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REVIEW

SigB-regulated antioxidant functions in gram-positive bacteria

Hoai T. Tran[1] · **Carla Y. Bonilla**[1]

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Abstract
Oxidative stress can have lethal consequences if organisms do not respond and remediate the damage to DNA, proteins and lipids. Bacterial species respond to oxidative stress by activating transcriptional profiles that include biochemical functions to reduce oxidized cellular components, regenerate pools of reducing molecules, and detoxify harmful metabolites. Interestingly, the general stress response in Gram positive bacteria controlled by SigB is induced by oxidative stress from reactive oxygen and electrophilic species. The upregulation of SigB regulated genes during exposure to electrophilic and oxidative compounds suggests SigB contributes directly to the adaptations required for oxidative stress survival. A subset of the functions of SigB regulated genes can be categorized with antioxidant biochemical activities, such as redoxins, reductases and dehydrogenases, including regulation of low molecular weight thiols, yet their exact cellular role is not fully understood. Here, we present an overview of the predicted antioxidant biochemical functions regulated by SigB, with potential for biomedical research given the prevalence of oxidative stress during bacterial infection, as well as during industrial applications of large-scale production of compounds by microbes.

Keywords General stress response · Oxidative stress · SigB · *Bacillus subtilis*

Introduction

One mechanism of stress adaptation used by bacteria is activation of alternative sigma factors to change the transcriptional profile of cells in order to overcome growth limiting conditions. SigB, the general stress response sigma factor, is conserved across Firmicutes species and regulates the expression of hundreds of genes when cells experience environmental, oxidative and energy stress (Hecker et al. 2007; Price 2011). SigB is tightly regulated so that induction happens during conditions of stress and is maintained in an inactive state until stress is sensed (Benson and Haldenwang 1993). Then SigB binds to RNA polymerase recruiting it to the promoters of target genes collectively called the SigB regulon (Boylan et al. 1993; Petersohn et al. 1999, 2001; Price et al. 2001). These genes then confer resistance to a wide array of stressors such as ethanol, osmotic stress, heat shock and low pH (Hecker and Völker 1998; Hoper et al. 2005). Additionally, SigB is induced by oxidative stress and can provide cells with cross-protective properties that increase cell survival (Engelmann and Hecker 1996; Helmann et al. 2003; Reder et al. 2012). More recently, our laboratory showed that SigB activation by environmental and energy stress could also protect against other oxidants such as diamide and sodium nitroprusside that cause disulfide and nitrosative stress respectively, arguing for broad antioxidant properties encoded by SigB regulated genes (Tran et al. 2019).

Still, little attention has been paid to the mechanism of SigB activation by oxidative stress or the physiological significance of the genes induced; likely due to the presence of major oxidative stress systems that directly sense the stress and regulate a significant transcriptional network with defined antioxidant biochemical functions (Antelmann and Helmann 2011; Mongkolsuk and Helmann 2002; Zuber 2009). This review will focus on the lesser understood, predicted SigB antioxidant functions to understand the contributions of SigB to oxidative stress protection. We broadly defined antioxidant functions as the reactions carried out by gene products such as enzymes, the metabolites they produce or the modifications they promote; that provide a protective role in the presence of oxidative and electrophilic reactive

✉ Carla Y. Bonilla
bonilla@gonzaga.edu

[1] Biology Department, Gonzaga University, Spokane, WA, USA

species so that cells can (1) prevent formation of reactive molecules, (2) eliminate oxidative compounds, and (3) repair damaged cellular components. In our definition, SigB regulated genes with antioxidant functions should be induced during oxidative conditions. We performed a survey of the literature of SigB regulon members with increased expression during oxidative conditions in three Gram-positive model bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Listeria monocytogenes*. We systematically analyzed the literature on the transcriptional and proteomic responses regulated by SigB in the presence of hydrogen peroxide, diamide, methylglyoxal, allicin and hypochlorite to understand the protective functions induced due to reactive oxygen species (ROS) and reactive electrophilic species (RES). These bacterial species were chosen as they are powerful model organisms specifically the pathogens *S. aureus* and *L. monocytogenes* where the general stress response contributes to virulence (Jenul and Horswill 2018; Oliver et al. 2010). Knowledge gained about these pathogens' antioxidant defense mechanisms will aid in understanding the role of SigB in virulence.

SigB activation by environmental and energy stress

Two pathways control SigB activation in *B. subtilis*, the stressosome that transduces environmental stress (osmotic, temperature, pH stress) and the RsbP/RsbQ complex that communicates energy stress (conditions that lower ATP levels) (Akbar et al. 2001; Brody et al. 2001; Vijay et al. 2000) (Fig. 1). Under non-inducing conditions, SigB is kept in an inactive form bound to an anti-sigma factor, RsbW (Benson and Haldenwang 1993). When inducing conditions are encountered, SigB is freed from its interaction with RsbW by the anti-anti-sigma factor RsbV through a partner-switching mechanism. Specifically, dephosphorylated RsbV favors RsbW binding causing RsbW to switch partners and release SigB (Alper et al. 1996). This partner-switching mechanism of SigB activation is conserved in all three species which all encode the regulatory proteins in an operon with *sigB*. During environmental stress, activation of the stressosome causes the phosphatase activity of RsbU to dephosphorylate RsbV (Yang et al. 1996). And during low energy conditions, RsbP phosphatase activity is regulated by its partner RsbQ leading to RsbV dephosphorylation (Brody et al. 2001). Ultimately, both pathways converge on the anti-anti-sigma factor RsbV promoting SigB activity. Even though both stresses induce SigB in all three species, the signaling cascades differ amongst them (Fig. 1). The most upstream regulators of SigB in *S. aureus* remain unknown although the RsbU ortholog is required for physical stress and stationary phase SigB dependent gene induction (Pane-Fare et al. 2006). In *L. monocytogenes* which has more components in common with *B. subtilis*, namely the stressosome complex, RsbT

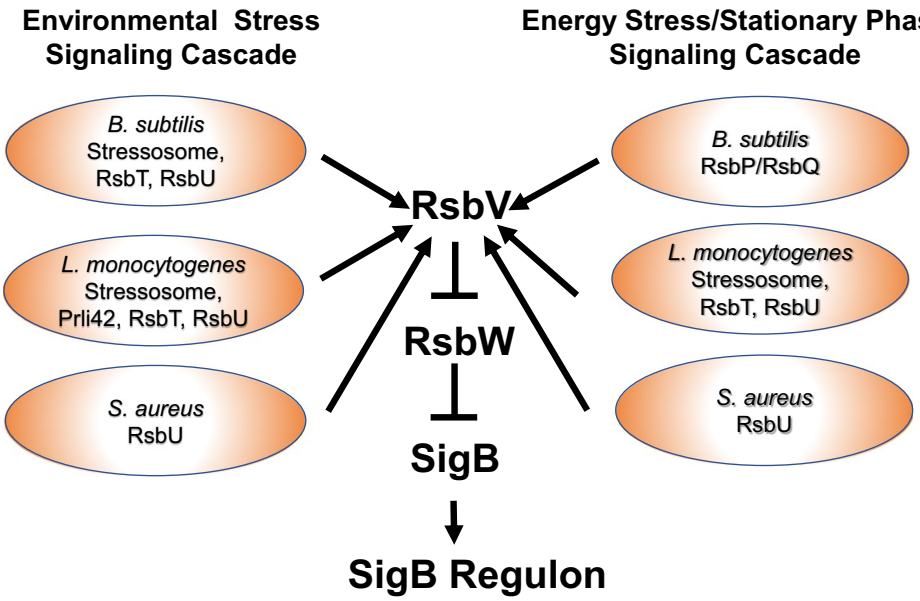

Fig. 1 SigB signaling cascade during environmental and energy stress. The two branches of the general stress response, environmental and energy/starvation stress, are shown along with the corresponding Regulators of Sigma B genes (Rsb). All three species share the anti-anti sigma factor RsbV, the anti-sigma factor RsbW and SigB. *B subtilis* uses two different complexes, the stressosome with RsbT and RsbU, and the RsbP/Q complex. *L. monocytogenes* uses the stressosome, RsbT and RsbU to transduce both types of stress but contains no RsbP/Q. *S. aureus*'s upstream regulators are unknown but RsbU is conserved and required for both environmental stress and for SigB induction during early stationary phase

and RsbU, both energy and environmental stress are sensed through the stressosome signaling cascade (Chaturongakul and Boor 2004; Martinez et al. 2010).

Possible mechanisms of SigB activation by oxidative stress

How is oxidative stress transduced to SigB? What is the signal? What is the sensor? It is possible that oxidative stress is transmitted through one of the two pathways although the experimental evidence needed to answer these questions is minimal. Interestingly, the energy stress phosphatase RsbP contains a conserved Per-Arnt-Sim (PAS) domain, which typically binds ligands such as heme groups and flavin adenine dinucleotide (FAD) that could serve as redox sensors during oxidative stress (Henry and Crosson 2011). In support of RsbP sensing oxidative stress, when *B. subtilis* was treated with nitric oxide in aerobic conditions, SigB induction was observed and was dependent on RsbP but no mechanism was determined (Moore et al. 2004). On the other hand, in the same study RsbU was required for SigB activation during stress induced by sodium nitroprusside, suggesting that both branches of the SigB regulatory circuit could be direct sensors of nitrosative stress, although through different molecular mechanisms.

A possible mechanism of oxidative stress sensing by the environmental stress branch could be envisioned through the components of the stressosome, RsbRA, RsbRB, RsbRC, RsbRD and YtvA. RsbR paralogs contain an N' terminal non-heme sensing domain, and YtvA has a specific type of PAS domain, a light-oxygen-voltage sensing domain (LOV), although it has been shown to be a light sensor instead (Akbar et al. 2001; Avila-Perez et al. 2006; Henry and Crosson 2011). RsbR proteins could bind a ligand created during oxidative stress or interact with another protein that directly senses oxidative stress.

While the *B. subtilis* scenarios are speculative, recent experimental evidence implicated the stressosome in *L. monocytogenes* in oxidative stress sensing through its direct interaction with a transmembrane miniprotein, Prli42. This suggests that oxidative stress can be sensed via the stressosome (Impens et al. 2017). Since the upstream regulatory proteins are not conserved, such as the stressosome or RsbP/Q, a conserved mechanism for oxidative stress signal transduction to SigB is unlikely. Still, induction by oxidative stress of the regulon happens in all three species. Therefore, we analyzed the published data in *B. subtilis*, *S. aureus* and *L. monocytogenes* to summarize the shared characteristics of the physiological responses by SigB during oxidative stress.

Sources of oxidative stress and commonly used compounds to study them

Metabolic reactions in the presence of oxygen have the potential to create stress by the essential reductive/oxidative (redox) reactions that are carried out inside the cell (Imlay 2019). Pathogenic bacterial species encounter strong oxidative stress when faced with the oxidative burst imposed by the immune system of the host. Phagocytes activate the production of reactive compounds such as hydrogen peroxide, nitric oxide, and hypochlorite as strong oxidants to kill the invading bacteria (Hurst 2012). Additionally, bacteria such as Bacilli species are widely used in industry for the synthesis of vitamins, detergent agents and other bio-medically relevant products and must successfully cope with oxidative stress during these energy demanding processes (Outtrup and Jorgensen 2008). The compounds chosen in this review are those most commonly used to induce different types of oxidative damage.

Hydrogen peroxide causes cytotoxicity via its potential to generate powerful reactive oxygen species like hydroxyl radicals (Juven and Pierson 1996). These free radicals can induce damage on DNA, proteins and lipids. Diamide increases intracellular disulfide crosslinking in proteins and readily reacts with low molecular weight thiols (Cumming et al. 2004). This results in protein misfolding and loss of function. Methylglyoxal has a variety of deleterious effects in cells. It promotes generation of advanced glycation end products in proteins via Millard reactions, which could impair functions of essential proteins. Moreover, methylglyoxal causes crosslinking between amino acids or between polymers, and it induces free radical formation such as superoxide anion (Chakraborty et al. 2014). The antibacterial characteristic of allicin lies in its interaction with thiol-containing proteins via formation of S-allylmercaptoglutathione, which could impair vital enzymes involved in metabolism and antioxidant mechanism in microbes (Muller et al. 2016). Additionally, evidence suggests DNA, protein, and RNA synthesis is inhibited by allicin (Ankri and Mirelman 1999). Finally, sodium hypochlorite oxidizes thiol groups on enzymes similar to allicin; and forms chlorinated amino acids which damage DNA (Fukuzaki 2006). It is possible that the choice of oxidants found in the literature caused bias in the observed sets of genes, since diamide, methylglyoxal and allicin can cause disulfide stress amongst other types of oxidative damage. A similar bias could be inferred from the transcriptomic data in *S. aureus* and *L. monocytogenes* giving us an incomplete view of the conserved antioxidant capacities of the SigB regulon in those species.

Literature search and selection criteria of oxidative stress induced genes

Google Scholar and PubMed were used to search the literature through a combination of the following keywords: *Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes*; transcriptome, transcriptomic; proteome, proteomic; SigB, Sigma B; oxidative stress, oxidation; hydrogen peroxide, diamide, methylglyoxal, hypochlorite and allicin. In addition, the databases SubtiWiki (Zhu and Stulke 2018), AureoWiki (Fuchs et al. 2018) were used to find information on nomenclature, gene function and regulation. Each article was screened for transcriptomic and proteomic data that were presented as microarray, RNA-seq or two-dimensional gel electrophoresis (2D-PAGE) followed by Mass Spectrometry (MS) under different oxidative stress treatments. All genes were chosen based on the original fold-change criteria set by the authors of each study to maintain fidelity. Among the genes and proteins identified, only those that met the following criteria were chosen. Firstly, genes must be members of SigB regulon. Secondly, genes must have been upregulated in samples with oxidant treatment in comparison to control samples since protective functions are unlikely to be downregulated during oxidative stress conditions. SigB-dependent genes whose expression was downregulated were left out since they likely do not provide protective functions. Although they are still interesting and represent clues into potentially damaging conditions that must be repressed during oxidative stress.

Bacillus subtilis. Six articles were used to identify genes upregulated in the five oxidants. Specifically, Helmann et al. (2003) used microarrays to measure gene expression in the presence of 8 and 58 μM hydrogen peroxide exposure (Helmann et al. 2003; Mostertz et al. 2004) found additional genes to be upregulated under 58 μM hydrogen peroxide (Mostertz et al. 2004). SigB-regulated genes were identified by Chi et al. (2019) via microarray analysis using 90 μM allicin treatment for 30 min (Chi et al. 2019). Chi et al. (2011) measured microarray data in a 10-min treatment with 50 μM sodium hypochlorite (Chi et al. 2011). Genes induced by methylglyoxal were identified by Nguyen et al. (2009) in a 10-min treatment under 2.8 and 5.6 mM methylglyoxal (Nguyen et al. 2009). In addition, microarray data from 1 mM diamide treatment for 5- and 15-min published by Leichert et al. identified diamide induced genes (Leichert et al. 2003). The SigB regulon was defined using the SubtiWiki database (Zhu and Stulke 2018).

Staphylococcus aureus. Genes induced under sodium hypochlorite and allicin were determined using 150 μM and 1 mM sodium hypochlorite and 300 μM allicin for 30 min (Loi et al. 2018, 2019). Chang et al. found genes induced in the presence of 10 mM hydrogen peroxide upon 10- and 20-min treatments (Chang et al. 2006). Two proteins were also identified by 2D-PAGE under 10 mM hydrogen peroxide and 1 mM diamide treatments (Posada et al. 2014; Wolf et al. 2008). Genes were determined by the authors in each individual publications, and cross referenced using the AureoWiki database and published SigB regulon members (Pane-Farre et al. 2006).

Listeria monocytogenes. Two articles were used to catalog SigB regulated genes with antioxidant function using our approach. Liu et al. identified genes in a thorough review of the literature based on phenotypic data (Liu et al. 2019), and Cortes et al. reported twenty-one SigB regulon genes with upregulation under 0.01 % hydrogen peroxide for 30 min (Cortes et al. 2019). We also used the core regulon identified by Oliver et al. and new members by Liu et al. 2017 to make sure we did not miss any genes (Liu et al. 2017; Oliver et al. 2010).

Summary of genes induced under multiple ROS and RES conditions

We compared the gene expression patterns for each species with every oxidant from the literature. They are organized in Supplementary Tables 1, 2 and 3. In *B. subtilis*, five genes were induced under all conditions: *clpC, mcsB, gabD, trxA* and *yraA*; and four genes induced under four out of five conditions: *ctsR, spx, mcsA* and *ygvN*. The analysis of *S. aureus* SigB regulon members is complicated by the multiple strains found in the literature, so we standardized the nomenclature and listed gene names based on predicted homolog names from AureoWiki (Fuchs et al. 2018). We found two genes, *hchA* and *SACOL2114*, were induced by three oxidants (NaOCl, diamide and allicin), and eleven genes induced by both hypochlorite and allicin only: *hxlA, hxlB, ktrB, rbfa, ribC, yvdD, yvgN, yceI, yflT*, SACOL2114 and SACOL2132. The SigB regulon differs in *L. monocytogenes* strains (Oliver et al. 2010). Therefore our analysis only applies to the strains used in the literature. Cortes et al. used strains 6179 and R479a to perform a transcriptomic study in the presence hydrogen peroxide and found twenty-one SigB-dependent induced genes and we added them to the list compiled by Liu et al. 2019. Given the minimal overlap between the regulons of each species, we discuss the antioxidant capabilities of SigB regulated genes by functional categories organized in Table 1. Categories like virulence, membrane transport and transcriptional regulation are not discussed in order to focus on oxidoreductases, protein quality and metabolism.

Antioxidant functional categories regulated by SigB

Oxidoreductases

Broadly speaking, proteins involved in redox reactions constitute the largest category, after genes of unknown function,

Table 1 Functional categories of SigB induced genes after oxidative stress

Functional categories	*Bacillus subtilis* (108)	*Staphylococcus aureus* (81)	*Listeria monocytogenes* (29)
oxidoreductases, low molecular weight thiol metabolism	*aldY, cypC, katE, mhqO, mhqP, ohrB, sodA, trxA, ycdF, ydaD, ydbP, ydaG, ydaP, ydbD, yerD, yhdN, yjgC, yjiB, ytkK, ytxJ, yvgN, yxbG, yxnA*	*crtI, ohr*, SACOL2114, SACOL2132, SACOL2553, SACOL2594, *trxA_3, ylbE, yvgN*, SACOL2717 (*bstA*)	LMRG_02813, *qoxABCD, sodA, ywnB, yqhD*, lmo0669, lmo1433
Control of protein quality	*clpP, clpC, ctsR, mcsA, mcsB, yfkM, yflG, ypuD, yraA*	*hchA, clpL, pfpI*	*hslO, htrA*
Metabolism and energy pathways	*gabD, guaD, luxS, ycdG, ydaE, yjiC, ykgA, yktC, yxaA*	*adh1, fabG, hutG, hxlA, hxlB, nagB, ribC*, SACOL0671, SACOL2597, SACOL2605, *suhB, crtO, gtrA, mvaK2*, SACOL0830, *nreB*	*dhaK, dhaL, dhaM, yqfL, coaX*
DNA repair and protection	*disA, dps, radA*	SACOL0742, *whiA*	*fri*
Control of transport across membrane	*bmr, bmrR, bmrU, corA, csbX, nhaX, ydzE, yqhB, ywtG*	*epiE, epiF, epiG, ktrB, nixA, opuD2*, SACOL0630, SACOL0921, SACOL2077	*bilEA*, lmo1422
Regulation of ribosome or tRNA modifying	*hpf, rpmE2, yacL, ydaF*	*rbfA*, SACOL0880, *truB*	
Transcriptional regulators	*spx, mgsR, rsbRB, rsbT, rsbS, rsbU, ydeC, ysdB*	*acrR, csoR*	*spxA* (LMRG_01641), ncRNA *sbrE, lisK*
Proteins of unknown function	*csbD, gspA, yaaI, ybyB, ycbP, ydaS, ydaT, ydhK, yebE, yfhD, yfhE, yfhF, yfhK, yfkR, yfkS, yflH, yflT, ygxB, yhcM, yjbC, yjgB, yjgD, ykzC, yocB, yocK, yqjL, ysnF, ytkL, ytxG, ytxH, yvgO, ywjC, ywmE, ywsB, ywzA, yxaB, yxjJ, yxzF, yycD, yydC, yyzH, gsiB, S1384*	*amaP, asp23*, SACOL0831, *yflT*, SACOL0039, SACOL0040, SACOL0156, SACOL0862, SACOL0863, SACOL0868, SACOL1788, SACOL1940, SACOL2076, SACOL2481, SACOL2621, *nreA*, SACOL0446, SACOL0741, SACOL1789, SACOL1802, SACOL2174, SACOL2596, *yceI, yvdD*, SACOL0085, SACOL0678	uspA (lmo0515), lmo1140, lmo1518, lmo2210, lmo2454, *sepA* (BDS), lmo0602, lmo2571, lmo2230
Virulence		*cap5C, cap5F, cap5L, eap, map-w, nuc, pnbA, ptpA*, SACOL2554 (*cidB*), SACOL2631	

Genes were organized using Gene Ontology (GO) nomenclature, biological pathway assignation from UniProt (UniProt 2019), or function identified by the authors. Genes under the same category do not imply sequence conservation between species since orthologs are not well conserved across the three species.

of SigB regulated genes induced by ROS and RES in *B. subtilis*, *S. aureus* and *L. monocytogenes*. This suggests that one main function of SigB during oxidative stress is prevention and management of oxidative damage through reductases and dehydrogenases to maintain an intracellularly balanced redox state. The specific activity of many SigB regulated reductases are untested and open to exploration. Dehydrogenases and reductases such as *aldY, ydaD, yfkM*, *yvgN*, *yhdN*, *ytxJ*, and *yxnA*, were induced by hypochlorite, hydrogen peroxide and diamide in *B. subtilis* (Table S1). Since all these oxidants cause multiple types of damage it is difficult to assign a specific function to each enzyme. However, through genetic analysis, sensitivity to oxidative compounds has been observed for mutants of some of these genes. Chandrangsu et al. found that cells lacking *yhdN* were more sensitive to methylglyoxal toxicity than wild type, while single mutants in *yfkM* and *yvgN*, were not affected (Chandrangsu et al. 2014). Deleting them in combination caused cells to become more sensitive suggesting redundant antioxidant pathways. Methylglyoxal causes lipid, protein and DNA oxidation (Lee and Park 2017), therefore each gene product could remediate a specific damage or degrade methylglyoxal intermediates through different mechanisms. The lack of transcriptional induction during methylglyoxal exposure complicates the interpretation. The difference between gene expression and sensitivity in the literature could be explained by the different growth conditions used in both experiments.

In. *B subtilis*, the aldo-keto reductase *yvgN* was induced in four out of the five oxidants from the literature, except methylglyoxal, suggesting antioxidant functions capable of reducing multiple substrates during exposure to oxidative stress (Lei et al. 2009). Similarly, in *S. aureus* SACOL2114, a predicted NAD + dependent aldehyde dehydrogenase, was induced under hypochlorite, diamide and allicin exposure. All three compounds are known to react with thiol-containing proteins so the reversal of this damage could require SACOL2114. In *L. monocytogenes* putative oxidoreductases, namely *ywnB*, *yqhD*, LMRG_02813, lmo0669, lmo2230 were induced by hydrogen peroxide yet each is predicted to carry out a different biochemical activity, so their molecular function remains unknown (Cortes et al. 2019; Liu et al. 2019). SigB regulated genes may not directly contribute to detoxification of damaged DNA or proteins, but instead could be responsible for maintaining the total antioxidant capacity of the cell through NAD(P)H-dependent dehydrogenases such as *ydaD, aldY*, and *yjgC* in *B. subtilis*. NAD(P)H is a coenzyme essential for cellular processes from metabolism to the degradation of oxidative compounds, and therefore contributes to the total antioxidant capacity of cells (Selles Vidal et al. 2018).

The well characterized enzymes encoded by *sodA*, *trxA* and *ohr* are differentially regulated in these three species, arguing that their regulation by SigB has evolved separately. Thioredoxin*s* in *B. subtilis* encoded by *trxA* was induced by all oxidants, and *ydbP* was induced by hypochlorite and diamide (Table S1), and *trxA-3* in *S. aureus* is induced by hypochlorite supporting the general role that thioredoxins play in ROS and RES scavenging and protection of oxidized proteins by their disulfide reducing activity (Lu and Holmgren 2014). The hydroperoxide resistance protein Ohr family members were induced in *B. subtilis* (*ohrB*) and *S. aureus* (*ohr*). These proteins are directly involved in protection against peroxide anions (Volker et al. 1998); and were also induced by hypochlorite (Table S1, S2). The superoxide dismutase *sodA* gene was induced in both *L. monocytogenes* and *B. subtilis*. Superoxide dismutase is responsible for the reaction that converts superoxide ions to hydrogen peroxide (Fridovich 1995). Its expression in the presence of hydrogen peroxide was expected, but it was also induced in hypochlorite and allicin, suggesting superoxide anions may be formed by these oxidants.

Low molecular weight thiol metabolism

An interesting predicted function of the SigB regulons of all three species is the regulation of low molecular-weight (LMW) thiols specifically glutathione in *L. monocytogenes* and bacillithiol in *B. subtilis* and *S. aureus*. In *L. monocytogenes* lmo1433, a putative glutathione reductase, in *S. aureus* SACOL2717, a putative bacillithiol-transferase, *bstA*; and in *B. subtilis ytxJ* a putative bacilliredoxin are all regulated by SigB (Table 1). LMW thiols such as glutathione and bacillithiol are important during oxidative stress for their multiple functions as cofactors used by oxidorectases, in protection of thiol-containing amino acids by direct thiolation, and as oxidation buffers themselves (Loi et al. 2015). Recycling of bacillihtiol to its reduced form during oxidative stress by a bacilliredoxin (*ytxJ*) would be important in *B. subtilis*. Although no experimental evidence exists of this function for *ytxJ*, it does suggest that SigB could directly contribute to the maintenance of reduced bacillithiol which could explain the need for SigB during oxidative stress.

Bacillithiol is also used in multiple reactions in the bacteria that produce it. One of its functions is to aid in the direct degradation of toxins by direct bacillithiolation by bacillithiol-transferase enzymes (*bst*) that carry out these reactions (Perera et al. 2014). In *S. aureus*, SACOL2717 encodes a bacillithiol transferase supporting the direct degradation of oxidants by the SigB regulon. Similarly, in *L. monocytogenes* which uses glutathione, the pool of the reduced form would need to be maintained due to ROS and RES and lmo1433 through a glutathione reductase activity could directly promote this. Thus, SigB in all three species appears to be directly involved in the metabolism of antioxidant molecules such as bacillithiol and glutathione.

Control of protein quality

One main function of SigB during oxidative stress is preventing the accumulation of oxidized proteins through their degradation by proteases and chaperones (Hecker and Völker 1998; Kruger et al. 1994). This is expected since ROS and RES cause direct protein damage such as protein oxidation and protein unfolding, and was one of the first characteristics of the general stress response identified. In *B. subtilis* most oxidants caused induction of the *clpC* protease and its regulators *ctsR, mcsA* and *mcsB* which are all induced in an operon (Derre et al. 1999). *yraA and yfkM* that encode glyoxylase III- like proteases were also induced in *B. subtilis*. Specifically, *yraA* was induced in the presence of all five oxidants suggesting it has a general proteolytic role. In support of *yraA* and *yfkM*'s function during RES a double mutant of these genes was sensitive to formaldehyde and methylglyoxal treatment compared to wild type (Nguyen et al. 2009). In *S. aureus hchA*, a predicted chaperon protein in the glyoxylase III family, was induced in hypochlorite, diamide and allicin stress conditions, and *clpL* was induced in hydrogen peroxide and hypochlorite exposure (Table S2). It appears that glyoxylase III-like proteins are a conserved feature of the SigB regulon. In *L. monocytogenes*, SigB-regulated proteases include serine protease *htrA* induced during hydrogen peroxide (Cortes et al. 2019), and a redox sensitive chaperonin, *hslO* (Table S3). Regulation of protein quality through chaperones and proteases is a shared feature of the SigB regulon.

Control of metabolism

Given that cellular respiration and ATP production are affected by oxidative agents, regulation of enzymes involved in metabolism of alternate sources of energy, electron acceptors, donors and co-factors are appropriate responses by these organisms. Consistently, in *B. subtilis gabD* which encodes succinic semi-aldehyde dehydrogenase involved in gamma-amino butyric acid (GABA) (Belitsky and Sonenshein 2002) was induced in all five conditions suggesting a general antioxidant role. GABA has been shown to have multiple roles during acid and oxidative stress in bacteria either by its effect on intracellular pH or by the production of NADPH during its reaction affecting the cellular redox potential (Feehily and Karatzas 2013). This could be a generalized response and main reason for metabolic gene induction during ROS and RES.

In *S. aureus*, the genes *hlxA, hxlB* were induced in two oxidative conditions (hypochlorite and allicin). They are involved in formaldehyde assimilation which could be important during detoxification of damaged metabolic intermediates (Chen et al. 2016). Similarly, predicted functions such as fatty acid biosynthesis by *fabG*, FAD synthesis by *ribC*, amino acid synthesis by *hutG*, mevalonate metabolism by *mvaK2*, and pyruvate oxidation by *cidC* (SACOL2553) were all induced although by different oxidants (Table S2). The putative regulator of gluconeogenesis, *yqfL*, was found to be induced during oxidative stress in *L. monocytogenes* (Cortes et al. 2019), consistent with other metabolic genes regulated by SigB in *B. subtilis* and *S. aureus.* As respiration is affected by oxidative stress, genes involved in alternate pathways could be necessary to control glycolytic or other metabolic pathways to aid in maintenance of appropriate redox conditions.

Conclusions

SigB is known as the general stress sigma factor, but oxidative stress protection is also one of its roles as is seen by the frequent induction of SigB-regulated genes under oxidative conditions in many bacterial species. Direct regulation of antioxidant genes by SigB could have come from the overlap between environmental and energy stress with oxidative stress as cells evolved a response to the constantly fluctuating conditions of life in natural environments. In fact, SigB is thought to increase resilience and promote higher stress tolerance by allowing cells to adjust and perform better under continued stress exposure (Guldimann et al. 2016). Gram-positive bacteria are used industrially for large-scale production of vitamins, enzymes, amino acids, etc. SigB is found in Gram-positive species of industrial and biomedical interest such as *Bacilli* species (Outtrup and Jorgensen 2008). Understanding the biochemical functions of SigB regulated genes could provide application avenues such as manipulating the appropriate SigB target(s) to optimize production by promoting higher stress tolerance in industrial conditions.

Higher resistance to oxidative stress caused during fermentation could be exploited to increase production yield in industrially used Gram-positive species. In *B. pumilis*, SigB targets in each of the categories in Table 1, *spxA*, *yfkM, trxA, ohrB*, *radA* and the *clp* proteases, were induced by hydrogen peroxide (Handtke et al. 2014). On the other hand, increased stress tolerance could be problematic during food production. Food sanitation often uses heat treatment and disinfectants such as hydrogen peroxide but depending on the amount, duration and sequence of these treatments, SigB could be activated giving food-borne pathogens an advantage, such as in the case of *L. monocytogenes*. Importantly, since food preservation aims to minimize bacterial contamination while maintaining nutritional properties, understanding the general stress response controlled by SigB will be important for designing effective protocols that successfully inhibit bacterial growth but do not promote the enhanced resistance (Bucur et al. 2018).

SigB plays a role in pathogenesis in both *S. aureus* and *L. monocytogenes* (Jenul and Horswill 2018; Liu et al. 2019). In *S. aureus sigB*-deleted cells were less effective at chronic intracellular persistence than their wild type counterparts (Tuchscherr et al. 2017). Chronic bacterial infection is characterized by the presence of persister cells that are resistant to antibiotics and are therefore a major concern in medical settings. Chronic infection requires a transcriptional program that can adapt to the hostile, intracellular environment of the host and SigB could play this role through the promotion of bacterial stress resilience contributing to the persister phenotype. Given the biochemical protective pathways associated with the SigB regulon, it would be interesting to characterize these predicted enzymes and their role in the persister phenotype as potential drug targets. In *L. monocytogenes*, SigB was not found to be a significant contributor to the persister phenotype in a culture assay, but it played a minor role in killing rate during early stationary phase when SigB is known to be active (Knudsen et al. 2013). The implication that the general stress response induced by SigB could be important for bacterial persistence makes it an important area of research.

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References

Akbar S, Gaidenko TA, Kang CM, O'Reilly M, Devine KM, Price CW (2001) New family of regulators in the environmental signaling pathway which activates the general stress transcription factor sigma(B) of *Bacillus subtilis* . J Bacteriol 183:1329–1338. https://doi.org/10.1128/JB.183.4.1329-1338.2001

Alper S, Dufour A, Garsin DA, Duncan L, Losick R (1996) Role of adenosine nucleotides in the regulation of a stress-response transcription factor in *Bacillus subtilis*. J Mol Biol 260:165–177. https://doi.org/10.1006/jmbi.1996.0390

Ankri S, Mirelman D (1999) Antimicrobial properties of allicin from garlic. Microbes Infect 1:125–129. https://doi.org/10.1016/s1286-4579(99)80003-3

Antelmann H, Helmann JD (2011) Thiol-based redox switches and gene regulation. Antioxid Redox Signal 14:1049–1063. https://doi.org/10.1089/ars.2010.3400

Avila-Perez M, Hellingwerf KJ, Kort R (2006) Blue light activates the sigmaB-dependent stress response of *Bacillus subtilis* via YtvA. J Bacteriol 188:6411–6414. https://doi.org/10.1128/JB.00716-06

Belitsky BR, Sonenshein AL (2002) GabR, a member of a novel protein family, regulates the utilization of gamma-aminobutyrate in *Bacillus subtilis*. Mol Microbiol 45:569–583. https://doi.org/10.1046/j.1365-2958.2002.03036.x

Benson AK, Haldenwang WG (1993) *Bacillus subtilis* sigma B is regulated by a binding protein (RsbW) that blocks its association with core RNA polymerase. Proc Natl Acad Sci USA 90:2330–2334. https://doi.org/10.1073/pnas.90.6.2330

Boylan SA, Redfield AR, Price CW (1993) Transcription factor sigma B of *Bacillus subtilis* controls a large stationary-phase regulon. J Bacteriol 175:3957–3963. https://doi.org/10.1128/jb.175.13.3957-3963.1993

Brody MS, Vijay K, Price CW (2001) Catalytic function of an alpha/beta hydrolase is required for energy stress activation of the sigma(B) transcription factor in *Bacillus subtilis*. J Bacteriol 183:6422–6428. https://doi.org/10.1128/JB.183.21.6422-6428.2001

Bucur FI, Grigore-Gurgu L, Crauwels P, Riedel CU, Nicolau AI (2018) Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing. Environ Front Microbiol 9:2700. https://doi.org/10.3389/fmicb.2018.02700

Chakraborty S, Karmakar K, Chakravortty D (2014) Cells producing their own nemesis: understanding methylglyoxal metabolism. IUBMB Life 66:667–678. https://doi.org/10.1002/iub.1324

Chandrangsu P, Dusi R, Hamilton CJ, Helmann JD (2014) Methylglyoxal resistance in *Bacillus subtilis*: contributions of bacillithiol-dependent and independent pathways. Mol Microbiol 91:706–715. https://doi.org/10.1111/mmi.12489

Chang W, Small DA, Toghrol F, Bentley WE (2006) Global transcriptome analysis of *Staphylococcus aureus* response to hydrogen peroxide. J Bacteriol 188:1648–1659. https://doi.org/10.1128/JB.188.4.1648-1659.2006

Chaturongakul S, Boor KJ (2004) RsbT and RsbV contribute to sigmaB-dependent survival under environmental, energy, and intracellular stress conditions in *Listeria monocytogenes*. Appl Environ Microbiol 70:5349–5356. https://doi.org/10.1128/AEM.70.9.5349-5356.2004

Chen NH, Djoko KY, Veyrier FJ, McEwan AG (2016) Formaldehyde stress responses in bacterial. Pathogens Front Microbiol 7:257. https://doi.org/10.3389/fmicb.2016.00257

Chi BK, Gronau K, Mader U, Hessling B, Becher D, Antelmann H (2011) S-bacillithiolation protects against hypochlorite stress in *Bacillus subtilis* as revealed by transcriptomics and redox proteomics. Mol Cell Proteomics 10:M111 009506. https://doi.org/10.1074/mcp.M111.009506

Chi BK et al (2019) The disulfide stress response and protein S-thioallylation caused by allicin and diallyl polysulfanes in *Bacillus subtilis* as revealed by transcriptomics and proteomics. Antioxidants. https://doi.org/10.3390/antiox8120605

Cortes BW, Naditz AL, Anast JM, Schmitz-Esser S (2019) Transcriptome sequencing of *Listeria monocytogenes* reveals major gene expression changes in response to lactic acid stress exposure but a less pronounced response to oxidative stress. Front Microbiol 10:3110. https://doi.org/10.3389/fmicb.2019.03110

Cumming RC, Andon NL, Haynes PA, Park M, Fischer WH, Schubert D (2004) Protein disulfide bond formation in the cytoplasm during oxidative stress. J Biol Chem 279:21749–21758. https://doi.org/10.1074/jbc.M312267200

Derre I, Rapoport G, Msadek T (1999) CtsR, a novel regulator of stress and heat shock response, controls clp and molecular chaperone gene expression in gram-positive bacteria. Mol Microbiol 31:117–131. https://doi.org/10.1046/j.1365-2958.1999.01152.x

Engelmann S, Hecker M (1996) Impaired oxidative stress resistance of *Bacillus subtilis* sigB mutants and the role of katA and katE. FEMS Microbiol Lett 145:63–69

Feehily C, Karatzas KA (2013) Role of glutamate metabolism in bacterial responses towards acid and other stresses. J Appl Microbiol 114:11–24. https://doi.org/10.1111/j.1365-2672.2012.05434.x

Fridovich I (1995) Superoxide radical and superoxide dismutases. Annu Rev Biochem 64:97–112. https://doi.org/10.1146/annurev.bi.64.070195.000525

Fuchs S et al (2018) AureoWiki The repository of the *Staphylococcus aureus* research and annotation community. Int J Med Microbiol 308:558–568. https://doi.org/10.1016/j.ijmm.2017.11.011

Fukuzaki S (2006) Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci 11:147–157. https://doi.org/10.4265/bio.11.147

Guldimann C, Boor KJ, Wiedmann M, Guariglia-Oropeza V (2016) Resilience in the face of uncertainty: sigma factor b fine-tunes gene expression to support homeostasis in gram-positive bacteria. Appl Environ Microbiol 82:4456–4469. https://doi.org/10.1128/AEM.00714-16

Handtke S et al (2014) *Bacillus pumilus* reveals a remarkably high resistance to hydrogen peroxide provoked oxidative stress. PLoS ONE 9:e85625. https://doi.org/10.1371/journal.pone.0085625

Hecker M, Völker U (1998) Non-specific, general and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the sigmaB regulon. Mol Microbiol 29:1129–1136

Hecker M, Pane-Farre J, Volker U (2007) SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. Annu Rev Microbiol 61:215–236. https://doi.org/10.1146/annurev.micro.61.080706.093445

Helmann JD, Wu MF, Gaballa A, Kobel PA, Morshedi MM, Fawcett P, Paddon C (2003) The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. J Bacteriol 185:243–253. https://doi.org/10.1128/jb.185.1.243-253.2003

Henry JT, Crosson S (2011) Ligand-binding PAS domains in a genomic, cellular, and structural context. Annu Rev Microbiol 65:261–286. https://doi.org/10.1146/annurev-micro-121809-151631

Hoper D, Volker U, Hecker M (2005) Comprehensive characterization of the contribution of individual SigB-dependent general stress genes to stress resistance of *Bacillus subtilis*. J Bacteriol 187:2810–2826. https://doi.org/10.1128/JB.187.8.2810-2826.2005

Hurst JK (2012) What really happens in the neutrophil phagosome? Free Radic Biol Med 53:508–520. https://doi.org/10.1016/j.freeradbiomed.2012.05.008

Imlay JA (2019) Where in the world do bacteria experience oxidative stress? Environ Microbiol 21:521–530. https://doi.org/10.1111/1462-2920.14445

Impens F et al (2017) N-terminomics identifies Prli42 as a membrane miniprotein conserved in Firmicutes and critical for stressosome activation in Listeria monocytogenes. Nat Microbiol 2:17005. https://doi.org/10.1038/nmicrobiol.2017.5

Jenul C, Horswill AR (2018) Regulation of *Staphylococcus aureus*. Virulence Microbiol Spectr. https://doi.org/10.1128/microbiolspec.GPP3-0031-2018

Juven BJ, Pierson MD (1996) Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation (dagger). J Food Prot 59:1233–1241. https://doi.org/10.4315/0362-028X-59.11.1233

Knudsen GM, Ng Y, Gram L (2013) Survival of bactericidal antibiotic treatment by a persister subpopulation of Listeria monocytogenes. Appl Environ Microbiol 79:7390–7397. https://doi.org/10.1128/AEM.02184-13

Kruger E, Volker U, Hecker M (1994) Stress induction of clpC in *Bacillus subtilis* and its involvement in stress tolerance. J Bacteriol 176:3360–3367. https://doi.org/10.1128/jb.176.11.3360-3367.1994

Lee C, Park C (2017) Bacterial responses to glyoxal and methylglyoxal: reactive electrophilic species. Int J Mol Sci 18:169. https://doi.org/10.3390/ijms18010169

Lei J, Zhou YF, Li LF, Su XD (2009) Structural and biochemical analyses of YvgN and YtbE from *Bacillus subtilis*. Protein Sci 18:1792–1800. https://doi.org/10.1002/pro.178

Leichert LI, Scharf C, Hecker M (2003) Global characterization of disulfide stress in *Bacillus subtilis*. J Bacteriol 185:1967–1975. https://doi.org/10.1128/jb.185.6.1967-1975.2003

Liu Y, Orsi RH, Boor KJ, Wiedmann M, Guariglia-Oropeza V (2017) Home alone: elimination of all but one alternative sigma factor in *Listeria monocytogenes* allows prediction of new roles for sigma. Front Microbiol 8:1910 https://doi.org/10.3389/fmicb.2017.01910

Liu Y, Orsi RH, Gaballa A, Wiedmann M, Boor KJ, Guariglia-Oropeza V (2019) Systematic review of the *Listeria monocytogenes* sigma(B) regulon supports a role in stress response virulence metabolism. Future Microbiol 14:801–828. https://doi.org/10.2217/fmb-2019-0072

Loi VV, Rossius M, Antelmann H (2015) Redox regulation by reversible protein S-thiolation in bacteria. Front Microbiol 6:187. https://doi.org/10.3389/fmicb.2015.00187

Loi VV et al (2018) Redox-sensing under hypochlorite stress and infection conditions by the Rrf2-family repressor HypR in *Staphylococcus aureus*. Antioxid Redox Signal 29:615–636. https://doi.org/10.1089/ars.2017.7354

Loi VV et al (2019) *Staphylococcus aureus* responds to allicin by global S-thioallylation—role of the Brx/BSH/YpdA pathway and the disulfide reductase MerA to overcome allicin stress. Free Radic Biol Med 139:55–69. https://doi.org/10.1016/j.freeradbiomed.2019.05.018

Lu J, Holmgren A (2014) The thioredoxin antioxidant system. Free Radic Biol Med 66:75–87. https://doi.org/10.1016/j.freeradbiomed.2013.07.036

Martinez L, Reeves A, Haldenwang W (2010) Stressosomes formed in *Bacillus subtilis* from the RsbR protein of *Listeria monocytogenes* allow sigma(B) activation following exposure to either physical or nutritional stress. J Bacteriol 192:6279–6286. https://doi.org/10.1128/JB.00467-10

Mongkolsuk S, Helmann JD (2002) Regulation of inducible peroxide stress responses. Mol Microbiol 45:9–15. https://doi.org/10.1046/j.1365-2958.2002.03015.x

Moore CM, Nakano MM, Wang T, Ye RW, Helmann JD (2004) Response of *Bacillus subtilis* to nitric oxide and the nitrosating agent sodium nitroprusside. J Bacteriol 186:4655–4664. https://doi.org/10.1128/JB.186.14.4655-4664.2004

Mostertz J, Scharf C, Hecker M, Homuth G (2004) Transcriptome and proteome analysis of *Bacillus subtilis* gene expression in response to superoxide and peroxide stress. Microbiology 150:497–512. https://doi.org/10.1099/mic.0.26665-0

Muller A et al (2016) Allicin induces thiol stress in bacteria through S-allylmercapto modification of protein cysteines. J Biol Chem 291:11477–11490. https://doi.org/10.1074/jbc.M115.702308

Nguyen TT et al (2009) Genome-wide responses to carbonyl electrophiles in *Bacillus subtilis*: control of the thiol-dependent formaldehyde dehydrogenase AdhA and cysteine proteinase YraA by the MerR-family regulator YraB (AdhR). Mol Microbiol 71:876–894. https://doi.org/10.1111/j.1365-2958.2008.06568.x

Oliver HF, Orsi RH, Wiedmann M, Boor KJ (2010) *Listeria monocytogenes* {sigma}B has a small core regulon and a conserved role in virulence but makes differential contributions to stress tolerance across a diverse collection of strains. Appl Environ Microbiol 76:4216–4232. https://doi.org/10.1128/AEM.00031-10

Outtrup H, Jorgensen ST (2008) The importance of bacillus species in the production of industrial enzymes. In: Applications and systematics of Bacillus and relatives. pp 206–218. https://doi.org/10.1002/9780470696743.ch14

Pane-Farre J, Jonas B, Forstner K, Engelmann S, Hecker M (2006) The sigmaB regulon in *Staphylococcus aureus* and its regulation. Int J Med Microbiol 296:237–258. https://doi.org/10.1016/j.ijmm.2005.11.011

Perera VR, Newton GL, Parnell JM, Komives EA, Pogliano K (2014) Purification and characterization of the *Staphylococcus aureus* bacillithiol transferase BstA. Biochim Biophys Acta 1840:2851–2861. https://doi.org/10.1016/j.bbagen.2014.05.001

Petersohn A, Antelmann H, Gerth U, Hecker M (1999) Identification and transcriptional analysis of new members of the sigmaB regulon in *Bacillus subtilis*. Microbiology 145(4):869–880. https://doi.org/10.1099/13500872-145-4-869

Petersohn A, Brigulla M, Haas S, Hoheisel JD, Volker U, Hecker M (2001) Global analysis of the general stress response of Bacillus subtilis. J Bacteriol 183:5617–5631. https://doi.org/10.1128/JB.183.19.5617-5631.2001

Posada AC et al (2014) Importance of bacillithiol in the oxidative stress response of *Staphylococcus aureus*. Infect Immun 82:316–332. https://doi.org/10.1128/IAI.01074-13

Price CW (2011) General stress response in *Bacillus subtilis* and related gram-positive bacteria. In: Bacterial stress responses, 2nd edn. American Society of Microbiology, pp 301–318

Price CW, Fawcett P, Ceremonie H, Su N, Murphy CK, Youngman P (2001) Genome-wide analysis of the general stress response in *Bacillus subtilis*. Mol Microbiol 41:757–774. https://doi.org/10.1046/j.1365-2958.2001.02534.x

Reder A, Hoper D, Gerth U, Hecker M (2012) Contributions of individual sigmaB-dependent general stress genes to oxidative stress resistance of Bacillus subtilis. J Bacteriol 194:3601–3610. https://doi.org/10.1128/JB.00528-12

Selles Vidal L, Kelly CL, Mordaka PM, Heap JT (2018) Review of NAD(P)H-dependent oxidoreductases: properties, engineering and application. Biochim Biophys Acta Proteins Proteom 1866:327–347. https://doi.org/10.1016/j.bbapap.2017.11.005

Tran V, Geraci K, Midili G, Satterwhite W, Wright R, Bonilla CY (2019) Resilience to oxidative and nitrosative stress is mediated by the stressosome, RsbP and SigB in *Bacillus subtilis*. J Basic Microbiol 59:834–845. https://doi.org/10.1002/jobm.201900076

Tuchscherr L, Geraci J, Loffler B (2017) Staphylococcus aureus regulator sigma B is important to develop chronic infections in hematogenous murine osteomyelitis model. Pathogens 6:31. https://doi.org/10.3390/pathogens6030031

UniProt C (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515. https://doi.org/10.1093/nar/gky1049

Vijay K, Brody MS, Fredlund E, Price CW (2000) A PP2C phosphatase containing a PAS domain is required to convey signals of energy stress to the sigmaB transcription factor of *Bacillus subtilis*. Mol Microbiol 35:180–188

Volker U, Andersen KK, Antelmann H, Devine KM, Hecker M (1998) One of two osmC homologs in *Bacillus subtilis* is part of the sigmaB-dependent general stress regulon. J Bacteriol 180:4212–4218. https://doi.org/10.1128/JB.180.16.4212-4218.1998

Wolf C, Hochgrafe F, Kusch H, Albrecht D, Hecker M, Engelmann S (2008) Proteomic analysis of antioxidant strategies of *Staphylococcus aureus*: diverse responses to different oxidants. Proteomics 8:3139–3153. https://doi.org/10.1002/pmic.200701062

Yang X, Kang CM, Brody MS, Price CW (1996) Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. Genes Dev 10:2265–2275

Zhu B, Stulke J (2018) SubtiWiki in 2018: from genes and proteins to functional network annotation of the model organism *Bacillus subtilis*. Nucleic Acids Res 46:D743–D748. https://doi.org/10.1093/nar/gkx908

Zuber P (2009) Management of oxidative stress in Bacillus. Annu Rev Microbiol 63:575–597. https://doi.org/10.1146/annurev.micro.091208.073241