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Original Articles

Phenotypic, Proteomic, and Genomic Characterization of a Putative ABC-Transporter Permease Involved in *Listeria monocytogenes* Biofilm Formation

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Abstract

The foodborne pathogen *Listeria monocytogenes* is able to form biofilms in food processing environments. Previously, we have reported that an lm.G_1771 gene (encoding a putative ABC-transporter permease) was involved in negative regulation of *L. monocytogenes* biofilm formation using LM-49, a biofilm-enhanced mutant isolated on Tn*917* mutagenesis (AEM 2008 p.7675–7683). Here, the possible action of this ABC-transporter permease in *L. monocytogenes* biofilm formation was characterized by phenotypic, proteomic, and genomic analyses using an lm.G_1771 gene deletant (Δ1771). The Δ1771 mutant exhibited the same enhanced ability for biofilm formation as the LM-49 strain using a crystal violet staining assay. DNA microarrays and two-dimensional gel electrophoresis revealed 49 and 11 differentially expressed (twofold or more) genes or proteins in Δ1771, respectively. The transcriptomics study indicated that lm.G_1771 could play a vital role in regulating candidate genes involved in biofilm formation such as genes encoding cell surface proteins (Dlt), cell surface anchor proteins (SrtA), and transcriptional regulators (GntR) contributing to negative reglution of biofilm formation by *L. monocytogenes.* The mutant Δ1771 was more sensitive to Triton X-100 and less resistant to cationic antibiotics, which might be explained by the down-regulation of *dlt* operon in this deletant and the fact that *dlt* involves the incorporation of D-alanine residues into lipoteichoic acids, resulting in a positive net charge on the teichoic acids. Therefore, lm.G_1771 is considered to be involved in negative regulation of biofilm formation, and the results from this work provide a possible molecular mechanism of biofilm formation regulated by lm.G_1771 in *L. monocytogenes.*

Introduction

The foodborne pathogen *Listeria monocytogenes* is able to attach to food surfaces, food-processing equipment, and other surfaces, including floor drains, storage tanks, hand trucks, and conveyer belts, and form biofilms (Zottola and Sasahara, 1994). *L. monocytogenes* biofilms are difficult to eliminate from contaminated environments. There is no doubt that *L. monocytogenes* biofilms increase the challenges to the production of safe food in food industries. Therefore, the understanding of the molecular basis of biofilm formation will contribute to the development of new strategies for cleaning and eliminating *L. monocytogenes.*

Bacterial biofilm formation is a complex process involving a number of biosynthetic pathways, and the process is subject to regulation by different signal transduction pathways. In *L. monocytogenes*, several genes or gene products are implicated in biofilm formation. First, motility proteins such as flagellins are important for bacterial adhesion onto a surface, the initial step in biofilm formation. It has been reported that flagellated *L. monocytogenes* cells readily colonized on glass or stainless steel, whereas adhesion by a nonflagellated mutant was markedly reduced (Vatanyoopaisarn *et al.*, 2000). Second, two bacterial signal transduction pathways were shown to regulate *L. monocytogenes* biofilm formation (Ren *et al.*, 2004). One involves the autoinducer 2 (AI-2) universal quorum sensing

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signalling molecule that is synthesized and transported by well-conserved pathways in bacteria. A key enzyme in this pathway is the AI-2 synthase, which is encoded by the *luxS* gene identified in the *L. monocytogenes* EGD-e genome (Ren *et al.*, 2004). A *luxS* mutant exhibited a biofilm-positive phenotype in *L. moncytogenes* (Sela *et al.*, 2006), which has also been found in several other bacterial species (Schauder and Bassler, 2001). The other signal transduction pathway is the accessory gene regulator (*agr*) system, which controls the expression of several virulence factors in *Staphylococcus aureus* (Shirtliff *et al.*, 2002). This system also regulates biofilm formation in *L. monocytogenes*, which was demonstrated with mutants with either *agrA* or *agrD* deleted from the *agrBDCA* operon showing markedly reduced adhesion to an abiotic surface (Rieu *et al.*, 2007). Third, a recent study by Harmsen *et al.* (2010) indicated that extracellular DNA was an important component for *L. monocytogenes* initial attachment and early biofilm formation through interactions with peptidoglycan (specifically N-acetyl glucosamine).

Recently, we characterized a Tn*917* insertion mutant LM-49 and identified that the mutant gene lm.G_1771 (encoding an ABC-transporter permease) was involved in negative regulation of biofilm formation by *L. mococytogenes* (Zhu *et al.*, 2008). The purpose of this study is to further determine the role of the lm.G_1771 gene in biofilm formation using an lm.G_1771 deletion mutant Δ1771 and its parent *L. monocytogenes* 4b G by genomic, proteomic, and phenotypic analyses. This research may provide insight into the possible mechanisms of biofilm formation by *L. monocytogenes*.

Materials and Methods

Bacterial strains, media, plasmids, and growth conditions

The *L. monocytogenes* 4b G strain was obtained from the Center for Disease Control of Hubei Province, China. The biofilm-positive mutant LM-49 was generated by Tn*917* mutagenesis (Chen and Shi, 2005), and the lm.G_1771 deletion mutant (Δ1771) was constructed according to the procedure described by Rieu *et al.* (2007). *L. monocytogenes* strains were grown in brain heart infusion broth (BD Company) or trypticase soy broth (Merck) at 37°C without shaking.

Preparation of protein samples and proteomic analyses

Total protein preparation for *L. monocytogenes* and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) were performed as described by Tremoulet *et al.* (2002). Protein samples were prepared from three independent cultures for each strain, and each sample was analyzed twice.

The scanned images for stained 2D gels were analyzed with the ImageMaster 2D Platinum software (release 5.0; Amersham Biosciences). Only the spots that exhibited significant differences between the mutant Δ1771 and the wild-type strain in at least *n*-1 gels (*n* representing the number of gels run for each condition) were selected for protein identification, and the significance was analyzed using the Student's *t*-test analysis (95% confidence interval).

Differentially expressed proteins were identified using a matrix-assisted lazer desorption ionization tandem time of flight (MALDI TOF/TOF) instrument (4800 plus analyzer; Applied Biosystems) as previously described (Lametsch *et al.*, 2002). Total spectra were generated and used to search the Swiss-Prot database for bacterial species by using MASCOT (Matrix Science), which revealed the identities of the differentially expressed proteins.

RNA isolation and sample preparation for NimbleGen GeneChip analysis

Three independent overnight cultures of *L. monocytogenes* 4b G strain and Δ1771 were grown in brain heart infusion medium and were harvested for RNA isolation. The preparation of total RNA was performed as previously described (Zhu *et al.*, 2008). Double-stranded cDNA synthesis, labeling, hybridization, and washing were carried out according to the NimbleGen Array gene expression analysis protocol (Roche, Mannheim, Germany). The cDNA labeled either with Cy3 or Cy5 was hybridized to a NimbleGen 4×72K 45-60-mer microarray slide (www.Nimblegen.Com/products/lit/index.html) covering 2821 genes of *L. monocytogenes* strain 4b F2365 (NC_002973). The statistical analyses were the average of six samples, and a cut off of twofold change in expression level was used, with a statistical significance of $p < 0.05$ and Student's *t*-test as determined by the significance analysis of microarray software (www-stat.stanford.edu/~tibs/SAM), with data expressed as Δ1771 expression relative to the wild-type strain (+= upregulated in Δ1771 or −= downregulated in Δ1771).

Real-time quantitative polymerase chain reacton

The preparation of total RNA was the same as for microarray analysis. One hundred ng of total RNA were added to a 25-μL polymerase chain reacton (PCR) reaction mixture employed for quantitative PCR according to the instructions provided with the One Step SYBR PrimeScript RT-PCR Kit (TaKaRa). Bio-Rad iCycler (Bio-Rad Laboratories) was used to detect fluorescence with the following protocol for the PCR: one cycle at 42°C for 5 min (reverse-transcript step) and 40 cycles at 95°C for 5 sec and 60°C for 31 sec. The PCR primers used for quantitative PCR are listed in Table 1. 16S rRNA gene fragments were amplified as an internal control, and three biological replicates were performed. Relative gene expression was calculated using the comparative critical threshold cycle ($\Delta\Delta C_T$) method with PCR efficiency (Livak and Schmittgen, 2001).

Results

Biofilm formation by the lm.G_1771 deletion mutant

To eliminate any possibility that the transposon vector (on LM-49) might contribute to the observed results and to generate a more suitable mutant for further study, an lm.G_1771 in-frame deletion mutant Δ1771was screened. The presence of lm.G_1771 mRNA was determined by the reverse transcription–PCR. The result showed that the lm.G_1771 mRNA was absent in Δ1771 but present in the wild-type strain (data not shown). Physiological characterization of this mutant indicated that it maintained the same cell and colony morphologies as well as growth rate as the wild-type strain (data not shown). However, observations on Δ1771 biofilm formation using the microplate crystal violet staining assay revealed that the deletion mutant exhibited an enhanced level of biofilm formation relative to the wild-type strain, which was consistent with the result obtained for the

Table 1. Real-Time Quantitative Polymerase Chain Reaction Oligonucleotide Primers Used in This Study

Gene no. or Gene	*Forward primer(5′-3′)*	*Reverse primer(5′-3′)*	*DNA product size (bp)*
LMOf2365_0994(*dltA*)	GCACCAATCAGGACAATTTCTC	TACAACACATACGGACCTACAG	191
LMOf2365_0993(*dltB*)	CCAACCATTGTAACATTGTCTCC	TCAGCAAGTCCAGTCCAAGG	200
LMOf2365_0992(*dltC*)	CGAGTTGTGTAATAATCATTTCAG	AGGCTTGCTTGATTCTATGG	131
LMOf2365_0991(*dltD*)	AGGCTTGCTTGATTCTATGG	GCCTTTACTGGTCGCATTCG	139
LMOf2365_0950(*srtA*)	GCTACCCTCTATGACATCTG	GCGGATAGTTACCCTTACC	188
LMOf2365_1458(*sod*)	GTGCTATGAAACTGGTGGAAC	GCCCTTGAAGTGCTAATTGTC	185
LMOf2365_1602(*usp*)	GATACAAGCATGGCAGACAAAG	TCTACAGCGTTAAGTCCAGTTG	203
LMOf2365_2147	TTCGGCATTACAGAAATCCTTG	AACGCATCATGTGTTACCATC	224
LMOf2365_2148	AAGATGCGTGCTGTTATTCC	GTAGATGATGCTTCCTGTTGC	147
LMOf2365_2274(*gntR*)	AATCAACCTTCAACCATGTCAC	CCTTCATAGCGAACCAACAAG	181
16S rRNA	TGCGTAGATATGTGGAGGAAC	AGGCGGAGTGCTTAATGC	189

Primers designed using Beacon Designer 7.0 software.

transposon mutant LM-49 (Fig. 1). This reinforced the conclusion that lm.G_1771 was involved in negative regulation of biofilm formation in *L. monocytogenens*, and the presence of Tn*917* did not influence regulation.

Identification of proteins differentially expressed in Δ1771 using 2D-PAGE and MALDI-TOF/TOF mass spectrometry

To gain insight into the mechanisms by which lm.G_1771 regulates biofilm formation, we examined differential protein expression in the deletion mutant and the wild-type strain. Approximately, 800 protein spots were obtained by 2D-PAGE from each protein gel using the ImageMaster 2D Platinum software (Fig. 2). Comparing the protein pattern of the Δ1771 mutant with that generated from the wild-type strain resulted in the identification of 15 differentially expressed proteins in the mutant, including 11 up-regulated proteins and 4 down-regulated ones (two- to eightfolds). Eleven of the protein spots were identified by MALDI TOF/TOF mass spectrometry performed after an in-gel trypsin digestion (Table 2). Unexpectedly, the fourth down-regulated protein spot in Δ1771 (i.e., the Lm.G_1771 permease) was not found in the wild-type by 2D-PAGE. One possible explanation was that the predicted isoelectric point (p*I*) of this Lm.G_1771 protein, which was 9.09, was beyond the pH range (pH 4–7) of the immobilized pH gradient (IPG) strips used.

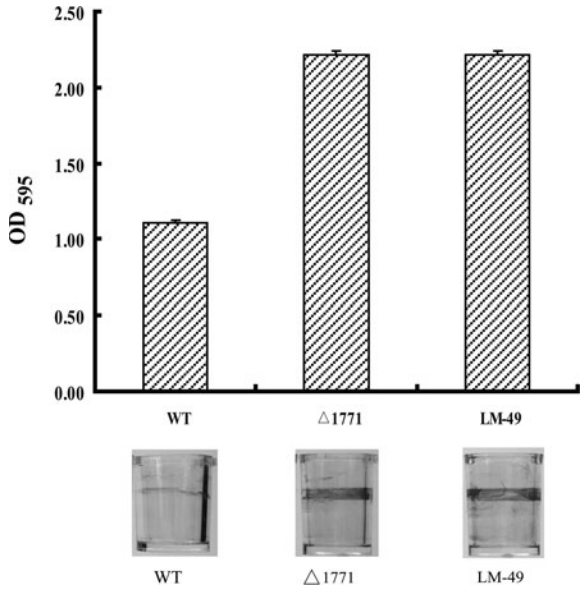

FIG. 1. Biofilm quantification using crystal violet staining. Pictures of biofilms formed in microplates were shown below each column. WT: wild-type strain; Δ1771: lm.G_1771 deletion mutant; LM-49: Tn*917* insertion mutant.

Functional grouping of differentially expressed genes in Δ1771

A high-density NimbleGene microarray constructed for *L. monocytogenes* strain 4b F2365 (containing probes for 2821 genes) was employed to characterize global differential gene expression in the deletion mutant. In this analysis, 49 genes (including lm.G_1771) showed differential expression at the mRNA level with the threshold of a minimum twofold difference ($p < 0.05$). These genes could be classified into 8 functional categories (Table 3). It was found that the number of genes identified as differentially regulated using microarrays was larger than that of proteins identified in the proteomic study. This result is not unexpected, as the pH range of the IPG strips used was pH 4–7 and the p*I* of many proteins was out of this range.

Evaluation of differentially expressed genes by real-time quantitative PCR

Ten genes (*dltA* to *dltD*, *srtA*, *sod*, *usp*, *gntR*, LMOf2365_2147 and 2148) that were differentially regulated were confirmed by real-time quantitative PCR with normalizing to 16S rRNA gene (Fig. 3). Relative quantitative values were obtained using the comparative threshold cycle method ($\Delta\Delta C_T$). The relative expression of the genes was determined in quadruplicate from total RNA extracted from three independent cultures. The resulting ratios were $\log_2$ values transformed and plotted against the $\log_2$ values transformed from microarray hybridization analysis. It was found that relative changes in gene expression obtained from microarray and real-time quantitative PCR experiments correlated in a linear relationship for each gene with $r > 0.9$, which was considered the threshold for strong correlation.

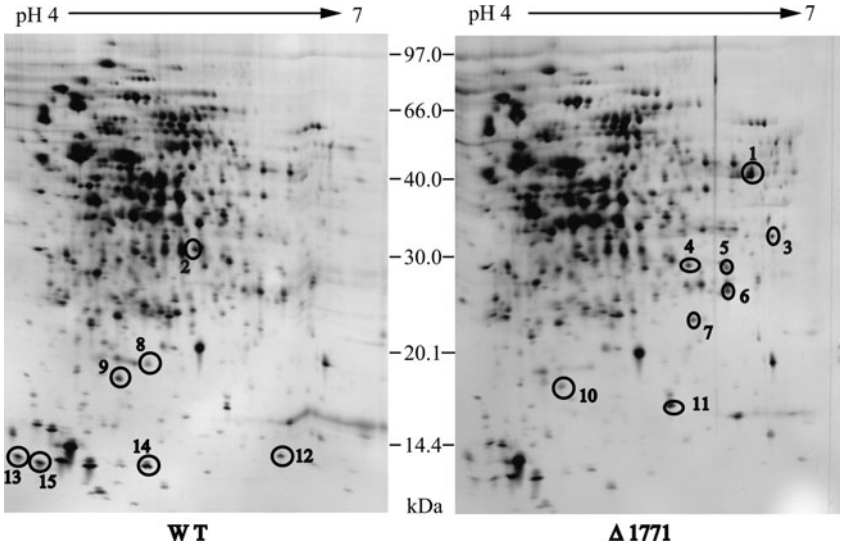

FIG. 2. Images of the silver-stained two-dimensional protein gels of *Listeria monocytogenes*. Circles and numbers indicate spots increased more than twofold between the wild-type *L. monocytogenes* and the Δ1771 mutant. The approximate positions of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis molecular mass (MW) standards are presented between the gel images. The proteins that were identified are described in Table 3.

Discussion

An lm.G_1771 deletion mutant (Δ1771) that showed the same increased ability to form biofilms as LM-49 was constructed for further study. Several genes or proteins were found to be possibly influenced by lm.G_1771 by proteomic and transcriptomic analyses in this work. Among the identified genes, several encoded for cell surface proteins. Therefore, lm.G_1771 could participate in a novel signal transduction pathway that regulates the expression of the identified cell wall surface proteins. Some valuable information regarding this potentially novel signal transduction pathway may have been lost in this study, as many proteins had *pIs* beyond the tested range. However, the data from microarray analyses suggested that the lm.G_1771 could participate in a novel signal transduction pathway (Lm.G_1771 does not export AI-2, data not shown).

Three *Listeria* cell surface proteins (Lsps, LMOf2365_0347, 0543 and 1974) exhibited more than twofold greater expression in the mutant, and they likely function as drivers of

Table 2. Differentially Expressed Proteins of the Mutant Δ1771 Identified by MALDI TOF/TOF Mass Spectrometry

Spots[a]	*Protein name*	*Accession no.*[b]	*Score*[c]	*S. C (%)*[d]	*Theor. Mr (kDa)/pI*[e]	*Esti. Mr (kDa)/pI*[f]	*Fuctional Class*[g]	*Expression In Δ1771*[h]
1	Pyruvate dehydrogenase (pdhA)	YP_013673	145	28	41.2/5.65	40.7/5.72	2.1.2	+
3	Glycosyl transferase	YP_015329	145	48	36.4/6.04	33.6/6.10	1.6	+
4	3-oxoacyl-(acyl-carrier-protein) reductase	YP_014428	444	60	26.3/5.61	27.1/5.73	2.4	+
5	Sortase family protein, SrtA	YP_013552	122	39	24.7/6.00	25.8/5.62	1.8	+
6	Uracil phosphoribosyltransferase	YP_015099	315	52	23.0/5.70	24.3/5.62	2.3	+
7	Superoxide dismutase, Mn (SOD)	YP_014056	631	77	22.6/5.23	22.5/5.64	4.2	+
10	Universal stress protein	YP_014200	550	57	17.0/4.98	17.2/5.23	4.1	+
11	Preprotein translocase, YajC sbunit	YP_014146	279	39	12.0/5.77	12.4/5.74	1.1	+
9	50S ribosomal protein L10	YP_012872	475	68	17.8/5.37	17.9/5.12	3.7.1	−
14	30S ribosomal protein S6	YP_012664	156	56	11.5/5.08	11.6/5.10	3.7.1	−
15	Aspartyl/glutamyl-tRNA, amidotransferase subunit C	YP_014375	98	36	10.6/4.63	10.9/4.39	3.7.2	−

[a]Spot number according to the position on the two-dimensional polyacrylamide gel electrophoresis (Fig. 3).
[b]Protein entries in National Center for Biotechnology Information.
[c]Protein Score based on MOWSE Score (Mascot Z score).
[d]S.C: sequence coverage, percentage of amino acids sequence covered by matching peptides.
[e]Theoretical molecular mass (M*r*) and p*I* of matched sequence. Theor.: theoretical; p*I*, isoelectric point.
[f]Estimated molecular mass (M*r*) and p*I* of protein spot from the gel by ImageMaster software. Esti.: estimated.
[g]Functional class codes according to Listilist *Listeria* genome database (http://genolist.pasteur.fr/Listilist/): 1.1 cell wall; 1.6 protein secretion; 1.8 cell surface proteins; 2.1.2 main glycolytic pathways; 2.2 metabolism of amino acids and related molecules; 2.3 metabolism of nucleotides and nucleic acids; 2.4 metabolism of lipids; 3.7.1 protein synthesis-ribosomal proteins; 3.7.2 aminoacyl-tRNA-synthetases; 4.1 adaptation to atypical conditions; 4.2 detoxification.
[h]Expression in Δ1771 cells: +, protein spot significantly ($p < 0.05$) increased in Δ1771; −, protein spot significantly ($p < 0.05$) reduced in Δ1771.

Table 3. List of Up- and Down-Regulated Genes in the *Listeria monocytogenes* Δ1771 Mutant Relative to Wild-Type

Group and locus ID	*Fold*	p-*Value*	*Gene product*
Cell envelopes			
LMOf2365_0991	−5.50	0.0049	DltD protein
LMOf2365_0992	−3.88	0.0195	D-alanine-poly(phosphoribitol) ligase subunit 2, DltC
LMOf2365_0993	−8.23	0.0135	DltB protein
LMOf2365_0994	−4.27	0.0035	D-alanine-D-alanyl carrier protein ligase, DltA
LMOf2365_0347	2.36	0.0289	Cell wall surface anchor family protein
LMOf2365_0543	2.31	0.0141	Cell wall surface anchor family protein
LMOf2365_1974	2.38	0.0057	Cell wall surface anchor family protein
LMOf2365_0950[a]	2.98	0.0133	Sortase family protein, SrtA
LMOf2365_1246	2.02	0.0326	Glutamate racemase
LMOf2365_2741[a]	2.69	0.0216	Glycosyl transferase, group 2 family protein
Stress response			
LMOf2365_1458[a]	2.29	0.0011	Superoxide dismutase, Mn Sod
LMOf2365_1602[a]	2.23	0.0023	Universal stress protein family
Protein fate			
LMOf2365_1548[a]	2.52	0.0201	Preprotein translocase, YajC subunit
LMOf2365_1289	2.39	0.0157	Signal peptidase I
LMOf2365_2810	2.07	0.0351	Peptidase, M20/M25/M40 family
Transporter and binding proteins			
LMOf2365_2147	23.34	0.0038	ABC transporter, ATP-binding protein
LMOf2365_2148	35.93	0.022	ABC transporter, permease protein
LMOf2365_0553	2.04	0.0496	Sulfate transporter family protein
LMOf2365_2158	2.88	0.0339	Maltose/maltodextrin ABC transporter, permease protein
LMOf2365_2809	2.17	0.024	Major facilitator family transporter
Regulation			
LMOf2365_0641	2.53	0.0192	Transcriptional regulator, MarR family
LMOf2365_1234	2.23	0.0482	Transcriptional regulator, MarR family
LMOf2365_2274	2.41	0.0145	Transcriptional regulator, GntR family
Ribosome components			
LMOf2365_0053[a]	−3.07	0.0453	30S ribosomal protein S6
LMOf2365_0262[a]	−2.78	0.0306	50S ribosomal protein L10
LMOf2365_2078	−2.32	0.0341	50S ribosomal protein L32
LMOf2365_2591	−2.36	0.0053	30S ribosomal protein S8
Synthesis and metabolism			
LMOf2365_0839	2.42	0.0162	Acetyltransferase, GNAT family
LMOf2365_2005	2.31	0.0006	Acetyltransferase, GNAT family
LMOf2365_2242	2.78	0.0225	Acetyltransferase, GNAT family
LMOf2365_1896	2.5	0.0043	Pyruvate phosphate dikinase
LMOf2365_1073[a]	2.11	0.0479	Pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase alpha subunit
ORFs with unknown function			
LMOf2365_0710	−2.87	0.0139	Hypothetical protein
LMOf2365_0723	−4.77	0.0162	Hypothetical protein
LMOf2365_0730	−31.07	0.031	Hypothetical protein
LMOf2365_0740	−10.43	0.0028	Hypothetical protein
LMOf2365_0760	−5.71	0.0094	Hypothetical protein
LMOf2365_0995	−4.83	0.0224	Hypothetical protein
LMOf2365_2209	−7.33	0.0145	Hypothetical protein
LMOf2365_2290	−3.30	0.0466	Hypothetical protein
LMOf2365_0618	2.06	0.0136	Hypothetical protein
LMOf2365_0926	2.2	0.0064	Hypothetical protein
LMOf2365_0927	2.1	0.0344	Hypothetical protein
LMOf2365_1219	2.83	0.0372	Hypothetical protein
LMOf2365_1448	2.14	0.0011	Hypothetical protein
LMOf2365_1747	2.03	0.0339	Hypothetical protein
LMOf2365_1895	2.32	0.035	Hypothetical protein
LMOf2365_1975	3.57	0.0408	Hypothetical protein

[a]Indicates the correlation between genes identified by microarray and the corresponding protein according to the proteomic analysis of the Δ1771 mutant.

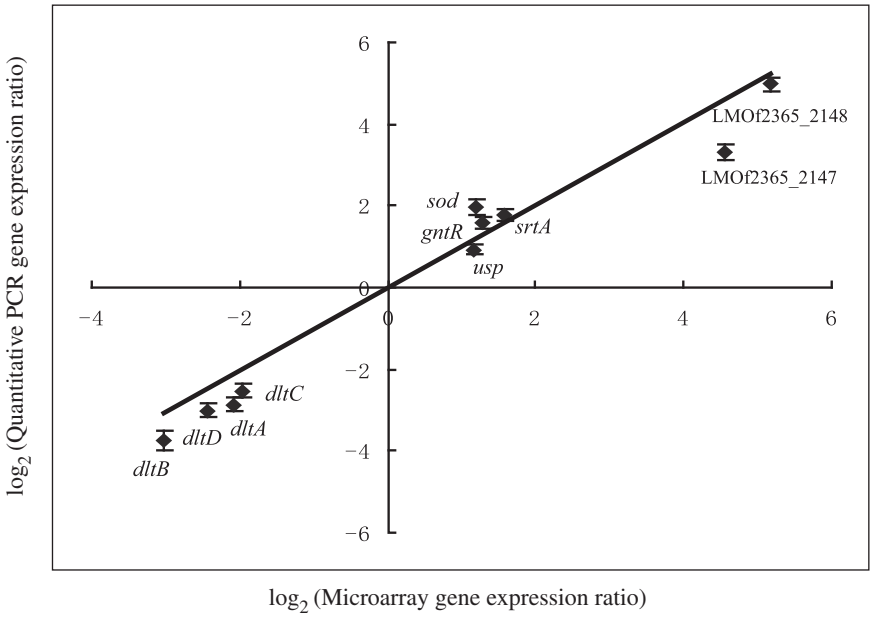

FIG. 3. Comparison between differential responses in gene expression in *L. monocytogenes* measured by real-time quantitative PCR and microarray data from RNAs obtained from the wild-type and Δ1771 strain. PCR, polymerase chain reaction.

biofilm formation in *L. monocytogenes* (Table 3). The characterization of some cell surface proteins in other bacteria has already demonstrated that they play important roles in biofilm formation. For example, the *Enterococcus faecalis* Esp cell surface protein was experimentally shown to be an important mediator of bacterial biofilm formation on a polystyrene surface (Toledo-Arana *et al.*, 2001). An up-regulated Lsp LMOf2365_1974 protein showed 39% amino acid sequence similarity to Esp cell surface protein, and its role in biofilm formation by *L. monocytogenes* is still unknown. Further, these three Lsps harbor a C-terminal sorting signal with an LPXTG motif, covalently linked to the cell wall peptidoglycan by a transamidase named sortase (Mazmanian *et al.*, 1999). Three other cell wall modifying enzymes (LMOf2365_0950, 1246 and 2741) were also expressed at a higher level in the mutant (Table 3). LMOf2365_0950 protein is a sortase A (SrtA) homolog in *L. monocytogenes*, as this protein shows 54% amino acid sequence similarity with SrtA from *Streptococcus mutans* (Levesque *et al.*, 2005), which suggests that this SrtA homolog could have a similar role in biofilm formation by translocation of the three Lsps with a LPXTG motif to cell surfaces. It would be very interesting to study whether these cell surface proteins function individually at different steps or they work in concert to mediate biofilm formation.

From the microarray data, we also observed that the expression of the *dlt* operon is down regulated in the Δ1771 mutant. Genes in the *dltA-dltD* operon are known to be involved the incorporation of positively charged D-alanine residues into lipoteichoic acids (LTAs). Reducing alanyl LTAs should increase the negative surface charges on bacterial cells (Heptinstall *et al.*, 1970) and the ester content determining the number of anionic sites on LTAs for autolysin binding (Wecke *et al.*, 1997). Therefore, this may explain why the Δ1771 mutant is more sensitive to cationic antibiotics and more easily lysed in Triton X-100 compared with the wild-type strain (data not shown). Alanylation of LTAs also affects biofilm formation in other bacteria. In *S. aureus*, inactivating a Dlt enzyme by gene knockout abrogates bacterial adhesion to plastic (Gross *et al.*, 2001), thereby negatively regulating biofilm formation. By contrast, a *Lactobacillus rhamnosus dltD* mutant exhibits a biofilm positive phenotype (Lebeer *et al.*, 2007). In the current study, reduced expression was observed for all *dlt* genes in the mutant by microarray data and was further confirmed by real-time quantitative PCR (Fig. 3), which suggests a reverse relation between *dlt* gene expression and biofilm formation in *L. monocytogenes*.

Genes encoding the putative transcription factors that were down-regulated in the lm.G_1771 deletion mutant were identified. One is the GntR-like transcription factor that has been shown to regulate biofilm formation in *E. faecalis* (Ballering *et al.*, 2009), and the other is MarR-like transcription factor. Therefore, functional analyses of the identified transcriptional factors and the cell wall surface proteins will yield important insights into the yet unknown signal transduction pathway involving lm.G_1771 and biofilm formation in *L. monocytogenes*. Further studies are needed to identify signaling molecules and signal transduction pathways that regulate biofilm formation in *L. monocytogenes*.

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Disclosure Statement

No competing financial interests exist.

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