The *Listeria monocytogenes* $\sigma^B$ Regulon and Its Virulence-Associated Functions Are Inhibited by a Small Molecule

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ABSTRACT The stress-responsive alternative sigma factor $\sigma^B$ is conserved across diverse Gram-positive bacterial genera. In *Listeria monocytogenes*, $\sigma^B$ regulates transcription of $>150$ genes, including genes contributing to virulence and to bacterial survival under host-associated stress conditions, such as those encountered in the human gastrointestinal lumen. An inhibitor of *L. monocytogenes* $\sigma^B$ activity was identified by screening $\sim57,000$ natural and synthesized small molecules using a high-throughput cell-based assay. The compound fluoro-phenyl-styrene-sulfonamide (FPSS) (IC$_{50}$ = 3.5 $\mu$M) downregulated the majority of genes previously identified as members of the $\sigma^B$ regulon in *L. monocytogenes* 10403S, thus generating a transcriptional profile comparable to that of a 10403S $\Delta$sigB strain. Specifically, of the 208 genes downregulated by FPSS, 75% had been identified previously as positively regulated by $\sigma^B$. Downregulated genes included key virulence and stress response genes, such as *inlA*, *inlB*, *bsh*, *hfg*, *opuC*, and *bile*. From a functional perspective, FPSS also inhibited *L. monocytogenes* invasion of human intestinal epithelial cells and bile salt hydrolase activity. The ability of FPSS to inhibit $\sigma^B$ activity in both *L. monocytogenes* and *Bacillus subtilis* indicates its utility as a specific inhibitor of $\sigma^B$ across multiple Gram-positive genera.

IMPORTANCE The $\sigma^B$ transcription factor regulates expression of genes responsible for bacterial survival under changing environmental conditions and for virulence; therefore, this alternative sigma factor is important for transmission of *L. monocytogenes* and other Gram-positive bacteria. Regulation of $\sigma^B$ activity is complex and tightly controlled, reflecting the key role of this factor in bacterial metabolism. We present multiple lines of evidence indicating that fluoro-phenyl-styrene-sulfonamide (FPSS) specifically inhibits activity of $\sigma^B$ across Gram-positive bacterial genera, i.e., in both *Listeria monocytogenes* and *Bacillus subtilis*. Therefore, FPSS is an important new tool that will enable novel approaches for exploring complex regulatory networks in *L. monocytogenes* and other Gram-positive pathogens and for investigating small-molecule applications for controlling pathogen transmission.

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*L. monocytogenes* causes a rare but potentially fatal foodborne disease called listeriosis. With its high fatality rate, listeriosis accounts for $\sim$10% of all deaths from food-borne diseases in the United States (1). *L. monocytogenes* can transition from a saprotroph existence under a wide range of environmental conditions (2) to intracellular infection in a diverse array of hosts (3). The ability of *L. monocytogenes* to transform from saprotroph to intracellular pathogen is influenced by regulatory networks that enable bacterial survival and control virulence gene expression in response to environmental signals (4).

Sigma B is one important component of a network that links environmental stress survival and virulence in *L. monocytogenes* (5, 6). Sigma factors are dissociable subunits of prokaryotic RNA polymerase. The association of a specific alternative sigma factor, e.g., $\sigma^B$, with core RNA polymerase under appropriate environmental conditions enables the rapid redirection of regulon transcription in response to environmental signals. More than 150 genes comprise the *L. monocytogenes* $\sigma^B$ regulon (7, 8).

$\sigma^B$ networks, including its interactions with PrfA, influence transmission of *L. monocytogenes* during both the gastrointestinal (9) and systemic stages of infection (5, 10). Complex interactions occur between $\sigma^B$ and PrfA-dependent gene regulation (5, 10); PrfA is the master regulator of *L. monocytogenes* virulence gene expression. $\sigma^B$ directly regulates prfA transcription via the P$_2^{prfA}$ promoter (11–13) and also indirectly regulates PrfA activity. Specifically, $\sigma^B$ downregulates PrfA activity in intracellular *L. monocytogenes*, thus moderating expression of PrfA-dependent virulence genes and thereby reducing host cell damage incurred by these virulence gene products (5).

A general strategy for exploring complex biological networks is to disrupt a targeted element of that network and then examine the consequences. High-throughput screening of small-molecule libraries has been used effectively to identify agents that disrupt specific bacterial targets, including an inhibitor of the virulence regulator ToxT in *Vibrio cholerae* (14). We screened multiple small-molecule libraries to identify an inhibitor of the stress response and virulence-associated regulator $\sigma^B$. The most promising small molecule was further assessed using an *L. monocytogenes*
whole-genome microarray, quantitative reverse transcription-PCR (qRT-PCR) of \( \sigma^B \)-dependent genes, and phenotypic profiling, including Caco-2 cell invasion assays and qualitative assessment of bile salt hydrolase activity. The compound also was evaluated for its ability to inhibit \( \sigma^B \) activity in \textit{B. subtilis}.

**RESULTS**

A high-throughput cell-based screening assay (HTS) was used to identify compounds that inhibit expression of the \( \sigma^B \)-dependent \textit{opuCA} promoter (15) without affecting \textit{L. monocytogenes} growth (Chembank Screening Project: SigBInhibition). Based on the primary screen, 41 putative inhibitors of \( \sigma^B \) activity were selected for secondary cell-based screening (Fig. S1). Compounds that induced \( \sigma^B \) activity were not analyzed further.

IC\(_{50}\) values, i.e., compound concentrations needed to inhibit 50\% of \( \sigma^B \) activity, were determined from secondary screening results for each of the 41 compounds. For 14 compounds, \( \sigma^B \) activity was inhibited at a concentration lower than that used in the primary screen; however, 11 compounds were eliminated from further consideration based on mammalian cell cytotoxicity data in ChemBank (http://chembank.broad.harvard.edu). The three remaining \textit{L. monocytogenes} \( \sigma^B \) inhibitors were 4-hydradzino[1]benzofuro[3,2-d]pyrimidine; 3-(cyclohexylacetyl)-4-hydroxy-2H-chromen-2-one; and (E)-N,2-diphenylethenesulfonamide. Among these, the most effective \( \sigma^B \) activity inhibitor, (E)-N,2-diphenylethenesulfonamide (IC\(_{50}\) = ~15 \( \mu \)M), which was a member of the ChemDiv3 library (Table S1), was not commercially available. Therefore, fluorophenyl-styrene-sulfonamide (FPSS), an analog of the original compound, was obtained for further study. Relative to (E)-N,2-diphenylethenesulfonamide, FPSS has a fluorine substituted for a hydrogen (Fig. 1). Based on quantitative reverse transcriptase PCR (qRT-PCR) results, FPSS was the most effective \( \sigma^B \) inhibitor among the three compounds. Data available in ChemBank indicated minimal evidence and no evidence of mammalian cell cytotoxicity for (E)-N,2-diphenylethenesulfonamide and FPSS, respectively.

We hypothesized that a small molecule that directly binds \( \sigma^B \) might also prevent \( \sigma^B \) from associating with core polymerase, thereby inhibiting \( \sigma^B \) activity. Therefore, the ability of various small molecules to bind \( \sigma^B \) was assessed using a small-molecule (SMM) screen with His-tagged \( \sigma^B \) (Fig. S2; Table S1). Of three putative ligands—i.e., 3-amino-4-oxo-N-(pyridin-3-ylmethyl)-1H-indole-3-carboxylate [Chemical Diversity], and 5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine [Maybridge]—none inhibited \( \sigma^B \) activity in the bile salt hydrolase activity assay, and therefore, none were evaluated further.

**Multiple lines of evidence support \( \sigma^B \) activity inhibition by FPSS.** Quantitative qRT-PCR assessment of the effects of FPSS concentrations from 1 \( \mu \)M to 64 \( \mu \)M on \( \sigma^B \)-dependent transcription showed that exposure to 64 \( \mu \)M FPSS resulted in a ~40-fold reduction in transcript levels for both \( \sigma^B \)-dependent genes \textit{opuCA} and \textit{gadA} relative to their transcript levels in cells not treated with FPSS (Fig. 2) \((P < 0.05, \text{GLM} \ [\text{general linear model}] \text{ with post-hoc Tukey's honestly significant difference [HSD] test}). \textit{opuCA} and \textit{gadA} transcript levels in cells treated with FPSS (ranging from 8 \( \mu \)M to 64 \( \mu \)M) were not significantly different from those in the \( \Delta\text{sigB} \) strain \((P > 0.05)\). At 4 \( \mu \)M, FPSS significantly reduced \textit{opuCA} and \textit{gadA} transcript levels compared to those in 10403S without FPSS \((P < 0.05)\) but not to levels equivalent to those in the \( \Delta\text{sigB} \) strain (Fig. 2). The FPSS concentration yielding half the maximal inhibition (IC\(_{50}\)) was calculated as 3.5 \( \mu \)M for \textit{opuCA} and 3.0 \( \mu \)M for \textit{gadA}. Importantly, absolute transcript levels for the housekeeping genes \textit{rpoB} and \textit{gap} were not different in \textit{L. monocytogenes} with and without exposure to FPSS, indicating that FPSS specifically inhibits transcription of \( \sigma^B \)-dependent genes without affecting transcription of housekeeping genes.

The phenotypic effects of various concentrations of FPSS on the activity of bile salt hydrolase, the product of the \( \sigma^B \)-dependent gene \textit{bsh}, which is required for \textit{L. monocytogenes} survival in vivo (6), were qualitatively assessed. \textit{L. monocytogenes} treated with 96 \( \mu \)M and 193 \( \mu \)M FPSS showed no bile salt hydrolase (BSH) activity, with no apparent effect on the ability of \textit{L. monocytogenes} to grow on brain heart infusion (BHI) agar. When treated with 290 \( \mu \)M FPSS, \textit{L. monocytogenes} produced no BSH activity but also grew poorly on BHI (data not shown).

**\textit{L. monocytogenes} whole genome microarray identified 208 genes downregulated by treatment with FPSS.** Transcriptional consequences of FPSS treatment were profiled using an \textit{L. monocytogenes} whole-genome microarray. FPSS treatment downregulated transcript levels for 208 genes and upregulated transcript levels for 52 genes (adjusted \( P \) value of <0.05 and an absolute fold change [FC] value of \( \geq 2 \)). In previous studies with \textit{L. monocytogenes} 10403S and EGD-e, 281 genes were identified as positively regulated by \( \sigma^B \) under at least one assay condition, and 137 genes as positively regulated by \( \sigma^B \) under two or more of the seven assay conditions examined (5–8, 16, 17) (Table S2). Overall, FPSS significantly reduced transcript levels of 56\% (156/281) of genes previously identified as being upregulated by \( \sigma^B \) in at least one study and of >91\% (125/137) of genes identified as being upregulated by \( \sigma^B \) in two or more studies (Table 1; Table S3). Of the 208 FPSS-downregulated genes, 115 were reported to be positively regulated by \( \sigma^B \) in both 10403S and EGD-e (5–8, 16, 17), with an additional 21 genes reported to be positively regulated by \( \sigma^B \) in 10403S (5, 7, 8, 17) and 20 reported to be positively regulated by \( \sigma^B \) in EGD-e (6, 16). FPSS downregulated transcript levels for >90\% of genes with previously reported hidden Markov model-identified \( \sigma^B \)-dependent promoters (17). A number of operons previously identified as being positively regulated by \( \sigma^B \) (8) were also significantly downregulated after treatment with FPSS, including \textit{inlAB}, which mediates entry into nonprofessional phagocytes (18), and \textit{opuABC} (18), which is involved in compatible solute transport. The autoregulated \textit{sigB} operon (7, 8, 19), consisting of...
lmo0893 to lmo0896 (rsbV, rsbW, sigB, and rsbX), was also downregulated by FPSS.

To evaluate FPSS effects on the function of other alternative sigma factors, transcript levels for genes in the σH and σE regulons were assessed. Among the 30 genes previously identified as σH dependent (with an FC ≥ 2.0), 14 were significantly downregulated by FPSS (adjusted P < 0.05, fold change ≤ −2); however, 12 of those 14 genes are also σB dependent. Gene set enrichment analysis (GSEA) showed that the σH-only regulon (i.e., genes that are regulated only by σH and not coregulated by σB) was not significantly enriched among the genes differentially transcribed as a result of FPSS treatment (false discovery rate [FDR] q = 0.472). GSEA also showed that the σE regulon was not significantly enriched as a result of treatment with FPSS (FDR q = 0.836).

FPSS treatment reduces transcript levels of σB-dependent opuCA and gadA. Normalized log-transformed opuCA (A) and gadA (B) transcript levels in L. monocytogenes 10403S exposed to 0.3 M NaCl to induce σB activity in the presence of FPSS at concentrations ranging from 1 to 64 μM; controls included strains 10403S and its isogenic ΔsigB mutant exposed to 0.3 M NaCl. Transcript levels were quantified by qRT-PCR, log10 transformed, and normalized to the geometric mean of the transcript levels for the housekeeping genes rpoB and gap. The data are means from three replicates; error bars show the standard deviations.

GSEA was used to determine if genes from specific biological role categories were overrepresented among those differentially affected by FPSS. Consistent with σB’s role in bacterial stress response, gene sets enriched among FPSS-downregulated genes included those classified as (i) “Cellular Processes: Adaptations to Atypical Conditions” and (ii) “Energy Metabolism (other)” (FDR q = 0.060 and q = 0.201, respectively). Previously identified σB-regulated genes also were significantly enriched among FPSS-downregulated genes (FDR q < 0.0001). Gene sets enriched among FPSS-upregulated genes included those classified as (i) “Cellular Processes: Chemotaxis and Motility,” (ii) “Protein Fate: Protein Folding and Stabilization,” and (iii) “Amino Acid Biosynthesis: Histidine Family” (FDR q < 0.0001, q = 0.008, and q = 0.031, respectively).
TABLE 1 Relationships between genes identified as differentially expressed after treatment with FPSS and genes identified previously as σB dependent

| Gene type                                      | Downregulated by FPSS | Upregulated by FPSS |
|-----------------------------------------------|-----------------------|---------------------|
| Identified previously as positively regulated by σB<sup>a</sup> | 152 (86) | 2 (0) |
| Identified previously as negatively regulated by σB<sup>b</sup> | 2 (0) | 7 (0) |
| Reported previously to be positively or negatively regulated under different conditions or in different studies<sup>d</sup> | 4 (0) | 0 |
| Having no previous evidence of σB-dependent transcript levels | 50 (0) | 23 (0) |
| Total                                          | 208 (86) | 32 (0) |

<sup>a</sup> Genes were classified with upstream σB-dependent promoters by in silico analysis using a hidden Markov model as described by Oliver et al. (17).

<sup>b</sup> Genes were classified as positively regulated by σB based on evidence from at least one of six previous microarray or RNA-Seq studies (5–8, 16, 17) (see Tables S2 and S3 for details).

<sup>c</sup> Genes were classified as negatively regulated by σB based on evidence from at least one of six previous microarray or RNA-Seq studies (5–8, 16, 17) (see Tables S2 and S4 for details).

<sup>d</sup> Genes reported as differentially regulated in previous microarray or RNA-Seq studies (5–8, 16, 17), including genes reported as negatively regulated by σB in one study and positively regulated by σB in another study and genes that were found to be negatively and positively regulated by σB under different conditions in the same study (see Table S2).

Of the 208 FPSS-downregulated genes, 126 were positively regulated by σB during infection in the murine intestine (6), including 17 genes that had been identified as σB-dependent only in the intestinal environment (Table S2). Among these 17 genes, 9 genes were of unknown or hypothetical function. Several FPSS-downregulated genes are recognized as contributing to virulence and host infection (i.e., inlA, inlB inlD, bilEAB, bsh, hfg, clpC, opuC, and gadA). Further, the PrfA regulon (i.e., genes regulated by the pleiotropic virulence gene regulator PrfA) was significantly enriched among the genes downregulated by FPSS (FDR q = 0.095). Interestingly, 19 genes that were previously classified as coregulated by PrfA and σB (10, 20) were both upregulated in the mouse spleen (10) and downregulated by FPSS, further supporting their σB-dependent transcription. Among these 19 genes, three were identified as potential virulence factors in murine and tissue culture models (10, 22), including lmo1601 (encoding a general stress protein), lmo1602 (encoding an unknown protein), and lmo2157 (encoding SepA, a metalloprotease in Staphylococcus epidermidis [21]), which is upregulated in L. monocytogenes during intracellular infection (22). PPSS also downregulated lmo0937, a PrfA-regulated gene that is upregulated in the mouse spleen at 48 h postinfection (10), and lmo0915, which encodes a component of a phosphotransferase system identified as a potential virulence factor by Camejo et al. (10); neither gene had been identified previously as σB dependent. Other σB-dependent genes downregulated by FPSS and upregulated during intracellular infection (22) include lmo0232 (clpC); lmo0445, which encodes a transcriptional regulator; lmo2672, which encodes a protein similar to a transcriptional regulator; and lmo0783, which is a member of an operon encoding mannose phosphotransferase system components.

FPSS-treated cells had lower transcript levels for a number of genes that encode cell wall-associated proteins previously shown to be upregulated under intracellular conditions (22) and in the murine intestine (6); these genes include inlA, inlD, lmo0610, lmo0880, and lmo2085, which all encode proteins with an LPXTG sorting motif for cell wall anchoring, and inlB, which encodes a protein with a GW domain that is important for binding host ligands (23).

Three genes important for glycerol utilization (i.e., lmo1538, lmo1539, and lmo1293) were also downregulated by FPSS; utilization of glycerol as a carbon source in intracellular environments (22) is required for intracellular survival (24). While lmo1538 (encoding a glycerol kinase) and lmo1539 (encoding a glycerol uptake facilitator) were downregulated by FPSS, they were previously reported to be negatively regulated by σB in stationary phase and under salt stress conditions (8). Interestingly, however, both genes were upregulated by σB in the intestine (6) and during intracellular replication (22). lmo1293 (glpD), which encodes a glycerol-3-phosphate dehydrogenase, was previously reported to be positively regulated by σB in L. monocytogenes exposed to salt stress (8) or grown intracellularly (24) and in the gastrointestinal tract (6) but was downregulated by σB in stationary-phase cells (8). Taken together, our data provide additional evidence supporting the hypothesis that the composition of the σB regulon is dynamically dependent on environmental conditions (7). Importantly, our data also demonstrate that a number of genes downregulated by FPSS are specifically regulated by σB in the gastrointestinal environment. For example, three additional genes downregulated by FPSS (i.e., lmo0642, lmo1251, and lmo1930) had higher transcript levels in the L. monocytogenes parent strain than in the ΔσB strain when both were grown in the murine intestine (6), but these genes did not appear to be σB-dependent under other in vitro conditions (5, 7, 8, 16, 17).

Only a small number of genes upregulated by FPSS have been identified previously as negatively regulated by σB. Overall, 32 genes were identified with significantly higher transcript levels in FPSS-treated L. monocytogenes than in untreated cells (Table 1), suggesting negative regulation of these genes by σB. While 264 genes were previously identified as negatively regulated by σB under at least one condition (5–8, 16, 17), only 7 of the 32 FPSS-upregulated genes were represented among these 264 genes. Further, only 14 genes were previously identified as negatively regulated by σB under at least two environmental conditions (5–8, 16, 17) and none of the 32 FPSS-upregulated genes were represented among these 14 genes. Very few genes appear to be consistently repressed by σB under various conditions, likely because these genes are indirectly rather than directly regulated by σB. Six of the 7 FPSS-upregulated genes previously identified as negatively regulated by σB encode proteins with known functions, including an ABC transporter (lmo2114), a posttranslation chaperone (prxA), a methyl-accepting chemotaxis protein (lmo1699 and lmo1700), NADP glutamate dehydrogenase (lmo0560), and a D-alanine-activating enzyme (dltA). While dlTA (the first gene in an operon encoding proteins that modify lipoteichoic and wall teichoic acids) was previously shown to be negatively regulated by σB, other genes in this operon (i.e., lmo0973 [dltB] and lmo0971 [dltD]) not previously identified as σB dependent were also significantly upregulated following FPSS treatment, suggesting that this entire operon is negatively regulated by σB, at least under some conditions. Two genes, lmo2568 (unknown function) and
Our recent studies (27) revealed that, in addition to its role as a promising tool for studying regulatory networks involving L. monocytogenes, the compound was tested for its ability to specifically inhibit \( \sigma^B \) activity in B. subtilis. FFPS (64 \( \mu \)M) significantly inhibited \( \sigma^B \)-dependent ctc-lacZ activity (P < 0.05; GLM with post-hoc Tukey’s HSD test) to levels equivalent to those in a \( \Delta \)sigB strain (Fig. 3) (P > 0.05) without reducing \( \beta \)-galactosidase activity from a \( \sigma^B \)-dependent lacZ fusion (30) (Fig. 3), further supporting FFPS specificity for inhibiting \( \sigma^B \) activity.

**DISCUSSION**

By using a high-throughput screen of approximately 57,000 small molecules, 41 candidate compounds were identified as potential inhibitors of L. monocytogenes \( \sigma^B \) activity. Through subsequent screens, we identified a compound designated FFPS that specifically inhibits \( \sigma^B \)-mediated transcription, as shown by qRT-PCR of \( \sigma^B \)-dependent genes and whole-genome microarray analysis of cells treated with the compound. This compound also significantly reduces L. monocytogenes invasion into human intestinal epithelial cells and inhibits \( \sigma^B \)-directed activity in the Gram-positive bacterium B. subtilis, indicating that this compound inhibits \( \sigma^B \)-mediated transcription across genera. Overall, our data show that FFPS (i) inhibits expression of the \( \sigma^B \) regulon with high specificity, yielding transcriptional profiles similar to those generated by a genetic null mutation of the \( \sigma^B \) gene, and (ii) specifically inhibits expression of \( \sigma^B \)-dependent genes important for virulence, stress response, and other functions associated with L. monocytogenes survival and growth in the gastrointestinal tract.

In combination with previous reports that identified small molecules that interfere with virulence factors and virulence activation and that show therapeutic promise (14, 31), our results suggest that, in addition to its role as a promising tool for studying regulatory networks involving \( \sigma^B \), FFPS may also represent a compound that can be developed into a therapeutic agent.
FPSS specifically inhibits expression of $\sigma^B$-dependent virulence, stress response, and other functions that are associated with 
$L. monocytogenes$ growth and survival in the gastrointestinal tract. $\sigma^B$ is well recognized as an important transcriptional regulator in multiple Gram-positive genera. For example, $\sigma^B$ regulates transcription of genes contributing to stress response, virulence, or both in low-GC Gram-positive microbes, including human pathogens such as 
*Bacillus cereus* (32, 33), *Bacillus anthracis* (34), *Staphylococcus aureus* (35, 36), and the opportunistic pathogen *S. epidermidis* (37). $\sigma^B$ activates transcription of a large number of target genes across the genera and species reported to date (e.g., 
*L. monocytogenes*, *Listeria innocua*, *S. aureus*, and *B. subtilis*) (8, 38, 39). FPSS treatment of 
*L. monocytogenes* affects expression of $\sigma^B$-dependent genes that are upregulated in the host intestine but that had not been identified previously as $\sigma^B$-dependent under other in vitro conditions. Specifically, among 172 genes in 
*L. monocytogenes* EGD-e that were upregulated by $\sigma^B$ in the murine intestinal lumen (6), FPSS treatment significantly downregulated 126 genes; 17 genes downregulated by FPSS had been identified as $\sigma^B$-dependent only in the intestinal lumen (6) but not in other in vitro test systems. Thus, identification of $\sigma^B$-dependent genes in 
*L. monocytogenes* treated with FPSS may provide new insight into $\sigma^B$-dependent gene regulation that may be critical during the gastrointestinal stage of infection. For example, the PrfA regulon was significantly enriched among genes downregulated by FPSS treatment, including two PrfA-dependent genes (i.e., *lmo0937* and *plcA*) that had not been identified previously as $\sigma^B$ regulated. These findings are consistent with $\sigma^B$'s role, via the P2$_{PrfA}$ promoter, in directly upregulating *prfA* transcription (11–13) and also support the idea that $\sigma^B$-dependent upregulation of *prfA* transcription plays a critical role during intestinal stages of infection. Activation of $\sigma^B$ in the intestinal lumen thus appears to increase expression of $\sigma^B$-dependent *inlA*, which is required for intestinal epithelial cell invasion (40), and also primes expression of PrfA, which is critical for regulating virulence gene expression during the subsequent intracellular stages of infection.

FPSS-treated 
*L. monocytogenes* also had higher transcript levels than nontreated cells for a number of genes involved in chemotaxis and motility. Several genes in a large operon encoding flagellar structural components were previously reported to be negatively regulated by $\sigma^B$ (8), and *sigB*-null mutants also exhibited increased swarming (6, 8). $\sigma^B$-dependent downregulation of transcripts encoding flagellar components and overall motility appear to be at least partially due to $\sigma^B$-dependent transcription of a long untranslated region (UTR) upstream of *mogR*, which encodes a negative regulator of 
*L. monocytogenes* motility. Reduced transcription of this UTR not only reduces *mogR* transcript levels (thereby increasing flagellar motility) but also appears to increase transcript levels for some flagellar genes, as the $\sigma^B$-dependent UTR also decreases flagellin gene transcripts through an antisense-RNA-type mechanism (6, 8). While 
*L. monocytogenes* flagellar motility appears to contribute to intestinal invasion (41), $\sigma^B$-dependent downregulation of flagellar expression in the intestinal lumen may be critical for subsequent stages of infection, as *Listeria* downregulates flagellar gene expression during infection (10) to evade the immune system; increased expression of flagellar components can induce potent proinflammatory effects via TLR5-mediated immunogenicity (42).

Inhibitors of alternative or factor activation represent potential avenues for development into therapeutics. In addition to its value as a compound that can be used to study regulatory pathways involving $\sigma^B$, FPSS also may provide a starting point for development of new therapeutic compounds that interfere with regulatory pathways critical for infection and virulence. Several small molecules that target transcription regulators inhibit virulence and virulence-associated characteristics in vitro and in vivo, suggesting that these targets are suitable for development of novel therapeutics against bacterial infections (43, 44). Importantly, prokaryotic transcriptional machinery, as represented by interactions between $\sigma^B$ and the $\beta'$ subunit of core RNA polymerase in 
*Escherichia coli*, can be disrupted by small molecules without affecting eukaryotic transcription (45). Consequently, the therapeutic potential of novel compounds that interfere with transcriptional regulation of bacterial virulence functions is of emerging interest.

Virstatin is an example of a small molecule with therapeutic potential that has been shown to inhibit transcriptional regulation in 
*V. cholerae*. Virstatin interferes with the virulence gene regulator ToxT, a member of the AraC family of transcriptional regulators, thus showing potential for treatment of 
*Vibrio* infections (14). Small-molecule inhibitors also have been identified for other members of the AraC transcription factor family, e.g., MarA, SoxS and Rob in *E. coli* (43) and LcrF in *Yersinia* spp. (44). As with $\sigma^B$, AraC-type regulators typically contribute to transcription of multiple stress response (46) and virulence factors (47, 48); therefore, inhibition of these and similar transcriptional regulators can result in broad physiological consequences for the affected microbes (43).

The small molecule identified here, FPSS, inhibits $\sigma^B$ activity at an IC$_{50}$ of 3 to 3.5 $\mu$M. By comparison, the ToxT inhibitor virstatin (14) has an MIC between 3 and 40 $\mu$M, depending on the target strain. Minimal bactericidal concentrations of gentamicin, ampicillin, and streptomycin against 
*L. monocytogenes* range from 2 to 46 $\mu$M (49). In addition to its promising IC$_{50}$ prior to structural optimization, FPSS produces highly specific, genome-wide reduction of $\sigma^B$-directed activity, including inhibited expression of $\sigma^B$-dependent virulence genes such as *inlA*, *bsh*, *bilE*, *clpC*, and *hfq* (5–8, 16, 17). Furthermore, *opaC* (50) and *gadA* (51), which are important for gastrointestinal survival in the host, are also significantly downregulated by FPSS. FPSS clearly inhibits transcription of a number of genes with functions in virulence and infection, thus increasing its therapeutic potential over compounds that target only one virulence factor (43). The contributions of $\sigma^B$ to 
*L. monocytogenes* virulence are also supported by phenotypic evidence, including reduced virulence of a $\Delta$sigB strain in a guinea pig model of infection (9) and reduced invasion of human Caco-2 cells by a $\Delta$sigB strain (9, 40), consistent with the reduced invasiveness for FPSS-treated 
*L. monocytogenes* observed here. Importantly, $\sigma^B$ also contributes to establishment of infection and virulence in other Gram-positive pathogens, including 
*B. anthracis* and 
*S. aureus*. A 
*B. anthracis* sigB mutant is less virulent than the parent strain, producing a 1-log reduction in 50$\%$ lethal dose, perhaps because $\sigma^B$ enhances the ability of 
*B. anthracis* to persist in the bloodstream of a mammalian host (34). In 
*S. aureus*, $\sigma^B$ directly and indirectly modulates global regulatory elements involved in virulence functions (52). Functional loss of $\sigma^B$ results in decreased 
*S. aureus* virulence in central venous catheter-related diseases manifested by significantly reduced multorgan infection (53). Similar to 
*B. anthracis*, $\sigma^B$ is suggested to promote 
*S. aureus* survival in the bloodstream, preventing clearance and
allowing establishment of infection (54). Further development and optimization of FPSS thus may provide an opportunity to develop novel therapeutics for some important Gram-positive pathogens.

**MATERIALS AND METHODS**

**Strain and media selection.** Strains used in this study included the *L. monocytogenes* parent strain 10403S (serotype 1/2a) (55), its otherwise isogenic ΔsigB derivative (FSL A1-254) (56), a reporter strain for σB activity (FSL S1-063 [10403S opuCA-gus]) (7, 57, 58), and a negative-control reporter strain for σB activity (FSL S1-059 [ΔsigB opuCA-gus]).

To evaluate the effectiveness of a selected small molecule to inhibit σB activity in a Gram-positive organism other than *Listeria*, *B. subtilis* strains bearing reporter fusions for either σB or σA activity and a ΔsigB negative-control reporter strain (Table S5) were also tested. To achieve low background fluorescence, a chemically defined minimal medium (59) with 25 mM glucose (DMG) (60) was used for the high-throughput screen. Cells were grown in brain heart infusion broth (BHI; Difco, Sparks, MD) for phenotypic and transcriptional profiling assays.

**Primary high-throughput cell-based small-molecule screen.** The *L. monocytogenes* opuCA-gus fusion strain FSL S1-063 was used in a cell-based high-throughput screen (HTS) against ~57,000 compounds. As reported at http://ChemBank.Broad.Harvard.edu, the libraries included as the following: (i) known bioactive compounds, including FDA-approved drugs (i.e., the SPBio and SMP libraries); (ii) synthetic compounds from diversity-oriented synthesis (e.g., the CMLD, ICCB, PK04, Aldi.1-H, and Sulfit 1.1-A libraries); (iii) natural products (i.e., the PhIELX and IBCGeX libraries); and (iv) commercially available compounds (e.g., the ChemDiv, Maybridge, and TimTec1 libraries). Table S1 contains a complete listing of libraries screened for this study.

Multidrop liquid-handling robots (Matrix, Thermo Fisher) were used to dispense 27 μl of DMG into black-walled clear-bottom 384-well plates (Nunc, Rochester, NY), and then 100 nl of each small-molecule stock was transferred from the library stock or source plate to the assay plates with a CyBi-Well Vario pipettor (CyBio AG, Jena, Germany). Final experimental concentrations of the small molecules used in the assays were determined to be dependent on each stock concentration [e.g., (E)-N,2-diphenylethenesulfonamide had a stock concentration of 19.3 mM, producing a 64.3 μM final concentration in each well]. Each source plate contained approximately 15 wells to which only dimethyl sulfoxide (DMSO) was added, as the small molecules were dissolved in DMSO; these wells are referred to as DMSO-only negative-internal-control wells. All source plates were prepared in duplicate to provide experimental replicates (i.e., plates A and B). Two plates in which all wells contained medium with DMSO and *L. monocytogenes* (inoculated as detailed below) were included as external plate controls. *L. monocytogenes* strains were grown to an optical density at 600 nm (OD600) of approximately 0.4 (3 hours) in BHI, cultures were diluted 1:50 with DMG, and then 3 μl of the appropriate diluted culture was added to each well. As a control, a custom assay plate containing 192 wells of the 10403S opuCA-gus strain FSL S1-063 and 192 wells of the otherwise isogenic ΔsigB opuCA-gus strain FSL S1-059 was treated with only DMSO.

All plates were sealed and incubated for 18 h at 37°C. To determine bacterial growth or inhibition in the presence of the compounds, absorbance (OD600) was measured using a Synergy HT multimode microplate reader (Biotek Instruments, Winooski, VT) after ~18 h of incubation. To measure fluorescence for β-glucuronidase (GUS) activity determination, black seals (PerkinElmer, Waltham, MA) were affixed to the bottoms of the plates after the absorbance readings were completed. Cells were then lysed using 5 μl of 2X CelllyticB (Sigma, St. Louis, MO) and a protease inhibitor cocktail mixture (1 ml 2X CelllyticB and 0.05 ml protease inhibitor cocktail; Sigma), immediately prior to the addition of 4 μl of 1.6 mg/ml 4-methylumbelliferyl β-D-glucuronide hydrate (4-MUG; Sigma) in DMSO. Reaction mixtures were incubated in the dark for 1 h at room temperature (−23°C), and reactions were stopped by the addition of 0.2 M Na2CO3. Fluorescence was read using a Wallac 2102 EnVision Multilabel Reader (PerkinElmer) with an excitation wavelength of 355 nm and an emission of 460 nm.

**Statistical analysis of primary screen data.** To identify compounds that inhibited σB activity without affecting *L. monocytogenes* growth, opuCA-directed GUS activity in the presence of each compound was calculated by dividing relative fluorescence units (RFU) by cell density in OD600 units (RFU/OD) (61). Statistical analyses were conducted in collaboration with the Broad Institute and performed as previously described (62, 63). Raw and analyzed data were deposited in ChemBank (64, 65). The software package Spotfire DecisionSite Analytics (TIBCO Spotfire, Somerville, MA) was used for two-dimensional data visualization.

**Secondary screen and dose response curve.** Forty-one compounds that appeared to inhibit σB activity (Z score of ≤−3 in both replicates) were selected for secondary cell-based screening using the assay and reporter fusion described above to calculate initial IC50. Each compound was diluted in DMSO in a series of six 1/5 dilutions of the initial stock concentration [e.g., starting from 19.3 mM stock, (E)-N,2-diphenylethenesulfonamide was diluted in a series of six to 5 dilutions, yielding concentrations of 3.86 mM to 1.24 μM]. The small molecules at these concentrations were then dispensed into the assay plates.

**Small-molecule microarray screens.** Two different arrays, each printed with 8,500 small-molecule (SM) spots and 1,500 DMSO control spots, were used to screen for binding of σB to the small molecules. Small-molecule microarrays (SMMs) were printed on glass slides at the Broad Institute as described previously (66–68). The immobilized SMs included 8,500 compounds created by diversity-oriented synthesis and 8,500 compounds representing natural products, FDA-approved drug-like compounds, commercial compounds, and known bioactive compounds (Table S1) (68, http://ChemBank.broadinstitute.org). SMM screening (three replicates) was performed as described by Bradner et al. (66). His-tagged σB was purified from *E. coli* M15, kindly provided by W. Goebel (69). Data analyses included (i) assessment of signal-to-noise ratio (SNR) of the spot feature; (ii) Z score calculations based on comparison of signals from compound spots to signals from DMSO control spots within a slide; and (iii) composite Z score calculations for data from the three replicates. Spotfire Analytics software was used for three-dimensional data visualization.

**FPSS.** (E)-N,2-diphenylethenesulfonamide, the compound identified by HTS as being responsible for the greatest inhibition of σB activity, was not commercially available. Therefore, the analog fluoro-pheno-styrene-sulfonamide [IUPAC name (E)-N-(4-fluorophenyl)-2-phenylethenesulfonamide; ChemBank ID, 2063822; MW 277.3] was obtained from Enamline Ltd. (Kiev, Ukraine). FPSS was dissolved in DMSO to a concentration of 10 mM. The solution was filtered sterilized using with a 0.1-μm filter (Omnipore membrane filter; Millipore Corporation, Billerica, MA) and a Swinnex stainless 13-mm holder for syringe filtration (Millipore Corporation).

**Bile salt hydrolase (BSH) activity assay.** As *L. monocytogenes* bsh, which encodes bile salt hydrolase, is σB dependent (5, 7, 15), a qualitative BSH activity assay was used to determine the FPSS concentration needed for σB inhibition. Four-well multidiaphragm plates (26 mm by 33 mm; Nunc) containing 6 ml of either BHI agar or de Man, Rogosa and Sharpe (MRS) agar medium (BD Biosciences, San Jose, CA) containing 0.5% (wt/vol) glycocolyoxycylic acid sodium (GDCA) salt (Calbiochem, San Diego, CA) (70) with either no FPSS or 96, 193, or 290 μM of FPSS (1.5, 3, or 4.5 times the 64.3 μM concentration used for (E)-N,2-diphenylethenesulfonamide in the HTS) were prepared and allowed to dry overnight. *L. monocytogenes* 10403S and ΔsigB were grown in BHI broth to exponential phase, defined as an OD600 of 0.4, and then 4 μl of culture was spotted in parallel on MRS and BHI agars. The MRS agar plates were incubated anaerobically using the BD-BBL GasPak anaerobic system (Becton Dickinson, Franklin Lakes, NJ), while BHI plates were incubated aerobically. Both sets of plates were incubated for 48 h at 37°C and then were visually assessed for growth (BHI plates) or the presence of a white precipitate comprised of deconjugated
bile salts indicating BSH activity (MRS plates). The assay was performed three times.

**RNA isolation.** For RNA isolation, *L. monocytogenes* 10403S and ΔigB strains were initially grown overnight in 5 ml of BHI broth at 37°C with shaking (230 rpm), followed by subculturing twice, each time at an OD_{600} of 0.4, using a 1% (vol/vol) transfer into 5 ml of prewarmed BHI. When the second subculture reached an OD_{600} of 0.4, cells were treated with a total volume of 76 μl comprising FPSS (to yield final concentrations ranging from 1 μM to 128 μM) and/or DMSO, followed immediately by addition of 324 μl of either 5 M NaCl (to yield a final concentration of 0.3 M NaCl, an osmotic stress that induces σB activity [8]) or (ii) sterile distilled water. Treated cultures were then incubated at 37°C with shaking (230 rpm) for 10 min, followed by addition of 2 volumes of RNAprotect (Qiagen Inc., Valencia, CA) and subsequent incubation at room temperature for 10 min. The cells were harvested following centrifugation for 10 min at 5,000 × g, and cell pellets were stored at −80°C until RNA was extracted and DNase treated using an Ambion RiboPure kit (Ambion, Austin, TX) according to the manufacturer’s instructions. RNA concentrations and purity were assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE), RNA quality was analyzed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and only RNA with an integrity number of ≥8 was used. Each treatment was performed 3 times.

**TaqMan qRT-PCR.** Transcript levels for the σB-dependent genes *apuCA* and *gadA* and the housekeeping genes *rpoB* and *gap* were quantified with TaqMan primers and probes [13, 58, 71] using RNA prepared as described above and an ABI Prism 7000 sequence detection system (Applied with TaqMan primer and probes (13, 58, 71) using RNA prepared as described above and an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) as a control) and (ii) DMSO as well as NaCl (0.3 M final concentration) as described above, with treated cultures being incubated at 37°C for 30 min. After this incubation, the OD_{600} was recorded, and 0.2 ml of the culture was added to a tube containing 2.8 ml Z buffer, followed by the addition of 20 μl tolune to permeabilize the cells. A prewarmed 0.4 ml volume of 4-mg/ml ortho-nitrophenyl-β-galactoside (ONPG) was added, and the time of addition was noted. After 85 min, 1 ml of 1 M sodium carbonate was added to stop the reaction, and the OD_{420} was measured. Miller units were calculated as previously described (76), β-Galactosidase activity results were analyzed using one-way ANOVA and Tukey’s studentized range (HSD) test.

**Microarray data accession numbers.** Data from microarray experiments were submitted to the Gene Expression Omnibus (GEO) database, assigned accession number GSE16887, and approved.

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**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00241-11/-/DCSupplemental.

**REFERENCES**

1. Scallan E, et al. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17:7–15.
2. Oliver HF, Boor KJ, Wiedmann M. 2007. Environmental reservoir and transmission into the mammalian host, p 111–138. In Goldfine H, Shen H

2,000 members. Genes were classified into sets based on the TIGR Com-

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Rauch M, Luo Q, Müller-Altrock S, Goebel W. Schwab U, Bowen B, Nadon C, Wiedmann M, Boor KJ. November/December 2011 Volume 2 Issue 6 e00241-11

Knudsen GM, Olsen JE, Dons L. Oliver HF, et al. 17.

Becker LA, Cetin MS, Hutchins RW, Benson AK. 2009. Identification of the gene encoding the alternative sigma factor σB from Listeria monocytogenes and its role in osmotolerance. J. Bacteriol. 187:800–804.

Schwab U, Bowen B, Nadon C, Wiedmann M, Boor KJ. 2005. The Listeria monocytogenes prfA2 promoter is regulated by sigma B in a growth phase dependent manner. FEMS Microbiol. Lett. 245:329–336.

Hurt NG, Shakhnovich EA, Pierso E, Mekalano JS. 2005. Small-molecule inhibitor of Vibrio cholerae virulence and intestinal colonization. Science 310:670–674.

Chatterjee SS, et al. 2006. Invasiveness is a variable and heterogeneous phenotype in Listeria monocytogenes serotype strains. Int. J. Med. Microbiol. 296:277–286.

Becker LA, Cetin MS, Hutchins RW, Benson AK. 1998. Identification of the gene encoding the alternative sigma factor σB from Listeria monocytogenes and its role in osmotolerance. J. Bacteriol. 180:4547–4554.

Milohanic E, et al. 2003. Transcriptome analysis of Listeria monocytogenes identifies three groups of genes differently regulated by PrfA. Mol. Microbiol. 47:1613–1625.

Lai Y, et al. 2007. The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in Staphylococcus aureus. Mol. Microbiol. 63:497–506.

Chatterjee SS, et al. 2006. Invasiveness is a variable and heterogeneous phenotype in Listeria monocytogenes serotype strains. Int. J. Med. Microbiol. 296:277–286.

Becker LA, Cetin MS, Hutchins RW, Benson AK. 1998. Identification of the gene encoding the alternative sigma factor σB from Listeria monocytogenes and its role in osmotolerance. J. Bacteriol. 180:4547–4554.

Milohanic E, et al. 2003. Transcriptome analysis of Listeria monocytogenes identifies three groups of genes differently regulated by PrfA. Mol. Microbiol. 47:1613–1625.

Lai Y, et al. 2007. The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in Staphylococcus aureus. Mol. Microbiol. 63:497–506.

Chatterjee SS, et al. 2006. Intracellular gene expression profile of Listeria monocytogenes. Infect. Immun. 74:1323–1338.

Marino M, Banerjee M, Jonquères R, Cossart P, Ghosh P. 2002. GW domains of the Listromyxoides invasion protein InlB are SH3-like and mediate binding to host ligands. EMBO J. 21:5623–5634.

Joseph B, et al. 2006. Identification of Listeria monocytogenes genes contributing to intracellular replication by expression profiling and mutant screening. J. Bacteriol. 188:556–568.

Williams T, Joseph B, Beier D, Goebel W, Kuhn M. 2005. Response regulator DegU of Listeria monocytogenes regulates the expression of flagella-specific genes. FEMS Microbiol. Lett. 252:287–298.

Knudsen GM, Olsen JE, Dons L. 2004. Characterization of DegU, a response regulator in Listeria monocytogenes, involved in regulation of motility and contributes to virulence. FEMS Microbiol. Lett. 240:171–179.

Bennett H, et al. 2007. Characterization of relA and codY mutants of Listeria monocytogenes: identification of the CodY regulon and its role in virulence. Mol. Microbiol. 63:1453–1467.

Gründling A, Burrack LS, Bouwer HG, Higgins DE. 2004. Listeria monocytogenes regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. Proc. Natl. Acad. Sci. U. S. A. 101:12318–12323.

Shen A, Higgins DE. 2006. The MogR transcriptional repressor regulates non hierarchical expression of flagellar motility genes and virulence in Listeria monocytogenes. PLoS Pathog. 2:e30.

Wise AA, Price CW. 1995. Four additional genes in the sigB operon of Bacillus subtilis that control activity of the general stress factor sigma B in response to environmental signals. J. Bacteriol. 177:123–133.

Lieberman LA, Higgins DE. 2009. A small-molecule screen identifies the antipsychotic drug pimozide as an inhibitor of Listeria monocytogenes infection. Antimicrob. Agents Chemother. 53:756–764.

van Schaik W, Tempelaars MH, Wouters JA, de Vos WM, Abeet T. 2004. The alternative sigma factor σB of Bacillus cereus: response to stress and role in heat adaptation. J. Bacteriol. 186:316–325.

van Schaik W, et al. 2007. Identification of the σB regulon of Bacillus cereus and conservation of σB-regulated genes in low-GC-content Gram-positive bacteria. J. Bacteriol. 189:4384–4390.

Fouet A, Nanny O, Lambert G. 2000. Characterization of the operon encoding the alternative σB factor from Bacillus anthracis and its role in virulence. J. Bacteriol. 182:5036–5045.

Chen HY, et al. 2011. Vancomycin activates σB in vancomycin-resistant Staphylococcus aureus resulting in the enhancement of cytotoxicity. PLoS One 6:e224472.

Kusch K, et al. 2011. The influence of SaRS and σB on the expression of superantigens in different Staphylococcus aureus isolates. Int. J. Med. Microbiol. 301:488–499.

Knobloch JK, et al. 2001. Biofilm formation by Staphylococcus epidermidis depends on functional RsbU, an activator of the sigB operon: differential activation mechanisms due to ethanol and salt stress. J. Bacteriol. 183:2624–2633.

Bischoff M, et al. 2004. Microarray-based analysis of the Staphylococcus aureus σB regulon. J. Bacteriol. 186:4085–4099.

Hecker M, Volker U. 2001. General stress response of Bacillus subtilis and other bacteria. Adv. Microb. Physiol. 44:35–91.

Kim H, Boor KJ, Marquis H. 2004. Listeria monocytogenes σB contributes to invasion of human intestinal epithelial cells. Infect. Immun. 72:7374–7378.

O’Neil HS, Marquis H. 2006. Listeria monocytogenes flagella are used for motility, not as adhesins, to increase host cell invasion. Infect. Immun. 74:6675–6681.

Way SS, et al. 2004. Characterization of flagellin expression and its role in Listeria monocytogenes infection and immunity. Cell. Microbiol. 6:145–154.

Bowers TE, et al. 2007. Novel anti-infection agents: small-molecule inhibitors of bacterial transcription factors. Bioorg. Med. Chem. Lett. 17:5652–5655.

Kim OK, et al. 2009. N-Hydroxybenzimidazolide inhibitors of the transcription factor LcrF in Yersinia: novel antivirulence agents. J. Med. Chem. 52:3626–5634.

Glasner BT, Bengendahl V, Thompson NE, Olson B, Burgess RR. 2007. LTB-based HTS of a small-compound library for inhibitors of bacterial RNA polymerase. Assay Drug. Dev. Technol. 5:759–768.

Li Z, Dempke B. 1994. SoxS, an activator of superoxide stress genes in Escherichia coli. Purification and interaction with DNA. J. Biol. Chem. 269:18371–18377.

Bina J, et al. 2003. ToxR regulon of Vibrio cholerae and its expression in vibrios shed by cholera patients. Proc. Natl. Acad. Sci. U. S. A. 100:7077–7080.

Finlay BB, Falkow S. 1997. Common themes in microbial pathogenicity revisited. Microbiol. Mol. Biol. Rev. 61:136–169.

Moellinger RC, Jr., Medoff G, Leech I, Wennersten C, Kunz LJ. 1972. Antibiotic synergism against Listeria monocytogenes. Antimicrob. Agents Chemother. 1:30–34.

Sealot RD, Wouters J, Gahan CGM, Abeet T, Hill C. 2001. Analysis of the role of OpuC, an osmolyte transport system, in salt tolerance and
virulence potential of *Listeria monocytogenes*. Appl. Environ. Microbiol. 67:2692–2698.

51. Cotter PD, Gahan CGM, Hill C. 2001. A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. Mol. Microbiol. 40: 465–475.

52. Bischoff M, Entenza JM, Giachino P. 2001. Influence of a functional sigB operon on the global regulators sar and agr in *Staphylococcus aureus*. J. Bacteriol. 183:5171–5179.

53. Lorenz U, et al. 2008. The alternative sigma factor sigma B of *Staphylococcus aureus* modulates virulence in experimental central venous catheter-related infections. Microbes Infect. 10:217–223.

54. Jonsson I-M, Arvidson S, Foster S, Tarkowski A. 2004. Sigma factor B and RsbU are required for virulence in *Staphylococcus aureus*-induced arthritis and sepsis. Infect. Immun. 72:6106–6111.

55. Bishop DK, Hinrichs DJ. 1987. Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of *in vitro* stimulation on lymphocyte subset requirements. J. Immunol. 139:2005–2009.

56. Wiedmann M, Arvik TJ, Hurley RJ, Boor KJ. 1998. General stress transcription factor σB and its role in acid tolerance and virulence of *Listeria monocytogenes*. J. Bacteriol. 180:3650–3656.

57. Ferreira A, Sue D, O’Byrne CP, Boor KJ. 2003. Role of *Listeria monocytogenes* σB in survival of lethal acidic conditions and in the acquired acid tolerance response. Appl. Environ. Microbiol. 69:2692–2698.

58. Sue D, Fink D, Wiedmann M, Boor KJ. 2004. σB-dependent gene induction and expression in *Listeria monocytogenes* during osmotic and acid stress conditions simulating the intestinal environment. Microbiology 150:3843–3855.

59. Premaratne RJ, Lin WJ, Johnson EA. 1991. Development of an improved chemically defined minimal medium for *Listeria monocytogenes*. Appl. Environ. Microbiol. 57:3046–3048.

60. Ferreira A, O’Byrne CP, Boor KJ. 2001. Role of σB in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. Appl. Environ. Microbiol. 67:4454–4457.

61. Shen A, Higgins DE. 2005. The 5’ untranslated region-mediated enhancement of intracellular listeriolysin O production is required for *Listeria monocytogenes* pathogenicity. Mol. Microbiol. 57:1460–1473.

62. Kelly KA, Clemons PA, Yu AM, Weissleder R. 2006. High-throughput identification of phage-derived imaging agents. Mol. Imaging 5:24–30.

63. Kim YK, et al. 2004. Relationship of stereochemical and skeletal diversity of small molecules to cellular measurement space. J. Am. Chem. Soc. 126: 14740–14745.

64. Seiler KP, et al. 2008. ChemBank: a small-molecule screening and cheminformatics resource database. Nucleic Acids Res. 36:D351–D359.

65. Strausberg RL, Schreiber SL. 2003. From knowing to controlling: a path from genomics to drugs using small molecule probes. Science 300: 294–295.

66. Bradner JE, McPherson OM, Koehler AN. 2006. A method for the covalent capture and screening of diverse small molecules in a microarray format. Nat. Protoc. 1:2344–2352.

67. Bradner JE, et al. 2006. A robust small-molecule microarray platform for screening cell lysates. Chem. Biol. 13:493–504.

68. Duffner JL, Clemons PA, Koehler AN. 2007. A pipeline for ligand discovery using small-molecule microarrays. Curr. Opin. Chem. Biol. 11: 74–82.

69. Böckmann R, Dickneite C, Middendorf B, Goebel W, Sokolovic Z. 1996. Specific binding of the *Listeria monocytogenes* transcriptional regulator PrfA to target sequences requires additional factor(s) and is influenced by iron. Mol. Microbiol. 22:643–653.

70. Dashkevica MP, Feighner SD. 1989. Development of a differential medium for bile salt hydrolase-active *Lactobacillus* spp. Appl. Environ. Microbiol. 55:11–16.

71. Kazmierczak MJ, Wiedmann M, Boor KJ. 2006. Contributions of *Listeria monocytogenes* σB and PrfA to expression of virulence and stress response genes during extra- and intracellular growth. Microbiology 152: 1827–1838.

72. Chan YC, Boor KJ, Wiedmann M. 2007. σB-dependent and σB-independent mechanisms contribute to transcription of *Listeria monocytogenes* cold stress genes during cold shock and cold growth. Appl. Environ. Microbiol. 73:6019–6029.

73. Subramanian A, et al. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. 102:15545–15550.

74. Boylan SA, Rutherford A, Thomas SM, Price CW. 1992. Activation of *Bacillus subtilis* transcription factor sigma B by a regulatory pathway responsive to stationary-phase signals. J. Bacteriol. 174:3695–3706.

75. Boylan SA, Redfield AR, Brody MS, Price CW. 1993. Stress-induced activation of the σB transcription factor of *Bacillus subtilis*. J. Bacteriol. 175:7931–7937.

76. Zhang X, Bremer H. 1995. Control of the *Escherichia coli* rrnB P1 promoter strength by ppGpp. J. Biol. Chem. 270:11181–11189.