Tolerance of *Listeria monocytogenes* to Cell Envelope-Acting Antimicrobial Agents Is Dependent on SigB

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**Mutation of sigB impairs the ability of *Listeria monocytogenes* to grow in sublethal levels, and to survive in lethal concentrations, of the bacteriocins nisin and lacticin 3147 and the antibiotics ampicillin and penicillin G. SigB may therefore represent an attractive target for the development of new control and treatment strategies for this important pathogen.**

*Listeria monocytogenes* is the etiological agent of listeriosis, an opportunistic infection that affects primarily pregnant and immunocompromised individuals. Food is the major source of infection, and those foods most frequently implicated include soft cheeses, dairy products, salads, and refrigerated ready-to-eat products (6). The β-lactams ampicillin and penicillin G are the antibiotics of choice in the treatment of listeriosis (12); however, despite their therapeutic use, up to one-third of patients die (16). As a result, listeriosis is a significant cause of mortality due to food-borne disease; it is estimated to be responsible for approximately 27.6% of food-related deaths in the United States annually (16). *L. monocytogenes* is recognized as a serious risk to public health and food safety, and the bacterium was responsible for 71% of all recalls of food products due to bacterial contamination in the United States between 1993 and 1998 (25).

*L. monocytogenes* must overcome the numerous environmental extremes encountered during food processing, handling, and storage and in vivo following consumption. One important mediator of the bacterium’s stress responses is the alternative sigma factor SigB. It has been shown to assist the in vitro survival of cells under a variety of environmental insults, including low pH, high osmolarity, and elevated bile concentrations, and during oxidative stress and carbon starvation (1–3, 7, 8). It is also becoming increasingly evident that SigB regulates stress loci important for intrahost survival, such as bile salt hydrolase (*bsh*) (3, 22), and also assists in the regulation of dedicated virulence factors such as the principal virulence regulator PrfA (19, 20).

**Bioinformatic analysis of the SigB regulon.** The entire *L. monocytogenes* SigB regulon has not yet been defined; however, initial efforts using a 208-gene microarray identified 55 genes that were regulated (GTTTN_3_17;GGGWAT was entered as a “Pattern search” in the genome web server ListiList [http://genolist.pasteur.fr/ListiList/]), and the search was restricted to patterns located within 350 bp upstream of a predicted open reading frame and showing one mismatch to the consensus) revealed a number of loci which, based on homology searches, may contribute to tolerance of antimicrobial compounds. These loci encode putative efflux pumps, penicillin binding proteins, autolysins, or proteins involved in the

![Table 1. Genetic loci that are regulated/putatively regulated by SigB and that may contribute to the tolerance of antimicrobial agents](http://aem.asm.org/).

<table>
<thead>
<tr>
<th>Genetic locus*</th>
<th>Function/putative function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell wall related</strong></td>
<td></td>
</tr>
<tr>
<td>lmo2591</td>
<td>N-Acetylmuramidase</td>
</tr>
<tr>
<td>lmo2691</td>
<td>Autolysin</td>
</tr>
<tr>
<td>lmo2558</td>
<td>Autolysin (ami)</td>
</tr>
<tr>
<td>lmo1076</td>
<td>Autolysin</td>
</tr>
<tr>
<td>lmo0971–lmo0974</td>
<td></td>
</tr>
<tr>
<td>(dlt operon)</td>
<td>t-Alanine transfer in lipoteichoic acids</td>
</tr>
<tr>
<td>lmo2229</td>
<td>Penicillin binding protein</td>
</tr>
<tr>
<td>lmo2754</td>
<td>Penicillin binding protein</td>
</tr>
<tr>
<td>lmo0129</td>
<td>Autolysin</td>
</tr>
<tr>
<td>marD</td>
<td>UDP-N-acetylmuramoylalanine 2-glutamate ligase</td>
</tr>
<tr>
<td>lmo2238</td>
<td>N-Acetylglucosamine-6-phosphate isomerase</td>
</tr>
<tr>
<td>lmo2427</td>
<td>Cell division protein</td>
</tr>
<tr>
<td>lmo2504</td>
<td>Cell wall binding protein</td>
</tr>
<tr>
<td>enaC</td>
<td>Ethanolamine ammonia lyase</td>
</tr>
<tr>
<td>lmo0731</td>
<td>Mannose-specific PTS component*</td>
</tr>
<tr>
<td>meCA</td>
<td>Competence negative regulator</td>
</tr>
<tr>
<td>segA</td>
<td>Metallo-beta-lactamase superfamily protein</td>
</tr>
<tr>
<td>lmo0956</td>
<td>N-Acetylglucosamine-6-phosphate deacylase</td>
</tr>
</tbody>
</table>

**Efflux pumps**

| lmo1300 | Efflux pump |
| lmo1409 | | |
| lmo2463 | Efflux pump (MdrL) |
| lmo1226 | Efflux pump |

**Stress**

| lmo0227 | Molecular chaperone (HtrO) |
| lmo0292 | Molecular chaperone (HtrA) |
| lmo1860 | Peptidyl methionine sulfoxide reductase |

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* Numbers refer to the National Center for Biotechnology Information (NCBI) annotation numbers.

* Previously shown to be positively regulated by SigB.

* Previously shown to be negatively regulated by SigB.

* A consensus SigB promoter was identified using the *L. monocytogenes* EGDe genome web server ListiList (http://genolist.pasteur.fr/ListiList/), suggesting that these genes may be regulated by SigB.

* PTS, phosphotransferase system.
modification of the cell envelope (Table 1). It was therefore
decided to investigate whether the SigB regulon contributes to
the tolerance of bacteriocins (peptide antimicrobials) and
antibiotics (nonpeptide antimicrobials).

SigB contributes to bacteriocin tolerance. The growth rate of
a sigB mutant (a nonpolar internal deletion mutant) (24) was
comparable to that of the wild type when grown in brain heart
infusion (BHI) broth at 37°C, indicating that SigB is not required
for growth under normal physiological conditions (data not
shown). Addition of sublethal levels of nisin (a bacteriocin cur-
cently used as a biopreservative) (5) or lacticin 3147 (a bacteriocin
that shows potential as a biopreservative) (11) had noticeable
effects on growth (Fig. 1A and B). Survival assays performed with
lethal levels of each bacteriocin revealed more-dramatic differ-
cences at every concentration tested. Although there was an initial
kill of the parent strain at the levels shown in Fig. 1, it was
detected at high numbers over the 6-h experiment, whereas the
mutant was not detected after 1 h in the broth supplemented with
nisin or after 4 h in the broth supplemented with lacticin. At
bacteriocin levels higher than these, the parent was rapidly and
completely inactivated. Interestingly, agar well diffusion assays
(not shown), overlay assays (Fig. 1E and F), and MIC determi-
nations by the broth dilution method (using twofold serial dilu-
tions of the bacteriocins in BHI broth) (data not shown) did not
reveal any differences between the strains. However, these exper-
iments are analyzed at a single time point after 24-h incubation
periods, after which the numbers of wild-type and mutant bacteria
were identical in our experiments (data not shown). Overall, our
data strongly suggest an important role for SigB in bacteriocin
tolerance, which may be important for the survival of L. mono-
cytogenes in foods. The results also highlight the importance of
assay selection in the assessment of the contribution of a specific
locus to bacteriocin tolerance. For example, Moorehead and
Dykes (17) concluded that SigB did not play a role in L. mono-

FIG. 1. (A to D) Growth and survival of wild-type L. monocytogenes 10403S (■) and the sigB mutant (○)
in broth supplemented with bacteriocins. Overnight cultures were inoculated (3%) into BHI broth supplemented with either −45 AU/ml lacticin 3147 (A), 50 µg/ml nisin (B), −100 AU/ml lacticin 3147 (C), or 300 µg/ml nisin (D). (Lacticin was prepared as described in reference 14, and nisin powder was obtained from Sigma.) Cultures were incubated with shaking at 37°C. Cell growth was measured spectrophotometrically by determining the optical density at 600
nm. Viable cell counts were performed by serial dilution in one-quarter-strength Ringer’s solution and enumeration on BHI agar. Error bars,
standard deviations from three independent experiments. (E and F) Overlay assays of wild-type (wt) and sigB mutant (∆sigB) L. monocytogenes
with bacteriocin-producing strains. Ten microliters of the lacticin 3147 overproducer Lactococcus lactis subsp. cremoris MG1363 plus pMRC01 and
pOM02 (E) or the nisin producer Lactococcus lactis subsp. cremoris pNZ9700 (F) was spotted onto GM17 agar. After overnight incubation at 30°C,
spots were UV treated for 10 min and then overlaid with wild-type L. monocytogenes 10403S or the ∆sigB strain in BHI semisolid agar. Plates were
incubated overnight at 37°C.
cytogenes tolerance of the bacteriocins nisin and sakacin A as determined by overlay assays.

**SigB contributes to antibiotic tolerance.** The abilities of the wild type and the sigB mutant to withstand exposure to penicillin G and ampicillin were compared, because these are the antibiotics of choice in the treatment of listeriosis (12). Initial agar diffusion experiments with antibiotic disks (Oxoid) revealed that the diameters of the zones of bacterial growth inhibition surrounding the filter disks were similar for the two strains (Fig. 2E and F). In addition, there was no difference in MICs as determined by the broth dilution method (using twofold serial dilution of the antibiotics in BHI broth) (data not shown). However, detailed growth curves (Fig. 2A and B) and survival assays (Fig. 2C and D) revealed that the sigB mutant was significantly impaired in growth in sublethal levels of each antibiotic and was killed more rapidly at lethal levels. It is therefore possible that SigB contributes significantly to the survival of *L. monocytogenes* in clinical settings. Designing future therapies to target SigB may improve the treatment of listeriosis, which is presently inefficient.

**Role of SigB in the tolerance of antimicrobial agents.** The exact role of SigB in *L. monocytogenes* tolerance of antimicrobial agents has yet to be determined. It is likely that SigB plays a role in controlling membrane characteristics (e.g., charge or lipid composition) and that altering these properties significantly affects the cell’s ability to tolerate antimicrobial compounds. It is noteworthy that bacteriocins and antibiotics act on bacterial cell walls and that *L. monocytogenes* sigB mutants have been shown to be significantly more sensitive than parent cells to stresses that exert their effects on the cell wall, such as bile (3). In addition, cell surface alterations have previously been shown to be important in the tolerance of several bacteriocins and antibiotics (4, 9, 10, 18, 23). SigB may also regulate general stress proteins or proteins involved in extrusion of...
antimicrobials out of the cell. Indeed, hraA (lmo0292), which encodes a putative molecular chaperone that has been shown to be involved in tolerance of penicillin G (21), and mdrL (lmo1409), which encodes an antibiotic efflux pump (15), both possess consensus SigB binding sites. In conclusion, we report our novel observation that SigB contributes positively to L. monocytogenes tolerance of the bacteriocins nisin and lacticin 3147 and of the antibiotics ampicillin and penicillin G. Because SigB may contribute to the survival of L. monocytogenes both in the food-processing environment and in vivo during infection, it may represent an attractive target for the development of new control and treatment strategies for this important pathogen.

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REFERENCES


