

\section*{\textbf{σ^B Activation under Environmental and Energy Stress Conditions in Listeria monocytogenes}}

Soraya Chaturongakul and Kathryn J. Boor*

Department of Food Science, Cornell University, Ithaca, New York

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To measure σ^B activation in \textit{Listeria monocytogenes} under environmental or energy stress conditions, quantitative reverse transcriptase PCR (TaqMan) was used to determine the levels of transcripts for the σ^B-dependent \textit{opaCA} and \textit{clpC} genes in strains having null mutations in genes encoding regulator of sigma B proteins (\textit{rsbT} and \textit{rsbV}) and sigma B (\textit{sigB}) and in the \textit{L. monocytogenes} wild-type 10403S strain under different stress conditions. The ΔsigB, ΔrsbT, and ΔrsbV' strains previously exhibited increased hemolytic activities compared to the hemolytic activity of the wild-type strain; therefore, transcript levels for \textit{hly} were also determined. RsbT, RsbV, and σ^B were all required for \textit{opaCA} expression during growth under carbon-limiting conditions or following exposure to pH 4.5, salt, ethanol, or the protonophore carbonyl cyanide \textit{m}-chlorophenylhydrazon (CCCP). Expression of \textit{clpC} was RsbT, RsbV, and σ^B dependent in the presence of CCCP but not under the other conditions. \textit{hly} expression was not RsbT, RsbV, or σ^B dependent in the presence of either CCCP or salt. \textit{opaCA} transcript levels did not increase in the presence of rapidly lethal stresses (i.e., pH 2.5 or 13 mM cumene hydroperoxide) despite the enhanced survival of the wild type compared with the survival of the mutant strains under these conditions. These findings highlight the importance of complementing phenotypic characterizations with gene expression studies to identify direct and indirect effects of null mutations in regulatory genes, such as \textit{sigB}. Overall, our data show that while σ^B activation occurs through a single pathway under both environmental and energy stress conditions, regulation of expression of some stress resistance and virulence genes in the σ^B regulon (e.g., \textit{clpC}) appears to require networks involving multiple transcriptional regulators.

\textit{Listeria monocytogenes} is a non-spore-forming, gram-positive, facultative intracellular pathogen. The emergence of this organism as a difficult-to-control food-borne pathogen is at least in part due to its ability to survive in a broad range of ecological niches (13) and in many different hosts, including both animals and humans (11, 50). Contamination of foods with \textit{L. monocytogenes} raises both public health and economic concerns (33, 57). Although rare, listeriosis is a severe disease that results in death in 20 to 30% of reported cases (33). Infection in humans occurs predominantly among pregnant women, newborns, the elderly, and immunocompromised adults.

σ^B is a stress-responsive alternative sigma factor that has been identified in various low-G+C-content gram-positive bacteria, including the genera \textit{Bacillus}, \textit{Staphylococcus}, and \textit{Listeria}. In \textit{L. monocytogenes}, σ^B contributes to cell survival under stress conditions, including exposure to low pH, oxidative stress, carbon starvation, and growth at low temperatures (14, 15, 35, 54, 55). Loss of σ^B also reduces the ability of \textit{L. monocytogenes} to invade human intestinal epithelial cells (20, 29, 30, 31), as well as its virulence in the murine (36, 55) and guinea pig models (20). Emerging evidence suggests that σ^B contributes to virulence in several gram-positive pathogens (27). For example, σ^B contributes to \textit{Bacillus anthracis} virulence in the murine model (18). In \textit{Staphylococcus aureus}, σ^B plays a major role in mouse septic arthritis (24), although it is not essential for infection in the mouse abscess model, the mouse hematogenous pyelonephritis model, or the rat osteomyelitis model (7, 38).

The σ^B activation network in \textit{Bacillus subtilis} has been extensively studied (2, 3, 12, 26, 41, 53). While the \textit{sigB} operons in both \textit{B. subtilis} and \textit{L. monocytogenes} are comprised of seven regulators of sigma B (Rsb) activity (1, 16, 25, 55, 56), the initial genetic evidence that the σ^B operon products contribute to σ^B activation under energy stress conditions was first demonstrated in \textit{B. subtilis} (5, 51), no corresponding operon is present in \textit{L. monocytogenes} (22). To determine the roles of \textit{L. monocytogenes} Rsb in σ^B-mediated responses to various stresses, in-frame deletions were created in \textit{rsbT} and \textit{rsbV} (9), two genes predicted to encode positive regulators of σ^B activity. Phenotypic characterization of the \textit{L. monocytogenes} rsbT and \textit{rsbV} null mutants revealed that both mutants were similar to the ΔsigB strain in terms of the ability to survive under environmental or energy stress conditions, suggesting that RsbT and RsbV both convey environmental and energy stress signals to \textit{L. monocytogenes} σ^B (9). In \textit{B. subtilis}, RsbT contributes to regulation of σ^B activity in response to environmental stresses, while RsbV contributes to σ^B activation under both environmental and energy stress conditions (41). Taken together, these observations suggest that Rsb-dependent activation of σ^B activity in \textit{L. monocytogenes} is different than Rsb-dependent activation of σ^B activity in \textit{B. subtilis}.

In the present study, quantitative reverse transcriptase PCR (RT-PCR) (TaqMan) was used to compare the σ^B activity profiles in \textit{L. monocytogenes} wild-type strain 10403S and ΔsigB, ΔrsbT, and ΔrsbV' strains challenged with either environmental...
or energy stress conditions. As $\sigma^p$ can be present in either an active or inactive state (2), measurement of transcript levels for $\sigma^B$-dependent genes was used to indirectly quantify the activity of this protein. To do this, transcript levels were determined for $opuCA$, which encodes a glycine/betaine/carnitine/cholesterol ABC transporter (6, 19, 28, 47, 48), and for $clpC$, which encodes endopeptidase Clp ATP-binding chain C, a stress response protein that contributes to $L. monocytogenes$ phagosomal escape (30, 44, 45), in the $L. monocytogenes$ wild-type, $\Delta\sigma^B$, $\Delta\sigma^B \Delta\sigma^T$, and $\Delta\sigma^B \Delta\sigma^T \Delta\sigma^V$ strains under different stress conditions. The previously observed higher hemolytic activities in the $\Delta\sigma^B \Delta\sigma^T$, and $\Delta\sigma^B \Delta\sigma^V$ strains than in the wild-type strain (9) suggested that $\sigma^B$ might be expressed in a $\sigma^B$-dependent manner. Therefore, in the present study, transcript levels for the $\sigma^B$ virulence gene, which encodes listeriolysin O, were also determined under $\sigma^B$-inducing conditions in all four strains.

**MATERIALS AND METHODS**

**Bacterial strains.** The $L. monocytogenes$ strains used in this study were wild-type strain 10403S (4), 10403S $\Delta\sigma^B$ (FSL A1-254 [55]), 10403S $\Delta\sigma^B \Delta\sigma^T$ (FSL C3-015 [9]), and 10403S $\Delta\sigma^B \Delta\sigma^T \Delta\sigma^V$ (FSL C5-057 [9]). Stock cultures were stored at $-80°C$ in brain heart infusion (BHI) (Difco, Sparks, MD) broth with 15% glycerol and were streaked onto BHI agar plates prior to each experiment. The plates were incubated overnight at $37°C$ prior to use.

**Growth conditions and stress exposure.** For all experiments, overnight bacterial cultures were grown in BHI broth at $37°C$ with shaking (250 rpm) and then inoculated into 10 ml of BHI broth (1:100 dilution) and grown at $37°C$ with shaking until the optical density at 600 nm was 0.4 (representing the mid-log phase). Stationary-phase cells were prepared by inoculation into 10 ml of BHI broth (1:100 dilution) and grown at $37°C$ with shaking until the optical density at 600 nm was 0.4 (representing the mid-log phase). Stationary-phase cells were used for exposure to 13 mM cumene hydroperoxide (CHP) (14), since the number of log-phase cells dropped below the detection limit within 5 min after exposure. Stationary-phase cells were prepared by inoculating a 1:10 dilution of an overnight BHI broth culture (prepared as described above) into fresh BHI broth, followed by growth at $37°C$ with shaking for 12 h. Stationary-phase cells were exposed to CHP by using the procedures described above for exposure to ethanol and acid stresses; i.e., Rnaprotect was added either immediately following CHP addition or at 5 min after CHP addition, and then cell pellets were harvested by centrifugation following 10 min of incubation with Rnaprotect.

For energy stress studies, carbon starvation was induced by growing cells in defined medium (DM) (40) containing a growth-limiting concentration of glucose (0.01%, wt/vol) (9, 14). Cells were loaded into microtiter plates and grown at $37°C$ until the optical density at 560 nm was 0.4, whereupon cells were washed twice with medium (20 ml per well) and resuspended in fresh defined medium (DM) (40) containing a growth-limiting concentration of glucose (0.01%, wt/vol) (9, 14). Cells were loaded into microtiter plates and grown at $37°C$ until the optical density at 560 nm was 0.4, whereupon cells were washed twice with medium (20 ml per well) and resuspended in fresh defined medium (DM) (40) containing a growth-limiting concentration of glucose (0.01%, wt/vol) (9, 14). Cells were loaded into microtiter plates and grown at $37°C$ until the optical density at 560 nm was 0.4. Cells were then exposed to NaCl or CCCP for 5 min after exposure to the stressor, and then the pellets were resuspended in $\Delta\sigma^B$ strain to $37°C$, cultures were diluted to 0.4 (representing the mid-log phase) and then centrifuged at $3000 \times g$ for $5 \text{ min}$.

RNA isolation. RNA isolation was performed essentially as described by Sue et al. (48). Briefly, pellets of Rnaprotect-treated cells were lysed enzymatically using lysozyme (by addition of $200 \mu\text{g}$ of a 15-mg/ml solution and 10 min of incubation) and mechanically using sonication. Total RNA was purified using an RNeasy Midi kit (QIAGEN) and was treated with RNase-free DNase (QIAGEN). Purified RNA samples were stored in 0.3 M sodium acetate--100% ethanol at $-80°C$.

**Quantitative reverse transcriptase PCR** was performed as described by Sue et al. (49), using the ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Transcript levels were determined for $opuCA$, $clpC$, and $\sigma^B$, as well as for the housekeeping genes, $rpoB$ and $gap$, using TaqMan probes and primers as previously described (31, 42, 48). The RT-PCR mixtures (25 ml) contained 25 ng total RNA, 12.5 ml TaqMan One-Step RT-PCR Master Mix, 6.25 U Multiscribe reverse transcriptase, each primer (forward and reverse) at a concentration of $900 \text{ nM}$, and the appropriate TaqMan probe at a concentration of 250 nM. Duplicate reaction mixtures were collected by centrifugation (2,520 $\times g$, 5 min) either immediately or 5 min after incubation with RNAprotect, cells in these cultures were collected by centrifugation with RNAprotect, cells in these cultures were collected by centrifugation with RNAprotect (QIAGEN, Valencia, CA) to generate an average value for expression. The absolute copy numbers, calculated based on DNA standard curves, reflect mRNA levels for each target gene present in each RNA sample.

**Statistical analysis.** All statistical analyses were performed with S-Plus 6.2 (Insightful Corp., Seattle, WA). Standard regression diagnostics were computed for all models. Statistical significance was established at a $P$ value of $<0.05$. Initial data analysis indicated that the absolute mRNA transcript level data were heteroscedastic and strongly skewed. Consequently, all mRNA transcript level data were log$_{10}$ transformed to correct the skewness and to stabilize the variance. To determine the counts to detect the normality of the log$_{10}$ transcript level data, we performed an analysis of variance (ANOVA) was performed for the means of the transcript accumulation for the control genes under different physiological conditions. Therefore, housekeeping gene expression can also vary under different conditions (48, 49). Therefore, we used absolute copy numbers for two independent housekeeping genes ($rpoB$ [34, 48] and $gap$ [30, 31]) to generate an average value for transcript accumulation for the control genes under different physiological conditions. To ensure that the trends in expression of both housekeeping genes were similar, an analysis of variance (ANOVA) was performed for the means of the log-transformed mRNA transcript levels for $rpoB$ and $gap$ (i.e., $\log_{10}$ transcript $+$ log$_{10}$ mRNA transcript) with “strain” (wild type, $\Delta\sigma^B \Delta\sigma^T$, and $\Delta\sigma^B \Delta\sigma^T \Delta\sigma^V$) as factors. No significant difference in log-transformed mRNA transcript levels for $rpoB$ and $gap$ was observed among different strains and times under any stress conditions, except for growth in DM at 18 or 24 h. Thus, all statistical analyses were performed using the log-transformed (log$_{10}$) mRNA transcript levels normalized to the geometric mean for $rpoB$ and $gap$ mRNA transcript levels obtained under each experimental condition, unless indicated otherwise. NOVA models of the normalized mRNA transcript levels were used to investigate significant effects and interactions for each of the target genes using four factors, including “strain” (wild type, $\Delta\sigma^B \Delta\sigma^T$, and $\Delta\sigma^B \Delta\sigma^T \Delta\sigma^V$), “stress” (presence or absence of test condition), “time” (0 and 5 min or 6, 12, 18, and 24 h), and “replicate” (replicates 1, 2, and 3). For the “strain” factor, multiple comparisons were performed using Fisher’s least square difference (LSD). When
appropriate, individual t tests were also performed to compare normalized mRNA transcript levels for two specific samples.

RESULTS AND DISCUSSION

σ^B activation occurs through a single pathway under both environmental and energy stress conditions. The transcript levels for *opuCA*, a σ^B-dependent gene, were determined in four *L. monocytogenes* strains (wild type, ΔσB, ΔrsbT, and ΔrsbV) exposed to different environmental and energy stress conditions to measure the contributions of RsbT and RsbV to σ^B activation under conditions that have been shown to require both RsbT and RsbV (environmental stress) or only RsbV (energy stress) for induction of σ^B activity in *B. subtilis* (26, 52).

ΔσB, ΔrsbT, and ΔrsbV cells exposed to 0.3 M NaCl for 5 min, an environmental stress, had significantly lower *opuCA* transcript levels than the wild-type strain, and there was no difference in transcript levels among the three mutant strains (Fig. 1), showing that both RsbT and RsbV are required for induction of σ^B activity under salt stress conditions. Interestingly, compared to the *opuCA* transcript levels in the three mutant strains, the *opuCA* transcript levels in the wild-type strain were also significantly higher in cells that were not exposed to NaCl, as well as in NaCl-stressed cells collected by centrifugation immediately after exposure to the stress. Increased transcript levels in these cells likely resulted from σ^B activation from centrifugation prior to addition of RNAprotect, which is consistent with the recent observation that centrifugation induces expression of at least some σ^B-dependent genes (Y. Chan, K. J. Boor, and M. Wiedmann, unpublished results).

Statistical analyses revealed that there were significant (*P < 0.05, as determined by ANOVA) effects of “stress” (i.e., the presence of 0.3 M NaCl), “strain,” and “time” on *opuCA* transcript levels, supporting the hypothesis that there was significant salt induction of σ^B activity beyond the activity which may have resulted from cell handling practices under the experimental conditions used.

To further examine the contributions of RsbV and RsbT to induction of σ^B activity under environmental stress conditions, *opuCA* transcript levels were determined in bacterial cells exposed to ethanol or acid (pH 4.5). In contrast to what happened with NaCl exposure, direct addition of RNAprotect to cultures containing either ethanol or acid did not result in the formation of a precipitate; therefore, RNAprotect was added prior to collection of cells by centrifugation. The levels of the *opuCA* transcript present in the wild-type strain exposed to ethanol or acid under “no incubation” conditions (Fig. 2A and B) were lower than the levels in the wild-type strain exposed to NaCl that was centrifuged prior to addition of RNAprotect (Fig. 1), which was indicative of σ^B activation by centrifugation in the cells exposed to NaCl. Importantly, however, cells of the ΔσB, ΔrsbT, and ΔrsbV strains exposed to ethanol or pH 4.5 for 5 min had significantly lower levels of the *opuCA* transcript than the wild-type strain had, and there was no difference in *opuCA* transcript levels among the three mutant strains (Fig.
FIG. 3. Relative cDNA copy numbers for opuCA, expressed as log (opuCA mRNA copy number/mean housekeeping gene [HKG] copy number) under energy stress conditions (intracellular ATP depletion [BHI broth containing 32 μg/ml CCCP]). Following addition of the CCCP, cells were collected by centrifugation and treated as described in the legend to Fig. 1. The strains used were L. monocytogenes wild-type strain 10403S (solid bars) and the ΔsigB (shaded bars), ΔrsbT (cross-hatched bars), and ΔrsbV (open bars) strains. Comparisons of the four strains under each condition with Fisher’s LSD resulted in identification of strains whose opuCA transcript levels differed; the bars for strains whose opuCA transcript levels differed (within a given condition) are labeled with different letters.

2). Overall, ANOVA showed that the factors “strain” and “time” had significant (P < 0.01) effects on the normalized opuCA transcript levels under both acid and ethanol stress conditions. Our data show that both RsbT and RsbV are required for σB activation under environmental stress conditions, which is consistent with previous observations for B. subtilis (26, 53).

Although σB clearly enhances L. monocytogenes survival following exposure to some environmental stress conditions that are rapidly lethal, such as pH 2.5 or 13 mM CHP (9, 14, 15, 55), in this study exposure to these specific conditions did not result in increased levels of the opuCA transcript (data not shown), probably due to rapid death of both wild-type and mutant cells under both conditions. The findings of the present study suggest that the enhanced survival of wild-type L. monocytogenes compared to the ΔsigB strain following exposure to stresses that are rapidly lethal, as observed in previous studies (9, 14, 15, 55), reflects the presence of preformed σB-dependent regulon products in the cell rather than de novo synthesis of these products following exposure to the lethal stresses.

To characterize the contributions of RsbV and RsbT to σB activation under energy stress conditions, we initially determined opuCA transcript levels in cells exposed to CCCP for 5 min to induce intracellular ATP depletion (21). The ΔsigB, ΔrsbT, and ΔrsbV strain cells exposed to CCCP for 5 min had significantly lower levels of opuCA transcripts than the wild-type strain cells had, and there were no differences in the opuCA transcript levels among the three mutant strains (Fig. 3), indicating that both RsbT and RsbV are required for induction of σB activity under CCCP-induced energy stress conditions. Consistent with the NaCl stress data, compared to the opuCA transcript levels in the three mutant strains, the opuCA transcript levels in the wild-type strain were significantly higher in unexposed cells and in CCCP-exposed cells collected by centrifugation immediately after CCCP addition. Higher opuCA transcript levels in these cells probably reflected σB activation by centrifugation prior to addition of RNAprotect. Statistical analyses revealed that “stress” (exposure to CCCP) had a significant (P < 0.001) as determined by ANOVA) effect on opuCA transcript levels and that the opuCA transcript levels were significantly (P = 0.0003, as determined by a t test) higher in exposed wild-type cells than in unexposed wild-type cells, supporting the hypothesis that there was significant induction of σB activity in the presence of CCCP.

To further confirm the importance of both RsbT and RsbV for induction of σB activity under energy stress conditions, opuCA transcript levels were also determined in L. monocytogenes grown in glucose-limiting defined medium. Following growth for 6 or 12 h in this medium, the ΔsigB, ΔrsbT, and ΔrsbV strains had significantly lower opuCA transcript levels than the wild-type strain had, and there were no differences in the opuCA transcript levels among the three mutant strains (Fig. 4). Thus, we concluded that, in contrast to the σB activation pathway in B. subtilis (52), both RsbT and RsbV are necessary for σB activation in response to both environmental and energy stresses.

Loss of σB has global physiological consequences for L. monocytogenes during growth under carbon-limiting conditions. While we have previously shown that growth in glucose-limiting defined medium results in more rapid growth, larger maximal populations, and more rapid declines in numbers of viable cells in the ΔsigB, ΔrsbT, and ΔrsbV strains than in the wild-type strain (Fig. 5A) (9), determination of the numbers of opuCA, gap, and rpoB CDNA copies in the ΔsigB, ΔrsbT, ΔrsbV, and wild-type strains showed the critical role of σB and the regulators of σB (RsbS) during growth under energy-limiting conditions. Consistent with the importance of σB as an activator of transcription under energy stress conditions, high absolute levels of the opuCA transcript were observed in the wild-type strain at both 6 and 12 h after inoculation into DM with 0.04% glucose, but the levels decreased dramatically at 18 and...
24 h after inoculation (Fig. 5B). Interestingly, the absolute transcript levels for the \(rpoB\) and \(gap\) housekeeping genes also were lower at 18 and 24 h after inoculation than at 6 and 12 h after inoculation (Fig. 5C and D). Consistent with the optical densities at 600 nm, which increased more rapidly up to 12 h and decreased more rapidly after 12 h in the \(/H9004\) sigB, \(/H9004\) rsbT, and \(/H9004\) rsbV strains than in the wild-type strain, the housekeeping gene transcript levels also decreased more rapidly in the mutant strains than in the wild-type strain after 12 h. Overall, the ANOVA results supported the hypothesis that the factors “strain” and “time” had a significant effect on \(rpoB\) and \(gap\) transcript levels. As a consequence of the differences in housekeeping gene expression patterns between the wild-type and mutant strains at 18 and 24 h, only data from 6 and 12 h (Fig. 5C and D) were used to quantify the relative expression patterns of \(opuCA\) in the different strains, as shown in Fig. 4.

Our data provide further evidence that housekeeping gene expression can change with physiological changes in the cell (48, 49) and highlight the conclusion that expression data for housekeeping genes should be obtained under all test conditions to ensure that the data obtained for these genes can legitimately be used for normalization of target gene data. Our results also clearly demonstrate that \(\sigma^H\) makes important contributions to \(L.\) monocytogenes gene expression during exponential growth in glucose-limiting defined media, as reflected by the high levels of \(opuCA\) mRNA in the wild-type strain at 6 and 12 h (Fig. 4 and 5B). We hypothesize that energy expenditures necessary for production of these stress response transcripts and the resulting proteins (or other possible negative effects associated with expression of high levels of stress proteins) may contribute to the increased doubling time for the wild-type strain compared with the doubling times of the mutants, as suggested previously for \(E.\) coli and \(B.\) subtilis (39, 46). However, accumulation of stress response proteins also may contribute to enhanced survival of the wild-type strain compared to the mutant strains at later times (e.g., 18 or 24 h) (Fig. 5A), consistent with the notion that the presence of preformed \(\sigma^H\)-dependent regulon products contributes to survival of the wild-type strain in the presence of lethal stresses.

**Appropriate regulation of stress response and virulence gene expression appears to require networks involving multiple transcriptional regulators.** Increasing evidence supports the hypothesis that some \(L.\) monocytogenes regulons (28, 34, 36, 48), including the \(\sigma^H\) and positive regulatory factor A (PrfA) regulons, the latter of which includes most of the well-recognized \(L.\) monocytogenes virulence genes, have overlapping functions. In particular, the prfA P2 promoter is \(\sigma^H\) dependent (36), and a number of PrfA-dependent genes are also regulated by \(\sigma^H\) (28, 34, 48). Interestingly, hemolytic activities were previously reported to be significantly higher in \(L.\) monocytogenes \(/H9004\) sigB, \(/H9004\) rsbT, and \(/H9004\) rsbV culture supernatants than in wild-type culture supernatants (9, 36), suggesting a possible role for \(\sigma^H\) in hemolysin expression. Therefore, to determine if \(\sigma^H\) contributes to transcriptional regulation of \(hly\), which encodes the \(L.\) monocytogenes hemolysin listeriolysin O, we determined \(hly\) transcript levels in the wild-type, \(/H9004\) sigB, \(/H9004\) rsbT, and \(/H9004\) rsbV strains at 37°C under selected environmental (NaCl) and energy (CCCP exposure) stress conditions. Al-
though it has been suggested that \( \sigma^B \) influences hemolysin expression at the transcriptional level in \( S. aureus \) (23), we found that \( L. monocytogenes \) hly transcript levels were identical in the \( \Delta sigB, \Delta rsbT, \Delta rsBV \), and wild-type strains under \( \sigma^B \)-inducing conditions. The higher apparent hemolysin activities in the \( \Delta sigB, \Delta rsbT, \Delta rsBV \) strains observed in previous studies may have resulted from indirect effects of a loss of \( \sigma^B \), possibly reflecting alterations in the translation rate of hly mRNA, hemolysin stability, or cellular structure (28). The findings obtained in this study highlight the importance of conducting expression analyses to identify the direct and indirect contributions of different transcriptional regulators to virulence and the stress response in bacterial pathogens under defined conditions.

In addition to \( \sigma^B \) and PrfA, a number of other transcriptional regulators also control expression of genes that contribute to virulence and the stress response, including \( L. monocytogenes \) CtsR (8, 37). Interestingly, mutations in the CtsR-regulated gene \( clpC \), which encodes an ATPase that belongs to a class of heat shock proteins involved in stress tolerance, result in attenuated \( L. monocytogenes \) virulence and bacterial susceptibility to multiple stresses, including high temperature, high osmolarity, and iron limitation (43, 44, 45). Since expression of \( clpC \) is \( \sigma^B \)-dependent in both \( B. subtilis \) (32) and \( L. monocytogenes \) (30), we hypothesized that the CtsR regulon also overlaps the \( L. monocytogenes \) \( \sigma^B \) regulon. Therefore, we determined \( clpC \) transcript levels under different environmental and energy stress conditions. We observed no differences in \( clpC \) transcript levels among the \( \Delta sigB, \Delta rsbT, \Delta rsBV \), and the wild-type strains when cells were exposed to acid (pH 4.5), ethanol, or salt, which is consistent with results reported by Conte et al. (10), who showed that \( clpC \) expression was not induced by exposure to acid. Interestingly, \( clpC \) transcript levels increased in a \( \sigma^B \)-dependent manner following addition of CCCP (Fig. 6); this finding was supported by an ANOVA, which showed that the factors “time,” “strain,” and “stress” (presence or absence of CCCP) had significant (\( P < 0.05 \)) effects on normalized \( clpC \) transcript levels. However, for cells grown in DM with 0.04% glucose, the \( clpC \) transcript levels of the \( \Delta sigB, \Delta rsbT, \Delta rsBV \), and wild-type strains did not differ, indicating that \( \sigma^B \), RsBV, and RsbT contribute to \( clpC \) transcription only under specific stress conditions.

While existing genomic sequence data for \( L. monocytogenes \) EGDe-e (22) do not support the presence of an apparent \( \sigma^B \)-dependent promoter immediately upstream of \( clpC \), a putative \( \sigma^B \)-dependent promoter (GGTGG-27 nucleotides-GGGAT) is located 71 nucleotides upstream of the ctsR start codon, which is the first gene in the operon encoding \( clpC \).

Overall, our data demonstrate that \( L. monocytogenes \) \( \sigma_B \) has a complex activation network which differs from that in the closely related gram-positive model organism \( B. subtilis \). Furthermore, we provide evidence that \( L. monocytogenes \) \( \sigma_B \) has both direct and indirect effects on virulence and the stress response in this important food-borne pathogen.

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REFERENCES


FIG. 6. Relative cDNA copy numbers for \( clpC \), expressed as log (\( clpC \) mRNA copy number/mean housekeeping gene [\( HKG \)] copy number) following exposure to CCCP. Following addition of the CCCP, cells were collected by centrifugation and treated as described in the legend to Fig. 1. The strains used were \( L. monocytogenes \) wild-type strain \( 10403 \) (solid bars) and the \( \Delta sigB \) (shaded bars), \( \Delta rsbT \) (cross-hatched bars), and \( \Delta rsBV \) (open bars) strains. Comparisons of the four strains under each condition with Fisher’s LSD resulted in identification of strains whose \( clpC \) transcript levels differed; the bars for strains whose \( clpC \) transcript levels differed (within a given condition) are labeled with different letters.


33.营造良好的环境有助于生物的生存。Microbiol. 150:3843–3855.


