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Contributions of Individual σ^B-Dependent General Stress Genes to Oxidative Stress Resistance of *Bacillus subtilis*

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The general stress regulon of *Bacillus subtilis* comprises approximately 200 genes and is under the control of the alternative sigma factor σ^B. The activation of σ^B occurs in response to multiple physical stress stimuli as well as energy starvation conditions. The expression of the general stress proteins provides growing and stationary nonsporulating vegetative cells with nonspecific and broad stress resistance. A previous comprehensive phenotype screening analysis of 94 general stress gene mutants in response to severe growth-inhibiting stress stimuli, including ethanol, NaCl, heat, and cold, indicated that secondary oxidative stress may be a common component of severe physical stress. Here we tested the individual contributions of the same set of 94 mutants to the development of resistance against exposure to the superoxide-generating agent paraquat and hydrogen peroxide (H_2O_2). In fact, 62 mutants displayed significantly decreased survival rates in response to paraquat and/or H_2O_2 stress compared to the wild type at a confidence level of an α value of $\leq$0.01. Thus, we were able to assign 47 general stress genes to survival against superoxide, 6 genes to protection from H_2O_2 stress, and 9 genes to the survival against both. Furthermore, we show that a considerable overlap exists between the phenotype clusters previously assumed to be involved in oxidative stress management and the actual group of oxidative-stress-sensitive mutants. Our data provide information that many general stress proteins with still unknown functions are implicated in oxidative stress resistance and further support the notion that different severe physical stress stimuli elicit a common secondary oxidative stress.

The genome of the Gram-positive model organism *Bacillus subtilis* comprises approximately 4,250 genes; about 1,300 of these genes encode proteins of still unknown functions (6). By the use of comparative transcriptome and proteome analyses of wild-type strains and mutant strains defective in regulatory proteins, it has become possible to define several stimulon, regulon, modulon, as well as operon structures in *B. subtilis*. These approaches also led to the characterization of the general stress regulon that is under the control of the alternative sigma factor σ^B and comprises approximately 200 genes (19, 35, 38, 40). The activation of σ^B occurs "generally" in response to various stress stimuli. These stimuli include physical stress, such as high and low temperatures, salt, ethanol, low pH, the nitrogen oxide (NO) donor sodium nitroprusside (SNP), and direct exposure to NO gas; irradiation with blue light; the addition of antibiotics such as bacitracin and vancomycin as well as starvation for glucose, phosphate, and oxygen; or treatment with compounds such as azide, mycophenolic acid, or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which cause a deprivation of ATP and GTP levels (15, 39). It has been shown that the σ^B-dependent stress response is important for survival against severe ethanol, heat, salt, and alkaline shocks as well as repeated freezing-thawing cycles (14, 21, 44). Furthermore, it has been demonstrated that σ^B is necessary for growth as well as stationary-phase survival during prolonged periods of low temperatures (8, 21, 28). Thus, the characteristic function of the general stress proteins is to provide the cell with comprehensive cross-protective and preemptive stress resistance (14, 16, 21, 39, 44): (i) cross-protective, because it also confers resistance to oxidative (2, 12, 17) or alkaline (14) stresses that are not typical σ^B-inducing stimuli, and (ii) preemptive, because the protective functions of the general stress proteins also equip a nongrowing vegetative and nonsporulating cell against possible future stresses (17, 21, 44). The importance of the general stress response in cross-protection against oxidative stress was recognized about 15 years ago (3, 12–14, 16). Nevertheless, primary oxidative stress plays only a minor role in the activation of σ^B (35), and previous reports showed that oxidative stress induces only a subset of the σ^B regulon members (18, 32).

Although the overall physiological significance of the σ^B regulon for the stress adaptation and survival of *B. subtilis* is obvious, more than one-third of the general stress genes encode proteins with still unknown functions. The first attempt to comprehensively characterize the contribution of these general stress proteins with undefined functions to resistance against one or more specific stresses was made by Höper et al. (21). A phenotype screening analysis of 94 mutations of individual general stress genes was carried out. The mutants were exposed to heat (54°C), ethanol (10%), cold (4°C and 12°C), and hyperosmotic (10% NaCl) stresses to assign them to specific stress clusters on the basis of sensitive phenotypes. Notably, most of the mutants exhibited multiple stress management defects, indicating that different stress stimuli must cause a related damage to the cell that is counteracted by the same set of general stress gene products. In this context, many mutants with severe multiple-stress phenotypes could be associated with protection against oxidative damage. Thus, it was assumed that secondary oxidative stress may be a common component of multiple severe growth-inhibiting and σ^B-inducing stress stimuli (21).

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The idea of secondary oxidative stress is corroborated by the identification of MgsR, a paralogue of the global regulator of the diamide stress response, Spx (41). The expression of MgsR is driven by σ^B and is necessary to achieve the full induction of a subregulon within the general stress response whose members can be linked to oxidative stress management (41). Recently, it was demonstrated that the regulator MgsR is activated by a redox switch beyond the primary decision of σ^B activation caused by the physical stress stimulus (A. Reder, D. Pöther, U. Gerth, and M. Hecker, unpublished data). Thus, the MgsR protein senses and integrates secondary oxidative stress signals caused by the imposition of ethanol stress.

Several studies further supported the idea that σ^B-inducing stress stimuli trigger secondary oxidative stress within the cell. Acid stress has been reported to induce the oxidative stress response in *B. subtilis* (46) and to cause hydroxyl radical ($OH^{\bullet}$) and peroxynitrite ($ONOO^-$) formation in *Bacillus cereus* (31). A radical-mediated mechanism for cell death caused by bactericidal antibiotics such as bacitracin was proposed previously (26), and the formation of superoxide and hydroxyl radicals was demonstrated to occur upon heat stress in *B. subtilis* (29) and *B. cereus* (30). Furthermore, ethanol, salt, and cold stresses have been shown to induce PerR-regulated genes in *B. subtilis* (20, 21, 24), and the respiratory chain of mitochondria was reported to be the origin of the extensive generation of reactive oxygen species (ROS) during ethanol metabolism in hepatocytes (1, 5).

Due to this increasing amount of data pointing at the significance of secondary oxidative stress management by the general stress proteins, we extended the previous analysis of Höper et al. (21) by monitoring the effect of the superoxide-generating agent paraquat as well as hydrogen peroxide (H_2O_2) stress on the survival of 94 mutants with singular defects in σ^B-dependent general stress genes. The results confirm that many general stress proteins are indeed involved in the oxidative stress resistance of *B. subtilis*. This functional genomics approach provides a valuable basis for more detailed biochemical investigations of the exact functions of general stress proteins to counteract secondary oxidative stress.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The two reference strains for this study were *Bacillus subtilis* wild-type strain 168 (10) and *sigB* mutant strain ML6 (22). Apart from two exceptions, the same set of 92 strains with mutations in single general stress genes described previously by Höper et al. was used (21). Mutants were constructed by the disruption of the reading frames of the general stress genes by the Campbell-type insertion of nonreplicative plasmids (pMutin1 to pMutin4) (25) or deletions internal to the structural gene *ctc*::*spc* (strain GF500) (42). The two pMUTIN mutant strains of *spx* (*yjbD*) (strain 2842) and *mgsR* (*yqgZ*) (strain 4773) that were used previously (21) have been replaced here by the Δ*mgsR* (BAR1) (41) and Δ*spx* (*spx*::*spc*) (strain BAR8) (this study) deletion mutants. For the creation of the *spx* mutant, a modified two-step-fusion PCR protocol (45) was used to generate linear DNA fragments carrying a central spectinomycin resistance marker flanked by homologous sequences of the chromosomal up- and downstream regions of *spx*. The following primers were used: *spx*_up_for (GATGAAGGCAAACATCATATT) and *spx*_up_rev (TATTAATTTGTTCGTATGTATTCATTCATCTTCACTC CTCTAATTAGT) to generate the upstream fragment, *spx*_do_for (TAA CAGATTAAAAAAATTATAATAGATCGTATCATCAAAAG) and *spx*-_do_rev (TTATTCTCGGAACATTTATTGC) to generate the downstream fragment with *B. subtilis* wild-type chromosomal DNA as the template, as well as *spec*_for (ATGAATACATACGAACAAATTAATA) and *spec*_rev (TTATAATTTTTTTAATCTGTTATTT) for the spectinomycin resistance marker with plasmid pUS19 (7) as the template. The purification and fusion of the PCR products were carried out as described previously (41). For all experiments, a synthetic medium (43) was used. Growth was monitored by measuring the optical density at 500 nm (OD_{500}). Fifty milliliters of prewarmed medium was inoculated with exponentially growing cells to obtain a starting OD_{500} of 0.06. Cultures were routinely grown in 500-ml Erlenmeyer flasks in a shaking water bath at 180 rpm at 37°C.

Exposure to stress and viability assays. When the cultures reached an OD_{500} of 0.4, 18 ml was transferred into a prewarmed 100-ml flask, and the cells were stressed with 2% (vol/vol) ethanol to induce the general stress response for 20 min. After this preadaptation period, the cells were stressed with final concentrations of either 100 mM paraquat or 5 mM H_2O_2. Samples were taken prior to the imposition of oxidative stress (time zero [t_0] control) and after 60, 120, 180, and 240 min for paraquat-stressed cells and after 30, 60, and 90 min for H_2O_2-stressed cells. Aliquots (100 μl) of the samples were diluted in a 0.9% (wt/vol) NaCl solution, appropriate dilutions of the cultures were plated onto LB agar and incubated overnight at 37°C, and the CFU were counted. All experiments were performed at least in triplicate, and at least two technical replicates from each dilution step were utilized to determine the number of CFU. The analysis of the survival experiment data was carried out as described previously by Höper et al. (21).

***In silico* analysis.** The amino acid sequences of the respective proteins were downloaded from the GenoList website (27) and were analyzed with the BLASTP protein-protein blast tool at the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS AND DISCUSSION

Impact of superoxide and peroxide stress on survival of *B. subtilis* wild-type and Δ*sigB* mutant cells. In the present study, we characterized the stress resistance profiles of 94 strains with mutations of individual general stress genes in response to superoxide and peroxide stresses. To be able to observe sensitive phenotypes for the individual mutants and to compare them with those of the *B. subtilis* wild-type strain and *sigB* mutant strain ML6, we used stress conditions that generated a maximum difference between the two reference strains without killing one of them completely. Hence, we introduced a preadaptation period by the imposition of 2% (vol/vol) ethanol stress 20 min prior to exposure to the detrimental oxidative stress. The preadaptation step equips the wild type with the protective functions of all general stress proteins and provides it with a maximal selective advantage (21). After preadaptation, cells were exposed to either 100 mM paraquat or 5 mM H_2O_2. Both stress conditions produced statistically significant differences in the survival rates between the wild-type and the *sigB* mutant strains (Fig. 1). The *sigB* mutant displayed a clear survival disadvantage compared to the wild-type strain upon either stress stimulus. Exposure to 100 mM paraquat reduced the viability of the wild type to approximately 10% after 240 min. The survival rates of the *sigB* mutant, however, decreased to a final level of 0.6% after 240 min (Fig. 1A). The imposition of 5 mM H_2O_2 did not severely affect the wild type but produced a strong and immediate decline in the survival rates of the *sigB* mutant to approximately 1% compared to wild-type levels throughout the time course (Fig. 1B). Thus, the results shown here again demonstrate that the general stress proteins provide *B. subtilis* with functions necessary for the efficient management of and survival against oxidative stress. Furthermore, the chosen stress levels lead to an appropriately wide range of selectivity between the wild type and the *sigB* mutant that

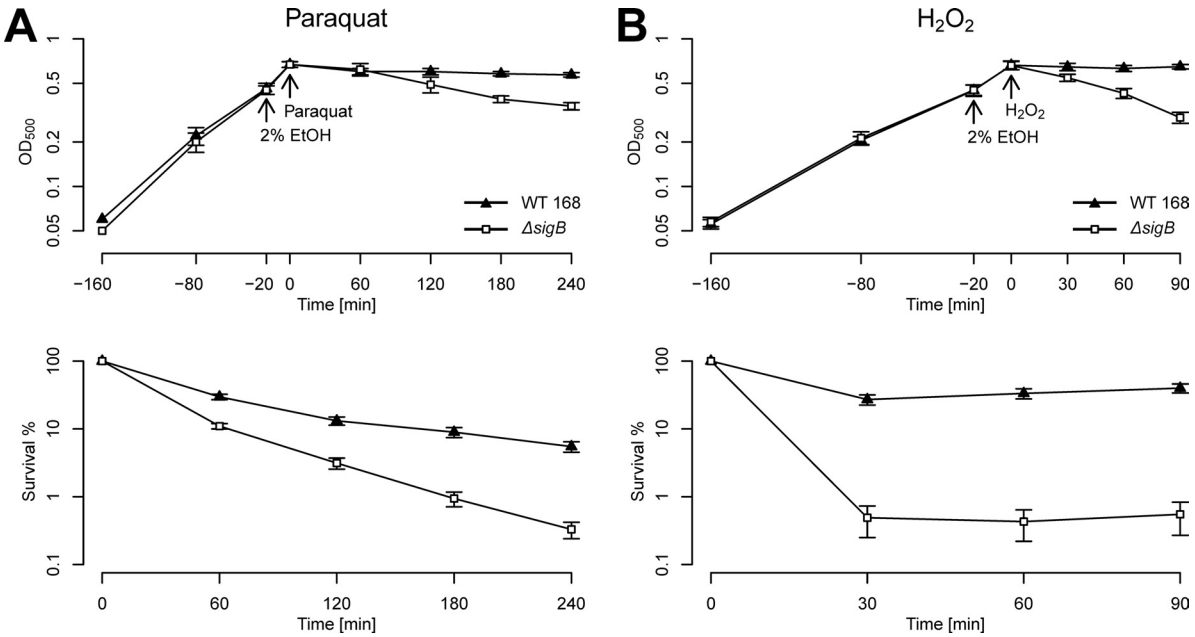

FIG 1 Comparative analysis of the oxidative stress resistances of wild-type (WT) strain 168 and its isogenic *sigB* mutant strain ML6. The upper graphs indicate the growth curves, and the lower graphs indicate the survival rates. Both strains were cultivated in synthetic medium, and growth was monitored by measuring the OD_{500}. At an OD_{500} of 0.4 (t of −20 min), the cells were treated with 2% ethanol (EtOH) (as indicated), followed by a preadaptation period of 20 min. After preadaptation (t_0), the cells were stressed with 100 mM paraquat (A) or 5 mM H_2O_2 (B) (as indicated). Survival rates were determined by plating appropriate dilutions of control samples taken before oxidative stress treatment at t_0 and after 60, 120, 180, and 240 min for the paraquat-stressed cells and after 30, 60, and 90 min for the H_2O_2-stressed cells. The values are arithmetic means and standard errors of the means.

allowed us to assess the effect of single mutations of general stress genes on survival against oxidative stress.

Paraquat and H_2O_2 stress resistances of mutants defective in individual general stress genes. In total, 92 of the 94 mutants from the previous study (21) were used in our experimental setup. Only two mutant strains that were used previously, the *spx* (*yjbD*) (strain 2842) and *mgsR* (*yqgZ*) (strain 4773) mutants, were replaced here by the Δ*mgsR* (BAR1) and Δ*spx* (BAR8) deletion mutants. Thus, the same 94 gene defects described previously by Höper et al. (21) were tested here for paraquat- and H_2O_2-sensitive phenotypes under the same conditions as those described above for the *B. subtilis* wild-type and Δ*sigB* reference strains. The survival rates of each mutant were determined, compared to those of the wild-type and *sigB* mutant strains, and tested for statistically significant differences (Table 1).

A total of 56 mutants exhibited a significant ($\alpha \leq 0.01$) paraquat-sensitive phenotype, and 15 strains were sensitive to H_2O_2 treatment compared to the wild type (Table 1). Nine of the 15 H_2O_2-sensitive strains were also paraquat sensitive. These data demonstrate that a large fraction of the general stress gene products is either directly or indirectly involved in oxidative stress management. With regard to this, Höper et al. (21) previously reported a broad overlap of the ethanol-sensitive as well as the salt-sensitive and cold-sensitive (4°C) groups (summarized in Table 1). It was proposed that these three stress stimuli or a combination of these stresses could result in the generation of reactive oxygen species representing a secondary overall or common stress. Notably, 41 of the paraquat- and/or H_2O_2-sensitive mutants are indeed ethanol sensitive. Furthermore, 13 of 17 strains sensitive to ethanol and cold (4°C) stresses as well as 13 of 22 ethanol- and salt-sensitive mutants were also sensitive to the oxidative stress treatment. This represents a remarkable overlap between the ethanol, salt, and cold stress phenotype clusters that were previously assumed to be involved in secondary oxidative stress management with the identified group of paraquat- and H_2O_2-sensitive mutants (Table 1).

Moreover, it is noteworthy that gene mutations known to be directly involved in oxidative stress resistance mechanisms belong to these phenotype clusters: the *dps* gene encodes a miniferritin and a paralogue of the DNA-binding and -protecting protein MrgA (3, 11). MrgA is part of the PerR regulon and is strongly induced by sublethal levels of H_2O_2 (9, 11). The Dps protein was shown previously to be required for the increased H_2O_2 resistance of glucose-starved stationary *B. subtilis* cells (3), and the study by Höper et al. (21) revealed that this mutant was also highly sensitive to ethanol and cold (4°C) stresses. In addition to this, we demonstrate here that the *dps* mutant also exhibits a severe sensitivity to paraquat treatment (Table 1, and see Fig. S1A in the supplemental material). The stronger survival disadvantage of the *dps* mutant in response to H_2O_2 treatment than of the *sigB* mutant may be related to the fact that the *dps* gene is also constitutively expressed from a second upstream promoter (3). Furthermore, mutations of *spx* and *mgsR*, encoding two redox-sensitive regulatory proteins, were also part of this study. The Δ*spx* strain, lacking the global regulator of the diamide stress response, Spx (33, 34), was reported previously to be salt and cold (4°C) sensitive (21) and was identified here to be paraquat sensitive as well (Table 1, and see Fig. S1B in the supplemental material). σ^B induces the *spx* gene as part of the bicistronic *yjbC-spx* operon (4, 37). The *yjbC* mutant was also identified to be ethanol and salt sensitive in the previous study and in addition was proven here to be paraquat sensitive (Table 1, and see Fig. S1C in the supplemental material). Further-

TABLE 1 Quantitative evaluation of the survival rates of *B. subtilis* wild-type strain 168, the *sigB* mutant (ML6), and 94 individual general stress gene mutants in response to paraquat and H_2O_2 stresses[a,b,c,d,e,f]

	gene [a]	strain [b]	function/ putative function [c]	Ethanol	NaCl	4°C	12,5°C	54°C	Paraquat	H_2O_2 [d]	paraquat [e] 60 min	paraquat [e] 120 min	paraquat [e] 180 min	paraquat [e] 240 min	H_2O_2 [f] 30 min	H_2O_2 [f] 60 min	H_2O_2 [f] 90 min
1	wild-type	168									100 (0,37)	100 (0,24)	100 (0,11)	100 (0,06)	100 (0,02)	100 (0,01)	100 (0,01)
	sigB	ML6	general stress response sigma factor SigB								**37 (1,00)**	**24 (1,00)**	**11 (1,00)**	**6 (1,00)**	**2 (1,00)**	**1 (1,00)**	**1 (1,00)**
	yaaH	BFS4195	spore peptidoglycan hydrolase								125 (0,30)	148 (0,16)	103 (0,10)	140 (0,04)	82 (0,02)	67 (0,02)	71 (0,02)
	yceE A2.4	BFS4342	unknown, putative tellurium resistance protein TerD-family								122 (0,30)	202 (0,12)	235 (0,04)	370 (0,02)	32 (0,06)	48 (0,03)	51 (0,03)
5	*yfhF* C1.3	BFS4692	unknown, putative nucleotide binding protein								94 (0,40)	118 (0,20)	144 (0,07)	195 (0,03)	130 (0,01)	98 (0,01)	82 (0,02)
	yvgN	BFS2491	glyoxal/methylglyoxal reductase								72 (0,52)	60 (0,40)	39 (0,27)	51 (0,12)	58 (0,03)	68 (0,02)	68 (0,02)
	yflA	BFS4617	unknown, putative aminoacid transporter								**13 (2,95)**	**8 (2,89)**	**6 (1,63)**	**6 (0,96)**	73 (0,02)	119 (0,01)	165 (0,01)
	yfhE C2.3	BFS4691	unknown, hypotetical protein								**65 (0,57)**	**45 (0,53)**	**23 (0,46)**	**19 (0,33)**	96 (0,02)	92 (0,01)	84 (0,02)
	yxiS	BFS4043	unknown, conserved hypotetical protein								**49 (0,76)**	**34 (0,71)**	**21 (0,50)**	**18 (0,34)**	91 (0,02)	82 (0,02)	72 (0,02)
10	*ohrB*	BFS 1818	organic hydroperoxide resistance reductase B								87 (0,43)	113 (0,21)	60 (0,17)	75 (0,08)	118 (0,02)	116 (0,01)	184 (0,01)
	yjgB	BFS 834	unknown, hypotetical protein								**59 (0,63)**	**66 (0,36)**	**40 (0,26)**	**34 (0,18)**	134 (0,01)	119 (0,01)	140 (0,01)
	aag	BFS 4002	putative 3-alkylated purines and hypoxanthine DNA								**91 (0,41)**	**131 (0,18)**	**21 (0,49)**	**21 (0,29)**	52 (0,03)	99 (0,01)	85 (0,02)
	yfkM	BFS 4627	unknown, putative DJ-1/PfpI family intracellular protease								**19 (1,98)**	**21 (1,11)**	**19 (0,55)**	**16 (0,39)**	102 (0,02)	92 (0,01)	96 (0,01)
	yjbC G1.2	BFS 2841	unknown, putative thiol oxidation management factor								**21 (1,74)**	**18 (1,34)**	**12 (0,91)**	**10 (0,62)**	106 (0,02)	109 (0,01)	103 (0,01)
15	*yjgC* H1.2	BFS 836	unknown, putative formate dehydrogenase α-subunit								**66 (0,56)**	**65 (0,36)**	**37 (0,29)**	**46 (0,13)**	56 (0,03)	63 (0,02)	52 (0,03)
	yjgD H2.2	BFS 860	unknown, conserved hypotetical protein								**53 (0,70)**	**56 (0,43)**	**30 (0,36)**	**31 (0,19)**	61 (0,03)	47 (0,03)	50 (0,03)
	ykgA	BFS 1820	unknown, putative aminohydrolase								**66 (0,56)**	**80 (0,30)**	**39 (0,27)**	**38 (0,16)**	103 (0,02)	62 (0,02)	142 (0,01)
	ymzB	BFS 2673	unknown, conserved hypotetical protein								**37 (1,00)**	**35 (0,68)**	**31 (0,34)**	**30 (0,21)**	85 (0,02)	99 (0,01)	88 (0,02)
	yqhQ	BFS 4754	unknown, conserved hypothetical protein								**49 (0,75)**	**57 (0,41)**	**34 (0,31)**	**30 (0,20)**	91 (0,02)	91 (0,01)	73 (0,02)
20	*ywsB*	BFS 1316	unknown, conserved hypotetical protein								**67 (0,55)**	**65 (0,36)**	**42 (0,25)**	**41 (0,15)**	106 (0,02)	257 (0,01)	200 (0,01)
	yxjJ	BFS 4032	unknown, hypotetical protein								**32 (1,16)**	**31 (0,77)**	**17 (0,62)**	**13 (0,46)**	97 (0,02)	93 (0,01)	93 (0,01)
	csbB	BFS 4700	unknown, putative glycosyl transferase								**36 (1,03)**	**26 (0,91)**	**28 (0,37)**	**24 (0,25)**	**39 (0,05)**	**49 (0,03)**	**51 (0,03)**
	katX	BFS 4003	major catalase in spores, detoxification of hydrogen								106 (0,35)	124 (0,19)	109 (0,10)	117 (0,05)	**30 (0,06)**	**32 (0,04)**	**40 (0,03)**

	ydbD	BFS 4442	unknown, putative manganese-containing catalase						117 (0,32)	294 (0,08)	155 (0,07)	151 (0,04)	**43 (0,04)**	**45 (0,03)**	**48 (0,03)**
25	*yacL*	BFS 4227	unknown, conserved hypotetical membrane protein						45 (0,82)	52 (0,46)	36 (0,30)	45 (0,14)	111 (0,02)	122 (0,01)	102 (0,01)
	yflT	BFS 4597	unknown, conserved hypotetical protein						65 (0,57)	78 (0,31)	71 (0,15)	55 (0,11)	101 (0,02)	104 (0,01)	107 (0,01)
	yhdN	BFS 1659	aldo/keto reductase specific for NADPH						96 (0,38)	89 (0,27)	57 (0,19)	66 (0,09)	117 (0,02)	126 (0,01)	117 (0,01)
	yhxD	BFS 1702	unknown, putative oxidoreductase (SDR) family						86 (0,43)	83 (0,29)	50 (0,21)	60 (0,10)	72 (0,02)	92 (0,01)	131 (0,01)
	ytaB	BFS 601	unknown, putative receptor of the TspO/MBR family						61 (0,61)	80 (0,30)	57 (0,18)	37 (0,17)	156 (0,01)	155 (0,01)	141 (0,01)
30	*yxaB*	BFS 4120	unknown, putative exopolysaccharide pyruvyl						102 (0,36)	109 (0,22)	63 (0,17)	43 (0,14)	102 (0,02)	92 (0,01)	82 (0,02)
	yxnA	BFS 4117	unknown, putative short chain oxidoreductase						97 (0,38)	99 (0,24)	53 (0,20)	42 (0,14)	78 (0,02)	109 (0,01)	107 (0,01)
	sodA	BFS 4792	superoxide dismutase, detoxification of oxygen						**0,08(2060)**	**0,1 (234)**	**0,0**	**0,0**	139 (0,01)	124 (0,01)	103 (0,01)
	yfkS F1.2	BFS 4619	unknown, hypotetical protein						**11 (3,48)**	**9 (2,61)**	**11 (0,97)**	**15 (0,40)**	46 (0,04)	100 (0,01)	117 (0,01)
	yfhK D1.3	BFS 4697	unknown, SH3 domain protein (signal transduction						**46 (0,81)**	**46 (0,52)**	**35 (0,31)**	**31 (0,19)**	107 (0,02)	128 (0,01)	101 (0,01)
35	*yfhL* D2.3	BFS 4698	SdpC immunity factor (SdpI superfamily)						56 (0,66)	70 (0,34)	52 (0,20)	41 (0,15)	**51 (0,04)**	**42 (0,03)**	**44 (0,03)**
	dps	BFS 625	mini-ferritin, DNA-protecting protein						**19 (1,93)**	**15 (1,54)**	**12 (0,92)**	**4 (1,73)**	**0,01 (230,02)**	**0,01 (88,54)**	**0,03 (46,68)**
	mgsR	BAR 1	transcriptional regulator of the Spx-subfamily						**22 (1,72)**	**11 (2,21)**	**7 (1,43)**	**6 (1,10)**	**14 (0,13)**	**7 (0,17)**	**5 (0,25)**
	ycbK	BFS 4316	unknown, putative efflux transporter						**62 (0,60)**	**50 (0,48)**	**35 (0,30)**	**32 (0,19)**	**33 (0,06)**	**43 (0,03)**	**67 (0,02)**
	ydaE B2.2	BFS 4424	unknown, putative D-lyxose ketol-isomerase						**66 (0,56)**	**49 (0,48)**	**39 (0,27)**	**26 (0,23)**	89 (0,02)	110 (0,01)	111 (0,01)
40	*ydaG*	BFS 4426	unknown, putative FMN-binding split barrel domain-						**53 (0,70)**	**42 (0,57)**	**27 (0,40)**	**25 (0,24)**	76 (0,02)	105 (0,01)	109 (0,01)
	ydaP	BFS 4435	unknown, putative pyruvate oxidase						**63 (0,58)**	**78 (0,30)**	**33 (0,32)**	**28 (0,21)**	41 (0,04)	55 (0,02)	72 (0,02)
	ydaT	BFS 4438	unknown, conserved hypotetical protein						**54 (0,69)**	**49 (0,48)**	**35 (0,30)**	**35 (0,17)**	91 (0,02)	105 (0,01)	92 (0,01)
	yfhD C3.3	BFS 4690	unknown, conserved hypotetical protein						**52 (0,71)**	**39 (0,60)**	**32 (0,33)**	**24 (0,26)**	135 (0,01)	130 (0,01)	114 (0,01)
	yocB	BFS 684	unknown, conserved hypotetical protein						**61 (0,60)**	**68 (0,35)**	**35 (0,30)**	**29 (0,21)**	63 (0,03)	79 (0,02)	78 (0,02)
45	*yaaI*	BFS 4196	unknown, putative isochorismatase						112 (0,33)	148 (0,16)	180 (0,06)	181 (0,03)	76 (0,02)	80 (0,02)	97 (0,01)

(Continued on following page)

TABLE 1 (continued)

	gene [a]	strain [b]	function/ putative function [c]	Ethanol	NaCl	4°C	12,5°C	54°C	Paraquat	H_2O_2 [d]	paraquat [e] 60 min	paraquat [e] 120 min	paraquat [e] 180 min	paraquat [e] 240 min	H_2O_2 [f] 30 min	H_2O_2 [f] 60 min	H_2O_2 [f] 90 min
	ybyB	BFS 4282	unknown, conserved hypotetical protein								65 (0,57)	63 (0,38)	55 (0,19)	76 (0,08)	104 (0,02)	103 (0,01)	69 (0,02)
	ycdF	BFS 4337	unknown, putative glucose 1-dehydrogenase								125 (0,30)	205 (0,12)	208 (0,05)	244 (0,02)	78 (0,02)	110 (0,01)	97 (0,01)
	yceD A1.4	BFS 4341	unknown, putative tellurium resistance protein TerD-family								221 (0,17)	380 (0,06)	298 (0,04)	422 (0,01)	65 (0,03)	90 (0,01)	68 (0,02)
	yugU	BFS 1412	unknown, conserved hypotetical protein								**36 (1,04)**	**33 (0,73)**	**22 (0,49)**	**27 (0,23)**	121 (0,01)	92 (0,01)	115 (0,01)
50	*ysnF*	BFS 2438	unknown, conserved hypotetical protein								**50 (0,75)**	**49 (0,48)**	**34 (0,31)**	**21 (0,29)**	**34 (0,05)**	**45 (0,03)**	**57 (0,02)**
	cypC	BFS 4281	fatty acid beta-hydroxylating cytochrome P450								**25 (1,50)**	**21 (1,16)**	**26 (0,40)**	**20 (0,31)**	**23 (0,08)**	**35 (0,04)**	**41 (0,03)**
	yfkH E3.3	BFS 4632	unknown, putative inner membrane ribonuclease fold								**65 (0,57)**	**38 (0,62)**	**30 (0,35)**	**28 (0,22)**	**41 (0,04)**	**45 (0,03)**	**54 (0,03)**
	guaD	BFS 1819	guanine deaminase, deamination of guanine to								**60 (0,62)**	**54 (0,44)**	**29 (0,37)**	**22 (0,27)**	67 (0,03)	80 (0,02)	81 (0,02)
	rnr	BFS 1121	3'-5' exoribonuclease R								**28 (1,33)**	**17 (1,43)**	**13 (0,79)**	**10 (0,58)**	72 (0,02)	78 (0,02)	76 (0,02)
55	*ydhK*	BFS 4532	unknown, conserved hypotetical protein								**48 (0,78)**	**49 (0,49)**	**24 (0,44)**	**21 (0,29)**	32 (0,06)	58 (0,02)	78 (0,02)
	yfkJ E1.3	BFS 4630	Protein-tyrosine-phosphatase								**21 (1,74)**	**21 (1,15)**	**27 (0,39)**	**36 (0,17)**	57 (0,03)	55 (0,02)	51 (0,03)
	yoxC	BFS 2043	unknown, conserved hypotetical protein								**73 (0,51)**	**64 (0,37)**	**37 (0,28)**	**31 (0,19)**	119 (0,02)	166 (0,01)	125 (0,01)
	yvgO	BFS 2490	unknown, conserved hypotetical protein								**71 (0,52)**	**66 (0,36)**	**35 (0,30)**	**28 (0,22)**	161 (0,01)	165 (0,01)	165 (0,01)
	yfhM D3.3	BFS 4699	unknown, putative alpha/beta hydrolase								103 (0,36)	94 (0,25)	172 (0,06)	126 (0,05)	**122 (0,01)**	**30 (0,04)**	**31 (0,04)**
60	*yceF* A3.4	BFS 4343	unknown, putative TerC family membrane transport protein								141 (0,26)	217 (0,11)	204 (0,05)	246 (0,02)	74 (0,02)	80 (0,02)	95 (0,01)
	yceG A4.4	BFS 4344	unknown, putative tellurium resistance protein								75 (0,49)	90 (0,26)	77 (0,14)	83 (0,07)	67 (0,03)	57 (0,02)	61 (0,02)
	yocK	BFS 675	unknown, similar to DnaK suppressor protein DksA								75 (0,50)	60 (0,40)	41 (0,26)	36 (0,17)	126 (0,01)	158 (0,01)	154 (0,01)
	ywmE	BFS 205	Unknown, hypotetical protein								66 (0,56)	70 (0,34)	52 (0,20)	42 (0,14)	106 (0,02)	114 (0,01)	121 (0,01)
	yxkO	BFS 4012	unknown, putative carbohydrate kinase								70 (0,53)	60 (0,39)	49 (0,21)	45 (0,14)	65 (0,03)	67 (0,02)	60 (0,02)
65	*yycD*	BFS 4145	unknown, conserved hypotetical protein								100 (0,37)	135 (0,18)	119 (0,09)	205 (0,03)	95 (0,02)	98 (0,01)	84 (0,02)
	spx G2.2	BAR 8	transcriptional regulator Spx of the thiol stress response								**21 (1,74)**	**18 (1,34)**	**12 (0,91)**	**10 (0,62)**	106 (0,02)	109 (0,01)	103 (0,01)
	gabD	BFS 4401	succinate-semialdehyde dehydrogenase NAD(P)								**66 (0,56)**	**65 (0,36)**	**40 (0,26)**	**33 (0,18)**	51 (0,04)	52 (0,02)	55 (0,02)

	csbD	BFS 214	unknown, conserved hypotetical protein					81 (0,46)	77 (0,31)	72 (0,15)	55 (0,11)	115 (0,02)	97 (0,01)	95 (0,01)
	yflH	BFS 4610	unknown, conserved hypotetical protein					59 (0,63)	53 (0,45)	56 (0,19)	44 (0,14)	119 (0,02)	108 (0,01)	112 (0,01)
70	*corA*	BFS 4770	magnesium transporter, LexA repressed					**60 (0,61)**	**70 (0,34)**	**36 (0,30)**	**32 (0,19)**	263 (0,01)	288 (0,00)	426 (0,00)
	csbC	BFS 4101	unknown, putative pentose transporter					**42 (0,87)**	**37 (0,64)**	**22 (0,48)**	**14 (0,44)**	98 (0,02)	144 (0,01)	133 (0,01)
	yerD	BFS 2262	unknown, putative glutamate synthase, paralogue of GltA					**32 (1,17)**	**23 (1,01)**	**13 (0,80)**	**17 (0,36)**	232 (0,01)	325 (0,00)	566 (0,00)
	rpmEB	BFS 620	50S ribosomal protein L31					76 (0,48)	90 (0,26)	79 (0,13)	81 (0,07)	200 (0,01)	169 (0,01)	165 (0,01)
	yvrE	BFS 2477	unknown, putative senescence marker protein-30 family					69 (0,54)	84 (0,28)	71 (0,15)	58 (0,10)	134 (0,01)	163 (0,01)	154 (0,01)
75	*ywlB*	BFS 230	unknown, conserved hypotetical protein					66 (0,56)	70 (0,34)	54 (0,20)	35 (0,17)	80 (0,02)	95 (0,01)	89 (0,02)
	yhcM	BFS 1625	unknown, hypotetical protein					**42 (0,88)**	**29 (0,81)**	**27 (0,39)**	**19 (0,31)**	62 (0,03)	83 (0,02)	70 (0,02)
	ydaD B1.2	BFS 4423	unknown, putative NAD(P) dependent dehydrogenase					187 (0,20)	220 (0,11)	324 (0,03)	409 (0,01)	**22 (0,08)**	**39 (0,03)**	**46 (0,03)**
	yqhB	BFS 4771	unknown, putative membrane bound 2-oxo acid					70 (0,53)	69 (0,34)	56 (0,19)	47 (0,13)	**14 (0,13)**	**7 (0,17)**	**5 (0,25)**
	ltaSA	BFS 4568	minor lipoteichoic acid synthetase					60 (0,61)	56 (0,43)	58 (0,18)	37 (0,16)	56 (0,03)	60 (0,02)	62 (0,02)
80	*yqjL*	BFS 4728	unknown, putative hydrolase					82 (0,45)	54 (0,44)	40 (0,26)	48 (0,13)	97 (0,02)	92 (0,01)	84 (0,02)
	yvyD	BFS 809	ribosome-associated sigma 54 modulation protein					**17 (2,13)**	**15 (1,54)**	**13 (0,80)**	**14 (0,43)**	**43 (0,04)**	**32 (0,04)**	**20 (0,07)**
	ysdB	BFS 2402	unknown, conserved hypotetical protein					**50 (0,74)**	**54 (0,44)**	**39 (0,27)**	**40 (0,15)**	94 (0,02)	79 (0,02)	92 (0,01)
	aldY	BFS 4022	unknown, putative dehydro-genase, GabD paralogue					**24 (1,53)**	**27 (0,89)**	**22 (0,48)**	**28 (0,22)**	**38 (0,05)**	**40 (0,03)**	**41 (0,03)**
	csbA	BFS 877	unknown, conserved hypotetical membrane protein					**44 (0,84)**	**39 (0,61)**	**23 (0,46)**	**26 (0,23)**	60 (0,03)	62 (0,02)	63 (0,02)
85	*ctc*	GF500	50S ribosomal protein L25					**24 (1,52)**	**30 (0,79)**	**19 (0,56)**	**29 (0,21)**	135 (0,01)	87 (0,01)	90 (0,02)
	ydaS	BFS 4437	unknown, conserved hypotetical protein					**71 (0,52)**	**62 (0,38)**	**38 (0,28)**	**36 (0,17)**	65 (0,03)	62 (0,02)	59 (0,02)
	yfkI E2.3	BFS 4631	unknown, conserved hypotetical protein					**22 (1,69)**	**18 (1,35)**	**12 (0,87)**	**16 (0,38)**	47 (0,04)	40 (0,03)	45 (0,03)
	yfkT F2.2	BFS 4618	unknown, putative spore germination protein					**27 (1,37)**	**32 (0,75)**	**35 (0,30)**	**37 (0,16)**	90 (0,02)	107 (0,01)	116 (0,01)
	yitT	BFS 3044	unknown, conserved hypotetical inner membrane					**51 (0,73)**	**19 (1,23)**	**13 (0,82)**	**8 (0,79)**	75 (0,02)	82 (0,02)	74 (0,02)
90	*ylxP*	BFS 2629	unknown, conserved hypotetical protein					**70 (0,53)**	**33 (0,71)**	**28 (0,37)**	**27 (0,23)**	103 (0,02)	74 (0,02)	89 (0,02)
	yraA	BFS 410	unknown, putative DJ-1/PfpI family intracellular protease					**48 (0,77)**	**32 (0,75)**	**20 (0,54)**	**13 (0,45)**	44 (0,04)	53 (0,02)	57 (0,02)

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TABLE 1 (continued)

gene [a]	strain [b]	function/ putative function [c]	Ethanol	NaCl	4°C	12,5°C	54°C	Paraquat	H_2O_2 [d]	paraquat [e]				H_2O_2 [f]		
										60 min	120 min	180 min	240 min	30 min	60 min	90 min
ytkL	BFS 72	unknown, putative hydrolase of the β-lactamase superfamily								**50 (0,74)**	**44 (0,54)**	**28 (0,37)**	**22 (0,28)**	64 (0,03)	46 (0,03)	51 (0,03)
ywiE	BFS 1244	cardiolipin synthetase								**55 (0,67)**	**64 (0,37)**	**28 (0,38)**	**29 (0,21)**	69 (0,03)	91 (0,01)	88 (0,02)
ycbP	BFS 4321	unknown, putative integral inner membrane protein								103 (0,36)	80 (0,30)	59 (0,18)	70 (0,09)	139 (0,01)	114 (0,01)	121 (0,01)
yvaA	BFS 1058	unknown, putative oxidoreductase								68 (0,55)	81 (0,29)	60 (0,17)	79 (0,08)	126 (0,01)	117 (0,01)	121 (0,01)
ywtG	BFS 1239	unknown, putative carbohydrate transporter								56 (0,66)	57 (0,42)	29 (0,37)	47 (0,13)	104 (0,02)	152 (0,01)	134 (0,01)

[a] The genes are ordered by sensitivity clusters according to reference 21. The genes of each cluster are in alphabetical order. The operon affiliation of the respective genes is indicated at the top right. The code A1.4, for example, means that this gene is the first of four genes of operon A.
[b] Mutant strains of the respective genes used in this study.
[c] Known or putative function of the respective gene products.
[d] Summary of all sensitive phenotypes determined previously by Höper et al. (21) and in this study. The list was sorted according to clusters of sensitive phenotypes in the following order: ethanol (pink), NaCl (brown), 4°C (light blue), 12.5°C (dark blue), 54°C (red), paraquat (green), and H_2O_2 (orange).
[e] Survival rates of the mutants in response to 100 mM paraquat are expressed relative to those of the wild type and *sigB* mutant strain ML6 for each time point tested. The wild-type values were defined as 100% for direct comparison with the mutant strains; thus, the survival rates of the mutants reflect percent survival rates based on the wild-type level. The comparison with the *sigB* mutant (values in parentheses) represents the fold difference compared to the single mutant strains. Thus, values lower than 1 indicate that the mutant strain was less sensitive than the *sigB* mutant; and values higher than 1 indicate that the mutant was more sensitive than the *sigB* mutant. The values for direct comparisons of the wild type and the *sigB* mutant are given in the first and second rows. Boldface type indicates significant differences with a confidence level of an α value of ≤0.01. Values that passed an even more stringent confidence level of an α value of ≤0.001 are shaded in light gray.
[f] Survival rates of the mutants in response to 5 mM H_2O_2 are expressed relative to those of the wild type and *sigB* mutant strain ML6 for each time point tested (see above).

more, our experimental setup also included 4 genes, (i) *ytkL*, (ii) *gabD*, (iii) *sodA*, and (iv) *yraA*, that belong to both the σ^B-dependent general stress regulon (35) and the Spx-regulated diamide/thiol stress regulon (34).

For the *ytkL* mutant, no sensitive phenotype could be described previously (21), but here it could be assigned to the paraquat-sensitive group (Table 1).

The *gabD* mutant exhibited a salt- and cold (4°C)-sensitive phenotype in the previous study and is shown here to be paraquat sensitive (Table 1, and see Fig. S1D in the supplemental material). The *gabD* gene encodes an $NADP^+$-dependent succinate-semialdehyde dehydrogenase. Thus, GabD may be necessary for the generation of reduction equivalents (NADPH) under conditions of oxidative stress. Notably, an *aldY* mutant strain encoding a paralogue of *gabD* was also identified here to be sensitive to paraquat and H_2O_2 treatment (Table 1).

The *sodA* mutant strain was shown to be ethanol and cold (4°C and 12°C) sensitive previously and displayed a strong paraquat-sensitive phenotype in this study (Table 1, and see Fig. S1E in the supplemental material). Although the latter observation was not surprising due to the fact that *sodA* encodes the superoxide dismutase SodA, which is directly involved in the detoxification of oxygen radicals and necessary for oxidative stress resistance (23), this is another good example of the correlation between known enzyme functions, stress phenotypes, and regulon clusters observed in this study. Nevertheless, due to the fact that the mutation of *sodA* produced a stronger negative effect on the survival rate under paraquat stress conditions than the *sigB* mutant, it is necessary to emphasize that *sodA* expression is under the control of multiple systems, only one of which is σ^B.

The *yraA* mutant strain was one of 14 mutants without a defined sensitive phenotype in the previous study (21). Here we show that the *yraA* mutant is sensitive to paraquat stress (Table 1, and see Fig. S1F in the supplemental material). Besides positive regulation by σ^B and Spx, the *yraA* gene was also reported previously to be specifically upregulated by AdhR as part of the *adhA-yraA* operon in response to aldehyde stress (36). In this context, it was proposed that *yraA*, encoding a putative DJ-1/Pf1 family protease, may be involved in the degradation or repair of oxidatively damaged proteins (36). The *yfkM* gene, encoding a paralogue of YraA, is also a σ^B regulon member and was tested here. Interestingly, the *yfkM* mutant also displayed an ethanol-sensitive, salt-sensitive (21), and paraquat-sensitive phenotype (Table 1, and see Fig. S1G in the supplemental material), further strengthening the hypothesis that these two putative proteases are specifically needed under conditions of oxidative stress.

As mentioned above, the Δ*mgsR* mutant strain as well as 11 strains with mutations in MgsR target genes were also included in this screening. It is known from a previous study that MgsR is a paralogue of Spx that controls a subregulon within the framework of the general stress response (41). Recently, MgsR was shown to be activated by a redox switch in response to ethanol stress, integrating secondary oxidative stress signals into a σ^B- and MgsR-mediated regulatory cascade (Reder et al., unpublished). Due to these observations, it has been assumed that the products of the MgsR target genes are needed for secondary oxidative stress management (41; Reder et al., unpublished). With regard to this, we show here that the *mgsR* mutant strain is sensitive not only to ethanol and cold stresses (21) but also to both paraquat and H_2O_2 stresses (Table 1, and see Fig. S1H in the supplemental material).

Furthermore, our experimental setup included 11 strains with mutations of known MgsR target genes. Of this group, only three mutations, *yhxD*, *yxaB*, and *yflH* (Table 1) did not have oxidative stress management defects. The remaining 8 strains, with mutations of *yjgB*, *yjgC*, *yjgD*, *ydbD*, *ydaE*, *ydaG*, *ysnF*, and *ydaD*, were all sensitive to paraquat and/or H_2O_2 stress (Table 1). For some of these gene products, it is possible to predict a putative function that would also be in line with their determined phenotypes. For example, *ydaD* encodes a putative $NADP^+$-dependent dehydrogenase. YjgC represents a putative formate dehydrogenase that may also be involved in the generation of NADH or NADPH. The *ydaG* gene encodes a potential flavin mononucleotide (FMN)-binding protein that may participate in redox processes. Finally, *ydbD* most likely encodes a manganese-dependent catalase that may be involved in the detoxification of H_2O_2. Notably, the catalase *katX* mutant and the putative catalase *ydbD* mutant are the only two strains that exhibited identical sensitivity patterns for all tested stimuli (Table 1, and see Fig. S1I and S1J in the supplemental material).

Conclusion. Because an increasing number of reports pointed to an involvement of *B. subtilis* general stress proteins in oxidative stress management, this follow-up study assessed the effects of individual mutations of a large set of σ^B-dependent general stress genes on resistance against severe paraquat (superoxide) and H_2O_2 stresses. We demonstrate here that a substantial number of general stress genes are indeed involved in the development of oxidative stress resistance, which is very likely a common "secondary" component of multiple "primary" σ^B-inducing physical stress stimuli. Together with the results of the previous study by Höper et al. (21), all but 3 strains, the *ycbP*, *ywtG*, and *yvaA* mutants, could be associated with at least one or more stress resistance defects. Although it is obvious that some of the general stress proteins exert functions that are related primarily to relieving oxidative stress, the contributions of others may be more general, with a secondary oxidative stress component among them.

Because most of the general stress proteins are crucial for the survival of *B. subtilis* but are still of undefined functions, these functional genomics data are of great importance. One example where the results of Höper et al. (21) provided the basis for a detailed analysis and guided experimental design was the characterization of the regulator MgsR and the identification of its subregulon within the σ^B response (41; Reder et al., unpublished). Therefore, we believe that the information gained by detailed phenotypic screening analyses is a first and important step for directed assays to determine the exact biochemical functions of uncharacterized proteins of *B. subtilis* involved in stress management.

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All authors discussed the results and implications and commented on the manuscript at all stages. We declare no competing financial interests.

REFERENCES

1. **Albano E.** 2006. Alcohol, oxidative stress and free radical damage. Proc. Nutr. Soc. **65**:278–290.
2. **Antelmann H, Engelmann S, Schmid R, Hecker M.** 1996. General and oxidative stress responses in *Bacillus subtilis*: cloning, expression, and mutation of the alkyl hydroperoxide reductase operon. J. Bacteriol. **178**:6571–6578.
3. **Antelmann H, et al.** 1997. Expression of a stress- and starvation-induced dps/pexB-homologous gene is controlled by the alternative sigma factor sigmaB in *Bacillus subtilis*. J. Bacteriol. **179**:7251–7256.
4. **Antelmann H, Scharf C, Hecker M.** 2000. Phosphate starvation-inducible proteins of *Bacillus subtilis*: proteomics and transcriptional analysis. J. Bacteriol. **182**:4478–4490.
5. **Bailey SM, Pietsch EC, Cunningham CC.** 1999. Ethanol stimulates the production of reactive oxygen species at mitochondrial complexes I and III. Free Radic. Biol. Med. **27**:891–900.
6. **Barbe V, et al.** 2009. From a consortium sequence to a unified sequence: the *Bacillus subtilis* 168 reference genome a decade later. Microbiology **155**:1758–1775.
7. **Benson AK, Haldenwang WG.** 1993. Regulation of sigma B levels and activity in *Bacillus subtilis*. J. Bacteriol. **175**:2347–2356.
8. **Brigulla M, et al.** 2003. Chill induction of the SigB-dependent general stress response in *Bacillus subtilis* and its contribution to low-temperature adaptation. J. Bacteriol. **185**:4305–4314.
9. **Bsat N, Herbig A, Casillas-Martinez L, Setlow P, Helmann JD.** 1998. *Bacillus subtilis* contains multiple Fur homologues: identification of the iron uptake (Fur) and peroxide regulon (PerR) repressors. Mol. Microbiol. **29**:189–198.
10. **Burkholder PR, Giles NH.** 1947. Induced biochemical mutations in *Bacillus subtilis*. Am. J. Bot. **34**:345–348.
11. **Chen L, Helmann JD.** 1995. *Bacillus subtilis* MrgA is a Dps (PexB) homologue: evidence for metalloregulation of an oxidative-stress gene. Mol. Microbiol. **18**:295–300.
12. **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.
13. **Engelmann S, Lindner C, Hecker M.** 1995. Cloning, nucleotide sequence, and regulation of *katE* encoding a sigma B-dependent catalase in *Bacillus subtilis*. J. Bacteriol. **177**:5598–5605.
14. **Gaidenko TA, Price CW.** 1998. General stress transcription factor σ^B and sporulation transcription factor σ^H each contribute to survival of *Bacillus subtilis* under extreme growth conditions. J. Bacteriol. **180**:3730–3733.
15. **Hecker M, Pané-Farré J, Völker U.** 2007. SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. Annu. Rev. Microbiol. **61**:215–236.
16. **Hecker M, Völker U.** 2001. General stress response of *Bacillus subtilis* and other bacteria. Adv. Microb. Physiol. **44**:35–91.
17. **Hecker M, Völker U.** 1998. Non-specific, general and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the σ^B regulon. Mol. Microbiol. **29**:1129–1136.
18. **Helmann JD, et al.** 2003. The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. J. Bacteriol. **185**:243–253.
19. **Helmann JD, et al.** 2001. Global transcriptional response of *Bacillus subtilis* to heat shock. J. Bacteriol. **183**:7318–7328.
20. **Höper D, Bernhardt J, Hecker M.** 2006. Salt stress adaptation of *Bacillus subtilis*: a physiological proteomics approach. Proteomics **6**:1550–1562.
21. **Höper D, Völker 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.
22. **Igo M, et al.** 1987. Genetic studies of a secondary RNA polymerase sigma factor in *Bacillus subtilis*. J. Bacteriol. **169**:3464–3469.
23. **Inaoka T, Matsumura Y, Tsuchido T.** 1999. SodA and manganese are essential for resistance to oxidative stress in growing and sporulating cells of *Bacillus subtilis*. J. Bacteriol. **181**:1939–1943.
24. **Kaan T, Homuth G, Mäder U, Bandow J, Schweder T.** 2002. Genome-wide transcriptional profiling of the *Bacillus subtilis* cold-shock response. Microbiology **148**:3441–3455.
25. **Kobayashi K, et al.** 2003. Essential *Bacillus subtilis* genes. Proc. Natl. Acad. Sci. U. S. A. **100**:4678–4683.
26. **Kohanski MA, Dwyer DJ, Collins JJ.** 2010. How antibiotics kill bacteria: from targets to networks. Nat. Rev. Microbiol. **8**:423–435.
27. **Lechat P, Hummel L, Rousseau S, Moszer I.** 2008. GenoList: an integrated environment for comparative analysis of microbial genomes. Nucleic Acids Res. **36**:D469–D474. doi:10.1093/nar/gkm1042.
28. **Méndez MB, Orsaria LM, Philippe V, Pedrido ME, Grau RR.** 2004. Novel roles of the master transcription factors Spo0A and sigma(B) for

survival and sporulation of *Bacillus subtilis* at low growth temperature. J. Bacteriol. **186**:989–1000.
29. **Mols M, Abee T.** 2011. Primary and secondary oxidative stress in *Bacillus*. Environ. Microbiol. **13**:1387–1394.
30. **Mols M, Pier I, Zwietering MH, Abee T.** 2009. The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus*. Int. J. Food Microbiol. **135**:303–311.
31. **Mols M, van Kranenburg R, van Melis CC, Moezelaar R, Abee T.** 2010. Analysis of acid-stressed *Bacillus cereus* reveals a major oxidative response and inactivation-associated radical formation. Environ. Microbiol. **12**: 873–885.
32. **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.
33. **Nakano S, Erwin KN, Ralle M, Zuber P.** 2005. Redox-sensitive transcriptional control by a thiol/disulphide switch in the global regulator, Spx. Mol. Microbiol. **55**:498–510.
34. **Nakano S, Küster-Schöck E, Grossman AD, Zuber P.** 2003. Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U. S. A. **100**:13603–13608.
35. **Nannapaneni P, et al.** 2012. Defining the structure of the general stress regulon of *Bacillus subtilis* using targeted microarray analysis and random forest classification. Microbiology **158**:696–707.
36. **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.
37. **Petersohn A, et al.** 1999. Identification of σ^B-dependent genes in *Bacillus subtilis* using a promoter consensus-directed search and oligonucleotide hybridization. J. Bacteriol. **181**:5718–5724.
38. **Petersohn A, et al.** 2001. Global analysis of the general stress response of *Bacillus subtilis*. J. Bacteriol. **183**:5617–5631.
39. **Price CW.** 2000. Protective function and regulation of general stress response in *Bacillus subtilis* and related Gram-positive bacteria, p 179–197. *In* Storz G, Hengge-Aronis R (ed), Bacterial stress responses. ASM Press, Washington, DC.
40. **Price CW, et al.** 2001. Genome-wide analysis of the general stress response in *Bacillus subtilis*. Mol. Microbiol. **41**:757–774.
41. **Reder A, et al.** 2008. The Spx paralogue MgsR (YqgZ) controls a subregulon within the general stress response of *Bacillus subtilis*. Mol. Microbiol. **69**:1104–1120.
42. **Schmalisch M, Langbein I, Stülke J.** 2002. The general stress protein Ctc of *Bacillus subtilis* is a ribosomal protein. J. Mol. Microbiol. Biotechnol. **4**:495–501.
43. **Stülke J, Hanschke R, Hecker M.** 1993. Temporal activation of β-glucanase synthesis in *Bacillus subtilis* is mediated by the Gtp pool. J. Gen. Microbiol. **139**:2041–2045.
44. **Völker U, Maul B, Hecker M.** 1999. Expression of the σ^B-dependent general stress regulon confers multiple stress resistance in *Bacillus subtilis*. J. Bacteriol. **181**:3942–3948.
45. **Wach A.** 1996. PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in *S. cerevisiae*. Yeast **12**:259–265.
46. **Wilks JC, et al.** 2009. Acid and base stress and transcriptomic responses in *Bacillus subtilis*. Appl. Environ. Microbiol. **75**:981–990.