.10	-3.24	-2.10		A	lmo2344	Unknown, similar to B. subtilis YtnI protein
					-1.39	C	lmo2345	Unknown, conserved hypothetical protein
0.61		-1.34	-2.93	-1.32		A	lmo2345	Unknown, conserved hypothetical protein
					-1.72	C	lmo2348	Unknown, similar to amino acid ABC-transporter (permease)
0.55	0.06	-2.06	-2.87	-1.58		A	lmo2348	Unknown, similar to amino acid ABC-transporter (permease)
					-2.92	C	lmo2349	Unknown, similar to amino acid ABC transporter (binding protein)
0.70			-3.44	-2.19		A	lmo2349	Unknown, similar to amino acid ABC transporter (binding protein)
					-2.51	C	lmo2350	Unknown, similar to B. subtilis YtmI protein
0.47	-0.60	-3.33		-1.58		A	lmo2350	Unknown, similar to B. subtilis YtmI protein
					-1.32	C	lmo2408	Unknown, similar to repressor protein
-0.84	-1.19	-1.28	-0.73	-0.54		A	lmo2408	Unknown, similar to repressor protein
					-1.30	C	lmo2433	Unknown, similar to acetylesterase
-0.26	-1.11	-1.81	-1.37	-1.29		A	lmo2433	Unknown, similar to acetylesterase
					-1.17	C	lmo2526	
-0.79	-1.20	-1.88	-1.95	-0.35		A	lmo2526	
					1.11	C	lmo2593	Unknown, similar to transcription regulators (MerR family)
1.32	2.16	2.83	3.77	0.91		A	lmo2593	Unknown, similar to transcription regulators (MerR family)
					1.01	C	lmo2667	Unknwon, similar to PTS system galactitol-specific enzyme IIA component
	1.24	1.90	1.24			A	lmo2667	Unknwon, similar to PTS system galactitol-specific enzyme IIA component
					-1.80	C	lmo2209	Unknown
-1.03	-0.85	0.60				A	lmo2209	Unknown
					1.73	C	lmo1868	unknown, similar to conserved hypothetical proteins
	1.07	1.18	0.81			A	lmo1868	unknown, similar to conserved hypothetical proteins
					-1.81	C	lmo0865	Unknown, similar to phosphomannomutase
-1.27	-1.74	-1.99	-2.28			A	lmo0865	Unknown, similar to phosphomannomutase
					-2.54	C	lmo0723	Unknown, similar to metyl-accepting chemotaxis protein
-0.60	-0.74	-0.64	-2.05	-1.43		A	lmo0723	Unknown, similar to metyl-accepting chemotaxis protein
					3.03	C	lmo0488	Unknown, similar to transcriptional regulator (LysR family)
0.65	0.70	1.06	1.60			A	lmo0488	Unknown, similar to transcriptional regulator (LysR family)
					-1.30	C	lmo0013	QoxA, AA3-600 quinol oxidase subunit II
-0.28	-0.61	-1.26	-1.55			A	lmo0013	QoxA, AA3-600 quinol oxidase subunit II

Tab. S-11: *L. monocytogenes* genes, which showed significant change in transcription levels in all DNA-microarray experiment.

*Identifier: **A**-acid shock at 25°C, **B**-acid shock at 37°C, **C**-temperature shift (from 25 to 37°C).

15min	30min	60min	120min	adapt.	temp. shift	Id*	LmoNr.	Function
					1.33	C	lmo0053	50S ribosomal protein L9
bad	1.76	2.60	2.50	bad		A	lmo0053	50S ribosomal protein L9
bad	2.79	1.50	0.54	bad		B	lmo0053	50S ribosomal protein L9
					-1.01	C	lmo0162	Unknwon, similar to *B. subtilis* DNA polymerase III (delta subunit)
-2.22	-3.37	-3.76	-3.45	bad		A	lmo0162	Unknwon, similar to B. *subtilis* DNA polymerase III (delta subunit)
-1.95	-1.57	-1.03	bad	bad		B	lmo0162	Unknwon, similar to *B. subtilis* DNA polymerase III (delta subunit)
					1.29	C	lmo0211	Ctc, similar to *B. subtilis* general stress protein
1.44	2.39	2.82	3.18	0.99		A	lmo0211	Ctc, similar to *B. subtilis* general stress protein
0.81	1.81	1.69	1.89	0.71		B	lmo0211	Ctc, similar to *B. subtilis* general stress protein
					1.69	C	lmo0223	CysK, highly similar to cysteine synthase
1.20	2.63	2.73	2.04	bad		A	lmo0223	CysK, highly similar to cysteine synthase
1.32	1.95	1.35	1.52	1.03		B	lmo0223	CysK, highly similar to cysteine synthase
					1.40	C	lmo0782	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIC
-1.60	bad	1.87	1.31	bad		A	lmo0782	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIC
1.52	2.14	1.21	bad	2.65		B	lmo0782	Unknown, similar to mannose-specific phosphotransferase system (PTS) component IIC
					1.27	C	lmo1138	Unknown, similar to ATP-dependent Clp protease proteolytic component
bad	0.87	1.75	2.16	bad		A	lmo1138	Unknown, similar to ATP-dependent Clp protease proteolytic component
0.97	2.18	1.48	0.49	bad		B	lmo1138	Unknown, similar to ATP-dependent Clp protease proteolytic component
					2.04	C	lmo1293	
bad	2.29	2.95	2.56	bad		A	lmo1293	
2.51	2.14	1.72	2.41	2.81		B	lmo1293	
					-1.20	C	lmo1355	
-0.55	-0.77	-1.43	-2.42	bad		A	lmo1355	
-0.92	-1.26	-1.14	bad	bad		B	lmo1355	
					1.22	C	lmo1381	Unknown
0.79	1.42	1.95	2.80	bad		A	lmo1381	Unknown
bad	1.55	1.30	1.71	0.90		B	lmo1381	Unknown
					-1.02	C	lmo1545	
-1.74	-2.82	-2.89	-2.40	bad		A	lmo1545	
bad	-1.66	-1.41	bad	0.59		B	lmo1545	
					1.12	C	lmo1630	
1.10	1.52	1.66	1.05	bad		A	lmo1630	

2.51	0.86	0.75	bad	0.74		B	lmo1630	
					1.04	C	lmo1962	Unknown, similar to transcription regulators (TetR family)
bad	1.53	2.16	1.52	bad		A	lmo1962	Unknown, similar to transcription regulators (TetR family)
bad	1.16	0.75	1.33	bad		B	lmo1962	Unknown, similar to transcription regulators (TetR family)
					1.07	C	lmo2006	
1.61	3.11	3.80	3.19	1.30		A	lmo2006	
2.62	2.59	2.57	2.80	0.80		B	lmo2006	
					1.63	C	lmo2206	
1.51	2.54	2.53	2.03	bad		A	lmo2206	
2.84	2.78	1.79	0.54	bad		B	lmo2206	
					-1.58	C	lmo2335	
bad	0.13	-0.38	-2.00	-0.47		A	lmo2335	
0.56	-0.94	-1.64	-2.14	-1.39		B	lmo2335	
					-4.05	C	lmo2337	Unknown, similar to regulatory protein DeoR family
bad	-0.34	-1.89	-4.90	-0.51		A	lmo2337	Unknown, similar to regulatory protein DeoR family
1.77	-1.95	-1.64	bad	-1.87		B	lmo2337	Unknown, similar to regulatory protein DeoR family
					1.06	C	lmo2432	Unknown
2.47	3.40	3.70	4.64	bad		A	lmo2432	Unknown
2.87	4.57	4.52	4.22	0.87		B	lmo2432	Unknown
					1.04	C	lmo2696	Unknown, similar to hypothetical dihydroxyacetone kinase
bad	2.40	3.75	4.22	bad		A	lmo2696	Unknown, similar to hypothetical dihydroxyacetone kinase
0.84	2.46	1.67	1.18	0.80		B	lmo2696	Unknown, similar to hypothetical dihydroxyacetone kinase
					1.21	C	lmo2741	Unknown, similar to drug-efflux transporters
0.82	1.44	2.18	2.32	bad		A	lmo2741	Unknown, similar to drug-efflux transporters
bad	1.40	1.05	bad	0.77		B	lmo2741	Unknown, similar to drug-efflux transporters
					1.27	C	lmo2792	Unknown
1.48	2.60	3.02	3.35	bad		A	lmo2792	Unknown
bad	1.77	1.98	1.79	bad		B	lmo2792	Unknown