Genes that are Affected in High Hydrostatic Pressure Treatments in a Listeria Monocytogenes Scott A ctsR Deletion Mutant

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Abstract

Listeria monocytogenes is a food-borne pathogen of significant threat to public health. High Hydrostatic Pressure (HHP) treatment can be used to control L. monocytogenes in food. The CtsR (class three stress gene repressor) protein negatively regulates the expression of class III heat shock genes. In a previous study, a spontaneous ctsR L. monocytogenes deletion mutant 2-1 that was able to survive under HHP treatment was identified; however, there is only limited information about the mechanisms of survival and adaptation of this mutant in response to high pressure. Microarray technology was used to monitor the gene expression profiles of ctsR mutant 2-1 under pressure treatments (450 Mpa, 3min). Some of the gene expression changes determined by microarray assays were confirmed by real-time RT-PCR analyses. Compared to non-pressure-treated ctsR mutant 2-1, 14 genes were induced (> 2-fold increase) in the ctsR deletion mutant whereas 219 genes were inhibited (< -2-fold decrease) by pressure treatments. The induced genes included genes encoding proteins involved in synthesis of purines, pyrimidines, nucleosides, and nucleotides, transport and binding, transcription, cell membrane, DNA and energy metabolism, protein synthesis, and unknown functions. The inhibited genes included genes encoding proteins for transport and binding, cell envelope, transcription, amino acid biosynthesis, regulatory functions, cellular processes and central intermediary metabolism. The information concerning L. monocytogenes survival under HHP at the molecular level may contribute to improved HHP treatments for food processing.

Keywords: Listeria monocytogenes; Scott A; microarray and real-time PCR; High hydrostatic pressure (HHP); ctsR mutant

Introduction

L. monocytogenes is a Gram-positive bacterium that can cause listeriosis in animal and human populations. Listeriosis is a foodborne disease with a high mortality rate (approximately 20 to 30% of cases) and occurs mostly in susceptible individuals such as pregnant women, newborns, the elderly, and immune-compromised patients. Outbreaks of listeriosis have been associated with the consumption of contaminated food products including ready-to-eat (RTE) meats and dairy products [1-2]. Because L. monocytogenes is widely distributed in the environment and survive under very harsh conditions, it is very difficult to eliminate this pathogen from foods and/or food processing plants.

High Hydrostatic processing (HHP) is a process that can inactivate microorganisms without significant deterioration of food quality. Foods treated with HPP generally have better sensory and nutritional qualities than products processed in more traditional ways. HHP has been used as a non-thermal preservation technique for processing of meats and dairy products to control L. monocytogenes and extend product shelf-life. In the food industry, pressures within the range of 300 to 600 MPa are used to inactivate vegetative cells of microorganisms, including pathogens such as L. monocytogenes. However, the efficiency of HHP depends on the pressure, time, and composition of the food [3]. For example, the inactivation of L. monocytogenes by HHP (600 MPa, 5min) ranged from 1.82 to 3.85 Log units, depending on the type of dry-cured ham [4].

The pressure tolerance of L. monocytogenes is also growth-stage dependent. Stationary-phase cells are often more resistant to pressure than the exponential-phase cells [5]. High pressure resulted in changes in viability, morphology, and physiology in bacteria such as E. coli and L. monocytogenes [6-9]. However, the molecular survival mechanisms of L. monocytogenes under high pressure remain unknown.

Microarrays have been used to study differential gene expression of L. monocytogenes and E. coli during HPP and some important genes have been identified [10-13].

The ctsR gene encodes a transcriptional regulator that represses the class III heat shock genes. CtsR has been shown to be related to high pressure since several pressure-tolerant mutants contained mutations in this gene [14-20]. L. monocytogenes Scott A ctsR mutant 2-1 exhibiting a higher level of viability under HPP and was less virulent, non-motile, heat and acid resistant, and sensitive to nisin [15]. Compared to the wild-type L. monocytogenes, genes that were differentially expressed in ctsR mutant 2-1 under high pressure treatment were identified [12]. However, why the ctsR mutant 2-1 survives better under HHP treatments is unknown.

In the present paper, we compared gene expression of the ctsR mutant 2-1 under HHP treatment vs. normal conditions. Our purpose was to explore what other genes contribute to the barotolerance in the ctsR mutant 2-1. Since ctsR mutants are most frequently isolated under high pressure treatments [18, 20], they represent a critical challenge in the tailing effect of HHP. Understanding the survival mechanism of the
**Materials and Methods**

**Bacterial strains and HHP treatments**

The *L. monocytogenes* Scott A *ctsR* mutant 2-1 and *L. monocytogenes* strain ScottA (wild-type) obtained from Dr. Joerger at University of Delaware were treated with high pressure (450 Mpa, 3 min) as described previously [12]. After pressure treatments, the suspension was centrifuged and the pellets were resuspended in RNA later and followed by RNA isolation according to Liu et al., [12]. The *L. monocytogenes* Scott A *ctsR* mutant 2-1 using as control samples was held at room temperature at atmospheric pressure for 3 min before centrifugation.

**RNA isolation, microarray chip design, hybridization, and data analysis**

Total RNA was isolated and quantified as described previously [12]. A whole genome microarray was constructed as described previously [12]. All samples (both wild-type and the *ctsR* mutant 2-1) were hybridized twice with one experiment (chip 1) using Alexa Fluor 647 to label the cDNA under normal conditions and Alexa Fluor 647 to label cDNA under pressure treatment and in the reciprocal experiment (chip2). Alexa Fluor 647 was used to label the cDNA under normal conditions and Alexa Fluor 555 to label the cDNA under pressure treatment. Microarray hybridization and washing was performed and the microarray slide was scanned, quantified as described previously [12]. A minimum threshold of a 2-fold change in gene expression with a p-value of <0.01 was used as the cut-off value.

**cDNA synthesis, primer design and real-time PCR analysis**

Synthesis of cDNA was carried out using Invitrogen’s SuperScript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA) following the manufacturer’s instructions. Primers selected based on the gene sequences of *L. monocytogenes* F2365 strain (GenBank accession#AE017262) were designed using Primer3 (v.0.4.0) software. Primer sequences are listed in Table 1 and Table 2. The housekeeping gene (*spoG*) was used as the internal control gene for real-time PCR analysis (Primer sequences 5’TGACGGTGAATTCCGTGATA3’; 3’TCAGCAGAAACGGATTCAGA5’), since this gene had the least variation among other housekeeping genes including 16S rRNA and 5’TCAGCAGAAACGGATTCAGA3’). The *ctsR* of *L. monocytogenes* strain Scott A (GenBank accession #AE017262) was designed following the manufacturer’s instructions. Primers selected based on the gene sequences of *L. monocytogenes* F2365 strain (GenBank accession#AE017262) were designed using Primer3 (v.0.4.0) software.

| GENE | Forward primer sequence | Reverse primer sequence | Amplicon size (bp) |
|------|-------------------------|-------------------------|-------------------|
| LMO2365_0019 | TTACATTTCTGCTGTTATCA | GATAAATGCGCGGAAATA | 111 |
| LMO2365_0992 | GCCGCTGGATTTGATTATA | ATTTGAGAAGCGTTTGGT | 146 |
| LMO2365_0993 | AATACTAATACGCGGAACCA | AGGTTCAGCGTCTTTGCA | 150 |
| LMO2365_1036 | CTTAGTTCCCCCGTTGGAT | CGCCGAGAAATCAGTGCCT | 135 |
| LMO2365_1075 | TCGCGCACTACAGCGACCA | CCGCAGACCGGATATTAG | 178 |
| LMO2365_1076 | CGCCAAATAACAGAGAAAT | AGGCCGATTTTGGGTGAT | 177 |
| LMO2365_1438 | GCAGCAGTAGAATACACATG | GATTITAGGTTCCCAGCAAT | 122 |
| LMO2365_1515 | CGGTGTTGCGGTGATCAC | ATGACCCATAGGGAAAG | 145 |
| LMO2365_1844 | AATCAGTCTCCGGTTAACAAA | TTAGGTTGCGGTCAACCAG | 103 |
| LMO2365_1920 | TCAGCGATCAACAAATGACAC | TAGCGTCCAAAGACATAA | 134 |
| LMO2365_1948 | AAGAAAACACCTCTCGGCACAT | GACAGCATTAAAGCAACATG | 119 |
| LMO2365_2230 | TAGACCGCGTTCTTAAATG | GCGTACGAGACGCGACTA | 109 |
| LMO2365_2305 | AAATCTGTGTTCTCAGGTT | AACAGGCAACGACAAGAAAG | 119 |
| LMO2365_2584 | TCGCGCATCTAATACCACT | ACGTCTACGAGTCTTGGA | 100 |

**Table 1: Oligonucleotides used for real-time PCR to evaluate induced genes.**

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LMO2365_1986 encoding for a transcriptional regulator in the Fur family was expressed at a moderate level in the *ctsR* mutant 2-1 under pressure treatment. The genes that were expressed at higher levels in the wildtype under pressure were also identified using microarray assays (Data not shown). There are 7 genes present in both wildtype and the *ctsR* mutant 2-1. The 7 unmatched genes that were only present in the *ctsR* mutant 2-1 are highlighted in boldface (Table 3). The unmatched genes are proposed to be pressure-resistant genes due to *ctsR* deletion. These genes are grouped into the following categories: genes encoding for proteins involved in transcription, regulatory functions, cell envelope, DNA and energy metabolism, and unknown functions.
of the 219 genes, 112 genes were repressed under pressure treatment were also identified by microarray analysis the metabolism, central intermediary metabolism, transport and binding, groups and carriers, protein synthesis, fatty acid and phospholipid transcription, energy metabolism, biosynthesis of cofactors, prosthetic nucleosides, and nucleotides, DNA metabolism, regulatory functions, supplement table). These genes encode proteins involved in amino strain under HPP treatment. For example, LMOf2365_0345 encoding for a putative membrane protein (LMOf2365_1438) and a dehydragenase, respectively. Both genes are highly expressed in the ctsR mutant 2-1 under HPP treatment. LMOf2365_1075 to 1076 encode for dihydrolipoamide acetyltransferase and dihydrolipoamide

decreased replication-dependent recombination [24], indicating that this gene is required for recombination. Consistent with this, LMO2365_1920 that encodes for recombination protein U is also expressed highly in the ctsR mutant 2-1. Elevation of this gene suggests that HHP directly damages DNA in the ctsR mutant 2-1. Interestingly, a DNA recombination and repair gene (recD) has been shown to be essential for high pressure growth in a deep-sea bacterium [25,26].

Gene related to energy metabolism were induced in the ctsR mutant 2-1 under HPP treatment. LMO2365_1075 to 1076 encode for dihydrolipoamide acetyltransferase and dihydrolipoamide dehydrogenase, respectively. Both genes are highly expressed in the ctsR mutant 2-1. Other relatively highly induced genes included genes encoding for a putative membrane protein (LMO2365_1438) and a hypothetical protein with unknown function (LMO2365_2230). Why these genes are induced in the ctsR mutant 2-1 under pressure remains unknown.

Repressed genes in L. monocytogenes Scott A ctsR mutant 2-1 strain under HPP treatment

Microarray analysis identified 219 genes that were repressed in the ctsR mutant 2-1 under high pressure treatment (Please see the supplemental table). The genes that were repressed in the wildtype under pressure treatment were also identified by microarray analysis (Data not shown). Of the 219 genes, 112 genes were repressed only in the ctsR mutant 2-1, not in the wildtype (Please see the supplement table). These genes encode proteins involved in amino acid biosynthesis, cell membrane, synthesis of purines, pyrimidines, nucleosides, and nucleotides, DNA metabolism, regulatory functions, transcription, energy metabolism, biosynthesis of cofactors, prosthetic groups and carriers, protein synthesis, fatty acid and phospholipid metabolism, central intermediary metabolism, transport and binding, and hypothetical proteins with unknown function. Only genes that encode for proteins involved in amino acid biosynthesis, cell envelope, transcription, transport and binding, regulatory functions, central intermediary metabolism and cellular processes were confirmed using real-time PCR assays. The 18 genes that were only repressed in the ctsR mutant 2-1 under pressure are highlighted in boldface (Table 4). These unmatched genes are likely to be pressure-resistant genes due to ctsR deletion.

Expression of a gene related to flagella synthesis (LMO2365_lmo0742) was reduced significantly in the ctsR mutant 2-1 under pressure (Table 4), this correlates with the absence of flagella and non-motile characteristics of ctsR mutant 2-1 [12]. Consistent with our findings, flagella mRNA and protein were also reduced in the ctsR mutant AK01 [17].

The expression of the RNA polymerase σ-70 factor gene (LMO2365_0255) was inhibited in the ctsR mutant 2-1 (-10-fold in the microarray and -5-fold by real-time PCR), possibly suggesting compensation for HPP-induced inhibition of RNA synthesis. Bacterial σ-70 factor directs RNA polymerase (RNAP) to specific promoter sites and starts transcription [27]. Interestingly, RNA polymerase σ-70 factor was required for stabilization of a deep-sea piezophillic bacterium under high-pressure conditions [28]. Furthermore, several transcription-associated genes were also inhibited, including those encoding proteins involved in transcription regulation, and termination/antitermination activities (Table 4).

Some genes related to the cell envelope were inhibited in the ctsR mutant 2-1 under HPP treatment. For example, LMO2365_0345 encoding for a leucine rich repeat domain/LPXTG-motif cell wall anchor domain protein was inhibited (-5-fold in microarray and 10-

| GENE     | Forward primer sequence | Reverse primer sequence | Amplicon size (bp) |
|----------|-------------------------|-------------------------|--------------------|
| LMO2365_0040 | AAAAGTGGTTCGGGCAAGTTAG | TAAATGCAGACTGTTGGGT | 101                |
| LMO2365_0255 | CTAGCAGGAGACGCGGTGCT | CTTTGAAGAGCGCACTATC  | 145                |
| LMO2365_0267 | GTATATCGAGAGCAGCGGATT | CAGCTAGTTCTGCAAGGTC | 118                |
| LMO2365_0305 | CGGGTCAACTTGTATCAAA | GTGGAAGAAAAAGCAAGGAA | 109                |
| LMO2365_0342 | TTCCCTACACTGAGTACAGAA | CTACAGATTCGCCCTTCTCA | 105                |
| LMO2365_0694 | AAGGACGGCGTGATTTTTC | ATCGCCGAGATTTTCGTT  | 123                |
| LMO2365_0742 | TTGGGATGTCGTGGTCCGA | TGATTTTACGCTCGATT  | 180                |
| LMO2365_1092 | TTTGGGATGTCGTGGTCCGA | TGATTTTACGCTCGATT  | 129                |
| LMO2365_1144 | CTCGCGATCGTTCAATAC  | CCGAAAGGTTGGGAAATCG | 108                |
| LMO2365_1370 | CCCCCAAGCCAGAAATGCT | CCGAAAGGTTGGGAAATCG | 122                |
| LMO2365_2333 | TACGCTGCGACGATAAAAA | TATGTTTTTCGCTGGGTTA | 127                |
| LMO2365_2500 | AAGTCACGCTACGGTTCCCA | ACCTGGTCGCTGGAAAGAAG | 150                |
| LMO2365_2550 | CTCGCTCAGCGAACTCTGGT | CACGGCGGTTCGTAATAGGT | 102                |
| LMO2365_2646 | CGCTGCGATCGTTCAATAC  | CCGAAAGGTTGGGAAATCG | 140                |
| LMO2365_2647 | CGTGGGAAAGGTTGGTCCAC | AAAAGGATCTGGGGTTTC | 142                |
| LMO2365_2742 | TGGTGGCTGCCCTGTAATGGA | AGCAATGGGCTATCTCTG | 126                |
| LMO2365_2749 | CACGCTCTCTAATCTCACA | GTTGATAGCCTGGTGGTAC | 130                |
| LMO2365_2763 | TTTGGCAGATCGAAGAATCTC | GTTTCAGAAACAGGTTGTT | 138                |
| LMO2365_2868 | CAGAAGTTCTGCTGATTCTCT | TGGGCTAGGCAGTAAAGTC | 115                |
| LMO2365_2868 | ATCTGCGAAATACCTCTCAA | CCAAGGAAAGCGATTATTA | 108                |

Table 2: Oligonucleotides used for real-time PCR to evaluate repressed genes.
fold by real-time PCR) in the ctsR mutant 2-1. Deletion of this gene in *L. monocytogenes* EDG strain resulted in greater susceptibility to nisin [29].

A number of genes encoding transport and binding proteins were inhibited in the ctsR mutant 2-1 under HHP treatment (Table 4). Interestingly, three of the five PTS systems that were inhibited in the ctsR mutant 2-1 are beta-glucoside-specific, indicating inhibition of the uptake of beta-glucosides under pressure. Gene expression of several ABC transporters was also inhibited under pressure. The substrates for these ABC transporters remain to be characterized.

Three genes encoding for amino acid biosynthesis were inhibited in the ctsR mutant 2-1 under pressure. Thus, reduction in amino acid synthesis may be related to the survival of the ctsR mutant 2-1 under pressure.

**Discussion**

In this study, microarrays were used to identify genes that are differentially expressed in a pressure tolerant ctsR mutant strain 2-1 under HPP treatment. The ctsR mutant 2-1 held under normal conditions (no high pressure treatment) was used as a control. The wildtype under normal and pressure-treated conditions were also investigated. After comparison, the genes that were induced or repressed only in the ctsR mutant 2-1 (highlighted in boldface in Supplement (Table, Table 3 and Table 4) were proposed to be pressure-resistant related genes due to ctsR deletion. All of the induced genes identified by microarray analysis in ctsR mutant 2-1 were confirmed by quantitative reverse transcriptase real-time PCR (qRT-PCR). Some of the repressed genes identified by microarray analysis were confirmed by qRT-PCR. The gene expression changes in the ctsR mutant 2-1 only may contribute to the barotolerance and adaptation/survival of the ctsR mutant 2-1 under pressure.

We chose our HPP treatment to mimic HPP exposure procedures and exposure times typically used for food processing. The conditions we used (450 Mpa, 3 minutes) resulted in a 6.7 log reduction of the *L. monocytogenes* Scott A. The gene expression levels of the house-keeping gene (*spoG*) in the ctsR mutant 2-1 remained the same under HPP vs. normal conditions, suggesting that RNA synthesis was not inhibited under these conditions. However, it has been shown that with increased pressure levels HHP combined with extended exposure times in *L. monocytogenes* [10], resulted in inhibition of RNA synthesis [30].

A problem observed during high pressure treatment is that a small portion of a bacterial population can be relatively resistant after a certain

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**Table 3:** Genes induced in *L. monocytogenes* strain Scott A ctsR mutant 2-1 under pressure treatment (450 Mpa, 3 minutes) as identified by microarray and real-time PCR analysis. Gene induced only in the ctsR mutant 2-1 (not in the wildtype) are in boldface.

| Category/Gene | Functiona | Fold changeb Microarrayc | RT-PCRd |
|---------------|-----------|--------------------------|---------|
| Genes encoding proteins involved in transport and binding | | | |
| LMO2365_2305 | PTS system; fructose-specific; IIABC component | 6.5 | 34.3 |
| LMO2365_1036 | glycerine betaine/L-proline ABC transporter; permease protein | 2.1 | 7.7 |
| Genes encoding proteins involved in cell envelope | | | |
| LMO2365_0992 | D-alanyl carrier protein | 5.0 | 4.6 |
| LMO2365_0993 | DltB protein | 3.2 | 7.0 |
| LMO2365_1438 | putative membrane protein | 3.4 | 6.3 |
| Genes encoding proteins involved in DNA metabolism | | | |
| LMO2365_1920 | recombination protein U | 3.2 | 2.7 |
| Genes encoding hypothetical or unknown function proteins | | | |
| LMO2365_2230 | hypothetical protein | 2.3 | 2.4 |
| Genes encoding proteins involved in transcription | | | |
| LMO2365_1515 | transcription elongation factor GreA | 2.5 | 2.5 |
| Genes encoding proteins involved in regulatory functions | | | |
| LMO2365_1986 | transcriptional regulator, Fur family | 2.0 | 2.8 |
| Genes encoding proteins of purines, pyrimidines, nucleosides, and nucleotides | | | |
| LMO2365_2584 | adenylate kinase | 3.4 | 3.2 |
| Genes encoding proteins involved in protein synthesis | | | |
| LMO2365_1844 | ribosomal protein L28 | 2.2 | 1.8 |
| Genes encoding proteins involved in energy metabolism | | | |
| LMO2365_1075 | dihydrolipoamide acetyltransferase | 2.6 | 1.9 |
| LMO2365_1076 | dihydrolipoamide dehydrogenase | 2.6 | 4.1 |
| LMO2365_0019 | cytochrome aa3 quinol oxidase, subunit IV | 1.2 | 119 |

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*a Only the genes that met the stringent criteria for being up-regulated in the ctsR mutant 2-1 of *L. monocytogenes* Scott A (i.e., fold change ≥2 fold; p<0.01) are listed here.

b Gene functions are based on annotations provided by TIGR (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi).

c Fold change indicates the transcript ratios of the ctsR mutant 2-1 between pressure treatment (450 Mpa, 3 minutes) and normal conditions as determined by microarray and real-time PCR.

d Numbers are average values from two independent experiments.

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applied pressure. This phenomenon is called the tailing effect [31], and it is a major challenge for the food industry. There are indications that high pressure results in genetic changes in the pressure-resistant subpopulation. A majority of pressure-resistant mutants contained mutations in the ctsR gene [16,18-20], indicating the involvement of this gene in the tailing effect. Understanding how the ctsR mutant 2-1 survives under HHP may help develop better strategies to eliminate the tailing effect of HHP in food processing. For example, LMO2365_0345 encoding for a leucine rich repeat domain/LPXTG-motif cell wall anchor domain protein was repressed in the ctsR mutant 2-1. Since deletion of this gene resulted in greater sensitivity to nisin [29], the reduced expression of LMO2365_0345 in ctsR mutant 2-1 provides an explanation for the sensitivity of this mutant to nisin under high pressure. This suggests that combination of a nisin and HPP treatment may inhibit the growth of L. monocytogenes. This notion has been supported by a study showing that a combination of high pressure treatment with nisin inhibited the growth of L. monocytogenes [4], in dry-cured ham, therefore, preventing the tailing effect.

Although the enhanced barotolerance of the ctsR mutant 2-1 made

### Table 4: Genes repressed in L. monocytogenes strain Scott A ctsR deletion mutant under pressure treatment as identified by microarray and real-time PCR analysis. Gene repression only in the ctsR mutant 2-1 (not in the wildtype) is in boldface.

| Category/Gene | Function | Fold changea | Microarrayb | RT-PCRc |
|---------------|----------|--------------|-------------|---------|
| Amino acid biosynthesis |          |              |             |         |
| LMO2365_0624 | O-acetylhomoserine (thio)lysyl-tRNA-synthetase | -2.3 | -2.0 |         |
| LMO2365_1705 | S-methyltetrahydropteroylglutamylhydrolysase-homocysteine S-methyltransferase | -4.3 | -3.3 |         |
| LMO2365_2285 | Aspartate aminotransferase | -3.6 | -2.5 |         |
| Cell envelope |          |              |             |         |
| LMO2365_0342 | Putative lipoprotein | -4.0 | -1.4 |         |
| LMO2365_0345 | Leucine rich repeat domain/LPXTG-motif cell wall anchor domain protein | -6.3 | -10 |         |
| LMO2365_0694 | Cell wall surface anchor family protein | -2.3 | -10 |         |
| LMO2365_1102 | Glycosyltransferase, group 2 family protein | -3.6 | -1.4 |         |
| LMO2365_2550 | Putative lipoprotein | -2.9 | -28.6 |         |
| LMO2365_2610 | Putative lipoprotein | -3.0 | -5.0 |         |
| LMO2365_2742 | D-alanyl-D-alanine carboxypeptidase | -2.9 | -87.8 |         |
| Transport and binding proteins |          |              |             |         |
| LMO2365_0267 | Sugar ABC transporter, sugar-binding protein | -5.6 | -2.0 |         |
| LMO2365_0305 | D-methionine ABC transporter, D-methionine-binding protein | -3.6 | -2.0 |         |
| LMO2365_0390 | PTS system, beta-glucoside-specific, IIB component | -2.8 | -1.4 |         |
| LMO2365_1001 | ABC transporter, permease protein | -5.0 | -3.3 |         |
| LMO2365_1056 | PTS system, beta-glucoside-specific, IIA or IIABC component | -7.7 | -2.5 |         |
| LMO2365_1445 | Glycine betaine/L-proline ABC transporter, glycine betaine/L-proline-binding protein | -5.6 | -10 |         |
| LMO2365_1744 | PTS system, beta-glucoside-specific, IIB component | -6.3 | -2.5 |         |
| LMO2365_2247 | ABC transporter, permease protein | -2.0 | -1.7 |         |
| LMO2365_2260 | ABC transporter, ATP-binding protein | -5.0 | -2.5 |         |
| LMO2365_2333 | Amino acid antiporter | -3.6 | -2.0 |         |
| LMO2365_2646 | Putative PTS system, galactitol-specific, IIB component | -3.4 | -2.0 |         |
| LMO2365_2647 | PTS system, IIA component | -4.0 | -5.0 |         |
| LMO2365_2749 | ABC transporter, ATP-binding protein | -2.3 | -1.4 |         |
| Transcription |          |              |             |         |
| LMO2365_0255 | RNA polymerase sigma-70 factor | -7.1 | -5.0 |         |
| Regulatory functions |          |              |             |         |
| LMO2365_0040 | Transcriptional regulator, LacI family | -3.3 | -1.3 |         |
| LMO2365_0344 | Putative transcriptional activator | -2.3 | -1.2 |         |
| LMO2365_1051 | Transcriptional regulator, LicT family | -2.3 | -1.4 |         |
| LMO2365_2407 | Transcription antiterminator LicT | -2.1 | -5.0 |         |
| LMO2365_2763 | Transcription antiterminator, BglG family | -3.0 | -2.5 |         |
| LMO2365_2805 | Transcriptional regulator, TetR family | -2.9 | -1.4 |         |
| Central intermediary metabolism |          |              |             |         |
| LMO2365_2328 | Putative glucosamine-6-phosphate isomerase | -2.3 | -2.0 |         |
| Cellular processes |          |              |             |         |
| LMO2365_0742 | Putative flagellar hook-associated protein FlgL | -2.1 | -2.5 |         |

aOnly the genes that met the stringent criteria for being up-regulated in the ctsR mutant 2-1 of L. monocytogenes Scott A (i.e., fold change >2 fold; p<0.01) are listed here.
bGene functions are based on annotations provided by TIGR (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi).
cFold change indicates the transcript ratios of the ctsR mutant 2-1 between pressure treatment (450 Mpa, 3 minutes) and normal conditions as determined by microarray and real-time PCR.
dNumbers are average values from two independent experiments.
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it difficult to eliminate the HHP tailing effect, the enhanced stress tolerance feature of the ctsR mutant can be beneficial to lactic acid bacteria. Various ctsR deletion mutants have been used successfully in food and beverage fermentation. For example, the ctsR deletion mutant of Lactobacillus plantarum was shown to survive better under ethanol stress [32], suggesting that this mutant can be potentially used for making wine. In another study, a ctsR deletion mutant of Lactobacillus sakei improved raw sausage fermentation since it grew better under stress [33,34].

Several lines of evidence suggest that the ctsR mutant 2-1 and deep-sea bacteria are similar in terms of pressure tolerance. First, the stress related genes were expressed under normal conditions, i.e. no pressure treatment. In the ctsR mutant 2-1, the clpC operon was highly expressed; whereas in a deep-sea bacterium, stress related genes were also highly expressed [35]. Although the expressed stress genes were different in the ctsR mutant 2-1 and a deep-sea bacterium, they may represent the same mechanism to compromise the environment. Second, some genes that were induced in the ctsR mutant 2-1 under pressure were also found to be necessary in deep-sea bacteria under pressure, e.g. genes encoding for respiratory chain [35] and recombinant proteins [25]. This indicates that they may share some adaptation/survival strategies under high pressure.

In the present study, whole-genome microarrays were used to identify multiple genes that were induced or inhibited by HHP treatment in ctsR mutant 2-1. The induced genes and a portion of the repressed genes were confirmed by real-time PCR. Identification of these genes begins to reveal the molecular mechanisms responsible for the adaptation and survival of ctsR mutant 2-1 under HHP treatment. Our results will provide a useful list of genes as novel candidates encoding for respiratory chain [35] and recombinant proteins [25]. A ctsR deletion mutant under HHP treatment. In the present study, whole-genome microarrays were used to identify multiple genes that were induced or inhibited by HHP treatment in ctsR mutant 2-1. The induced genes and a portion of the repressed genes were confirmed by real-time PCR. Identification of these genes begins to reveal the molecular mechanisms responsible for the adaptation and survival of ctsR mutant 2-1 under HHP treatment. Our results will provide a useful list of genes as novel candidates encoding for respiratory chain [35] and recombinant proteins [25]. This indicates that they may share some adaptation/survival strategies under high pressure.

Acknowledgments

We thank Amy Ream for performing real-time PCR assays. We are grateful to Anna Porto-Fett, John Luchansky, Brad Shoyer, and Jeffery Call for their work on HHP treatments. We appreciate Dr. Pina Fratamico and Dr. James Smith (USDA, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA) for critical reading of the manuscript.

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