JOURNAL OF BACTERIOLOGY, Oct. 2001, p. 5617–5631 Vol. 183, No. 19
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.19.5617–5631.2001

Global Analysis of the General Stress Response of *Bacillus subtilis*

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Received 16 March 2001/Accepted 9 July 2001

Gene arrays containing all currently known open reading frames of *Bacillus subtilis* were used to examine the general stress response of *Bacillus*. By proteomics, transcriptional analysis, transposon mutagenesis, and consensus promoter-based screening, 75 genes had previously been described as σ^B-dependent general stress genes. The present gene array-based analysis confirmed 62 of these already known general stress genes and detected 63 additional genes subject to control by the stress sigma factor σ^B. At least 24 of these 125 σ^B-dependent genes seemed to be subject to a second, σ^B-independent stress induction mechanism. Therefore, this transcriptional profiling revealed almost four times as many regulon members as the proteomic approach, but failure of confirmation of all known members of the σ^B regulon indicates that even this approach has not yet elucidated the entire regulon. Most of the σ^B-dependent general stress proteins are probably located in the cytoplasm, but 25 contain at least one membrane-spanning domain, and at least 6 proteins appear to be secreted. The functions of most of the newly described genes are still unknown. However, their classification as σ^B-dependent stress genes argues that their products most likely perform functions in stress management and help to provide the nongrowing cell with multiple stress resistance. A comprehensive screening program analyzing the multiple stress resistance of mutants with mutations in single stress genes is in progress. The first results of this program, showing the diminished salt resistance of *yjbC* and *yjbD* mutants compared to that of the wild type, are presented. Only a few new σ^B-dependent proteins with already known functions were found, among them SodA, encoding a superoxide dismutase. In addition to analysis of the σ^B-dependent general stress regulon, a comprehensive list of genes induced by heat, salt, or ethanol stress in a σ^B-independent manner is presented. Perhaps the most interesting of the σ^B-independent stress phenomena was the induction of the extracytoplasmic function sigma factor σ^W and its entire regulon by salt shock.

Almost 15 years ago we began to analyze the response of *Bacillus subtilis* cells to stress and starvation because these unfavorable conditions are the rule in natural ecosystems and adaptation to stress and starvation is crucial for survival in nature. We used the highly sensitive two-dimensional gel electrophoresis technique to visualize global changes in the gene expression pattern (24, 25, 38). These studies revealed a large group of stress proteins that seemed to be induced together by physical stress such as heat, salt, ethanol, or acid stress, as well as by glucose, oxygen, or phosphate starvation. This complex induction profile encouraged us to suggest that these proteins may have a rather nonspecific, but nevertheless very essential, protective function in response to stress or starvation, regardless of the specific stress stimulus. Therefore, the proteins were called nonspecific or general stress proteins (24, 25, 38).

Subsequently, stress induction of this protein group was shown to be mediated by the alternative sigma factor σ^B, the general stress sigma factor of gram-positive bacteria. W. G. Haldenwang and R. Losick discovered σ^B more than 20 years ago (22), but its role and physiological function remained matters of speculation for more than a decade. In the early 1990s the laboratories of W. G. Haldenwang and C. W. Price independently discovered that the *sigB* operon was induced by the same stimuli as the general stress proteins, namely, either heat, ethanol, or salt stress or entry into the stationary-growth phase, and that this induction was achieved by σ^B itself (6, 7, 9, 11). These findings strongly suggested that the genes encoding the general stress proteins belong to the σ^B regulon. Identification of numerous general stress proteins by N-terminal sequencing or matrix-assisted laser desorption ionization–time-of-flight mass spectrometry and subsequent detailed analysis of gene regulation proved that σ^B indeed controls induction of the general stress genes. By use of transposon mutagenesis C. W. Price and coworkers investigated the effects of σ^B on transcription and identified eight σ^B-dependent genes (for reviews see references 26 and 37).

Finally, analysis of the *B. subtilis* genome for σ^B-dependent promoters was used to identify additional members of the σ^B regulon. This computer-aided identification of new general stress genes became feasible because of the highly conserved and distinct consensus sequence of σ^B-dependent promoters. Screening of the potential target genes by oligonucleotide hybridization revealed more than 20 new genes that are probably under σ^B control (36). The three approaches described above and additional genetic and transcriptional studies have led thus far to the identification of 75 σ^B-dependent general stress genes. The number of genes identified by each of these approaches is given in Table 3.

Many of the general stress genes display basal level tran-

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scription from vegetative σ^A-dependent promoters. However, activation of σ^B activity following metabolic or environmental stress dramatically increases the transcription of the general stress genes. As a result of this massive induction of the regulon, the fraction of total translational capacity utilized for production of general stress proteins rises from approximately 1% in growing cells to 20% or even more in starved or stressed bacteria (8). During exponential growth σ^B is kept in an inactive complex by binding to its anti-sigma factor, RsbW (5). Activation of σ^B requires the dephosphorylation of an antagonist protein, RsbV, which then forms a complex with RsbW and releases σ^B from its inhibition (1, 17). During exponential growth RsbV is phosphorylated and inactivated by RsbW (17, 52), but after the imposition of stress or starvation two specific PP2C type phosphatases, RsbU and RsbP, can shift the equilibrium from RsbV~P to RsbV and consequently trigger stress gene activation (48, 55).

Comparative phenotypic studies of *sigB* mutants and wild-type bacteria have meanwhile proven that high-level expression of the general stress regulon provides stressed or starved cells with multiple, nonspecific, prospective stress resistance in anticipation of "future stress" (18, 19, 51). This protective function is particularly important for cells that are no longer able to grow (51). Therefore, the general stress response might be an essential alternative for all resting *Bacillus* cells that do not sporulate efficiently either because the cell density is too low (21) or because stress conditions (e.g., osmotic stress, oxygen limitation) do not allow sporulation (28, 39).

Analysis of the precise function of the general stress regulon in stress management will undoubtedly profit from a comprehensive description of all σ^B-dependent genes. Therefore, we decided to use DNA macroarrays for transcriptional profiling of stress adaptation in *B. subtilis* to detect the still missing members of the σ^B regulon. By this approach more than 60 new σ^B-dependent genes were discovered. The screening was also utilized for the characterization of σ^B-independent stress gene induction. Interestingly, these studies showed salt shock induction of the regulon of the extracytoplasmic function (ECF) sigma factor σ^W.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The following *B. subtilis* strains were used: 168 (*trpC2*), BSA46 (*trpC2* SPβ *ctc*::*lacZ*), ML6 (*trpC2 sigB*::Δ*Hin*dIII-*Eco*RV::*cat*), BSA272 (*trpC2 sigB*::Δ*Hin*dIII-*Eco*RV::*cat*), and BSA386 (*trpC2 rsbX*::*spc sup20a* SPβ *ctc*::*lacZ*; obtained from W. G. Haldenwang). The *yjbC* (BFA2841) and *yjbD* (BFA2842) mutants were constructed by inserting the nonreplicative plasmid pMUTIN4, carrying fragments of the *yjbC* and *yjbD* structural genes, respectively, lacking the ribosome binding site and the first N-terminal codons, into the corresponding genes via a Campbell type single-crossover event (41). Strains were grown with vigorous agitation at 37°C in a synthetic medium with 0.2% (wt/vol) glucose as the carbon source (strains 168, ML6, BFA2841, and BFA2842) (44) or in Luria-Bertani medium (strains BSA46, BSA272, and BSA386). Ethanol or osmotic stress was imposed by adding ethanol or NaCl to exponentially growing cells to a final concentration of 4% (vol/vol or wt/vol, respectively). For heat stress, the temperature was shifted from 37 to 48°C.

Survival of growth-preventing salt stress was examined by transferring exponentially growing cultures into minimal medium containing an initial NaCl concentration of 4% (wt/vol). After a preadaptation period of 30 min, this was raised to a final NaCl concentration of 10% (wt/vol).

Cell lysis and RNA isolation. RNA was isolated either according to the acid phenol method of Majumdar et al. (34), with the modifications previously described (49), or after mechanical disruption of the cells as described by Hauser et al. (23). In the latter case sedimented cells were resuspended in 200 μl of growth medium and immediately frozen in a small Teflon vessel of a grinding mill (B. Braun Biotec Int., Melsungen, Germany) in liquid nitrogen. After addition of a tungsten carbide bead, the frozen drops were mechanically broken for 2 min at top speed. The frozen powder was instantly taken up in guanidine thiocyanate buffer (4 M guanidine thiocyanate, 25 mM sodium acetate [pH 5.2], 0.5% [wt/vol] *N*-lauroylsarcosinate) and was extracted three times with 1 volume of acid phenol-chloroform-isoamyl alcohol solution (25:24:1, vol/vol/vol), and twice with chloroform-isoamyl alcohol (24:1, vol/vol). After ethanol precipitation and washing with 70% ethanol, the RNA pellet was dried and dissolved in diethyl pyrocarbonate-treated distilled water.

Preparation of labeled cDNA, array hybridization, and DNA macroarray regeneration. Prior to cDNA synthesis, the quality of the RNA was routinely verified by standard Northern blot analysis with digoxigenin-labeled antisense RNA probes specific for known general stress genes. For cDNA synthesis, 2 μg of total RNA was mixed with 4 μl of a commercially available primer mix (Sigma-Genosys Ltd.) and 3 μl of 10× hybridization buffer (100 mM Tris [pH 7.9], 10 mM EDTA, 2.5 M KCl) in a total volume of 30 μl. The primer mix consisted of 4,107 specific oligonucleotide primers complementary to the 3′ ends of all *B. subtilis* mRNAs (Sigma-Genosys Ltd.). The sample was heated to 95°C for 10 min and subsequently cooled to 42°C for primer annealing. Reverse transcription was performed in a total volume of 60 μl with SuperScript II reverse transcriptase and [α-^{33}P]dCTP in the appropriate buffer for 1 h (Life Technologies, GmbH, Karlsruhe, Germany). After addition of 2 μl of 1% sodium dodecyl sulfate (SDS), 2 μl of 0.5 M EDTA (pH 8.0), and 6 μl of 3 M NaOH, the remaining RNA was hydrolyzed by incubation at 65°C for 30 min and at room temperature for 15 min. Prior to ethanol precipitation, the cDNA solution was neutralized with 20 μl of 1 M Tris (pH 8.0) and 6 μl of 2 N HCl. After a wash with 70% ethanol the pellet was carefully dried and resolved in 100 μl of distilled water. Labeling efficiency was determined with a liquid scintillation counter. This study was performed with Panorama *B. subtilis* gene arrays from Sigma-Genosys Ltd., which carry duplicate spots of PCR products representing 4,107 currently known *B. subtilis* genes. cDNA denaturation, probe hybridization, and washing of filters were performed as described by Hauser et al. (23).

The arrays were exposed for 2 and 4 days to storage phosphor screens (Molecular Dynamics, Sunnyvale, Calif.) and scanned with a Storm 840/860 PhosphorImager (Molecular Dynamics) at a resolution of 50 μm and a color depth of 16 bits.

Bound cDNA was stripped off the membranes by a short (1-min) washing step with 250 ml of boiling buffer (5 mM sodium phosphate [pH 7.5], 0.1% SDS), incubation in 250 ml of fresh buffer at 95°C for 20 min, and two additional wash steps with fresh boiling buffer.

Data analysis. Hybridization signals were quantified with ArrayVision software (Imaging Research Inc.) after direct import of the PhosphorImager files. After subtraction of the background, which was defined as the median of signals surrounding the entire spot fields, the overall spot normalization function of ArrayVision was used to calculate the normalized intensity values of individual spots, thus facilitating the comparison of results from different hybridizations and filters. Briefly, this procedure involved two steps: (i) calculation of the intensity of an average spot by dividing the sum of the intensities of all PCR product specific signals on the array by the total number of spots and (ii) dividing the intensity of the individual spot by the intensity of this average spot.

For each growth condition mRNA was prepared from two independent cultivations and then used for independent cDNA synthesis and DNA array hybridizations. For exponentially growing bacteria and ethanol treatment, three entirely independent replicates were processed. In total, 32 array hybridizations were performed. For each gene the average of the normalized intensity values from all the replicate experiments was calculated. To avoid extreme intensity ratios for genes close to or below the detection limit, the average normalized intensity for these low values was arbitrarily set to a value corresponding to a signal-to-noise ratio of 2. These average values were then used to calculate expression ratios for the following comparisons: (i) stressed (ethanol, salt, or heat shock applied for 10 min) versus exponentially growing wild-type strains, (ii) stressed (ethanol, salt, or heat shock applied for 10 min) versus exponentially growing *sigB* mutant cells, (iii) stressed wild-type cells versus stressed *sigB* mutant cells (both treated with ethanol, salt, or heat shock for 10 min), and (iv) the *rsbX sup20a* hyperexpression mutant versus the *sigB* mutant 60 min after ethanol addition. *rsbX* mutants lack an essential negative regulator of the σ^B regulatory cascade, fail to restrict the σ^B response, and therefore display artificially high and extended σ^B activity (50). In the suppressor mutant (*rsbX sup20a*) artificially high σ^B activity is compatible with growth (W. G. Haldenwang, unpublished data).

Experiments involving ethanol, salt, or heat stress (10 min) were performed with the isogenic *B. subtilis* strain pair (168 and ML6). In order to substantiate

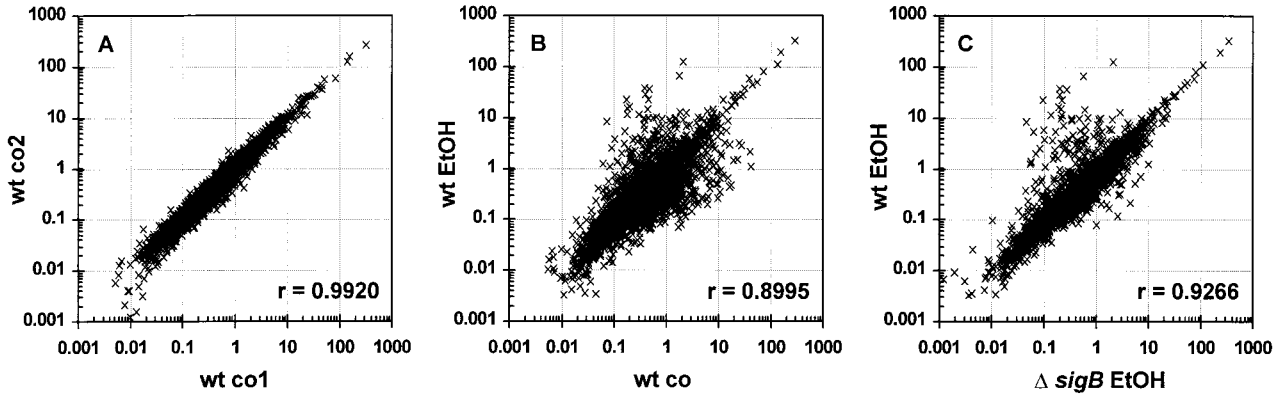

FIG. 1. Scatter diagrams of normalized spot intensities. (A) Spot intensities of two array hybridizations with two different unstressed samples from the wild-type strain 168 (wt co1 versus wt co2). (B) Spot intensities of array hybridizations from a nonstressed probe of strain 168 (wt co) and an ethanol-stressed sample of the same strain (wt EtOH). (C) Comparison of spot intensities of filters hybridized with probes of wild-type strain 168 (wt EtOH) and its isogenic *sigB* mutant ML6 (Δ*sigB* EtOH), both treated with ethanol. For the presentation, spot intensities of the 4,107 genes have been normalized and the duplicate spots on the filter have been averaged as described in Materials and Methods. r, Pearson correlation coefficient.

the results and to minimize the number of false positives, experiments involving treatment with ethanol for 10 min were also performed with an independent strain pair, BSA46 and BSA272, and Panorama *B. subtilis* gene arrays (Sigma-Genosys Ltd.) from a different batch. Due to the different strain pair and the different array batches, hybridizations involving this strain pair did not produce exactly the same induction ratios but confirmed the candidates identified in this study with the strain pair 168 and ML6.

The ratios of the expression levels obtained from the averaged normalized intensities of all replicate experiments were imported into GeneSpring 3.2.12 software (Silicon Genetics, Redwood City, Calif.) and used to find additional σ^B-dependent and σ^B-independent stress genes. Seventy-five genes have previously been assigned to the σ^B regulon. Twelve of these (*clpC*, *ctsR*, *opuE*, *sms*, *trxA*, *yacH*, *yacI*, *yacK*, *ytxG*, *ytxH*, *ytxJ*, and *yvyD*) exhibit an additional stress induction mechanism, but the remaining 63 (*aldY*, *bmr*, *bmrR*, *bmrU*, *bofC*, *clpP*, *csbA*, *csbB*, *csbD*, *csbX*, *ctc*, *dps*, *gsiB*, *gspA*, *gtaB*, *katB*, *katX*, *nadE*, *rsbV*, *rsbW*, *rsbX*, *sigB*, *yacL*, *ycdF*, *ydaD*, *ydaE*, *ydaG*, *ydaP*, *ydaS*, *ydaT*, *ydbD*, *ydbP*, *ydhK*, *yfhK*, *yfkM*, *yflA*, *yflT*, *yhdF*, *yhdN*, *yhxD*, *yjbC*, *yjgB*, *yjgC*, *ykgA*, *ykzA*, *yocK*, *yotK*, *yoxA*, *yoxC*, *ypuB*, *yqhA*, *yqhQ*, *yqxL*, *yrvD*, *ysnF*, *ytkL*, *yugU*, *yvrE*, *yxaB*, *yxbG*, *yxcC*, *yxkO*, and *yycD*) should display clear σ^B-dependent induction in response to all three stresses. These genes were also used to obtain a consensus sequence of σ^B-dependent promoters.

The DNA sequences preceding genes with potential σ^B-dependent stress induction were subsequently inspected for the occurrence of the characteristic −35 and −10 boxes recognized by the RNA holoenzyme carrying σ^B with the MotivFinder program (Decodon GmbH, Greifswald, Germany).

World Wide Web access. The complete data sets for all the growth conditions investigated are available online (http:///www.uni-marburg.de/mpi/voelker/functional-genomics).

RESULTS

Identification of σ^B-dependent general stress genes. DNA macroarrays that contain all currently known genomic open reading frames of *B. subtilis* were used to record the comparative transcriptional profiles of exponentially growing cells and cultures exposed to mild ethanol, heat, or salt stress. Bacteria were exposed to the stresses for 10 min in order to achieve maximal transcriptional induction.

Prior to quantification of the array data, the reproducibility of the array experiments was estimated by comparing the normalized spot intensities in scatter diagrams (Fig. 1). Array data from hybridizations of independent samples from the same cultivation condition always yielded high Pearson correlation coefficients (see Fig. 1A for an example; $r = 0.992$). As expected, Pearson correlation coefficients calculated for comparison of untreated and stressed samples of the wild type or for comparison of ethanol-stressed samples from a wild-type strain and from the corresponding *sigB* mutant were lower ($r = 0.8995$ and $r = 0.9266$, respectively), and the scatter diagrams displayed genes induced and genes repressed by ethanol stress (Fig. 1B and C).

In this study a gene was considered to require σ^B for stress induction when it complied with all of the following three criteria. (i) Expression of the gene had to be induced more than twofold by at least two of the three stresses in the wild type. (ii) The ratio of induction had to be at least 2 in three of the four mutant comparisons employed, i.e., wild type versus *sigB* mutant after heat, salt, or ethanol stress and expression in the *rsbX sup20a* mutant versus expression in the *sigB* mutant 60 min after exposure to ethanol stress. (iii) A σ^B-dependent promoter had to be located in front of the gene or the transcriptional unit to which the gene belonged. These criteria clearly differentiated between specific and general σ^B-dependent stress genes.

Table 1 lists the 101 genes that met these selection criteria. Fifty-one genes belong to the group of 75 σ^B-dependent genes already described in the literature, and 50 genes correspond to potential new members of the σ^B regulon.

However, it was apparent that we preferentially missed σ^B-dependent genes subject to an additional σ^B-independent stress induction mechanism, because those genes would not always display much stronger induction in the wild type than in the *sigB* mutant. Therefore, we applied a modified two-step discrimination protocol to the same set of data to hunt for this class of genes. Table 2 displays the results of this search, which required twofold stress induction by at least two of the three stress factors in the wild type and the *sigB* mutant. Potential candidates passing this first test were subsequently screened for the presence of the conserved σ^B-dependent promoter. This search strategy identified 24 additional genes, 11 of which had previously been described as general stress genes. The latter group includes *yvyD*, which remained inducible by etha-

TABLE 1. Summary of σ^B-dependent general stress genes

Gene[a]	Function or nearest homolog (E value)[b]	Regulatory region or potential promoter sequence[c]	Operon structure		Induction ratio[f]				
			Predicted[d]	Validated[e]	EtOH, 10 min/co	EtOH, wt/*sigB*	Heat, wt/*sigB*	Salt, wt/*sigB*	EtOH, *rsbX*/*sigB*
yaaH	Cortical fragment-lytic enzyme SleL, *Bacillus cereus* (e-120)	Potential *yaaIH* operon	2nd		4.3	3.3	1.9	3.0	7.4
yaaI	Isochorismatase (EntB) homolog, *A. fulgidus* (1e-21)	CAAA**GTTTTT**TCATTGCCTAAAAA**GGCTAC**ATAT-N_{33}-**TTG**	1st		16	15	2.1	3.5	1.9
ctc	Probable ribosomal protein L25, *Pseudomonas aeruginosa* (1e-18)	CGAG**GTTTAA**ATCCTTATCGTTAT**GGGTAT**TGTTTGTAAT(**A**)GG	1st	+	8.2	27	9.1	8.4	21
spoVC	Peptidyl-tRNA hydrolase (Pth), stage V sporulation protein C (1e-106)	*ctc spoVC* operon	2nd	+	8.4	8.4	2.1	3.2	2.7
ybdT	Fatty acid beta-hydroxylating cytochrome P450 (CypC) (<1e-180)	GTCG**GAAGAT**TAACGTGAAAATAGA**GGGTAA**AAAG-N_{17}-**ATG**	m		6.5	6.3	4.2	4.1	39
ybyB	Unknown conserved protein BH2666, *B. halodurans* (7e-08)	ACAG**GTTTAG**CAATTT.CCAAAAC**GGGAAT**GATA-N_{38}-**ATG**	m		40	120	53	1.7	273
ycbK	Probable integral membrane protein CJ0860, *Campylobacter jejuni* (2e-07)	AAAG**GTGTTA**TTCTGACTGCTCAA**GGATAT**ACGC-N_{55}-**ATG**	m		8.0	6.3	2.5	6.3	1.2
ycbP	Conserved hypothetical protein YndM, *B. subtilis* (6e-11)	TAAG**GTTTAA**CTTTTT.ACATTTG**AGGAAT**TATA-N_{34}-**ATG**	m		20	31	14	23	12
ycdF	Glucose 1-dehydrogenase II, *B. megaterium* (2e-66)	TGCT**GTTTTC**AACTCGGAAAAACA**GGGTAT**TTTC-N_{35}-**ATG**	1st		16	15	6.3	21	191
ycdG	Oligo-1,6-glucosidase (EC 3.2.1.10), *B. thermoglucosidasius* (<1e-180)	Potential *ycdFG* operon	2nd		7.4	5.2	2.1	3.2	22
nadE	NH_3-dependent NAD^+ synthetase (EC 6.3.5.1)	TCAT**GATTCA**TTTTCATTGATTTA**GGGAAA**TGATCAGT(**A**)ATA	m	+	3.2	2.3	1.4	8.7	3.1
ydaD	Hypothetical oxidoreductase YhdF, *B. subtilis* (e-113)	CCAT**GTTTAT**CCAAAGAGTGTGTG**AGGTAC**ACAACAAA(**TA**)GA	1st	+	132	110	16	59	168
ydaE	No similarity	*ydaDE* operon	2nd	+	66	52	9.9	36	7.3
ydaG	Probable general stress protein 26, *D. radiodurans* (7e-04)	TCTT**GTTTAA**ATCTTCCCCGGATG**TGGAAA**AGTAACAG(**C**)GGA	m	+	6.4	9.0	3.4	15	59
ydaP	Pyruvate dehydrogenase-related protein, *T. acidophilum* (e-103)	CCGG**GTTTTA**AAGCCTTTCTCCTG**TGGTAT**TGAAAAAA(**GG**)AA	m	+	22	30	9.8	18	155
ydaS	Conserved hypothetical membrane protein YwzA, *B. subtilis* (5e-06)	AAGT**GTTTCG**AAATGATCAGGAGC**GGTTAT**TGAT-N_{29}-**ATG**	m		5.3	3.2	1.4	2.7	6.7
ydaT	No similarity	ATGC**GTTTTT**ATTTTT.CACCTGC**GGGTAC**CATT-N_{27}-**ATG**	m		3.9	3.6	2.4	4.4	9.6
gsiB	Embryonic abundant protein Em1, *Arabidopsis thaliana* (4e-18)	GTTT**GTTTAA**AAGAAT.TGTGAGC**GGGAAT**ACAACAAC(**CA**)AC	m	+	61	64	16	44	263
ydbD	Manganese-containing catalase BH2190, *B. halodurans* (e-102)	TTTC**GTTTAT**CTTTCT.ATCGATC**GGAAAT**ATAA-N_{31}-**ATG**	m		83	75	9.8	18	454
rsbV	Anti-anti-sigma factor of SigB	AAAG**GTTTAA**CGTCTGTCAGACGA**GGGTAT**AAAGCCAACT(**A**)G	1st	+	4.8	6.0	4.6	15	12
rsbW	Switch protein/serine kinase, anti-sigma factor of SigB	*rsbVW sigB rsbX* operon	2nd	+	7.1	8.6	3.8	13	18
sigB	RNA polymerase general stress sigma factor	*rsbVW sigB rsbX* operon	3rd	+	7.1	15	7.3	8.7	51
rsbX	PP2C type serine phosphatase	*rsbVW sigB rsbX* operon	4th	+	5.8	9.9	6.2	8.2	16
yfnI	Anion-binding protein homolog, YflE, *B. subtilis* (<e-180)	TGTT**GTGTGA**TGCCTTGATTTTGTT**TGGAAAA**GAAG-N_{34}-**ATG**	m		5.6	12	2.3	6.8	14
yflT	No similarity	ATCT**GTTTCA**GGTACA.GACGATC**GGGTAT**GAAA-N_{33}-**ATG**	m		105	202	58	283	646
yflH	Unknown conserved protein BH2390, *B. halodurans* (5e-08)	GCAG**GTTTAA**CCTCCTCCAATTGC**AGGTAA**AGCA-N_{28}-**ATG**	m		13	5.5	2.2	2.5	11
yflA	Amino acid transporter BH4033, *B. halodurans* (8e-68)	AAAG**GTTTAT**GTTTTTCCATCTAT**GGGAAA**TGAT-N_{25}-**ATG**	m		3.3	5.2	2.7	7.4	3.6
yfkM	General stress protein BH3025, *B. halodurans* (3e-64)	GAAT**GTTTAT**TTTAGT.GTGGCTG**GGGTAG**AGTG-N_{36}-**ATG**	m		24	26	5.9	17	171
yfkJ	Protein-tyrosine-phosphatase BH2238, *B. halodurans* (5e-46)	GAAG**GTTTCT**TTTTAGAGAAATAG**GGGCAA**AGAA-N_{17}-**ATG**	1st		11	8.7	2.1	14	6.0
yfkI	No similarity	Potential *yfkJIH* operon	2nd		21	14	3.0	38	11
yfkH	Hypothetical protein YfkH, *B. cereus* (5e-59)	Potential *yfkJIH* operon	3rd		4.3	3.3	1.3	3.7	8.5
yfhD	No similarity	Potential *yfhFED* operon	3rd		6.8	4.9	3.2	7.1	9.2
yfhE	No similarity	Potential *yfhFED* operon	2nd		2.3	2.9	1.5	2.2	4.9
yfhF	Cell division inhibitor SLR1223, *Synechocystis* sp. (6e-54)	ACGC**GTTTTC**TTTTAT.TACAATG**AGGTAA**AGTA-N_{30}-**ATG**	1st		4.0	2.8	1.9	2.7	3.9
yfhK	Conserved hypothetical protein YwsB, *B. subtilis* (1e-22)	TTAT**GTTTGG**CTTTGCAAACAAAG**GGGAAT**AGGA-N_{84}-**TTG**	1st		8.5	11	7.2	52	43
yfhL[g]	Hypothetical protein YvaZ, *B. subtilis* (5e-05)	Potential *yfhKLM* operon	2nd		5.3	3.4	2.0	1.8	1.2
yfhM	Epoxide hydrolase-related protein DR2549, *D. radiodurans* (9e-61)	Potential *yfhKLM* operon	3rd		9.8	3.4	2.7	2.1	1.5
yhcM	ORF DG1040 (fragment), *D. discoideum* (4e-06)	TAAC**GGTTAA**TTTGTCTAACGAGG**GGGAAA**ATAT-N_{23}-**ATG**	m		8.9	7.3	2.1	5.6	52
ygxB	CmpX, *Pseudomonas fluorescens* (6e-10)	ATCT**GTTTGC**TTATTG.GATAAGT**GGGTAA**CAC-N_{31}-**TTG**	m		27	21	6	18	90
yhdF	Oxidoreductase (short chain DH/Red.) BH1511, *B. halodurans* (e-104)	TGGC**GTTTAT**TCATTTCTGTCGTG**TGGTAA**CGTTCAGT(**A**)TC	m		11	5.1	2.1	2.4	0.9
yhdN	Probable oxidoreductase PA1127, *P. aeruginosa* (e-112)	AAAG**GTTTAA**CATTTTTTCCAGAG**GGGAAA**AGAT-N_{25}-**ATG**	m	+	2.9	8.8	4.9	9.4	32
yheK	YxiE, *B. subtilis* (5e-12)	AAAG**GTTAAT**TGTGCT.CAAATTC**GGGTAG**TAGT-N_{30}-**ATG**	m		8.1	13	5.1	28	33
yhxD	Ribitol dehydrogenase, *Z. mobilis* (6e-66)	ACAT**GTTTTT**CTGCTTATGCTCAG**GGGTAC**ACAT-N_{35}-**ATG**	m		4.8	7.9	2.4	1.9	15
yitT	Unknown conserved protein BH2916, *B. halodurans* (6e-99)	TCGT**GTTCAA**ATATTT.GTTTTAA**GGGAAA**ACAT-N_{30}-**ATG**	m		6.2	4.9	1.2	5.1	7.9
yjcE	No similarity	TGCC**GTTTTA**CAAGAA....ACAC**GGGTAT**CGCG-N_{122}-**ATG**	m		3.2	6.3	3.8	36	5.7
yjgB	No similarity	GGCT**GTTTAA**ACAAGA.AGAAATG**GGGTAT**ATCT-N_{38}-**ATG**	m		37	38	4.8	12	36
yjgC	Formate dehydrogenase homolog YrhE, *B. subtilis* (<e-180)	GTAT**GTTTTA**TTGAGTTGTTGTAA**GGGAAC**TGAA-N_{40}-**ATG**	1st		34	24	5.2	17	47
yjgD[h]	Conserved hypothetical protein YrhD, *B. subtilis* (4e-11)	*yjgCD* operon	2nd		83	88	18	64	120
ykgA	Hypothetical protein BH1779, *B. halodurans* (3e-64)	AATG**GTTTAA**TGATTTTCATGATGA**GGGAAT**AATA-N_{33}-**ATG**	m		20	26	3.8	11	6.7
ykzA	Organic hydroperoxide resistance protein Ohr, *P. aeruginosa* (2e-21)	GCAT**GTTTAA**AAAGAT..CAGAAA**GGGAAA**TATAACAACT(**A**)G	m	+	79	147	36	162	238
yknA	Putative deoxycytidylate deaminase, *C. arietinum* (1e-27)	GAAT**GTTCTT**TGCATT..CTTTTC**GGCTAT**ACTA-N_{29}-**ATG**	m		4.1	4.1	1.4	2.0	2.1
ykuT	Unknown conserved protein BH2666, *B. halodurans* (4e-72)	AAAC**GTTTAA**CATGGTCATGTACA**GGGTAA**CTAG-N_{38}-**ATG**	m		2.0	1.7	3.2	9.9	2.5
ykzI	Unknown conserved protein BH2636, *B. halodurans* (2e-06)	GCGC**GTTTGA**AGTAAGGAGAAATG**TGGTAA**TAAA-N_{139}-**ATG**	m		13	24	6.5	38	17
ylxP	Unknown conserved protein BH2412, *B. halodurans* (2e-21)	AGAA**GTTTCA**CAAGGCTATGAATG**TGGTAT**TACA-N_{71}-**GTG**	m		4.6	10	1.6	2.3	18
ymzB	Unknown conserved protein BH1784, *B. halodurans* (5e-04)	TCCC**GTGTAA**AAGATGCAGCAATT**GGGAAT**AGTA-N_{24}-**TTG**	m		6.1	9.2	1.4	2.7	23

yoxC	No similarity	TTCT**GATTAA**AAAAAC.GGATACA**GGGTAA**TGAC-N_{20}-**ATG**	m		16	26	3.4	10	14
yocB	No similarity	TCAG**GTTTGA**TCGTTT.TTAAGAG**AGGAAA**AAGA-N_{30}-**ATG**	m		29	31	12	7.4	60
yocK	DnaK suppressor DksA, *Chlamydia pneumoniae* (3e-16)	CCAT**GTTTGA**CAGAAG.GCAAAAC**GGGAAA**CAGG-N_{20}-**ATG**	m		14	36	5.3	3.0	27
bmrU	Multidrug resistance protein	CTTC**GTTTAC**TGCTTACAGAAAAA**GGGGAT**TATATAACC(**A**)GA	1st	+	5.8	3.8	1.2	4.4	3.3
***bmr*[g]**	Multidrug efflux transporter	*bmrU bmr bmrR* operon	2nd	+	1.1	1.1	1.1	2.7	0.7
***bmrR*[g]**	Transcriptional regulator (MerR family)	*bmrU bmr bmrR* operon	3rd	+	4.3	1.5	1.1	1.5	3.4
yqxL	Mg^{2+} and Co^{2+} transport protein CorA, *A. aeolicus* (2e-11)	ACTG**GTTTAG**TGACGC.GGTTATT**GGGCAA**TTAA-N_{33}-**ATG**	m		8.0	11	3.6	12	25
yqhB	Putative membrane protein with hemolysin domain, *C. jejuni* (3e-68)	ACAT**GTTTTA**TGAGCATTTTCAGG**TGGTAT**GGAA-N_{29}-**ATG**	m		5.2	6.7	3.0	6.0	12
yqgZ[h]	Arsenate reductase BH3485, *B. halodurans* (3e-16)	AATG**GTTTAA**ATGAAAAATGATCC**GGGTAG**TTAT-N_{37}-**ATG**	m		5.4	101	76	257	337
bofC	Forespore regulator of the sigma K checkpoint	Potential *csbX bofC* operon	2nd	+	5.2	6.4	2.7	3.6	60
csbX	α-Ketoglutarate permease	ACAG**GATTAC**AATTCAGCAAGCTT**GGGTAT**ATACTCCATT(**G**)A	1st	+	7.9	8.9	4.0	4.3	5.1
ysnF	Hypothetical protein DR1314, *D. radiodurans* (1e-13)	TTTT**GTTTAA**TTCAAA.GAACAGC**GGGAAT**TACA-N_{46}-**ATG**	m	+	116	194	16	40	398
ytxJ	General stress protein BH3013, *B. halodurans* (7e-11)	*ytxGHJ* operon	3rd	+	8.8	5.6	5.4	23	5.2
ytxH	Seed maturation protein PM30, *Glycine max* (6e-04)	*ytxGHJ* operon	2nd	+	7.7	4.4	7.4	26	6.8
ytxG	General stress protein BH3245, *B. halodurans* (2e-19)	ACAT**GTTTAT**GATTGA.AGAAAAC**GGGTAA**ACAGCAG(**T**)ATAT	1st	+	7.7	5.0	10	38	5.7
dps	DNA-protecting protein	AGAG**GTTTTA**GCGTAG.ATATTAA**GGGTAT**ACATAGTCAT(**A**)T	m	+	20	47	11	111	31
ytiA	Ribosomal protein L31, *B. halodurans* (9e-30)	TGAG**GTTTAC**GATGTG.AAACAGA**GGGAAG**GATA-N_{75}-**ATG**	m		13	25	6.1	16	1.0
ytaB	ORF168 protein, *A. rubrum* (6e-07)	GGGG**GTTTGA**TATTTATAAGATAAA**GGGTAA**TTAA-N_{21}-**ATG**	m		9.3	12	2.6	12	28
yugU	Unknown conserved protein BH3498, *B. halodurans* (2e-48)	TCGG**GTTTAT**GAGAGCGGTTTAACA**GGAAAA**AAAA-N_{21}-**ATG**	m		3.0	3.2	1.9	3.5	3.8
yuzA	Hypothetical conserved protein BH3345, *B. halodurans* (7e-22)	GATG**GTTTTA**TTTTGCAAGGTGCT**GGGAAA**GAAG-N_{113}-**ATG**	m		13	11	2.6	3.4	1.6
yvrE	Senescence marker protein-30 (SMP30-fam.), *Xenopus aevis* (2e-50)	AGTG**GTTTGG**ACACCT.CTTTGCC**GGGAAT**AACA-N_{32}-**ATG**	m		12	12	4.0	14	33
yvgO	Hypothetical 19.6-kDa protein, *B. amyloliquefaciens* (1e-53)	TTGA**GATTAC**AAATACATTGAGCA**GGGTAT**GCCT-N_{36}-**TTG**	m		10	7.0	3.8	5.1	0.9
yvaA	Hypothetical oxidoreductase YdgJ, *Escherichia coli* (2e-76)	TTAG**GTTTTA**CCATTTGATCAGGA**GGGTAT**ATAC-N_{35}-**GTG**	m		10	12	6.3	13	20
yvaJ	3′-to-5′ exoribonuclease RNase R	*yvaK yvaJ* operon	2nd		7.1	5.1	1.7	3.0	12
yvaK	Carboxylesterase precursor (EC 3.1.1.1), *B. stearothermophilus* (2-107)	AAAC**GTTTTT**TTCTGATTAAACTG**TGGAAA**ACTA-N_{28}-**ATG**	1st		6.6	7.8	1.5	3.5	10
gtaB	UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9)	AAAT**GTGTAA**AAACATATTGAAAA**GGGTAA**ATGTGCTGT(**A**)GT	m	+	5.2	4.4	2.0	6.1	4.6
ywtG	D-Xylose-proton symporter XylT, *L. breyis* (e-113)	AAAG**GTTTAA**TGGCCGGAAAAAGA**GGCTAA**AAGA-N_{60}-**ATG**	m		4.7	4.5	1.8	3.5	79
ywsB	Hypothetical protein YfhK, *B. subtilis* (2e-22)	TGAT**GTTTAG**GAACCTGCGATAAC**GTGAAT**AGAG-N_{42}-**ATG**	m		18	61	20	159	60
csbD	No similarity	GAAT**GTTTAT**TGCCTC.TCAGATC**GGGAAG**TTAA-N_{34}-**ATG**	m		60	240	66	245	21
ywmE	No similarity	ATTG**GTTTAA**AAAACAG.TTTGGGC**GGGAAT**GATA-N_{21}-**ATG**	m		8.4	6.5	3.1	4.1	6.3
ywjC	No similarity	ATAG**GTTTAC**GACTTGTCAGCTTT**GGGAAC**TTAG-N_{36}-**ATG**	m		7.2	16	10	13	69
ywiE	Cardiolipin synthetase BH2858, *B. halodurans* (e-115)	CAAG**GTTTAT**CGATTAGAAAAAAG**AGGTAA**TACA-N_{32}-**ATG**	m		2.4	2.3	1.3	2.6	2.6
ywzA	Conserved hypothetical protein YdaS, *B. subtilis* (2e-06)	AGTT**GTTTAT**CTTATACAAAAAAG**AGGAAT**GATA-N_{130}-**ATG**	m		88	140	29	208	459
gspA	Putative glycosyl transferase LgtC, *P. multocida* (6e-30)	ACGT**GTTTAT**TTTTTT.GAAAAA**GGGTAT**GTAACTTGT(**A**)CA	m	+	42	88	33	57	395
yxzF	No similarity	Potential *yxlJ yxzF* operon	2nd		2.6	2.3	1.6	2.0	13
yxlJ	*N*-Methylpurine-DNA glycosylase, *Mus musculus* (3-e21)	AGCC**GTTTTT**TTTGAT.CTGCTTC**GGGAAT**GGAT-N_{36}-**GTG**	1st		4.2	3.2	1.6	3.0	5.3
katX	Catalase X (EC 1.11.1.6)	GGCT**GTTTTA**AAATCTTTCCATTCA**GGGAAT**ATTGTTAC(**C**)GT	m	+	5.2	3.8	1.6	2.0	11
yxkO	Hypothetical protein TM0922, *T. maritima* (2-e30)	TTTT**GTTTGA**AAAAGAAAAGGGAC**AGGAAA**AATA-N_{27}-**ATG**	m		3.9	2.4	2.0	2.1	37
yxiS	No similarity	*katB yxiS* operon	2nd	+	3.6	4.0	3.0	7.9	9.2
katB	Catalase 2 (EC 1.11.1.6)	AGCA**GTTTAT**ATGAAGAACGCCAC**GGGTAA**ATGTGCTGT(**A**)GA	1st	+	2.2	2.1	1.3	2.6	11
csbC	Metabolite transport protein homolog YwtG, *B. subtilis* (e-115)	AAAT**GTTTCA**AATGAGATAGGAAAT**GGGTAC**TAATCT(**A**)TTAA	m	+	4.3	3.6	2.2	6.6	73
yxbG	Probable short-chain dehydrogenase PA1649, *P. aeruginosa* (1e-30)	GCAT**GTTTAT**CACTGC.ACATAGC**GGGAAG**ACAA-N_{23}-**ATG**	m		2.7	2.0	1.4	3.0	8.7
yxnA	Glucose 1-dehydrogenase II (EC 1.1.1.47), *B. megaterium* (4e-24)	AGGG**GTAAGA**CCCTTC..CGGATG**GGGTAA**TGTA-N_{29}-**ATG**	m		4.8	3.1	1.6	3.1	23
yxaB	EpsL, *S. thermophilus* (4e-32)	CAAT**GCATAG**CCATCCTTCTTTTT**GGGTAG**AGAC-N_{45}-**ATG**	m		8.5	8.5	1.9	3.6	7.1
yycD	No similarity	GATC**GTTTCG**GACAGTAACAAGGC**GGGAAA**AATG-N_{27}-**ATG**	m		33	34	8.3	14	104

[a] Genes are listed according to their order in the *B. subtilis* genome. Genes in bold letters were known from the literature to be σ^B dependent prior to the DNA macroarray analysis.

[b] The function of the protein encoded is given if it has been supported by genetic or biochemical data. Otherwise the nearest homolog of the protein encoded is listed. The expect values (E values) given in parentheses were obtained with BLAST 2.0 searches using the BLOSUM 62 matrix against the SWALL-none redundant protein database. "No similarity" indicates that the E value was above e-03. In cases where the best homolog has not been characterized yet, the next possible homolog with known function is given instead.

[c] The presumed −35 and −10 regions of potential σ^B-dependent promoters and the start codon of the coding region are shown in boldface. The experimentally determined 5′ ends of the mRNAs are represented by bold letters in parentheses. If the gene is not the first gene in an operon, the operon is shown instead of the regulatory region.

[d] Potential operon structures derived from the *B. subtilis* genome sequence. m, monocistronic; ordinal numbers indicate the position in the potential operon.

[e] Plus sign indicates verification of operon structure by Northern blot analysis.

[f] Calculated by dividing the normalized hybridization signals of individual genes in the induced sample or wild type (wt) by the relative intensities of the untreated controls. Values reflect averages of at least two independent array hybridizations. EtOH 10 min/co, RNA was prepared from a wild-type strain either immediately before or 10 min after treatment with 4% (vol/vol) ethanol; EtOH, heat, or salt, wt/*sigB*, ratios were obtained with RNAs prepared from the wild-type strain and the *sigB* mutant treated for 10 min with 4% (vol/vol) ethanol, 48°C, or 4% (wt/vol) NaCl; EtOH, *rsbX*/*sigB*, the ratios were obtained with RNA prepared from an *rsbX* suppressor mutant (BSA386) and the *sigB* mutant (BSA272) 60 min after addition of ethanol to a final concentration of 4% (vol/vol).

[g] Operon internal genes that do not fulfill the stringent expression criteria are included here if the flanking genes display σ^B-dependent expression or if the operon structure has been proven by Northern blot analysis.

[h] The expression pattern has been verified by Northern blot analysis.

TABLE 2. Summary of putative σ^B-dependent genes subject to additional σ^B-independent stress induction[a]

Gene	Function or nearest homolog (E value)	Regulatory region or potential promoter sequences	Operon structure		Induction ratio[b]					
					wt			*sigB*		
			Predicted	Validated	EtOH	Heat	Salt	EtOH	Heat	Salt
ctsR	Negative transcriptional regulator of class III stress genes	TCAG**GTTTTG**TGGACCGGGAAAAT**GGAAAT**AATGAAGG(**A**)T(**A**)	1st	+	9.0	10	4.2	6.5	8.1	2.8
mcsA	Modulator of CtsR repression	*ctsR mcsAB clpC sms yacK* operon	2nd	+	3.3	5.4	1.9	4.3	4.4	1.5
mcsB	Modulator of CtsR repression	*ctsR mcsAB clpC sms yacK* operon	3rd	+	10	16	5.4	14	15	3.4
clpC	Class III stress response-related ATPase	*ctsR yacHI clpC sms yacK* operon	4th	+	5.8	19	5.0	7.4	13	2.3
sms	DNA repair protein homolog BH0104, *B. halodurans* (e-178)	*ctsR yacHI clpC sms yacK* operon	5th	+	6.2	4.6	4.2	4.5	3.0	3.2
yacK	Putative DNA-binding protein SCE9403, *Streptomyces coelicolor* (1e-80)	*ctsR yacHI clpC sms yacK* operon	6th	+	3.5	3.2	3.7	4.5	2.2	3.3
yacL	Putative glutamyl-tRNA-transferase, *Listeria monocytogenes* (e-108)	TTTC**GGTTAA**AACCTTATGAATAC**GGGTAT**ATTAATGTT(**G**)GTT	m		2.4	3.2	2.5	2.5	2.2	1.9
yceC	Resistance protein CdrC, *C. acetobutylicum* (2e-43)	AAAC**GTATAT**ATTAGT.AATTTAC**GGCTTA**TTTT-N_{61}-**GTG**	1st		2.9	1.9	9.6	6.1	2.2	12
yceD	Resistance protein CdrC, *C. acetobutylicum* (7e-63)	Potential *yceCDEFGH* operon	2nd		3.2	1.8	6.8	5.7	1.7	8.8
yceE	Resistance protein CdrC, *C. acetobytylicum* (1e-74)	Potential *yceCDEFGH* operon	3rd		2.7	1.4	6.2	8.3	2.5	12
yceF	Toxic anion resistance protein YkoY, *B. subtilis* (4e-28)	Potential *yceCDEFGH* operon	4th		2.1	1.6	5.2	4.0	1.6	7.3
yceG	No similarity	Potential *yceCDEFGH* operon	5th		2.7	3.2	6.8	4.1	3.0	7.9
yceH	Tellurite resistance protein TelA, *R. sphaeroides* (1e-30)	Potential *yceCDEFGH* operon	6th		1.7	2.5	4.9	3.6	3.1	9.6
ycnH[c]	Succinate-semialdehyde DH GabD, *B. halodurans* (e-141)	AACG**GATTAC**TTTTGC.TGACAGC**GGGAAT**TAACGGTA(**A**)TATC	m		13	6.3	4.5	11	4.0	2.0
yjbC	Conserved protein BH2863, *B. halodurans* (8e-21)	GGCT**GTTTAA**ACAAGA.AGAAATG**GGGTAT**ATCTAAAAGT(**A**)TG	1st	+	8.9	3.8	9.2	18	3.2	18
yjbD	Conserved protein BH2861, *B. halodurans* (1e-56)	*yjbCD* operon	2nd	+	4.8	2.3	2.7	5.6	2.4	4.1
ypuD	No similarity	TACG**GTTTTT**TATTCATGAAAAAA**AGGAAT**AACT-N_{40}-**ATG**	m		2.2	2.3	1.6	3.4	1.8	2.5
yqjL	Probable hydrolase PA2934, *P. aeruginosa* (2e-05)	CCCT**GTTCAA**TACGAA.AGAGGCT**GGTAAT**GCCC-N_{104}-**ATG**	m		5.1	5.6	2.0	9.4	5.2	2.8
sodA	Superoxide dismutase (EC 1.15.1.1)	GCAG**GTTTAA**TGGGGCAGATTATC**GGTTAA**AGTG-N_{132}-**ATG**	m		5.3	3.1	3.0	5.4	3.7	1.3
yraA	Intracellular proteinase I PfpI, *P. furiosus* (4e-28)	GATT**GATATT**TCCACATTGTAATA**GGTTAG**TCCT-N_{118}-**ATG**	m		6.9	3.6	3.5	9.1	3.2	2.0
trxA	Thioredoxin	TCAG**GTTTTA**AAACAGCTCCGGCA**GGGCAT**GGTAAAGTAC(**A**)TG	m	+	6.9	3.9	3.9	4.8	2.9	1.7
yvgN	Putative morphine dehydrogenase YtbE, *B. subtilis* (e-116)	AAGC**GTATTA**TTGGTATCGGCTGAG**AGGAAT**GTGA-N_{44}-**GTG**	m		3.1	6.1	2.8	7.6	7.4	2.4
clpP	ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)	GATG**GTTTGA**ACCCCTGTATTTTTG**GGGAAA**ATGGGAAAA(**AG**)A	m	+	2.6	17	5.1	4.9	18	4.1
yvyD	Ribosomal protein S30AE family, *B. halodurans* (7e-62)	TTAT**GTTTCA**GCAGGAATTGTAAA**GGGTAA**AAGAGAAAT(**A**)GA	m	+	4.6	2.1	2.0	8.5	0.6	0.5

[a] For details on the data presentation, see Table 1, footnotes *a* through *e*.

[b] Calculated by dividing the relative hybridization signals of individual genes in the induced sample of the wild type (wt) or the *sigB* mutant by the respective control values. For experimental details, see Materials and Methods. EtOH, ethanol.

[c] Expression pattern has been verified by Northern blot analysis.

TABLE 3. Analysis of the frequency of retrieving known σ^B regulon members on gene arrays

Identification strategy	No. known	No. verified by DNA array analysis[a]
Proteome analysis	34	31 (+3)
Promoter- and oligonucleotide-based screening	24	12 (+2)
Transposon mutagenesis	8	6
Other	19	10 (+6)
Total	75	51 (+11)

[a] Number in parentheses is the number of genes subject to additional σ^B-independent stress induction.

nol stress in the *sigB* mutant at the σ^H-dependent promoter (16), as well as the *clpC* operon and *clpP,* both of which remained stress inducible in a *sigB* mutant at a σ^A-dependent promoter after inactivation of the CtsR repressor by stress (20, 32). *trxA* also belongs to this group, but the mechanism for stress induction in the *sigB* mutant has not been clarified yet (40). Assigning the other 13 genes to the σ^B regulon is more complicated because all of them still displayed stress induction in a *sigB* mutant (Table 2). In the case of the presumed *yceCDEFGH* operon this additional stress induction mechanism seems to involve the ECF sigma factor σ^W (see below). Detailed transcriptional analysis will be necessary to confirm σ^B dependency for each single gene listed in Table 2.

In total this DNA array analysis revealed 125 σ^B-dependent genes, 24 of which seem to be subject to a second, σ^B-independent stress induction. We confirmed 62 of the 75 σ^B-dependent genes known from the literature. Most notably, all the σ^B-dependent genes identified by the proteomics approach were confirmed by this array analysis (Table 3). This observation is not surprising, because the proteomics approach should have mainly detected genes displaying strong expression as well as clear transcriptional induction. Also, most of the genes identified by transcriptional studies (27, 37) or by the transposon-based approach of Price and coworkers (10, 12) were confirmed (Table 3). However, only 14 of 24 genes newly described as σ^B dependent by a promoter consensus search (36) were validated by DNA macroarrays. The reason for the surprisingly low validation rate of this group is not yet known. The list of genes already described in the literature as σ^B dependent but not confirmed by this DNA macroarray analysis includes *aldY*, *csbA*, *csbB*, *opuE*, *ydbP*, *ydhK*, *yotK*, *yoxA*, *ypuB*, *yqhA*, *yqhQ*, *yrvD*, and *ytkL.* We cannot exclude the possibility that a few of these genes constitute false positives that were described in earlier studies. However, we suspect that in some cases the apparent lack of detection by this DNA array approach might also be an artifact due to differences in the amount or quality of the PCR products on the membrane or in the primers utilized for the cDNA synthesis. One member of the latter group is certainly *opuE*, whose σ^B dependency has been unambiguously demonstrated (43).

Induction of σ^B-dependent genes ranged from twofold to several hundredfold. Such extreme induction ratios might be explained by the fact that σ^B, which is almost inactive during exponential growth, exclusively controls some σ^B-dependent genes. Frequently, general stress genes contain additional promoters, in most cases σ^A-dependent promoters, that allow significant basal expression level during growth. Accordingly, the stress induction ratios of genes in the latter group are lower than those for the former.

When the data were analyzed, it was apparent that salt and ethanol triggered much stronger induction of the σ^B regulon than heat stress, although heat stress was effective in inducing heat-specific stress proteins (see below). The reason for this difference is not clear but might be related to the influence of the stresses on growth and consequently their stringency. Both ethanol and salt reduced the growth rate slightly at the concentration used (final concentration, 4%), whereas a temperature shift from 37 to 48°C still stimulated growth.

Sometimes not all genes of an operon met the stringent criteria applied. If the missing genes were flanked by genes displaying a clear induction pattern (e.g., *yfhL*) or if the operon structure had been experimentally proven (e.g., the *bmrU bmr bmrR* operon), the genes were added to the table, since the failure of detection was most likely caused by the limitations of the array analysis described above. Applying stringent criteria to the searches will certainly minimize the detection of false-positive candidates, but at the risk of producing false negatives. Besides the genes listed in Tables 1 and 2, we uncovered a group of genes with either a less conserved σ^B-dependent promoter or a stress induction pattern just failing to fulfill the requirements outlined above. These 38 genes (*aldY*, *aroI*, *mtrA*, *purK*, *rbfA*, *spoIIQ*, *yabK*, *yacO*, *yazC*, *ycsE*, *ydcF*, *yddS*, *yeaC*, *yerD*, *yfjB*, *yfkC*, *yfkT*, *yfmG*, *yfmK*, *ygxA*, *yhdE*, *ykrS*, *ykrT*, *ykyB*, *ykzC*, *yrrU*, *ysdB*, *ytzB*, *ytzE*, *yumB*, *yusD*, *yusS*, *yutK*, *yvaM*, *ywdJ*, *ywdL*, *ywlB*, and *ywmF*) are currently the subject of detailed Northern blot analysis to clarify their potential σ^B dependence.

Locations and functions of new general stress proteins. The proteomic approach almost exclusively identified general stress proteins that were localized in the cytoplasm. The transposon mutagenesis as well as the promoter search- and oligonucleotide screening-based approaches have already indicated that the synthesis of membrane proteins is also subject to σ^B control, leading to the assumption that σ^B also contributes to the maintenance of the integrity of the cell envelope during stress (19, 36). Inspection of the σ^B-dependent gene products described in this study for membrane-spanning helices (MSH) revealed that 25 of them contain at least one potential MSH (Table 4). Furthermore, at least six of the general stress proteins seemed to contain signal sequences indicating an extracellular location. Four of those proteins are potential lipoproteins and are most likely attached to the outside of the cytoplasmic membrane (Table 5).

The functions of most of these newly described σ^B-dependent genes are still unknown. Probably the proteins encoded by those genes are involved, like the proteins already known, in the development of nonspecific multiple stress resistance in starving cells or in growing cells subject to harsh stress. In order to define the kind of stress resistance in which the individual genes are involved, a comprehensive screening program analyzing the stress resistance of mutants with mutations in single stress genes is in progress (41). This screening has already revealed a number of stress genes that have dramatic effects on resistance to specific stress factors. Figure 2 displays the sensitivities of two selected mutants to growth-preventing salt stress (see reference 51). The newly described *yjbCD*

TABLE 4. Membrane-localized specific and general stress proteins

No. of MSH[a]	Proteins[b]
1	YbbM, **YbyB**, YdjG, **YpuD**, YqfB, **YtxG**, **YtxH**, YuaG, YvlC, YxzE
2	YdbS, YdjH, **YfhL**, YjcE, YobJ, YqfA, **YqxL**, **YrvD**, YteJ, **YuzA**, YvlA, **YxiS**
3	**YdaS**, **YkuT**, YrkA, YuaF, YvlD, YvqI, YwrE
4	MrpB, **YcbP**, **YflA**, YknZ, YqeZ, **YqhB**, YwoA
5	OpuBB, OpuCB, OpuCD, YdbT, **YfnI**, **YitT**, YknW, **YtaB**
6	**YfkH**
7	YvgW
8	**YceF**, YthQ
11	YdaR, **YgxB**, YhaU, YhfA, **YwtG**
12	**Bmr**, **CsbC**, **CsbX**

[a] Determined by using either the dense alignment surface method of Cserzo et al. (15) or a hidden Markov model-based method introduced by Sonnhammer et al. (42). Proteins containing only one or two potential transmembrane helices (MSH) were classified as potential membrane proteins only if the MSH were predicted by both algorithms.

[b] Proteins displaying SigB-dependent stress-induced synthesis are shown in bold.

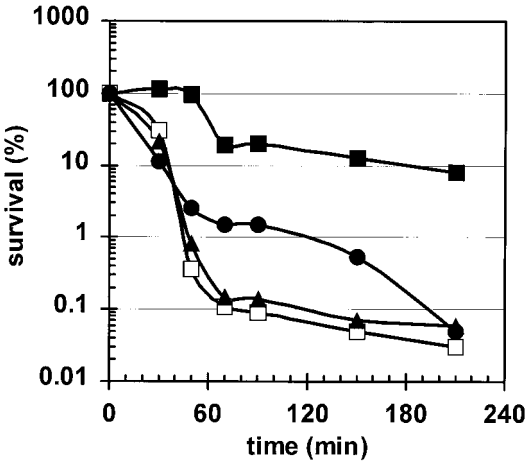

FIG. 2. Survival of *B. subtilis* strains during growth-preventing salt stress. The wild-type strain 168 (solid squares) and its isogenic mutants with mutations in *sigB* (ML6) (open squares), *yjbC* (BFA2841) (solid triangles), and *yjbD* (BFA2842) (solid circles) were grown in a synthetic medium and exposed to salt stress. Survival was determined by plating appropriate dilutions on Luria-Bertani agar plates. Cultures were pretreated with a mild salt stress of 4% NaCl for 30 min at time zero before the sodium chloride concentration was raised to 10% (wt/vol).

operon, which is at least in part under σ^B control (see Table 2), therefore codes for proteins that are somehow involved in salt resistance.

Identification of σ^B-independent stress genes. The DNA array data had thus far been used only for the identification of genes strictly requiring σ^B for stress induction or possessing a σ^B-dependent stress induction component. However, the same array data also provide a comprehensive picture of genes inducible by ethanol, salt, or heat stress independently of σ^B. Table 6 lists genes displaying significant induction by only one stress or a combination of two or all three stresses. In those cases stress induction is very likely σ^B independent, because similar or even stronger induction was observed in the *sigB* mutant and the corresponding transcriptional units seemed to lack σ^B-dependent promoters. Seven genes (*murG*, *sacC*, *yugJ*, *yutG*, *ywaC*, *ywnF*, and *ywoA*) seemed to lack a potential σ^B promoter and displayed at least twofold induction in the wild type and the *sigB* mutant under all stress conditions tested. For most of these genes, the precise biochemical functions of the products have not yet been determined.

In addition to σ^B-independent stress genes induced by all three stresses, there are proteins induced at least 2.5-fold by heat alone or heat plus ethanol (Table 6). Because ethanol may induce cellular signals similar to those induced by heat stress, genes induced by ethanol and heat stress might actually prove to encode specific heat stress proteins. The well-known members of the HrcA regulon belong to this group. Induction of the HrcA regulon by ethanol was more pronounced in the *sigB* mutant, most likely because σ^B-dependent stress gene induction did not compete for the limiting RNA polymerase core enzyme in this mutant.

The group of genes preferentially induced at least threefold by ethanol stress alone is somewhat surprising, since it was not recognized in the proteomic studies. For most of those genes neither an induction mechanism nor their functions in adaptation to ethanol can be inferred from the currently available data. Induction of the *ureAB* operon is most likely accomplished via σ^H, which has already been shown to be involved in stress induction of the *ytxGHJ* operon and the *yvyD* gene (16, 47).

Activation of the ECF sigma factor σ^W following salt shock. Investigation of the genes specifically induced after imposition of salt stress revealed 64 genes that were at least threefold induced in the wild type and the *sigB* mutant or that belonged to an operon fulfilling this criterion (Table 6). In accordance with expectations, screening for genes specifically induced by salt stress revealed four (*opuA*, *opuB*, *opuC*, and *proHJ*) of the already known osmoregulated operons of *B. subtilis* (13). Interestingly, the *gbsAB* operon, encoding proteins for the conversion of choline to the osmoprotectant glycine betaine (13), also displayed salt shock induction. Quite surprisingly, the gene coding for the ECF sigma factor σ^W was clearly induced following salt shock. Frequently, the genes of ECF sigma factors, *sigW* included, are subject to autoregulation (29). Consequently, the list of salt-induced genes included 23 genes previously described as σ^W dependent (30). Screening of the regulatory regions of the remaining salt-induced genes revealed that 11 of them either possessed a putative σ^W promoter or belonged to a potential σ^W-dependent transcriptional unit. Further extending this analysis, we inspected (i) the salt

TABLE 5. Extracellular specific and general stress proteins[a]

Protein class	Proteins
Predicted lipoproteins	...SacC, **YfhK**, YobJ, **YoxC**, YpuA, **YvgO**, **YwsB**, YvlA
Predicted extracellular proteins	.....**BofC**, OpuAC, OpuBC, OpuCC, **YjgB**, YknX

[a] All stress-induced genes were analyzed for the occurrence of a potential signal sequence with the SignalP algorithm of Nielsen et al. (35), and potential candidates were subsequently compared with a recent genome-based analysis of the secretome of *B. subtilis* by Tjalsma et al. (45). Proteins displaying σ^B-dependent stress-induced synthesis are shown in boldface.

TABLE 6. σ^B-independent stress gene induction in *B. subtilis*[a]

Gene[b]	Function or nearest homolog (E value)	Regulatory protein or sigma factor	Operon structure		Induction ratio					
					wt			*sigB*		
			Predicted	Validated	EtOH	Heat	Salt	EtOH	Heat	Salt
Induction by EtOH, heat, and salt shock										
murG	Undecaprenyl-PP-MURNAC-pentapeptide-UDPGlCNAc GlCNAc transferase		m		2.5	2.7	2.3	2.9	2.2	2.5
sacC	Levanase (EC 3.2.1.65)	CcpA, LevR	m		3.2	4.4	3.2	4.1	4.4	4.9
yugJ	Butanol dehydrogenase (EC 1.1.1.-), *C. acetobutylicum* (1e-97)		m		3.7	6.7	5.1	6.3	5.0	2.7
yutG	Low temperature requirement C protein, *B. halodurans* (4e-51)		m		2.2	8.4	3.9	3.0	7.6	2.4
ywoA	Probable bacitracin transport permease BcrC, *B. licheniformis* (4e-17)		m		2.5	3.1	2.0	4.1	2.5	2.4
ywnF	Unknown conserved protein, *B. halodurans* (6e-05)		m		4.7	5.0	5.9	6.0	3.5	3.1
ywaC	GTP pyrophosphokinase, *B. halodurans* (4e-36)		m		2.4	3.2	2.3	4.3	2.9	5.1
Induction by EtOH and heat shock										
groES	Class I heat shock protein (chaperonin)	HrcA	1st	+	7.2	11	1.0	16	16	1.6
groEL	Class I heat shock protein (chaperonin)		2nd	+	5.0	14	1.3	11	18	1.9
yhfA	Unknown conserved protein, *B. halodurans* (1e-41)		2nd		2.5	3.7	1.3	2.8	3.4	1.3
yhfI	Unknown conserved protein, *B. halodurans* (2e-59)		1st		2.6	2.7	1.1	3.4	2.6	0.8
yhfJ	Lipoate-protein ligase, *B. halodurans* (e-136)		2nd		2.8	3.2	1.2	3.3	2.8	0.8
yhfK	Unknown conserved protein BH1520, *B. halodurans* (3e-32)		3rd		3.3	3.0	1.9	5.2	2.8	1.3
yitW	dTDP-4-keto-*l*-rhamnose reductase RmlD, *S. mutans* (3e-31)		m		3.2	2.9	1.2	4.0	2.7	1.2
yjbG	Oligopeptidase F homolog, *B. subtilis* (<3e-180)		m		4.3	4.1	1.8	4.6	2.8	1.2
ykuV	Unknown conserved protein, *B. halodurans* (1e-59)		m		2.6	3.1	1.3	2.5	2.6	0.8
yoeB	No similarity		m		7.4	20	1.5	17	6.0	0.7
yqkF	Oxidoreductase BH1011, *B. halodurans* (e-102)		m		6.7	4.0	1.8	6.9	2.8	1.1
yqiG	NADH oxidase, *T. brockii* (2e-45)		m		2.5	4.6	1.7	5.1	5.4	1.6
hrcA[c]	Transcriptional repressor of class I heat shock genes	HrcA	1st	+	5.2	7.8	5	6.8	7.6	8.6
grpE	Heat shock protein (HSP-70 cofactor)		2nd	+	2.8	3.9	1.8	4.9	4.3	2.8
dnaK[c]	Molecular chaperone		3rd	+	1.4	5.5	1.8	3.2	6.4	3.7
yvrD	3-Hydroxybutyrate dehydrogenase, *S. meliloti* (1e-30)		m		3.1	4.8	2.4	4.0	4.3	1.5
iolS	Ion channel homolog YccK, *B. subtilis* (3e-94)		m		2.6	3.1	1.8	3.9	3.2	1.0
Induction by heat shock										
ycnD	NADPH-flavin oxidoreductase, *B. halodurans* (4e-49)		m		1.4	4.1	2.1	1.9	4.1	1.6
yjeA	Peptidoglycan GlcNAc deacetylase, *Streptococcus pneumoniae* (1e-48)		m		0.6	6.2	0.6	0.9	6.6	0.7
clpE	ATP-dependent Clp protease-like (class III stress gene)	CtsR	m	+	1.0	12	1.2	2.3	15	4.4
yqjM	NADH oxidase, *B. halodurans* (e-126)		m		1.6	6.5	1.4	2.3	5.4	1.1
yrkF	ORF H0532, *Halobacterium* sp. (5e-13)		3rd		1.2	9.0	2.8	5.7	6.1	1.8
yrkE	Hypothetical protein, *Staphylococcus aureus* (5e-36)		2nd		1.0	4.7	2.2	3.1	3.5	1.2
yrkD	Hypothetical protein, *S. aureus* (5e-14)		1st		0.8	3.5	1.5	2.8	3.4	1.1
Induction by EtOH shock										
ygaC	Unknown conserved protein BH3193, *B. halodurans* (6e-28)		m		3.1	1.2	2.3	3.2	1.2	2.8
yhcX	Nitrilase-related protein, *D. radiodurans* (1e-32)		2nd		3.8	2.5	2.2	3.9	1.8	1.2
yhgB	No similarity		1st		3.4	1.1	2.0	3.7	1.0	1.7
ykgB	Hypothetical protein YadB, *L. lactis* (1e-60)		m		4.9	1.9	1.6	3.8	1.8	0.7
yktC	*myo*-Inositol-1(or 4)-monophosphatase homolog, *B. subtilis* (e-153)		1st		3.4	1.6	2.7	3.4	1.3	1.1
ypiA	α-Acetolactate synthase Als, *L. lactis* (9e-34)		1st		4.2	2.2	1.9	6.7	1.0	1.8
yrzF	No similarity		1st		3.2	1.9	2.3	3.7	1.8	2.1
yrzG	No similarity		2nd		3.1	1.6	2.6	5.6	1.7	2.4
ytkL	No similarity		m		3.8	2.4	2.0	3.1	1.3	0.9
ytxK	Unknown conserved protein BH3193, *B. halodurans* (3e-76)		m		3.6	1.2	0.9	5.9	1.3	0.7
yuaE	No similarity		m		4.3	2.5	2.2	6.5	1.9	1.5
yvqH	Phage shock protein A, *E. coli* (8e-07)		2nd		3.6	1.1	0.6	23	1.2	0.9
yvqI	No similarity		1st		3.4	1.6	1.3	16	1.5	0.9
yvgW	Cation-transporting ATPase, P type (PacS) PAB0626, *P. abyssi*		m		3.5	1.4	1.0	4.0	1.6	0.8
ureB	Urease (gamma subunit) (EC 3.5.1.5)	SigA, SigH	1st		3.8	0.8	0.8	3.2	0.7	0.6
ureA	Urease (beta subunit) (EC 3.5.1.5)	Cod, GlnR	2nd		4.4	1.3	1.4	3.5	1.0	0.9
Induction by salt shock										
yaaN	Tellurite resistance protein, *R. sphaeroides* (2e-50)	SigW	2nd		1.4	1.3	4.7	2.2	1.2	5.7
sigW	RNA polymerase ECF type sigma factor	SigW	1st		1.3	0.8	3.3	1.4	0.7	3.7
ybbM	Anti-σ factor of SigW		2nd		1.2	0.6	4.2	2.7	1.2	8.6
ybfA	Ribosomal protein S18 alanine acetyltransferase homolog, *A. fulgidus* (1e-06)		m		2.2	1.9	3.7	3.8	2.1	6.0
opuAA[c]	Glycine betaine ABC transporter (ATP-binding protein)	SigA	1st	+	0.3	0.4	2.2	0.6	0.4	3.6

Continued on following page

TABLE 6—*Continued*

Gene[b]	Function or nearest homolog (E value)	Regulatory protein or sigma factor	Operon structure		Induction ratio					
					wt			*sigB*		
			Predicted	Validated	EtOH	Heat	Salt	EtOH	Heat	Salt
opuAB[c]	Glycine betaine ABC transporter (permease)		2nd	+	0.8	0.9	1.9	0.7	0.7	2.9
opuAC	Glycine betaine ABC transporter (glycine betaine-binding protein)		3rd	+	0.3	0.6	3.2	0.6	0.5	4.0
ydbS	Unknown conserved protein, *B. halodurans* (3e-28)	SigW	1st		2.1	1.3	6.7	2.8	1.3	5.3
ydbT	Unknown conserved protein BH1721, *B. halodurans* (9e-68)		2nd		1.4	0.8	7.1	2.2	0.7	4.9
ydjF	Phage shock protein A, *D. radiodurans* (4e-20)	SigW	m		2.3	0.9	9.1	3.9	1.1	14
ydjG	Unknown conserved protein, *B. halodurans* (6e-74)	SigW	1st		1.2	1.1	5.5	2.4	1.3	9.6
ydjH	Unknown conserved protein, *B. halodurans* (2e-11)		2nd		1.5	1.5	3.0	1.8	1.3	4.5
ydjI	Unknown conserved protein, *B. halodurans* (4e-5)		3rd		0.9	1.0	4.1	1.7	1.0	7.8
ydjO	No similarity	SigW	3rd		1.0	0.7	4.2	1.0	0.7	5.3
ydjP	Bromide peroxidase, *S. aureofaciens* (1e-18)		2nd		1.8	1.5	5.9	1.4	1.2	5.9
yeaA	No similarity		1st		1.4	1.2	4.8	1.2	1.1	4.8
spoOM	Sporulation control protein	SigH	m		3.7	0.8	6.1	4.2	0.9	6.6
yhaU	Putative transmembrane transport protein, *S. coelicolor* (8e-65)		3rd		0.9	0.9	7.3	0.8	0.7	4.5
yhaT[c]	Hypothetical protein YrvC, *B. subtilis* (4e-49)		2nd		1.1	1.0	3.7	1.2	1.2	2.6
yhaS	No similarity		1st		1.6	1.6	20	1.7	1.5	12
yhgD	Transcriptional regulator (TetR/AcrR family), *B. halodurans* (8e-32)		m		1.1	1.6	5.3	0.6	1.4	11
yjoB	FtsH, *Helicobacter pylori* (2e-20)	SigW	m		3.6	1.3	15	2.2	1.0	13
ykrL	Probable protease HtpX, *E. coli* (2e-51)		m		1.2	1.1	6.9	2.4	1.5	12
yknW	Unknown conserved protein, *B. halodurans* (4e-20)	SigW	1st		1.0	0.6	7.5	2.4	0.8	11
yknX[c]	ATP-binding cassette transporter-like protein TptB, *S. cristatus* (5e-23)		2nd		0.4	0.7	0.7	0.9	0.9	0.6
yknY	Putative ABC transporter YvrO, *B. subtilis* (3e-75)		3rd		1.2	0.6	3.4	3.2	0.8	5.5
yndN	Glutathione transferase FosB (EC 2.5.1.18), *S. epidermidis* (8e-48)	Potential SigW	m		1.3	1.1	4.5	2.2	1.1	6.4
proJ	Glutamate 5-kinase	SigA	2nd	+	1.3	1.3	4.9	1.2	1.2	4.4
proH	Pyrroline-5-carboxylate reductase		1st	+	1.4	1.2	4.6	1.1	0.9	4.1
yoaF	No similarity	Potential SigW	m		0.3	0.5	3.2	0.7	0.7	6.4
yobJ	No similarity	Sig W	m		1.2	0.5	5.2	3.9	0.9	10
yocL	No similarity		2nd		1.0	0.8	3.3	1.0	1.0	6.3
yocM	Small heat shock protein HspC, *B. japonicum* (2e-9)		1st		0.8	1.1	3.6	0.8	1.0	5.7
yozO	Hypothetical protein YjqA, *B. subtilis* (7e-10)	Potential SigW	m		0.6	0.6	6.1	0.6	0.6	6.4
yqfB	No similarity	Potential SigW	3rd		1.2	1.0	3.9	1.6	1.2	6.2
yqfA	Protein of unknown function ORF1, *B. megaterium* (1e-151)		2nd		1.7	0.9	24	2.8	1.1	22
yqeZ	No similarity		1st		1.3	0.9	17	1.8	1.0	17
yrkA	Integral membrane protein with hemolysin domain, *C. jejuni* (1e-73)		m		0.9	0.8	9.4	1.1	1.1	16
yteJ	Unknown conserved protein, *B. halodurans* (3e-31)	SigW	2nd		1.3	1.0	3.4	4.1	1.0	5.3
yteI	Proteinase IV, *A. aeolicus* (1e-37)		1st		2.4	0.9	3.6	5.3	0.8	4.7
ythQ	ABC transporter (permease), *B. halodurans* (6e-05)	Potential SigW	2nd		1.5	1.1	3.4	2.3	1.2	4.7
ytgB	ABC transporter, ATP-binding protein (TroB), *T. pallidum* (6e-79)		2nd		0.8	0.8	3.7	0.9	1.1	10
ytgA	ABC transporter, periplasmic binding protein (TroA), *T. pallidum* (9e-63)		1st		0.9	1.0	4.2	0.9	1.1	10
yuaI	Probable acetyltransferase, *D. radiodurans* (1e-15)	SigW	3rd		2.5	0.8	34	7.0	2.1	62
yuaG	Epidermal surface antigen, *B. halodurans* (4e-78)		2nd		3.6	0.9	57	7.2	1.9	68
yuaF	No similarity		1st		1.7	1.0	14	2.7	1.2	20
gbsB[g]	Alcohol dehydrogenase	SigA	2nd	+	1.0	1.1	2.0	1.0	1.2	3.8
gbsA	Glycine betaine aldehyde dehydrogenase		1st	+	1.0	1.7	4.6	1.2	2.1	15
mrpB	Na^+/H^+ antiporter BH1318, *B. halodurans* (2e-26)		2nd	+	0.8	1.7	3.1	0.7	1.6	6.9
opuBD[c]	Choline ABC transporter (membrane protein)	SigA	4th	+	1.2	1.2	2.6	0.9	1.2	4.2
opuBC[c]	Choline ABC transporter (choline-binding protein)		3rd	+	0.6	0.7	2.9	0.8	1.0	9.4
opuBB	Choline ABC transporter (membrane protein)		2nd	+	0.9	1.0	3.0	1.0	1.0	5.3
opuBA	Choline ABC transporter (ATP-binding protein)		1st	+	0.5	0.7	3.3	0.7	1.0	12
opuCD[c]	Glycine betaine/carnitine/choline ABC transporter (membrane protein)	SigA	4th	+	4.3	0.8	2.6	0.3	0.8	3.2
opuCC	Glycine betaine/carnitine/choline ABC transporter (binding protein)		3rd	+	0.3	0.7	15	0.5	1.0	28
opuCB	Glycine betaine/carnitine/choline ABC transporter (membrane protein)		2nd	+	0.7	0.9	9.8	1.2	1.1	16
opuCA	Glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)		1st	+	0.4	0.9	13	0.4	0.9	15
yvlD	Unknown conserved protein, *B. halodurans* (4e-23)	SigW	4th		1.5	1.2	6.0	2.7	1.3	9.3
yvlC[c]	Unknown conserved protein BH3592, *B. halodurans* (3e-11)		3rd		0.9	1.3	2.3	1.2	1.1	2.9
yvlB	Unknown conserved protein, *B. halodurans* (4e-63)		2nd		2.7	1.3	7.2	3.4	1.2	11
yvlA	No similarity		1st		2.0	1.3	6.1	3.1	1.3	9.2
yxjI	Hypothetical protein SCGD3.06, *S. coelicolor* (2e-05)	SigW	m		1.1	0.5	3.2	1.2	0.6	4.8
ahpF	Alkyl hydroperoxide reductase (large subunit) (EC 1.6.99.3)		2nd	+	0.9	1.7	3.7	1.0	1.6	3.6
ahpC[c]	Alkyl hydroperoxide reductase (small subunit)	SigA	1st	+	0.7	2.1	2.8	1.0	2.0	3.4

[a] See Table 1, footnotes b through e, and Table 2, footnote b, for explanations of data presentation.
[b] Genes are sorted according to their order in the *B. subtilis* genome. Genes previously known to be induced by the stimulus are shown in bold. EtOH, ethanol.
[c] Operon internal genes that do not meet the stringent expression criteria are included here if the flanking genes display σ^B-dependent expression or if the operon structure has been proven by Northern blot analysis.

induction pattern of other previously described members of the σ^W regulon (30) for their response to salt shock and (ii) genes displaying stress induction via σ^B and at least one other mechanism (Table 2) for putative σ^W promoters. This approach suggested 14 more genes that seemed to belong to a σ^W-dependent transcriptional unit displaying salt shock induction. An overview of the induction of the σ^W regulon by salt shock is presented in Table 7. Examination of the data indicates that many of these potential σ^W-dependent genes were also—although to a lesser extent—induced by ethanol (Tables 6 and 7), an effect that was more pronounced in the *sigB* mutant than in the wild type strain.

Twenty-three of the salt-induced proteins contain at least one putative MSH, and five seem to be exported or attached to the membrane as lipoproteins. These proteins are involved either in the acquisition of compatible solutes (the Opu-class of proteins) (13) or in the compensation of constraints imposed by salt stress on the membrane or the cell wall.

Because other stress stimuli such as oxidative, alkaline, or acid stress were not considered in this study, care should be taken in the classification of genes as stress specific from these data alone. AhpC and AhpF, for instance, should be considered oxidative stress proteins, because both are particularly induced by peroxide (3, 14). In this case induction by salt stress most likely reflects a secondary oxidative stress.

DISCUSSION

The σ^B-dependent general stress regulon is one of the largest regulons of *B. subtilis*. The discovery and functional characterization of almost all σ^B-dependent genes will be necessary for a comprehensive understanding of the physiological role of this huge regulon. Therefore, the DNA array technique was used to detect the candidates not yet found by proteomics, transcriptional analysis, consensus promoter-based transcriptional screening, or transposon mutagenesis (2, 8, 10, 12, 26, 36, 37). The DNA array induction pattern of the previously published σ^B-dependent genes (see Materials and Methods for a comprehensive list) was utilized to formulate the following criteria for identifying the remaining members of the regulon: (i) induction in the wild type by at least two of the three stresses analyzed (heat shock, salt stress, and ethanol stress), (ii) σ^B dependency of stress induction, that is, absence in the *sigB* mutant and/or presence for a prolonged time in an RsbX$^-$ suppressor mutant that displayed prolonged and increased σ^B activity following stress, and (iii) presence of a putative σ^B-dependent promoter in front of the gene or operon. This approach is validated by the fact that it detected 51 of the 64 genes already known to be strictly σ^B dependent. In addition to this large group, 50 new genes, all subject to the control of a putative σ^B-dependent promoter, were identified. In order to also facilitate the recognition of genes with an additional σ^B-independent stress induction component, target genes displaying stress induction in the wild type and the *sigB* mutant were screened for the presence of the typical σ^B promoter structure in the regulatory region. This adjustment of the data analysis revealed 11 already known σ^B-dependent genes for which complex regulation had been described previously as well as 13 new candidates.

In total we describe 125 genes that belong to the σ^B regulon in this study. For the new members of the regulon detected in this study, σ^B dependency is highly probable but has to be confirmed by additional transcriptional studies in each case. Northern blot hybridizations have been conducted and confirmed σ^B dependency for *ycnH*, *yjgD*, and *yqgZ*.

However, a few genes described as σ^B dependent in earlier studies, such as *aldY*, *csbA*, *csbB*, *opuE*, *ydbP*, *ydhK*, *yotK*, *yoxA*, *ypuB*, *yqhA*, *yqhQ*, *yrvD*, and *ytkL*, were not detected by our approach. The majority of these belong to a group of genes that had been found to be σ^B dependent by a consensus promoter-directed slot blot hybridization screening (36). Of the 24 new candidates identified by this strategy, only 14 could be confirmed in the present investigation. Possible reasons for this failure include (i) the complex control of genes, which could blur the σ^B dependency, especially if it was combined with a weak σ^B promoter that showed only a low induction rate, and, alternatively, (ii) false-positive candidates described in earlier studies. We suspect (iii) that some genes were not confirmed because of artifacts due to either the quality or quantity (or both) of the PCR product on the membrane or the quality of the primers utilized for synthesis of the labeled cDNA. *opuE* is a clear example of this class, because its σ^B-dependent stress induction has been unequivocally demonstrated (43, 53).

Therefore, it should be stressed that the real number of σ^B-dependent genes might be even higher. Thirty-eight genes displayed σ^B dependency but failed to comply with all the criteria applied in this study. Those genes had to be listed separately either because they did not display induction by multiple stresses or because they lacked the well-conserved σ^B-dependent promoter, although they exhibited much stronger induction in the wild type than in the *sigB* mutant. Failure to display an obvious σ^B promoter, for a gene that shows clear σ^B-dependent induction, might reflect indirect control, probably via a transcriptional regulator subject to σ^B-dependent induction. However, this hypothesis remains to be substantiated by experimental data. Besides additional σ^B-dependent stress genes, this list of 38 genes probably also contains some false-positive candidates. Detailed transcriptional analysis of each single gene or operon is currently being performed so that a final decision on their σ^B dependency can be made.

Although the limitations of the DNA array hybridization at best allow a semiquantitative comparison of the expression profiles of different genes, the variations in the expression level of the σ^B-dependent genes were striking. Most of the σ^B-dependent genes displaying the strongest induced signals on the DNA macroarrays have already been found by the proteomic approach, which should preferentially identify the strongly expressed genes. Examples of this group are *ctc*, *gsiB*, *clpP*, *ydaD*, *yflT*, and *ykzA*. Three other strongly expressed σ^B-dependent genes have not yet been identified on two-dimensional gels. This is not surprising, because one of them encodes a membrane protein (*ytxG*), and the other two most likely escaped detection by the proteomic approach because their small products have a very basic pI (*csbD* and *ywzA*). The reasons for the strong expression of these genes are not immediately apparent because most of their promoters do not show what we currently believe to be perfect −35 (GTTTAA) and −10 (GGGWAW) boxes. In the case of *gsiB* the strong ribosome binding site, leading to high stability of the mRNA, seems to be an additional factor contributing to a high expres-

TABLE 7. Induction of the σ^W regulon in *B. subtilis* by salt or ethanol stress[a]

Gene	Function or nearest homolog (E value)	Regulatory region or potential promoter sequences	Induction ratio			
			wt		*sigB*	
			EtOH	Salt	EtOH	Salt
xpaC	5-Bromo-4-chloro-indolyl phosphate hydrolysis protein	AAGA**TGAAAC**TTGTTTAAGGATTGAA**CGTA**GTAG-N_{41}-**ATG**	1.4	2.3	1.6	2.3
yaaN	Tellurite resistance protein TelA, *R. sphaeroides* (2e-50)	*xpaC yaaN* operon	1.4	4.7	2.2	5.7
sigW	RNA polymerase ECF type sigma factor	AAAT**TGAAAC**CTTTTGAAACGAAGCT**CGTA**TACATACA(**GA**)C	1.3	3.3	1.4	3.7
ybbM	Anti-σ factor	*sigW ybbM* operon	1.2	4.2	2.7	8.6
yceC	Resistance protein CdrC, *C. acetobutylicum* (2e-43)	TTTA**CGAAAC**TTTGATATAATAACAAA**CGTA**TATA-N_{83}-**GTG**	2.9	9.6	6.1	1.2
yceD	Resistance protein CdrC, *C. acetobutylicum* (7e-63)	Potential *yceCDEFGH* operon	3.2	6.8	5.7	8.8
yceE	Resistance protein CdrC, *C. acetobutylicum* (1e-74)	Potential *yceCDEFGH* operon	2.7	6.2	8.3	1.2
yceF	Toxic anion resistance protein YkoY, *B. subtilis* (4e-28)	Potential *yceCDEFGH* operon	2.1	5.2	4.0	7.3
yceG	No similarity	Potential *yceCDEFGH* operon	2.7	6.8	4.1	7.9
yceH	Tellurite resistance protein TelA, *R. sphaeroides* (1e-30)	Potential *yceCDEFGH* operon	1.7	4.9	3.6	9.6
ydbS	Conserved protein, *B. halodurans* (3e-28)	AGAA**TGAAAC**CTTTCTGTAAAAGAGA**CGTA**TAAATAA(**CG**)A	2.1	6.7	2.8	5.3
ydbT	Conserved protein BH1721, *B. halodurans* (9e-68)	*ydbST* operon	1.4	7.1	2.2	4.9
ydjF	Phage shock protein A DR1473, *D. radiodurans* (4e-20)	AAAG**TGAAAC**TTTTAACGATAATAAAT**AGTA**TATG-N_{40}-**ATG**	2.3	9.1	3.9	14
ydjG	Unknown protein BH1806, *B. halodurans* (6e-74)	*ydjFGHI* operon	1.2	5.5	2.4	9.6
ydjH	Unknown protein BH1807, *B. halodurans* (2e-11)	*ydjFGHI* operon	1.5	3.0	1.8	4.5
ydjI	Unknown protein BH1805, *B. halodurans* (4e-5)	*ydjFGHI* operon	0.9	4.1	1.7	7.8
ydjO	No similarity	*yeaA ydjPO* operon	1.0	4.2	1.0	5.3
ydjP	Bromide peroxidase, *S. aureofaciens* (1e-18)	*yeaA ydjPO* operon	1.8	5.9	1.4	5.9
yeaA	No similarity	TTTA**TGAAAC**CTTTGGCCCTATTTAT**CGTA**TTACGT(**A**)AAA	1.4	4.8	1.2	4.8
yfhL	Hypothetical protein YvaZ, *B. subtilis* (5e-05)	TGCA**TGAAAC**ATTTCTTCTTTCTGCA**CGTA**ACAATGA(**GA**)A	5.3	3.9	1.6	2.0
yfhM	Epoxide hydrolase-related protein, *D. radiodurans* (9e-61)	*yfhLM* operon	9.8	7.5	3.8	4.1
yjoB	FtsH, *H. pylori* (2e-20)	GGGA**TGAAAC**AAAATGCTATGTCAAT**CGTA**TATATAAC(**G**)T	3.6	15	2.2	13
yknW	Unknown conserved protein, *B. halodurans* (4e-20)	AACA**TGAAAC**TTTTTGATATCCTTCC**CGTA**CTATTTGT(**T**)**A**	1.0	7.5	2.4	11
yknX[b]	ABC-transporter-like protein TptB, *S. cristatus* (5e-23)	*yknWXYZ* operon	0.4	0.7	0.9	0.6
yknY	Putative ABC transporter YvrO, *B. subtilis* (3e-75)	*yknWXYZ* operon	1.2	3.4	3.2	5.5
yknZ	ABC transporter–ATP-binding protein NMB0549, *Neisseria meningitidis* (6e-52)	*yknWXYZ* operon	1.2	2.8	2.3	3.8
yndN	Glutathione transferase FosB (EC 2.5.1.18), *S. epidermidis* (8e-48)	TGTA**TGAAAC**TTTCTTATGAAAAAAGT**CGTA**TATG-N_{32}-**GTG**	1.3	4.5	2.2	6.4
yoaF	No similarity	ATAA**TGAAAC**CCGGAGTATGCCAAGCC**CGTA**TAAC-N_{28}-**ATG**	0.3	3.2	0.7	6.4
yobJ	No similarity	TATA**TGAAAC**CTTTTTTATTTTAGCC**CGTA**TTAAAAGT(**A**)A	1.2	5.2	3.9	10
yozO	Hypothetical protein YjqA, *B. subtilis* (7e-10)	ATAT**TGAAAC**TTTTTTCTCTATATGTG**CGTA**TTAC-N_{171}-**TTG**	0.6	6.1	0.6	6.4
yqfB	No similarity	*yqeZ yqfAB* operon	1.2	3.9	1.6	6.2
yqfA	ORF1, *B. megaterium* (1e-151)	*yqeZ yqfAB* operon	1.7	24	2.8	22
yqeZ	Conserved protein BH1356, *B. halodurans* (e-103)	AAAA**TGAAAC**CTTTGATACATTTGTTA**CGTA**TGAA-N_{42}-**TTG**	1.3	17	1.8	17
yteI	Proteinase IV, *A. aeolicus* (1e-37)	GAAG**TGAAAC**ATTTTTCATATTGAAT**CGTA**TAATGAG(**AG**)A	2.4	3.6	5.3	4.7
ythQ	ABC transporter (permease) EcsB, *B. halodurans* (6e-05)	*ythPQ* operon	1.5	3.4	2.3	4.7
ythP	ABC transporter–ATP-binding protein EcsA, *B. subtilis* (2e-45)	TTAA**GAAAAC**TTTTTTTATTCTATTT**CGTA**GTAA-N_{20}-**TTG**	1.3	2.5	1.3	2.6
yuaI	Probable acetyltransferase, *D. radiodurans* (1e-15)	*yuaFGI* operon	2.5	34	7.0	62
yuaG	Epidermal surface antigen, *B. halodurans* (4e-78)	*yuaFGI* operon	3.6	57	7.2	68
yuaF	No similarity	ATTT**TGAAAC**TTTTCCCGAGGTGTCT**CGTA**TAAATGGT(**A**)A	1.7	14	2.7	20
yvlD	Unknown conserved protein, *B. halodurans* (4e-23)	*yvlABCD* operon	1.5	6.0	2.7	9.3
yvlC	Conserved protein BH3592, *B. halodurans* (3e-11)	*yvlABCD* operon	0.9	2.3	1.2	2.9
yvlB	Unknown conserved protein, *B. halodurans* (4e-63)	*yvlABCD* operon	2.7	7.2	3.4	11
yvlA	No similarity	AATT**TGAAAC**CTGAAGAGATTTTAAA**CGTA**TAAATAA(**GT**)A	2.0	6.1	3.1	9.2
ywrE	No similarity	TTTA**TGAAAC**GTTTTTCCTTTTTCTT**CGTA**TAAAGGTA(**GA**)	0.7	2.4	0.7	3.5

[a] See Table 1, footnotes a through e, and Table 2, footnote b, for explanations of data presentation.
[b] Operon internal genes that do not meet our stringent expression criteria are included here if the flanking genes display σ^B-dependent expression or if the operon structure has been proven by Northern blot analysis.

sion rate (31). For the other genes the factors determining strong expression still need to be elucidated.

The complete description of all members of a regulon is only a prerequisite for a full understanding of its physiological role. Detailed biochemical and physiological studies must now follow to obtain substantially new information on the physiological role of the σ^B regulon. Previous studies showed that σ^B-dependent stress proteins provide the starved or stressed cell with oxidative, pH, salt, and heat stress resistance (3, 18, 19, 51). So far only Dps has been shown to be required for oxidative stress resistance (4), and the σ^B-dependent proteins essential for salt, heat, and acid resistance are not known. Because the *clpC* operon or the *clpP* gene remains heat inducible in a *sigB* mutant, a limiting amount of ClpC or ClpP should not be the main reason for the impaired heat stress resistance of a *sigB* mutant (for reviews see references 26 and 27). The newly identified σ^B-dependent genes do not immediately help to answer this question, because most of them encode proteins of thus far undefined function. However, many membrane proteins belong to this group, indicating an essential role in the maintenance of cell envelope or transport capacity, as already discussed by C. W. Price (19, 37). Experimental evidence for this suggestion has been provided by studies by E. Bremer's group, who showed that some genes encoding proteins involved in the uptake of compatible solutes are at least partly under σ^B control (43, 53). A few of the new σ^B-dependent genes seem to encode proteins with interesting functions. YfhF, a probable cell division inhibitor, might prevent division under conditions of severe stress, giving cells time to recover. Some of the products might be involved in detoxification, such as the products of the *yceCDEFGH* operon, which seems to encode toxic anion resistance proteins, or that of *yqgZ*, which encodes a potential arsenate reductase. Other proteins seem to perform functions in maintaining the redox balance of the cell, including the products of *yxnA*, *ycnH*, and *yvaA*, encoding a glucose-1-dehydrogenase, a potential succinate semialdehyde dehydrogenase, and a hypothetical oxidoreductase, respectively. The superoxide dismutase SodA is certainly required for detoxification of superoxide, whereas the potential intracellular proteinase YraA might be required to degrade proteins that cannot be repaired. However, detailed functional analysis will be necessary to ascertain the precise functions of these proteins in stress management.

One of the interesting findings of this study is the surprisingly large number of genes with unknown functions that belong to the σ^B regulon (see Tables 1 and 2). Of the 4,100 *B. subtilis* genes, about 1,700 code for proteins with still unknown functions. Elucidation of the functions of all these proteins is a great challenge for future research. Allocating unknown proteins to their regulation groups is a useful approach for a preliminary prediction of their functions. This approach indicates that almost 100 σ^B-dependent proteins with still unknown functions are probably involved in the development of multiple prospective stress resistance in cells entering the stationary-growth phase or in the development of heat or osmotic stress resistance. However, detailed phenotypic screening of mutants is necessary to assign each protein to a single facet of stress resistance as a first step and to uncover its exact function by detailed biochemical experiments. Such experiments are in progress in order to gain a more comprehensive picture of the physiological role of this huge regulon in stress adaptation. The first results of this screening are presented in Fig. 2. Obviously, inactivation of *yjbC* renders *B. subtilis* almost as sensitive to growth-preventing salt stress as a *sigB* null mutant. In the *yjbD* mutant, too, stress resistance is significantly diminished from that in the wild type. It is noteworthy that *yjbD*, the downstream gene of an operon (*yjbCD*) that is at least partially σ^B dependent, encodes a protein (YjbD) whose *Lactococcus lactis* homolog seems to affect degradation of nonnative proteins and thereby stress tolerance (H. Ingmer, personal communication). Further studies are in progress to analyze the precise physiological role of both *B. subtilis* proteins in more detail.

Despite some restrictions discussed above, this study clearly shows that the DNA array technique is a very useful approach for defining the structure and function of already known or still unknown regulons. Fewer than 30% of the regulon members had been identified thus far by a proteomics approach, and low-abundance proteins or proteins with membrane-spanning domains had been missed entirely. Even though the proteomics approach will still have wide application because the final and active products of gene expression, as well as information on posttranslational modification or protein sorting, can be visualized only by proteomics, it will be replaced by the DNA array technologies for the purpose of defining regulon structure. The latter approach is easier to handle and is certainly more comprehensive, but even such a sophisticated screening will require detailed follow-up experiments to validate the data, including quantification of the results.

Among σ^B-independent stress induction phenomena, the salt shock induction of the σ^W regulon was certainly the most interesting. σ^W is one of seven ECF type sigma factors of *B. subtilis*, the functions of all of which are still not well understood. In general this class of sigma factors controls uptake or secretion of specific molecules and ions or responses to a variety of stresses (33). σ^W in particular has been implicated in detoxification responses and the production of antimicrobial compounds (30, 46). Although a *sigW* mutant displays altered resistance to cell wall biosynthesis inhibitors (46), no difference in the zone of inhibition was observed when a *sigW* mutant and its corresponding wild type were exposed to HCl, NaOH, NaCl, EDTA, dithiothreitol, 2-mercaptoethanol, lysozyme, SDS, hydrogen peroxide, methyl viologen, metal ions, and various antibiotics (30). In this study we provide evidence that the σ^W regulon is induced by salt shock. This is most likely not an osmotic but an ionic effect, because none of the known osmoregulated genes of *B. subtilis* possesses a σ^W promoter. Probably salt shock interferes with the cell envelope or the transport capacity of the cell and thereby triggers induction of the σ^W regulon. Sensing of transport processes has already been implicated in triggering σ^W activity (46). Salt shock does not seem to be the only stress inducing the σ^W regulon. Recently, Schumann and coworkers discovered alkaline shock induction of the σ^W regulon (54). Alkaline shock could also interfere with the transport capacity of the cell and thus release σ^W from its inhibition by its anti-sigma factor RsiW.

ACKNOWLEDGMENTS

A. Petersohn and M. Brigulla contributed equally to this work.

We are grateful to W. G. Haldenwang for providing strain BSA386 and to Jeff Errington for construction of the BFA2841 and BFA2842

mutants. We thank N. Hauser, M. Scheideler, and T. Beißbarth for initial help with the gene array hybridizations and analysis of the data.

This work was supported by grants from the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, the BMBF, the European Union (GLG2-CT-1999-01455) and the Max-Planck-Gesellschaft to J.H., U.V., and M.H.

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