

# $\sigma^B$ and $\sigma^L$ Contribute to *Listeria monocytogenes* 10403S Response to the Antimicrobial Peptides SdpC and Nisin

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## Abstract

The ability of the foodborne pathogen *Listeria monocytogenes* to survive antimicrobial treatments is a public health concern; therefore, this study was designed to investigate genetic mechanisms contributing to antimicrobial response in *L. monocytogenes*. In previous studies, the putative bacteriocin immunity gene *lmo2570* was predicted to be regulated by the stress responsive alternative sigma factor,  $\sigma^B$ . As the alternative sigma factor  $\sigma^L$  controls expression of genes important for resistance to some antimicrobial peptides, we hypothesized roles for *lmo2570*,  $\sigma^B$ , and  $\sigma^L$  in *L. monocytogenes* antimicrobial response. Results from phenotypic characterization of a *L. monocytogenes lmo2570* null mutant suggested that this gene does not contribute to resistance to nisin or to SdpC, an antimicrobial peptide produced by some strains of *Bacillus subtilis*. While *lmo2570* transcript levels were confirmed to be  $\sigma^B$  dependent, they were  $\sigma^L$  independent and were not affected by the presence of nisin under the conditions used in this study. In spot-on-lawn assays with the SdpC-producing *B. subtilis* EG351, the *L. monocytogenes*  $\Delta sigB$ ,  $\Delta sigL$ , and  $\Delta sigB/\Delta sigL$  strains all showed increased sensitivity to SdpC, indicating that both  $\sigma^B$  and  $\sigma^L$  regulate genes contributing to SdpC resistance. Nisin survival assays showed that  $\sigma^B$  and  $\sigma^L$  both affect *L. monocytogenes* sensitivity to nisin in broth survival assays; that is, a *sigB* null mutant is more resistant than the parent strain to nisin, while a *sigB* null mutation in  $\Delta sigL$  background leads to reduced nisin resistance. In summary, while the  $\sigma^B$ -dependent *lmo2570* does not contribute to resistance of *L. monocytogenes* to nisin or SdpC, both  $\sigma^B$  and  $\sigma^L$  contribute to the *L. monocytogenes* antimicrobial response.

## Introduction

THE GRAM-POSITIVE, facultative intracellular foodborne pathogen *Listeria monocytogenes* is the causative agent of listeriosis, which has a human case-fatality rate >20% in the United States (Mead *et al.*, 1999). The vast majority of human listeriosis cases have been reported to occur via consumption of contaminated foods (Mead *et al.*, 1999); therefore, development of more effective methods for controlling the presence of *L. monocytogenes* in foods is a desirable goal. To that end, various antimicrobial peptides have been investigated as a potential means for inhibiting growth of *L. monocytogenes* in foods (Muriana, 1996; Cleveland *et al.*, 2001).

Bacteriocins are bacterially produced antimicrobial peptides that are generally most effective against other bacteria that are genetically similar and present in similar ecological niches. To enhance producer strain self-preservation, bacteriocin production is frequently coupled with production of cognate bacteriocin immunity proteins (Venema *et al.*, 1995; Cleveland *et al.*, 2001; Eijsink *et al.*, 2002). For example, NisI,

which provides immunity to nisin, is encoded downstream of the nisin biosynthesis genes in *Lactococcus lactis* (Engelke *et al.*, 1994). Previous studies have reported that bacteriocin production can be influenced by bacterial environmental stress response pathways such as the RecA-dependent SOS response and the ppGpp-dependent stringent response (de los Santos *et al.*, 2005). Bacteriocin production and immunity to antimicrobials are hypothesized to enhance the ability of producer bacteria to vie for limited nutrients in the presence of competitors (Nissen-Meyer and Nes, 1997).

Lactic acid bacteria are recognized as producers of various bacteriocins (Klaenhammer, 1993; Jack *et al.*, 1995). Lactic acid bacteria are commonly present in human foods; therefore, the bacteriocins that they produce, such as pediocin PA-1/AcH, enterocins, and/or sakacins, also may be present in foods (Cleveland *et al.*, 2001). Currently, only nisin, a class I lantibiotic bacteriocin produced by the lactic acid bacterium *L. lactis*, has generally recognized as safe status for intentional application as an antimicrobial in the U.S. food industry (Jack *et al.*, 1995). Nisin creates membrane-spanning pores in the

bacterial cell wall, which enable dissipation of the cell's proton motive force (Bruno *et al.*, 1992; Bonnet *et al.*, 2006). Although, in general, nisin has been demonstrated as an effective anti-listerial peptide (Benkerroum and Sandine, 1988), some strains of *L. monocytogenes* have developed resistance to both nisin and pediocin PA-1 (Gravesen *et al.*, 2002a, 2002b; Gandhi and Chikindas, 2007). *L. monocytogenes* resistance to nisin is a concern to the segments of the food industry (e.g., dairy and poultry) that currently use this peptide to control pathogen growth (Gandhi and Chikindas, 2007). A better understanding of the molecular mechanisms contributing to antimicrobial resistance in foodborne pathogens could lead to development of improved food safety intervention strategies. One means to that end is to identify and examine putative bacteriocin immunity genes and their physiological roles in protecting the producer strain against either endogenously or exogenously produced antimicrobial peptides.

In a previous study, Kazmierczak *et al.* (2003) identified *lmo2570* as a putative  $\sigma^B$ -dependent gene with 45% similarity to the *Bacillus subtilis* bacteriocin immunity gene *sdpI* (*yvaZ*) (cmr.jcvi.org), which encodes SdpI. SdpI is a membrane protein conferring resistance to the endogenously produced antimicrobial peptide SdpC (Butcher and Helmann, 2006; Ellermeier *et al.*, 2006). Butcher and Helmann (2006) found that while SdpI has a predominant role in conferring resistance to SdpC, the *B. subtilis* regulon controlled by  $\sigma^W$ , an extracytoplasmic function sigma factor, provides secondary immunity to this antimicrobial peptide. Taken together, these data indicate the importance in antimicrobial resistance of both immunity genes and transcriptional level regulatory mechanisms as mediated by alternative sigma factors.

We hypothesized that  $\sigma^B$  and  $\sigma^L$  contribute to antimicrobial response in *L. monocytogenes*.  $\sigma^B$  has been shown to regulate response to antimicrobial peptides in other Gram-positive bacteria. To illustrate, the *B. subtilis*  $\sigma^B$  regulon is upregulated after treatment with either bacitracin or vancomycin (Mascher *et al.*, 2003). In a collection of teicoplanin-resistant *Staphylococcus aureus* mutants, the majority of the mutations responsible for antimicrobial resistance mapped to *rsbW*, which encodes the RsbW anti-sigma factor that sequesters  $\sigma^B$  to prevent it from interacting with RNA polymerase. The teicoplanin-resistant strains with mutations in *rsbW* showed

increased  $\sigma^B$  activity relative to their parent strain (the MB33 *rsbU* mutant strain) or to other strains carrying the *rsbU* wild-type allele (Bischoff and Berger-Bachi, 2001), providing evidence of a link between  $\sigma^B$  activity and antimicrobial resistance.  $\sigma^B$  also has been shown to contribute to bacterial stress response regulation in *S. aureus* (Chan *et al.*, 1998). *L. monocytogenes* alternative sigma factor  $\sigma^L$  regulates expression of genes that mediate sensitivity to antimicrobials such as the class IIa bacteriocin, mesentericin Y105 (Robichon *et al.*, 1997); hence,  $\sigma^L$  also has been associated with antimicrobial response. Therefore, in the studies described below, phenotypic and genotypic assessments were used to determine the contributions of  $\sigma^B$ ,  $\sigma^L$ , and *Lmo2570* to the *L. monocytogenes* response to SdpC and nisin.

## Materials and Methods

### Bacterial strains and growth conditions

*L. monocytogenes* parent strain 10403S (serotype 1/2a) and otherwise isogenic *sigB* and *sigL* single and double null mutants ( $\Delta sigB$ , FSL A1-254;  $\Delta sigL$ , FSL B2-124;  $\Delta sigB/\Delta sigL$ , FSL B2-127) were used in this study. *Listeria innocua* FSL C2-008 (Woodling and Moraru, 2005), *Listeria ivanovii* FSL C2-010, *Listeria welshimeri* FSL N1-064, and *Listeria seeligeri* FSL N1-067 (Table 1) were used to assess intragenus competition with the *L. monocytogenes* parent and mutant strains. To examine the susceptibility of the *L. monocytogenes* parent and mutant strains to a closely related bacterium that produces an antimicrobial peptide, we used strains of *B. subtilis* that produce SdpC, the bacteriocin whose cognate immunity gene is predicted by sequence similarity to be homologous to *L. monocytogenes lmo2570*. These strains included *B. subtilis* prototroph (PY49) (Youngman *et al.*, 1984) and its mutant EG351 (PY79 *P<sub>spac</sub>-hy-sdpABC*) (gift from Dr. J. Helmann, Department of Microbiology, Cornell University), which expresses SdpC under control of an inducible promoter.

*L. monocytogenes* strains were grown in brain heart infusion (BHI) broth (Difco, Sparks, MD) at 37°C with shaking (250 rpm) overnight (16–18 h), then were subcultured (1:100) and grown as described below for each experiment. *B. subtilis* strains were grown in Luria-Bertani broth as described for *L. monocytogenes*, unless otherwise stated. The  $\Delta sigB/\Delta sigL$  strain grew more slowly than the 10403S,  $\Delta sigB$ , and  $\Delta sigL$

TABLE 1. STRAINS USED IN THIS STUDY

Strain	Characteristics	Reference or source
<i>Listeria monocytogenes</i> 10403S	Laboratory parent strain	Bishop and Hinrichs (1987)
<i>L. monocytogenes</i> FSL A1-254	10403S $\Delta sigB$	Wiedmann <i>et al.</i> (1998)
<i>L. monocytogenes</i> FSL P1-002	10403S $\Delta lmo2570$	This study
<i>L. monocytogenes</i> FSL B2-124	10403S $\Delta sigL$	Chaturongakul, unpublished
<i>L. monocytogenes</i> FSL B2-127	10403S $\Delta sigB/\Delta sigL$	Chaturongakul, unpublished
<i>Listeria innocua</i> FSL C2-008		Woodling and Moraru (2005)
<i>Listeria ivanovii</i> FSL C2-010		Wiedmann, unpublished <sup>a</sup>
<i>Listeria welshimeri</i> FSL N1-064		Fish processing plant environment
<i>Listeria seeligeri</i> FSL N1-067		Fish processing plant environment
<i>Bacillus subtilis</i> PY79	Prototroph, parent strain	Youngman <i>et al.</i> (1984)
<i>B. subtilis</i> EG351	PY79 <i>P<sub>spac</sub>-hy-sdpABC</i>	Butcher and Helmann (2006)

<sup>a</sup>Isolate kindly provided (as USDA 2717) by I. Wesley, USDA-ARS. USDA-ARS, U.S. Department of Agriculture's Agricultural Research Service.

strains, requiring an additional incubation time of ~30 min to reach the same OD<sub>600</sub>.

#### Mutant construction

An in-frame 543 base pair deletion within *lmo2570* was created in *L. monocytogenes* 10403S using splicing-by-overlap extension (SOE) polymerase chain reaction (PCR) and allelic exchange mutagenesis (Ho *et al.*, 1989). Primers used were 5'-GGA AGC TTT AAG GCA CTG TGA GCC TGG-3' (*lmo2570* SOEA), 5'-TCA TAC TAG GAA ATA TAC CAA C-3' (*lmo2570* SOEB), 5'-GTT GGT ATA TTT CCT AGT ATG ATT ATT GTT GTT G-3' (*lmo2570* SOEC), and 5'-GGG GTA CCT CAG GTT CAC TGG CAG CTA G-3' (*lmo2570* SOED). Primers were synthesized by IDT Technologies (Coralville, IA). Allelic exchange mutagenesis was confirmed through PCR and subsequent DNA sequencing, the latter of which was performed by the Cornell BioResource Center (Ithaca, NY). The  $\Delta$ *lmo2570* mutation did not affect growth rate of the mutant strain relative to the 10403S parent strain when both were grown in BHI at 37°C with shaking at 250 rpm (data not shown).

#### Spot-on-lawn assays

Spot-on-lawn assays were performed in triplicate as described by Butcher and Helmann (2006). Briefly, to create lawns, 100  $\mu$ L of a given strain (i.e., 10403S,  $\Delta$ *lmo2570*,  $\Delta$ *sigB*,  $\Delta$ *sigL*, or  $\Delta$ *sigB*/ $\Delta$ *sigL*) that had been grown to an optical density of OD<sub>600</sub> = 0.4 was inoculated into 2 mL of 0.7% Luria-Bertani soft agar that had been tempered at 50°C. To induce *P*<sub>spac</sub>-regulated transcription of *sdpABC* when EG351 was used as the spotting strain, 1 mM isopropyl- $\beta$ -D-thiogalactopyranoside also was added to the tempered agar that had been inoculated with bacteria. Each mixture was then poured into one well in an eight-well rectangular multidish (26 $\times$ 33 mm; Nunc, Rochester, NY). The plates were then dried in a laminar hood for 30 min. Subsequently, 4  $\mu$ L of the strain being assessed for bacteriocin production (e.g., 10403S, PY79, or EG351), which had been grown to an OD<sub>600</sub> = 0.6, was spotted on the agar in the middle of each well. The plates were covered with lids and incubated in a moist container at 37°C for 22–24 h. In addition to the 10403S, PY79, or EG351 test strains, isolates representing five *Listeria* species also were used as spotting strains to determine if the lawn strains would demonstrate sensitivity to bacteria representing different species within the same genus (Table 1). Sensitivity of lawns to potential bacteriocin producer strains was assessed by measuring the zone of inhibition (zoi) around the growth of the spotted strain. Radii of zoi were determined by measuring the diameters of both the spotted colony and the surrounding zoi in pixels (px). The diameter of the spotted colony was then subtracted from the diameter of the zoi, and the resulting product was divided by 2 (to yield a radius). Measurements were performed using Adobe® Photoshop® CS (Adobe Systems Incorporated, Mountain View, CA).

Radii of zoi produced on the various lawn strains were initially compared to zoi produced on the 10403S reference lawn using one-way analysis of variance (ANOVA) with Dunnett's *t*-test, using SAS® 9.0 (SAS Institute, Cary, NC). To determine if there were statistically significant interaction effects between the *sigB* and the *sigL* deletions, a two-way ANOVA (with Tukey's adjustment for multiple comparisons)

was performed. In this model, the dependent variable was zoi radius, and the independent variables included *sigB* + *sigL* + *sigB*\**sigL* + replicate. The factors *sigB* and *sigL* in the model indicate the presence or absence of that gene in the strains tested. An adjusted *p* < 0.05 was considered significant in this and all other statistical analyses.

#### Nisin minimum inhibitory concentration determination

The objective of this experiment was to determine a minimal inhibitory concentration of nisin for log-phase *L. monocytogenes* to enable selection of an appropriate sublethal concentration for subsequent quantitative reverse-transcriptase (qRT)-PCR experiments. Nisin's solubility and activity are optimal at pH 3 and 3.5, respectively (Abee and Delves-Broughton, 2003); therefore, nisin is typically dissolved in an acidified solution before use (Liu and Hansen, 1990; Huot *et al.*, 1996). As  $\sigma^B$  expression and activity are induced at low pH (Becker *et al.*, 1998; Sue *et al.*, 2004), we predicted that addition of nisin in an acidified solution to the various cultures would upregulate expression of the  $\sigma^B$  regulon, thus conferring a survival advantage to the wild type over the  $\Delta$ *sigB* strain (Wiedmann *et al.*, 1998; Ferreira *et al.*, 2001, 2003) and, hence, confounding interpretation of our experimental results. Therefore, to avoid induction of  $\sigma^B$  activity, nisin was dissolved in sterile distilled water (1000 AU/mL), and the pH of the final solution was adjusted to 7.0 using 0.01 N sodium hydroxide. Nisin solutions at pH 7.0 were used throughout these experiments. The nisin solutions were filter sterilized with a 0.2  $\mu$ m, 25 mm syringe filter (NALGENE®; Thermo Fisher Scientific, Waltham, MA) and diluted to the test concentrations. The minimum inhibitory concentration of nisin (Sigma, St. Louis, MO) was determined for all strains of *L. monocytogenes* by measuring absorbance at OD<sub>600</sub> using a Fusion™ Universal Microplate Analyzer (PerkinElmer, Shelton, CT). Strains were grown overnight, subcultured 1:100, and grown to OD<sub>600</sub> = 0.4. Strains were inoculated to a final concentration of 1 $\times$ 10<sup>4</sup> CFU/well into 96-well round bottom microplates (Costar, Corning, NY), and the edges were sealed with Parafilm® (Alcan Packaging, Neenah, WI) to prevent evaporation. OD<sub>600</sub> measurements were taken after 24 h incubation at 37°C with shaking. The lowest concentration that inhibited growth for all strains after a 24 h incubation in a 96-well plate format was 100 AU nisin/mL, as determined in three replicate trials. Therefore, a sublethal concentration of 75 AU nisin/mL was used for TaqMan qRT-PCR assays.

#### Total RNA isolation

*L. monocytogenes* 10403S,  $\Delta$ *sigL*, and  $\Delta$ *sigB* were grown to logarithmic phase (OD<sub>600</sub> = 0.4) and collected after (i) incubation for 10 min after addition of nisin in sterile distilled water to yield a final concentration of 75 AU/mL nisin, (ii) incubation for 10 min after addition of an equivalent volume of sterile distilled water without nisin, and (iii) incubation for 10 min without any addition. RNA isolation and purification was performed as previously described (Sue *et al.*, 2004; Raengpradub *et al.*, 2008), except that DNase treatments were performed using TURBO™ DNase (Ambion, Austin, TX) following the manufacturer's instructions. Total nucleic acid concentrations and purity were estimated using absorbance readings (260 nm/280 nm) on a NanoDrop™ ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE).

### qRT-PCR

Transcript levels of *lmo2570*, as well as of two housekeeping genes, *rpoB* and *gap*, were quantified using TaqMan primers and probes and the ABI Prism<sup>®</sup> 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) as previously described (Kazmierczak *et al.*, 2006). Data were analyzed using the ABI Prism 7000 Sequence Detection System software (Applied Biosystems) as previously described by Sue *et al.* (2004). Primer Express<sup>®</sup> 1.0 software (Applied Biosystems) was used to design oligonucleotide primers and TaqMan probes for *lmo2570*: forward primer (5'-AAG TGG CGG TGC ATT TCG-3'), reverse primer (5'-TAA GCC AAG CCA CTT TTG CAT-3'), and probe (6FAM 5'-ACG GAC TTC TCC CCA GAT-3' MGB-NFQ). Primers and probes for *gap* and *rpoB* were previously described (Sue *et al.*, 2004; Schwab *et al.*, 2005), respectively.

Transcript levels of *lmo2570*, as determined by qRT-PCR, were log<sub>10</sub> transformed and then normalized to the geometric mean of transcript levels from the housekeeping genes *rpoB* and *gap* as previously described (Kazmierczak *et al.*, 2006). Statistical analyses of normalized *lmo2570* transcript levels were performed using one-way ANOVA and Tukey's studentized range (honestly significant difference or HSD) test, performed in SAS 9.0 (SAS Institute).

### Nisin survival assay

The objective of this assay was to measure relative survival characteristics of stationary phase *L. monocytogenes* 10403S,  $\Delta sigB$ ,  $\Delta sigL$ ,  $\Delta sigB/\Delta sigL$ , and  $\Delta lmo2570$  strains in the presence of an initially lethal concentration of nisin (150 AU/mL nisin). Strains were grown in BHI at 37°C with shaking overnight, followed by a 1% subculture and growth to logarithmic phase (OD<sub>600</sub> = 0.4), followed by a second subculture and growth to stationary phase (OD<sub>600</sub> = 1.0 + 3 h), followed by a third 1% subculture (0.5 into 50 mL, final concentration of  $\sim 2 \times 10^7$  CFU/mL) in a 300 mL flask (Bellco, Vineland, NJ). Nisin (150 AU/mL, pH 7.0) was added to the BHI, and cultures were incubated at 37°C with shaking for an additional 9 h. Bacterial numbers were determined before and after the addition of nisin. Specifically, samples were taken at 0, 0.5, 1, 2, 3, 4, 5, 6, and 9 h postaddition and spiral plated on BHI agar using a Spiral Biotech Autoplate<sup>®</sup> 4000 (Spiral Biotech, Norwood, MA). Colonies were enumerated with a QCount<sup>™</sup> (Spiral Biotech) after 24 h incubation at 37°C. Colony counts were transformed to log<sub>10</sub> CFU/mL.

Data from the nisin survival assay were used to calculate two parameters: (i) bacterial reduction after 0.5 h of nisin exposure and (ii) growth rate during recovery and re-growth (between 1 and 9 h after nisin exposure). Linear regression was used to determine the slope representing the change in bacterial numbers for each strain from 1 to 9 h (i.e., the period when viable cell numbers were increasing [re-growth]); this value represents the bacterial growth rate in log<sub>10</sub> growth/h. Statistical analyses were then performed on both parameters. First, a one-way ANOVA (with Dunnett's *t*-test or Tukey's studentized range [HSD] test) was performed to determine if (i) bacterial reduction or (ii) growth rate differed between the mutant strains and the parent strain. To determine if there were statistically significant interaction effects between the *sigB* and the *sigL* deletions, a two-way ANOVA (with Tukey's adjustment for multiple comparisons) was performed. In this

model, the dependent variable was either (i) bacterial reduction after nisin exposure or (ii) growth rate during re-growth; the independent variables included  $sigB + sigL + sigB^* sigL + replicate$ . The factors *sigB* and *sigL* in the model indicate the presence or absence of that gene in the strains tested.

### Results

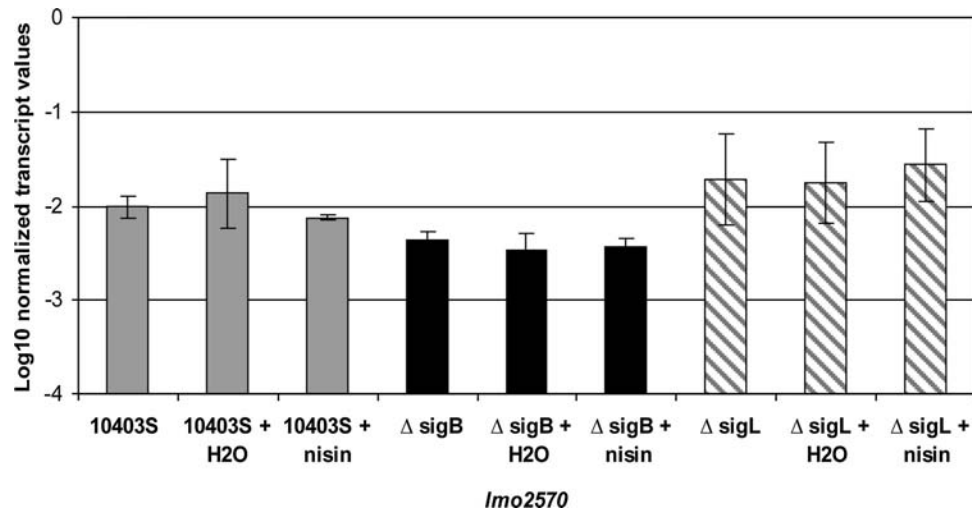
No intragenus competition was evident between *L. monocytogenes* and the other *Listeria* strains tested (Table 1). Specifically, no zoi occurred between any of the listerial species that were used as spotting strains (*L. innocua*, *L. ivanovii*, *L. welshimeri*, or *L. seeligeri*) and any of the *L. monocytogenes* lawn strains (10403S,  $\Delta sigB$ ,  $\Delta sigL$ , or  $\Delta sigB/\Delta sigL$ ; data not shown).

#### *lmo2570* is $\sigma^B$ , but not $\sigma^L$ dependent and does not contribute to resistance to nisin or SdpC

qRT-PCR was initially used to determine whether either  $\sigma^B$  or  $\sigma^L$  contributes to transcription of *lmo2570*, a putative bacteriocin immunity gene (Fig. 1). *lmo2570* transcript levels were consistently and significantly lower in the  $\Delta sigB$  strain as compared to the 10403S parent strain ( $p < 0.05$ ; Fig. 1), indicating  $\sigma^B$ -dependent transcription of *lmo2570*. While *lmo2570* transcript levels were consistently higher in the  $\Delta sigL$  strain as compared to the parent strain, this difference was not significant ( $p > 0.05$ ; Fig. 1). The presence of nisin at a sublethal concentration (75 AU/mL) did not affect *lmo2570* transcript levels (Fig. 1). Normalized *lmo2570* transcript levels in 10403S were low, ranging from 0.007 to 0.034. To put these low *lmo2570* transcript levels into biological context, in 10403S,  $\sim 0.02$  transcripts of *lmo2570* were present relative to the mean transcript levels of the highly expressed housekeeping genes, *rpoB* and *gap*, as indicated in Fig. 1. The *lmo2570* transcript levels observed in the present study are on the same order of magnitude as those reported previously for other  $\sigma^B$ -dependent genes [e.g., *opuCA* and *bsh* (Chan *et al.*, 2007)].

Spot-on-lawn assays were used to compare the sensitivities of *L. monocytogenes* 10403S and  $\Delta lmo2570$  to SdpC, the antimicrobial peptide whose cognate immunity gene shares amino acid similarity with *lmo2570*. Specifically, *B. subtilis* PY79 (which naturally produces SdpC) and *B. subtilis* EG351 (which overexpresses SdpC in the presence of isopropyl- $\beta$ -D-thiogalactopyranoside) were spotted on lawns of either 10403S or  $\Delta lmo2570$ . Zoi for 10403S and  $\Delta lmo2570$  (Table 2) did not differ significantly ( $p > 0.05$ ), indicating that *lmo2570* does not contribute to SdpC resistance. Neither 10403S nor  $\Delta lmo2570$  showed inhibition by 10403S (Table 2) or by any other *Listeria* species (data not shown), indicating absence of intragenus inhibition, at least among the strains tested.

To characterize the responses of stationary phase *L. monocytogenes* 10403S and  $\Delta lmo2570$  to nisin, we evaluated survival of  $\sim 2 \times 10^7$  CFU/mL 10403S or  $\Delta lmo2570$  in BHI in the presence of 150 AU nisin/mL (Fig. 2). Exposure to nisin for 30 min led to 4.0 and 3.9 log reductions in bacterial numbers for 10403S and  $\Delta lmo2570$ , respectively (Fig. 2), indicating no difference in nisin susceptibility between these strains. After the initial killing by nisin, bacterial numbers increased between 1 and 9 h post-nisin exposure, reflecting growth of cells that had survived nisin exposure. Specifically, after 9 h, bacterial numbers were 6.9 and 6.6 log for the parent strain and



**FIG. 1.** Normalized log-transformed *lmo2570* transcript levels for *Listeria monocytogenes* 10403S (gray bars),  $\Delta sigB$  (black bars), and  $\Delta sigL$  (hatched bars). Transcript levels were determined by quantitative reverse-transcriptase–polymerase chain reaction using RNA isolated from logarithmic phase ( $OD_{600} = 0.4$ ) *L. monocytogenes* that had been (i) incubated for 10 min after addition of nisin in sterile distilled water to yield a final concentration of 75 AU/mL nisin; (ii) incubated for 10 min after addition of an equivalent volume of sterile distilled water without nisin; or (iii) incubated for 10 min without any addition. Transcript levels were log transformed and normalized to the geometric mean of the transcript levels for the housekeeping genes *rpoB* and *gap*. Values represent mean transcript levels from three independent RNA collections; error bars indicate one standard deviation from each mean. Overall ANOVA showed a significant effect of the factor strain, but no effect of the factor condition (i.e., no addition, addition of water, or addition of nisin). Tukey’s test showed significantly lower transcript levels for *lmo2570* in the  $\Delta sigB$  strain as compared to the parent strain; transcript levels did not differ significantly between the  $\Delta sigL$  strain and the parent strain.

*Δlmo2570*, respectively, further supporting that *Δlmo2570* and 10403S susceptibilities to nisin do not differ.

**$\sigma^B$  and  $\sigma^L$  both contribute to resistance to the SdpC antimicrobial peptide produced by *B. subtilis***

Spot-on-lawn assays were used to compare the sensitivities of *L. monocytogenes* 10403S,  $\Delta sigB$ ,  $\Delta sigL$ , and  $\Delta sigB/\Delta sigL$  to the antimicrobial peptide SdpC. The zoi produced by *B. subtilis* PY79 did not differ between *L. monocytogenes* 10403S and  $\Delta sigB$  or  $\Delta sigL$ ; the zoi produced by PY79 on the  $\Delta sigB/\Delta sigL$  lawn was significantly larger than the zoi produced on 10403S ( $p < 0.05$ ; Table 2), suggesting the possibility that  $\sigma^B$  and  $\sigma^L$  contribute to SdpC resistance in an additive fashion. *B. subtilis* EG351 produced significantly larger zoi on  $\Delta sigB$  ( $28.2 \pm 1.6$  px),  $\Delta sigL$  ( $20.7 \pm 5.3$  px), and  $\Delta sigB/\Delta sigL$  lawns ( $32.0 \pm 2.6$  px), as compared to the zoi produced on 10403S ( $13.5 \pm 3.9$ ;

Table 2). Two-way ANOVA analyses of zoi data showed no significant “sigB\*sigL” interaction effect on SdpC sensitivity ( $p > 0.05$ ), further supporting the notion of additive (as compared to multiplicative) contributions of  $\sigma^B$  and  $\sigma^L$  to SdpC resistance. Overall, results from this assay indicate that alternative sigma factors  $\sigma^B$  and  $\sigma^L$  both contribute to resistance to SdpC.

**$\sigma^B$  and  $\sigma^L$  both contribute to response to the bacteriocin nisin**

A 30 min exposure to nisin (150 AU/mL) resulted in a 4.0 log reduction in bacterial numbers for stationary phase 10403S. By comparison, reduction of  $\Delta sigB$  bacterial numbers was significantly less (3.0 log reduction;  $p < 0.05$ ; Fig. 2), indicating increased nisin resistance of this strain relative to that of 10403S. Bacterial numbers for the  $\Delta sigL$  and  $\Delta sigB/\Delta sigL$

**TABLE 2. SPOT-ON-LAWN ASSAY RESULTS**

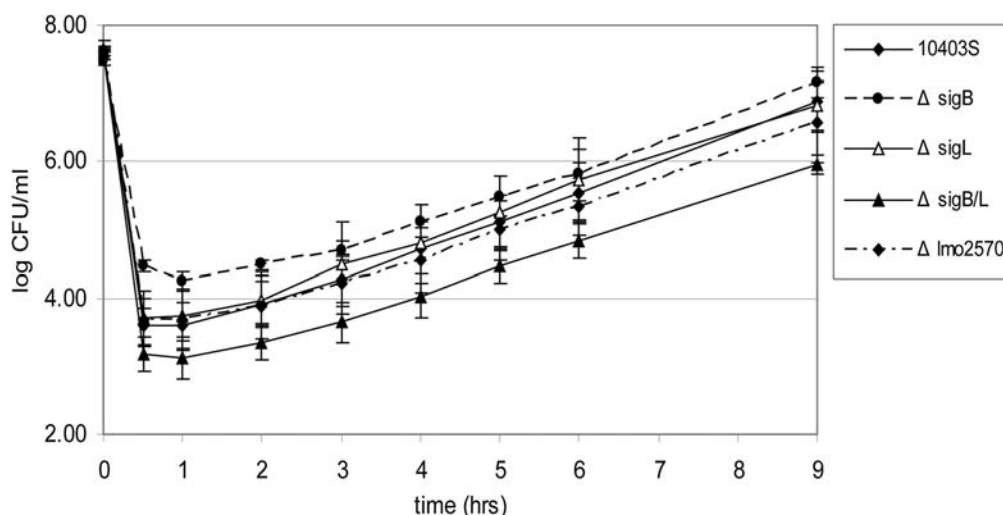
Strain spotted <sup>a</sup>	Mean zoi radii (SD) for <i>L. monocytogenes</i> strains <sup>b</sup>				
	10403S	$\Delta lmo2570$	$\Delta sigB$	$\Delta sigL$	$\Delta sigB/\Delta sigL$
<i>L. monocytogenes</i> 10403S	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
<i>B. subtilis</i> PY79	11.8 (1.6)	10.0 (2.2)	11.5 (4.9)	16.7 (0.6)	20.5 <sup>c</sup> (2.3)
<i>B. subtilis</i> EG351 (IPTG)	13.5 (3.9)	8.3 (2.9)	28.2 <sup>c</sup> (1.6)	20.7 <sup>c</sup> (5.3)	32.0 <sup>c</sup> (2.6)

<sup>a</sup>Strains spotted on the lawns are listed in left column; the average zoi radius around each spot is shown for each lawn, with SD in parentheses.

<sup>b</sup>Radii were determined from three independent experiments by measuring diameters of the zoi in pixels using Adobe® Photoshop® CS.

<sup>c</sup>Values that are significantly different ( $p < 0.05$ ; one-way ANOVA with Dunnett’s *t*-test) from the zoi produced on the *L. monocytogenes* 10403S lawn.

IPTG, isopropyl- $\beta$ -D-thiogalactopyranoside; zoi, zone of inhibition; SD, standard deviations; ANOVA, analysis of variance.



**FIG. 2.** Viable numbers of stationary-phase *L. monocytogenes* 10403S,  $\Delta lmo2570$ ,  $\Delta sigB$ ,  $\Delta sigL$ , and  $\Delta sigB/\Delta sigL$  at various time points after exposure to 150 AU/mL nisin. Values are reported as log CFU/mL. Data shown represent the average of four independent experiments; error bars represent one standard deviation from each mean.

strains were reduced by 3.9 and 4.5 log; these reductions were not significantly different from that of 10403S ( $p > 0.05$ ; Dunnett's t-test). Interestingly, log reduction for the  $\Delta sigB/\Delta sigL$  strain was significantly ( $p > 0.05$ ; Tukey's HSD) greater (4.5 log) as compared to the  $\Delta sigB$  strain (3.0 log reduction), indicating a significant effect of the *sigL* deletion on nisin killing in a  $\Delta sigB$  background. Based on the reductions in bacterial numbers between 0 and 0.5 h, two-way ANOVA analyses found a significant  $sigL \times sigB$  interaction effect on survival after nisin exposure, indicating that the effect of one sigma factor on survival differs depending on the presence or absence of the other sigma factor.

After initial killing by nisin, all strains showed re-growth between 1 and 9 h post-nisin exposure. Rates of re-growth, as represented by the slopes of the graphs between 1 and 9 h postexposure, were compared among the strains. While the growth rate for the  $\Delta sigL$  strain (0.39 log CFU/h) was not significantly different from that of the parent strain (0.41 log CFU/h), both the  $\Delta sigB$  and the  $\Delta sigB/\Delta sigL$  strains showed significantly slower growth rates (0.36 log CFU/h for both;  $p < 0.05$ , Dunnett's t-test) as compared to the parent strain. Although no significant  $sigB \times sigL$  interaction effect on nisin survival was identified by two-way ANOVA analysis, the *sigB* deletion had a significant effect on growth rate, with *sigB* deletion strains (i.e.,  $\Delta sigB$  and  $\Delta sigB/\Delta sigL$ ) showing slower growth rates as compared to the corresponding strains with intact *sigB* genes.

## Discussion

We hypothesized that alternative sigma factors  $\sigma^B$  and  $\sigma^L$  and the hypothetical bacteriocin immunity gene, *lmo2570*, contribute to *L. monocytogenes* antimicrobial response. This hypothesis was based on previous observations, including (i)  $\sigma^B$  and its homolog  $\sigma^F$  contribute to antimicrobial response in other Gram-positive organisms (Bischoff and Berger-Bachi, 2001; Mascher *et al.*, 2003; Michele *et al.*, 1999), (ii) *L. monocytogenes*  $\sigma^L$  controls sensitivity to class IIa bacteriocins, mesentericin Y105, pediocin PA-1, and enterocin A, and (iii) the

putative  $\sigma^B$ -dependent *lmo2570* has sequence homology to the *B. subtilis* bacteriocin immunity gene, *sdpI*. To test our hypothesis, we assessed the sensitivities of strains with null mutations in *sigB*, *sigL*, *sigB/sigL*, or *lmo2570* to the antimicrobial peptides SdpC and nisin. We also characterized transcription of *lmo2570* in 10403S,  $\Delta sigB$ , and  $\Delta sigL$  strains exposed to a subminimal inhibitory concentration of nisin. Our results show that (i) while *lmo2570* is  $\sigma^B$  dependent, it does not contribute to resistance to SdpC or nisin and (ii) both  $\sigma^B$  and  $\sigma^L$  contribute to resistance to the antimicrobial peptide SdpC, as shown by results from spot-on-lawn assays. In addition, both  $\sigma^B$  and  $\sigma^L$  affect *L. monocytogenes* sensitivity to nisin in broth survival assays. Specifically, while loss of only *sigB* renders the resulting strain more resistant to nisin than the parent strain, loss of *sigB* in a  $\Delta sigL$  background leads to reduced nisin resistance relative to the original parent strain.

The effects of antimicrobial peptides on *L. monocytogenes* appear to differ depending on the class of peptide, the strain, initial number of bacteria, growth phase, and the assay used for evaluation. To illustrate,  $\sigma^B$  was reported previously to contribute to *L. monocytogenes* tolerance to nisin or lactacin 3147 in broth assays (Begley *et al.*, 2006), but not to nisin, lactacin 3147, or sakacin A resistance in agar overlay assays (Moorhead and Dykes, 2003; Begley *et al.*, 2006). Moorhead and Dykes (2003) showed that an *L. monocytogenes* serotype 1/2a wild-type strain was less resistant to nisin than a serotype 4c wild-type strain, suggesting differences in antimicrobial sensitivities among strains. It is also likely that other environmental stresses (in addition to the presence of the antimicrobial peptide) imposed upon the cells also evoke differential phenotypic responses from the cells (e.g., exposure to low pH induces  $\sigma^B$  activity in *L. monocytogenes*), which may provide cross-resistance to multiple stresses (Ferreira *et al.*, 2003).

### *lmo2570* is $\sigma^B$ dependent, but does not contribute to antimicrobial resistance

*L. monocytogenes* *lmo2570* is 45% similar at the amino acid level to the *B. subtilis* immunity protein SdpI, which confers

immunity against SdpC (Butcher and Helmann, 2006; Ellermeier *et al.*, 2006); therefore, we hypothesized that *lmo2570* may play a role in antimicrobial immunity in *L. monocytogenes*. *lmo2570* was predicted as  $\sigma^B$  dependent in previous microarray experiments (Kazmierczak *et al.*, 2003). As previous reports have shown that bacteriocin immunity genes can contribute to resistance to multiple antimicrobials (Matsuno-Nakano and Kuramitsu, 2006), in addition to examining its role in SdpC resistance, we also tested the contributions of *lmo2570* to resistance to the commercially available bacteriocin, nisin. The  $\Delta lmo2570$  strain did not show reduced sensitivity to either SdpC or nisin. Exposure to nisin did not induce transcription of *lmo2570* in either the wild-type or any of the mutant strains. *lmo2570* thus does not appear to be important for SdpC or nisin resistance in *L. monocytogenes*. A role for this gene in resistance to other bacteriocins or in contributing to nisin and SdpC resistance under environmental conditions not tested here cannot be excluded by our data, however. Our confirmation of *lmo2570* as  $\sigma^B$  dependent suggests a role for *lmo2570* in *L. monocytogenes* survival or growth under conditions that remain to be defined.

#### $\sigma^B$ and $\sigma^L$ both contribute to *L. monocytogenes* response to SdpC and nisin

We found clear evidence that alternative sigma factors  $\sigma^L$  and  $\sigma^B$  both contribute to SdpC resistance. Specifically, as determined in a spot-on-lawn assay, we showed that both the  $\Delta sigB$  and the  $\Delta sigL$  strains were significantly more susceptible to the bactericidal effect of the antimicrobial peptide SdpC produced by *B. subtilis* EG351 than the otherwise isogenic 10403S parent strain. Characterization of a  $\Delta sigB/\Delta sigL$  double-mutant strain suggested that deletion of both genes had an additive, but not an interactive, effect on SdpC resistance. However, deletions of both  $\Delta sigB$  and  $\Delta sigL$  had an interactive effect on *L. monocytogenes* resistance to nisin. Specifically, while the  $\Delta sigB/\Delta sigL$  strain showed decreased resistance to nisin as compared to the  $\Delta sigB$  strain, the  $\Delta sigB$  strain showed increased resistance to nisin as compared to the parent strain, which has both *sigB* and *sigL* intact. The interactive effect observed after the loss of both *sigB* and *sigL* may indicate that at least some genes important for recovery and re-growth after nisin exposure are coregulated, either directly or indirectly, by these alternative sigma factors. We also found that re-growth of both the  $\Delta sigB$  and the  $\Delta sigB/\Delta sigL$  strains after nisin exposure was slower than that of the parent strain, consistent with previous observation that  $\sigma^B$  is important for *B. subtilis* recovery after rifampin treatment (Bandow *et al.*, 2002). The overall observation that a deletion of the gene encoding  $\sigma^L$  (i.e., a single deletion) does not affect *L. monocytogenes* resistance to nisin is consistent with observations by Dalet *et al.* (2000), who reported that  $\sigma^L$  (which has also been designated as RpoN) is not involved in *L. monocytogenes* nisin resistance. Relative to its otherwise isogenic parent, a  $\Delta rpoN$  *L. monocytogenes* strain (i.e., a strain lacking  $\sigma^L$ ) has previously shown increased resistance to the class IIa nonlantibiotic bacteriocins mesentericin Y105, pediocin PA-1, and enterocin A (Robichon *et al.*, 1997; Dalet *et al.*, 2000), consistent with our finding that  $\sigma^L$  contributes to resistance to some bacteriocins (i.e., SdpC). Our findings, as well as previous findings by others (e.g., Dalet *et al.*, 2000), thus support that different regulatory elements are critical for the ability of *L. mono-*

*cytogenes*, and other bacteria, to respond to different bacteriocins, a notion consistent with the diverse nature of this group of antibacterial compounds.

Overall, our data indicate that  $\sigma^B$  and  $\sigma^L$  both contribute to the ability of *L. monocytogenes* to respond to antimicrobials. Regulatory interactions among multiple alternative sigma factors also have been shown to contribute to antibiotic resistance in *B. subtilis*. Specifically, three ( $\sigma^M$ ,  $\sigma^W$ , and  $\sigma^X$ ) of the seven *B. subtilis* extracytoplasmic function alternative sigma factors have overlapping regulons that contribute to antibiotic resistance, as demonstrated by the greatly enhanced sensitivity of a triple MWX mutant to various antimicrobials, including nisin (Mascher *et al.*, 2007). Strains bearing single or double mutations in the genes encoding these alternative sigma factors displayed considerably less antimicrobial sensitivity than the strain with the triple mutation (Mascher *et al.*, 2007). Thus, in combination with previous studies, our data support a model in which multiple alternative sigma factors contribute to regulatory networks important for fine-tuning transcriptional regulation of gene expression to help optimize bacterial cell resistance to antimicrobial peptides.

#### Conclusions

Alternative sigma factors have been shown to regulate genes and operons critical for resistance to antimicrobials in various bacteria, including *B. subtilis*, *L. monocytogenes*, *Salmonella enterica* serovar Typhimurium, *S. aureus*, and *Vibrio cholerae* (Robichon *et al.*, 1997; Crouch *et al.*, 2005; Zhang *et al.*, 2005; Butcher and Helmann, 2006; Mathur *et al.*, 2007). Our data indicate that  $\sigma^B$  and  $\sigma^L$ , as well as the simultaneous presence of both  $\sigma^B$  and  $\sigma^L$ , contribute to antimicrobial response in *L. monocytogenes* in a manner that is dependent on the antimicrobial that is present. The results reported in this study provide further evidence of the importance of regulatory networks for fine-tuning *L. monocytogenes* responses to changing environmental conditions (Chaturongakul *et al.*, 2008).

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#### Disclosure Statement

No competing financial interests exist.

#### References

- Abee T and Delves-Broughton J. Bacteriocins—nisin. In: *Food Preservatives*. Russell NJ and Gould GW (eds.). New York City: Springer, 2003, pp. 146–169.
- Bandow JE, Brotz H, and Hecker M. *Bacillus subtilis* tolerance of moderate concentrations of rifampin involves the  $\sigma^B$ -dependent general and multiple stress response. *J Bacteriol* 2002;184:459–467.

- Becker LA, Cetin MS, Hutkins RW, and Benson AK. Identification of the gene encoding the alternative sigma factor  $\sigma^B$  from *Listeria monocytogenes* and its role in osmotolerance. *J Bacteriol* 1998;180:4547–4554.
- Begley M, Hill C, and Ross RP. Tolerance of *Listeria monocytogenes* to cell envelope-acting antimicrobial agents is dependent on SigB. *Appl Environ Microbiol* 2006;72:2231–2234.
- Benkerroum N and Sandine WE. Inhibitory action of nisin against *Listeria monocytogenes*. *J Dairy Sci* 1988;71:3237–3245.
- Bischoff M and Berger-Bachi B. Teicoplanin stress-selected mutations increasing  $\sigma^B$  activity in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001;45:1714–1720.
- Bishop DK and Hinrichs DJ. Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of *in vitro* stimulation on lymphocyte subset requirements. *J Immunol* 1987;139:2005–2009.
- Bonnet M, Rafi MM, Chikindas ML, and Montville TJ. Bioenergetic mechanism for nisin resistance, induced by the acid tolerance response of *Listeria monocytogenes*. *Appl Environ Microbiol* 2006;72:2556–2563.
- Bruno ME, Kaiser A, and Montville TJ. Depletion of proton motive force by nisin in *Listeria monocytogenes* cells. *Appl Environ Microbiol* 1992;58:2255–2259.
- Butcher BG and Helmann JD. Identification of *Bacillus subtilis*  $\sigma^W$ -dependent genes that provide intrinsic resistance to antimicrobial compounds produced by *Bacilli*. *Mol Microbiol* 2006;60:765–782.
- Chan YC, Boor KJ, and Wiedmann M.  $\sigma^B$ -dependent and  $\sigma^B$ -independent mechanisms contribute to transcription of *Listeria monocytogenes* cold stress genes during cold shock and cold growth. *Appl Environ Microbiol* 2007;73:6019–6029.
- Chan PF, Foster SJ, Ingham E, and Clements MO. The *Staphylococcus aureus* alternative sigma factor  $\sigma^B$  controls the environmental stress response but not starvation survival or pathogenicity in a mouse abscess model. *J Bacteriol* 1998;180:6082–6089.
- Chaturongakul S, Raengpradub S, Wiedmann M, and Boor KJ. Modulation of stress and virulence in *Listeria monocytogenes*. *Trends Microbiol* 2008;16:388–396.
- Cleveland J, Montville TJ, Nes IF, and Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol* 2001;71:1–20.
- Crouch M-L, Becker LA, Bang I-S, Tanabe H, Ouellette AJ, and Fang FC. The alternative sigma factor  $\sigma^E$  is required for resistance of *Salmonella enterica* serovar Typhimurium to antimicrobial peptides. *Mol Microbiol* 2005;56:789–799.
- Dalet K, Briand C, Cenatiempo Y, and Hechard Y. The *rpoN* gene of *Enterococcus faecalis* directs sensitivity to subclass IIa bacteriocins. *Curr Microbiol* 2000;41:441–443.
- de los Santos PE, Parret AHA, and de Mot R. Stress-related *Pseudomonas* genes involved in production of bacteriocin LlpA. *FEMS Microbiol Lett* 2005;244:243–250.
- Eijsink VG, Axelsson L, Diep DB, Havarstein LS, Holo H, and Nes IF. Production of class II bacteriocins by lactic acid bacteria; an example of biological warfare and communication. *Antonie Leeuwenhoek* 2002;81:639–654.
- Ellermeier CD, Hobbs EC, Gonzalez-Pastor JE, and Losick R. A three-protein signaling pathway governing immunity to a bacterial cannibalism toxin. *Cell* 2006;124:549–559.
- Engelke G, Gutowski-Eckel Z, Kiesau P, Siegers K, Hammelmann M, and Entian KD. Regulation of nisin biosynthesis and immunity in *Lactococcus lactis* 6F3. *Appl Environ Microbiol* 1994;60:814–825.
- Ferreira A, O'Byrne CP, and Boor KJ. Role of  $\sigma^B$  in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. *Appl Environ Microbiol* 2001;67:4454–4457.
- Ferreira A, Sue D, O'Byrne CP, and Boor KJ. Role of *Listeria monocytogenes*  $\sigma^B$  in survival of lethal acidic conditions and in the acquired acid tolerance response. *Appl Environ Microbiol* 2003;69:2692–2698.
- Gandhi M and Chikindas ML. *Listeria*: a foodborne pathogen that knows how to survive. *Int J Food Microbiol* 2007;113:1–15.
- Gravesen A, Jydegaard Axelsen AM, Mendes da Silva J, Hansen TB, and Knochel S. Frequency of bacteriocin resistance development and associated fitness costs in *Listeria monocytogenes*. *Appl Environ Microbiol* 2002a;68:756–764.
- Gravesen A, Ramnath M, Rechinger KB, Andersen N, Jansch L, Hechard Y, Hastings JW, and Knochel S. High-level resistance to class IIa bacteriocins is associated with one general mechanism in *Listeria monocytogenes*. *Microbiology* 2002b;148:2361–2369.
- Ho SN, Hunt HD, Horton RM, Pullen JK, and Pease LR. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* 1989;77:51–59.
- Huot E, Barrena-Gonzalez C, and Petitdemange H. Comparative effectiveness of nisin and bacteriocin J46 at different pH values. *Lett Appl Microbiol* 1996;22:76–79.
- Jack RW, Tagg JR, and Ray B. Bacteriocins of Gram-positive bacteria. *Microbiol Rev* 1995;59:171–200.
- Kazmierczak MJ, Mithoe SC, Boor KJ, and Wiedmann M. *Listeria monocytogenes*  $\sigma^B$  regulates stress response and virulence functions. *J Bacteriol* 2003;185:5722–5734.
- Kazmierczak MJ, Wiedmann M, and Boor KJ. Contributions of *Listeria monocytogenes*  $\sigma^B$  and PrfA to expression of virulence and stress response genes during extra- and intracellular growth. *Microbiology* 2006;152:1827–1838.
- Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* 1993;12:39–85.
- Liu W and Hansen JN. Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. *Appl Environ Microbiol* 1990;56:2551–2558.
- Mascher T, Hachmann A-B, and Helmann JD. Regulatory overlap and functional redundancy among *Bacillus subtilis* extracytoplasmic function  $\sigma$  factors. *J Bacteriol* 2007;189:6919–6927.
- Mascher T, Margulis NG, Wang T, Ye RW, and Helmann JD. Cell wall stress responses in *Bacillus subtilis*: the regulatory network of the bacitracin stimulon. *Mol Microbiol* 2003;50:1591–1604.
- Mathur J, Davis BM, and Waldor MK. Antimicrobial peptides activate the *Vibrio cholerae*  $\sigma^E$  regulon through an OmpU-dependent signalling pathway. *Mol Microbiol* 2007;63:848–858.
- Matsumoto-Nakano M and Kuramitsu HK. Role of bacteriocin immunity proteins in the antimicrobial sensitivity of *Streptococcus mutans*. *J Bacteriol* 2006;188:8095–8102.
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, and Tauxe RV. Food-related illness and death in the United States. *Emerg Infect Dis* 1999;5:607–625.
- Michele TM, Ko C, and Bishai WR. Exposure to antibiotics induces expression of the *Mycobacterium tuberculosis sigF* gene: implications for chemotherapy against Mycobacterial persisters. *Antimicrob Agents Chemother* 1999;43:218–225.
- Moorhead SM and Dykes GA. The role of the *sigB* gene in the general stress response of *Listeria monocytogenes* varies be-



- tween a strain of serotype 1/2a and a strain of serotype 4c. *Curr Microbiol* 2003;46:461–466.
- Muriana PM. Bacteriocins for control of *Listeria* spp. in food. *J Food Prot* 1996 Supplement;59:54–63.
- Nissen-Meyer J and Nes IF. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. *Arch Microbiol* 1997;167:67–77.
- Raengpradub S, Wiedmann M, and Boor KJ. Comparative analysis of the  $\sigma^B$ -dependent stress responses in *Listeria monocytogenes* and *Listeria innocua* strains exposed to selected stress conditions. *Appl Environ Microbiol* 2008;74:158–171.
- Robichon D, Gouin E, Debarbouille M, Cossart P, Ceniempo Y, and Hechard Y. The *rpoN* ( $\sigma^{54}$ ) gene from *Listeria monocytogenes* is involved in resistance to mesentericin Y105, an antibacterial peptide from *Leuconostoc mesenteroides*. *J Bacteriol* 1997;179:7591–7594.
- Schwab U, Bowen B, Nadon C, Wiedmann M, and Boor KJ. The *Listeria monocytogenes* *prfAP2* promoter is regulated by sigma B in a growth phase dependent manner. *FEMS Microbiol Lett* 2005;245:329–336.
- Sue D, Fink D, Wiedmann M, and Boor KJ.  $\sigma^B$ -dependent gene induction and expression in *Listeria monocytogenes* during osmotic and acid stress conditions simulating the intestinal environment. *Microbiology* 2004;150:3843–3855.
- Venema K, Venema G, and Kok J. Lactococcal bacteriocins: mode of action and immunity. *Trends Microbiol* 1995;3:299–304.
- Wiedmann M, Arvik TJ, Hurley RJ, and Boor KJ. General stress transcription factor  $\sigma^B$  and its role in acid tolerance and virulence of *Listeria monocytogenes*. *J Bacteriol* 1998;180:3650–3656.
- Woodling SE and Moraru CI. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surfaces. *J Food Sci* 2005;70:m345–m351.
- Youngman P, Perkins JB, and Losick R. Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. *Plasmid* 1984;12:1–9.
- Zhang H, Morikawa K, Ohta T, and Kato Y. *In vitro* resistance to the CS $\alpha\beta$ -type antimicrobial peptide ASABF- $\alpha$  is conferred by overexpression of sigma factor *sigB* in *Staphylococcus aureus*. *J Antimicrob Chemother* 2005;55:686–691.

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