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Analysis of the Role of RsbV, RsbW, and RsbY in Regulating σ^B Activity in *Bacillus cereus*

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The alternative sigma factor σ^B is an important regulator of the stress response of *Bacillus cereus*. Here, the role of the regulatory proteins RsbV, RsbW, and RsbY in regulating σ^B activity in *B. cereus* is analyzed. Functional characterization of RsbV and RsbW showed that they act as an anti-sigma factor antagonist and an anti-sigma factor, respectively. RsbW can also act as a kinase on RsbV. These data are in line with earlier functional characterizations of RsbV and RsbW homologs in *B. subtilis*. The *rsbY* gene is unique to *B. cereus* and its closest relatives and is predicted to encode a protein with an N-terminal CheY domain and a C-terminal PP2C domain. In an *rsbY* deletion mutant, the σ^B response upon stress exposure was almost completely abolished, but the response could be restored by complementation with full-length *rsbY*. Expression analysis showed that *rsbY* is transcribed from both a σ^A-dependent promoter and a σ^B-dependent promoter. The central role of RsbY in regulating the activity of σ^B indicates that in *B. cereus*, the σ^B activation pathway is markedly different from that in other gram-positive bacteria.

The gram-positive rod-shaped bacterium *Bacillus cereus* is a frequent cause of food-borne disease, which has vomiting or diarrhea as its relatively mild main symptoms (22). *B. cereus* can also cause dangerous nongastrointestinal infections, including periodontitis, fulminant endophthalmitis, and meningitis in immunocompromised patients (6, 9, 12, 15). *B. cereus* is part of a group of bacteria which has been named the *B. cereus* group (reviewed in reference 13). This group also includes *Bacillus thuringiensis*, which is an insect pathogen and therefore is widely used as a biopesticide, and *Bacillus anthracis*, which can cause the disease anthrax. Recent complete-genome sequence studies confirmed the close genetic relationships between the organisms in the *B. cereus* group (21). In several gram-positive bacteria, such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Listeria monocytogenes*, the alternative sigma factor σ^B plays an important role in redirecting gene expression under stress conditions (reviewed in references 10, 20, and 28). In *B. cereus*, σ^B is activated upon environmental stress and entry into stationary phase (5, 26). Phenotypic analysis of the *sigB* deletion mutant of *B. cereus* showed that σ^B is involved in the adaptive heat stress response (26).

Extensive studies in *B. subtilis* have addressed the topic of the regulation of σ^B activity (reviewed in reference 20). In nonstressed cells, σ^B is present in an inactive form by complexation with the anti-sigma factor RsbW. In this form, σ^B is unable to bind to RNA polymerase and thus cannot initiate the transcription of stress response genes. Under stress, an anti-sigma factor antagonist, RsbV, can bind to RsbW, thereby forming an RsbV-RsbW complex. This leads to the release of σ^B, which can then bind to RNA polymerase, leading to the transcription of σ^B-dependent genes. RsbW not only acts as an anti-sigma factor for σ^B but also is a kinase for RsbV, in which it phosphorylates a serine residue. The phosphorylated form of RsbV is unable to complex with RsbW and thus cannot release σ^B from its complex with RsbW. However, under stress conditions, a phosphatase which can dephosphorylate RsbV can be activated. Dephosphorylated RsbV can then form a complex with RsbW, leading to the release of σ^B.

There is considerable variation in the biochemical makeup of the phosphatases, which can dephosphorylate RsbV in the different bacteria (4, 28). A common theme is that they all have a C-terminal PP2C phosphatase domain, which is responsible for the dephosphorylation of RsbV. In *B. subtilis*, there are two PP2C phosphatases that act on phosphorylated RsbV (RsbV~P), which were termed RsbU and RsbP. RsbU has an N-terminal domain that can bind an upstream regulator (RsbT) (4). The second is RsbP, which is unique for *B. subtilis* and has an N-terminal PAS (Per-ARNT-Sim) domain (29). The RsbU homolog in the *B. cereus* group has an N-terminal CheY-like domain. The CheY domain is a widespread regulatory domain in prokaryotes. It is named after the single-domain CheY protein, which is involved in chemotaxis, but in many bacteria, the CheY domain is coupled with a C-terminal effector domain, which can have a wide variety of functions (8, 24, 30). We have earlier proposed the name RsbY for the RsbU homolog of *B. cereus* to reflect its structural differences with other PP2C phosphatases which perform the crucial role of dephosphorylating RsbV~P in the σ^B activation pathway in other bacteria (26).

In this study, we set out to characterize the roles of RsbV, RsbW, and RsbY in regulating the σ^B response of *B. cereus*. The bacterial strains, culture conditions, and genetic methods used in this study were described previously (26, 27). The oligonucleotides used are listed in Table 1.

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TABLE 1. Oligonucleotides used in this study

Oligonucleotide	Sequence (5′–3′)[a]
OERsbV-PagI-F	GGGCGGAAATCATGAATTTGGCAATAAA
OERsbV-XhoI-R	CTCCCTCGAGCCTTCTTTCTACTTTTTCAA
OERsbW-NcoI-F	GGTGCCATGGAGAGATTTGAAAAGATAG
OERsbW-XhoI-R	GTGGCTCGAGGTAAGATTCGTAGGTTGAGATTG
KORsbY-XbaI-F	GTTCTAGAGATTATGGATGCG
KORsbY-EcoRI-R	GGAGGAATTCCAATGCCAAATGATAAGGAAAAA
Erycas-SacI-F	CCCAGAGCTCGGTCCGCAAAAGAAAAAC
Erycas-EcoRI-R	CCACGAATTCCATACCTAATAATTTATCTAC
PEOrf4-R	TGTCCTTGTTCATCACTAAT
PrSigB-F	GAAATCGCAAATCATTTAGG
qPCRrsbY-F	TGCCTGAAATTGATGGACTTGA
qPCRrsbY-R	CGGCCAATTTATTTGCATCC
qPCRtufA-F	GCCCAGGTCACGCTGACTAT
qPCRtufA-R	TCACGTGTTTGAGGCATTGG
GSP1-rsbY	TGATCTTCTCTTAATGGGCTACTT
GSP2-rsbY	GATTTTCTTCTTGCTCTTTATGC
ComprsbY-HindIII-F	GGAGAAGCTTGCAGCGAAATTAAATATGACAGAG
ComprsbY-BamHI-R	CACCGGATCCACCCAATTTAATCCTAGTGAACAA

[a] Underlined nucleotides indicate introduced restriction sites.

Functional analysis of RsbV and RsbW of *B. cereus*. The functions of RsbV and RsbW in *B. cereus* were determined by performing in vitro transcription and phosphorylation reactions. For the in vitro study of the function of RsbV and RsbW of *B. cereus*, the genes encoding these proteins were cloned into pET28-b (by using the OERsbV and OERsbW primer pairs, respectively), resulting in a C-terminal His_6 tag. Further purification of the proteins was performed as described previously for σ^B (26), with the exception that RsbV and RsbW were dialyzed against 10 mM Tris-HCl (pH 8), 50 mM KCl, 10 mM $MgCl_2$, 0.4 mM dithiothreitol, and 20% glycerol (17). In vitro transcription reactions including purified *B. cereus* RNA polymerase (RNAP) at 30 nM, σ^B at 60 nM, RsbW at 0.3 μM, and RsbV at 1.5 μM were essentially performed as described previously (27). As a template for the in vitro transcription reaction, a PCR product generated with primers BcSigBF and PEOrf4, which contains the σ^B-dependent promoter upstream of *orf4*, was used at a concentration of 30 nM. For the reactions, the template, nucleotides, σ^B, and the regulators RsbV and RsbW were mixed and incubated at 30°C for 5 min before *B. cereus* core RNAP was added.

The in vitro transcription experiments confirmed the predicted functions of RsbW as an anti-sigma factor and RsbV as an anti-sigma factor antagonist (Fig. 1A). In *B. subtilis*, RsbW acts as a kinase on RsbV (7, 31). This property of RsbV and RsbW from *B. cereus* was also tested in an in vitro phosphorylation assay, which was performed according to previously described methodology (16, 31). In short, 1 μM RsbV, 1 μM RsbW, 40 μCi [γ-^{32}P]ATP (3,000 Ci/mmol), and 20 μM non-radioactively labeled ATP were mixed in kinase buffer (50 mM Tris-HCl, pH 7.6, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EDTA) and incubated at 30°C for 30 min, and the reaction was terminated by the addition of 2× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and heating at 85°C for 5 min. Control reactions in which RsbW was omitted were also performed. Samples were separated on an 18% polyacrylamide gel. This revealed that also in *B. cereus*, RsbW can phosphorylate RsbV (Fig. 1B). In conclusion, the basic functions of RsbV and RsbW in *B. cereus* are essentially identical to the *B. subtilis* homologs, even though their primary sequence homologies at the amino acid level are relatively limited (43% for RsbV and 56% for RsbW [26]).

RsbY has a crucial role in regulating σ^B activity of *B. cereus*. Previously, we have identified the *rsbY* gene, which is directly downstream of the *sigB* operon (26). Its C-terminal PP2C-domain, its close proximity to the *sigB* gene, and the absence of other genes with obvious homology to the important regulatory

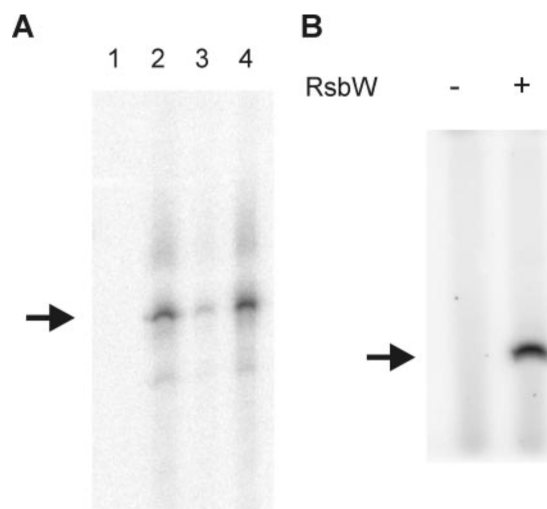

FIG. 1. Functional analysis of RsbV and RsbW of *B. cereus*. (A) In vitro transcription assays for the determination of the function of RsbV and RsbW. A PCR template containing the σ^B-dependent promoter site 5′ of *orf4* was used in the in vitro transcription reactions including *B. cereus* core RNAP (lane 1); core RNAP and σ^B (lane 2); core RNAP, σ^B, and RsbW (lane 3); and core RNAP, σ^B, RsbW, and RsbV (lane 4). After electrophoresis, runoff transcription products were visualized by autoradiography. The size of the σ^B-dependent transcription product is indicated with the arrow. (B) Phosphorylation of RsbV by RsbW. The phosphorylation reaction mixture contained 40 μCi [γ-^{32}P]ATP (3,000 Ci/mmol), 1 μM RsbV, and where indicated, 1 μM RsbW. Non-radioactively labeled ATP was added at a concentration of 20 μM. Proteins were separated on an 18% SDS-PAGE gel, and phosphorylated proteins were visualized by autoradiography. The position of RsbV (determined by running a sample of purified RsbV in parallel to the phosphorylation reaction mixtures) is indicated by the arrow.

protein RsbU in *B. subtilis*, *L. monocytogenes*, and *S. aureus* already suggested that RsbY has a role in regulating σ^B activity of *B. cereus*. To check this hypothesis, a deletion mutant of *rsbY* was constructed by allelic replacement of the *rsbY* gene with an erythromycin resistance cassette. A 3.2-kb product was amplified by PCR with the primers KORsbY-XbaI-F and KORsbY-EcoRI-R, digested with the appropriate restriction enzymes, and cloned into pATΔS28 (18), resulting in pATΔrsbY. Note that the XbaI site in this PCR product is a natural restriction site which lies in the *sigB* gene. Restriction of this site did not generate mutations in the *sigB* gene, as was confirmed by subsequent sequencing of the *sigB* gene in the *rsbY* deletion mutant. Subsequently, the plasmid pATΔrsbY was digested with SacI and MunI. These enzymes cut in the *rsbY* gene at positions 110 and 980, respectively (the complete *rsbY* gene is 1,143 bases long). Subsequently, the erythromycin resistance cassette of pUC18ERY (25) was amplified with the primers Erycas-SacI-F and Erycas-EcoRI-R and, upon restriction with SacI and EcoRI, cloned into the digested pATΔrsbY vector (note that digestion with MunI and EcoRI results in compatible sites), resulting in pATΔrsbYery. This plasmid was then transformed to *E. coli* HB101/pRK24, and the resulting strain was used in conjugation experiments with *B. cereus* to generate the *rsbY* deletion mutant *B. cereus* FM1401, according to previously described methodology (26). Subsequently, the activation of σ^B under various stress conditions was studied by immunoblotting with σ^B antiserum. Cultures of *B. cereus* ATCC 14579 and FM1401 in the mid-exponential growth phase (OD_{600}, 0.4 to 0.5) were stressed by a heat shock from 30°C to 42°C and by the addition of ethanol or NaCl to a final concentration of 4% (vol/vol) or 2.5% (wt/vol), respectively. Proteins were extracted before and 2.5, 5, 10, and 30 min after the stress exposure and immunoblotted anti-σ^B antiserum as described previously (26), with the modification that the resulting immunocomplexes were visualized by using the SuperSignal West Pico chemiluminescent substrate (Perbio, Etten-Leur, The Netherlands).

Under all tested conditions, an almost completely abolished σ^B response was observed in the *rsbY* null mutant (Fig. 2A). This clearly shows that RsbY is the key regulator of σ^B activity in *B. cereus*. The slight σ^B-activating effect upon stress exposure that remains in *B. cereus* FM1401 may be due to an as yet unidentified regulatory mechanism of minor importance. During stress exposure, proteins were isolated at regular intervals, and this allowed the study of the time course of σ^B activation. It is clear that in *B. cereus*, this response can be extremely rapid: already after 2.5 min, upon a heat shock, an increase in σ^B levels can be noted, and after 10 min, σ^B reached maximal levels. Also, during ethanol stress, there is a rapid increase of σ^B levels. The response of σ^B to osmotic stress is slower: only after 30 min can a strong activation of σ^B be observed.

The important role of RsbY in the σ^B activation pathway of *B. cereus* was confirmed by complementation of the *rsbY* deletion mutant in *trans* with a wild-type copy of *rsbY* expressed under the control of its own σ^A-dependent promoter (see below for the description of the promoter of *rsbY*). A PCR fragment, generated with the primers ComprsbY-HindIII-F and ComprsbY-BamHI-R, was cloned into the gram-positive shuttle vector pKSV7 (23), resulting in pKSV7-*rsbY*. *B. cereus* ATCC 14579 and FM1401 were transformed with the plasmids pKSV7 and pKSV7-*rsbY* by electroporation (1). Immunoblotting with anti-σ^B antiserum showed that the activation of σ^B upon a heat shock was restored in the *rsbY* deletion mutant upon complementation with the wild-type copy of *rsbY* (Fig. 2B).

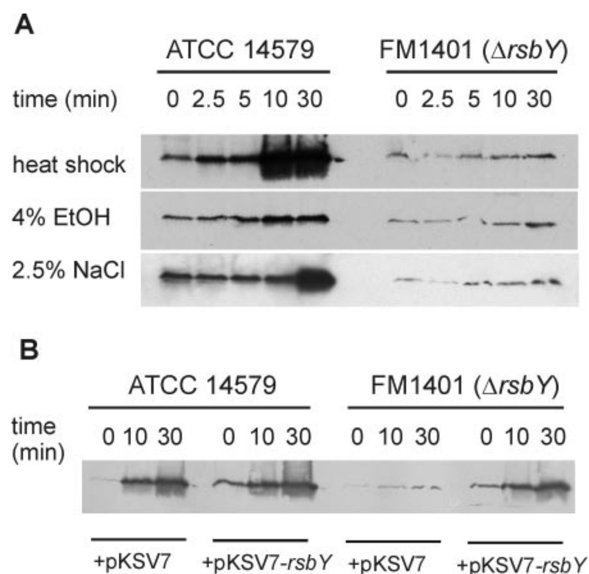

FIG. 2. The effect of deletion of *rsbY* on the stress-induced activation of σ^B in *B. cereus*. (A) Cellular σ^B levels in *B. cereus* ATCC 14579 and its *rsbY* deletion mutant FM1401 upon stress exposure. Bacterial proteins were extracted from cultures in the mid-exponential growth phase (time, 0 min) and upon exposure to the indicated stress for 2.5, 5, 10, and 30 min. Forty micrograms of protein of each sample was loaded on a 15% SDS-PAGE gel. σ^B was detected by immunoblotting with anti-σ^B antiserum as described in the text. EtOH, ethanol. (B) In *trans* complementation of the *rsbY* deletion mutant restores the activation of σ^B under stress. *B. cereus* ATCC 14579 and its *rsbY* deletion mutant FM1401 carrying the vector pKSV7 or pKSV7-*rsbY* (which contains full-length *rsbY* under the control of its natural promoter) were grown until mid-exponential growth phase and heat shocked from 30°C to 42°C. Proteins were extracted at the indicated times. Electrophoresis and the detection of σ^B were performed as described above.

Expression analysis of *rsbY*. The levels of expression of *rsbY* in *B. cereus* ATCC 14579 and its *sigB* deletion mutant (*B. cereus* FM1400) during the mid-exponential growth phase and upon a heat shock from 30°C to 42°C were determined. Northern analysis was first used to asses the expression of *rsbY*, but no transcripts could be visualized (data not shown), and consequently, real-time PCR was employed in follow-up experiments. RNA was extracted from two independent cultures of mid-exponential phase and heat-shocked *B. cereus* cells by using RNAwiz (Ambion, Huntingdon, United Kingdom). Residual DNA from the RNA preparations was enzymatically removed by using TURBO DNA-*free* (Ambion), and cDNA was synthesized from RNA by using Superscript III reverse transcriptase (Invitrogen, Breda, The Netherlands), 2 pmol of a *rsbY* gene-specific primer (qPCRrsbY-R), each deoxynucleoside triphosphate at a concentration of 0.5 mM, and 1 μg of total RNA. Reverse transcription and quantitative PCRs on the synthesized cDNAs by using an ABI Prism 7700 with SYBR green technology (PE Applied Biosystems, Nieuwekerk a/d IJssel, The Netherlands) were performed as described previously (2). Relative transcript levels were calculated by using the relative expression software tool (REST) (19). The expres-

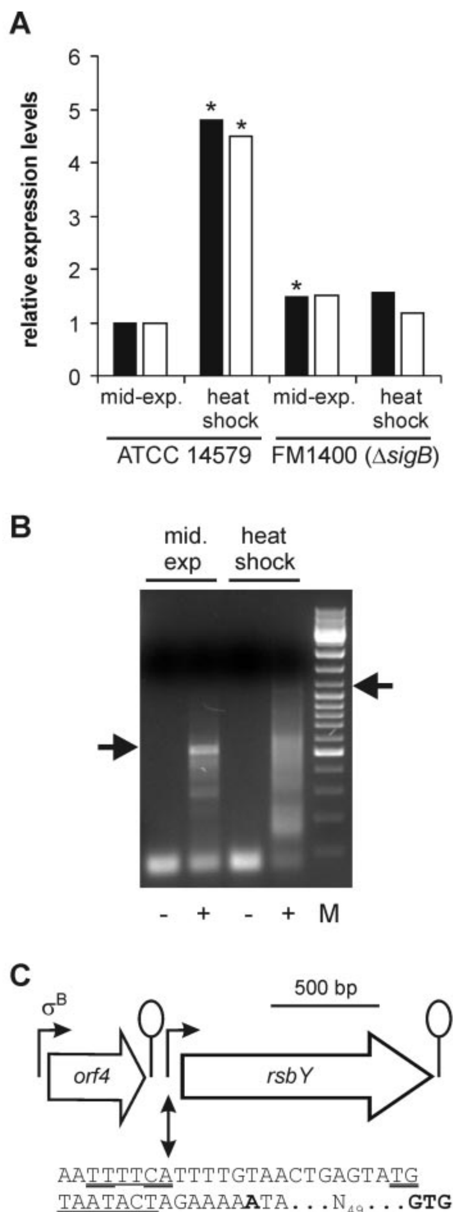

FIG. 3. Expression analysis of *rsbY*. (A) Real-time PCR quantification of *rsbY* transcript levels. Cultures of *B. cereus* ATCC 14579 and the *sigB* deletion mutant FM1400 were grown until the mid-exponential growth phase and exposed to a mild heat shock from 30°C to 42°C for 10 min. The results of RNA extraction from two independently treated cultures (black and white bars) are shown. The expression level of *rsbY* was set at 1 during the mid-exponential growth phase in *B. cereus* ATCC 14579, and the other conditions were compared to that condition by using REST (19). Expression levels of *rsbY* significantly different ($P < 0.05$) from the levels of expression of *rsbY* during mid-exponential growth phase in *B. cereus* ATCC 14579 are indicated with an asterisk. (B) 5′ RACE mapping of *rsbY* promoter sites. The RNA used in the 5′ RACE reactions was isolated from *B. cereus* ATCC 14579 under the same growth conditions as those described above. Agarose gel electrophoresis of PCR-amplified untailed (negative controls, indicated by the minus symbols) and poly(dC)-tailed (plus symbols) cDNA is shown. The marker (M) shown is the GeneRuler DNA Mix marker from MBI Fermentas GmbH, St. Leon-Rot, Germany. Fragments that were cloned and sequenced are indicated with the arrows. (C) Overview of the transcription of *rsbY* in *B. cereus* ATCC 14579. The σ^A-dependent promoter site directly upstream of *rsbY* is shown with the identified −35 and −10 regions underlined. Double-underlined residues indicate matches with the *B. subtilis* σ^A promoter consensus sequence (11). Bold type indicates the mapped transcriptional start site. The spacing to the GTG start codon of *rsbY* is also indicated. The *orf4* reading frame with its σ^B-dependent promoter upstream of *rsbY* is indicated. Lollipops indicate stem-loop structures.

sion of the *tufA* gene was used as a reference for the determination of induction levels. The expression of *rsbY* was found to be upregulated approximately 4.6-fold upon a heat shock in *B. cereus* ATCC 14579 but not in the *sigB* deletion mutant, which indicates that the expression of *rsbY* is upregulated under a heat shock in a σ^B-dependent fashion (Fig. 3A). Note that in one experiment, a small but borderline significant difference in the expression of *rsbY* in the mid-exponential growth between *B. cereus* ATCC 14579 and *B. cereus* FM1400 was observed. This effect could not be reproduced in the second experiment, leading to the conclusion that in the mid-exponential growth phase, the expression of *rsbY* is essentially identical in both strains.

The 5′ ends of the *rsbY* transcripts were mapped by using 5′ rapid amplification of cDNA ends (RACE) performed on RNA samples isolated from *B. cereus* ATCC 14579 with the 5′ RACE system (Invitrogen) using the GSP RsbY primers (Table 1) according to the manufacturer's instructions. During exponential growth, *rsbY* is transcribed from a promoter that is situated directly upstream from *rsbY* (Fig. 3B). This promoter is probably σ^A dependent, even though its sequence is somewhat different from the σ^A promoter consensus sequence from *B. subtilis*. These apparent mismatches may result in the low-level transcription of the *rsbY* gene under exponential growth conditions. With RNA that was isolated upon a heat shock, a smear was observed in the 5′ RACE reaction, which may indicate degradation of the mRNA. Upon the cloning and sequencing of an approximately 1.0-kb product, which appeared to be the largest fragment visible upon electrophoresis, a second promoter site was mapped. The 5′ RACE reaction product mapped to a position in *orf4*, the open reading frame 5′ of *rsbY*. However, the fact that no clear σ^B-dependent promoter site exists at this position leads to the conclusion that this is probably a degradation product of a transcript that originates from the σ^B-dependent promoter site upstream of *orf4*. The fact that transcripts originating from *orf4* contribute to *rsbY* expression is remarkable because directly downstream of *orf4*, a stem-loop structure with a calculated free energy of formation of −9.4 kcal/mol exists. Previous Northern analysis of the transcription of *orf4* did not show transcription proceeding beyond this structure (26). However, by use of a more sensitive PCR-based method, it was shown that transcription through this stem-loop structure occurs and that it contributes significantly to the upregulation of *rsbY* transcription under stress conditions.

A model for the regulation of σ^B activity in *B. cereus*. On the basis of the results obtained in this study, a model for the regulation of σ^B activity in *B. cereus* can be drawn up (Fig. 4). The main conclusions of this study are that RsbV and RsbW of *B. cereus* have functions identical to those of their homologs in other gram-positive bacteria and that the unique regulator RsbY is crucial for regulating σ^B activity in *B. cereus*. The

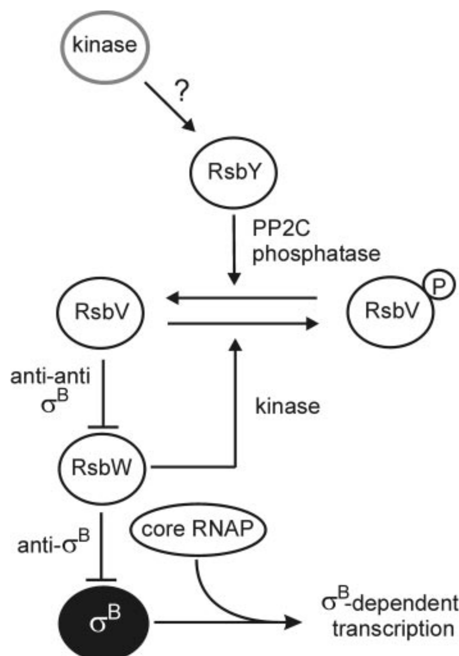

FIG. 4. Model for the regulation of σ^B activity in *B. cereus*. Under nonstress conditions, σ^B is kept in an inactive state by its anti-sigma factor, RsbW. This protein also functions as a kinase, which can phosphorylate (P) the anti-sigma factor antagonist, RsbV. When RsbV is dephosphorylated by the action of the RsbY phosphatase, RsbV can bind to RsbW. This leads to the release of σ^B from its complex with RsbW and, upon association of σ^B to core RNAP, to the transcription of σ^B-dependent genes. RsbY has an N-terminal CheY response regulator domain. This strongly suggests that an as-yet-unidentified kinase (shown in gray) can phosphorylate this domain, leading to the activation of the phosphatase.

N-terminal CheY response regulator domain of RsbY suggests that RsbY is activated through a mechanism which involves phosphorylation of a conserved aspartate residue in the CheY domain by an as yet unidentified kinase. The coupling of a CheY domain to a PP2C phosphatase domain in itself is not unique, but it is a rare occurrence, as most CheY domains are coupled to a C-terminal binding DNA output domain that activates or represses transcription of specific target genes (8). If the activation of σ^B in *B. cereus* is directly coupled with a sensor kinase, as in a classical two-component signal transduction cascade, this would mean a major difference with the σ^B activation pathway of *B. subtilis* in which more partner-switching units and large protein complexes form important parts of the sensing and signaling cascade (3, 14, 20). So it appears that even though *B. cereus* and *B. subtilis* are relatively closely related bacteria, two different sensing and signaling pathways leading to the activation of the σ^B have evolved. In this respect, it is also noteworthy that the *rsbY* gene is partially under transcriptional control of σ^B. In other gram-positive bacteria, the PP2C phosphatase that is responsible for the dephosphorylation of RsbV is constitutively transcribed. It remains to be determined if this positive feedback effect of σ^B levels on the expression of *rsbY* contributes to the process of σ^B activation in *B. cereus*.

The identification of mechanisms that regulate the activity of RsbY is currently under way in our laboratory and may provide important mechanistic clues on how stress conditions are sensed and signaled leading to the activation of σ^B in *B. cereus* and in closely related bacteria like *B. anthracis* and *B. thuringiensis*. This may provide indications on how the activation pathway of σ^B can be perturbed. A disruption of the stress response may lead to the sensitization of bacteria under stress conditions. This approach may then be used to counter the growth and survival of bacteria from the *B. cereus* group during food processing or pathogenesis.

REFERENCES

1. **Bone, E. J., and D. J. Ellar.** 1989. Transformation of *Bacillus thuringiensis* by electroporation. FEMS Microbiol. Lett. **49:**171–177.
2. **Bron, P. A., M. Marco, S. M. Hoffer, E. Van Mullekom, W. M. de Vos, and M. Kleerebezem.** 2004. Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters in vitro and in situ in the gastrointestinal tract. J. Bacteriol. **186:**7829–7835.
3. **Chen, C. C., R. J. Lewis, R. Harris, M. D. Yudkin, and O. Delumeau.** 2003. A supramolecular complex in the environmental stress signalling pathway of *Bacillus subtilis*. Mol. Microbiol. **49:**1657–1669.
4. **Delumeau, O., S. Dutta, M. Brigulla, G. Kuhnke, S. W. Hardwick, U. Volker, M. D. Yudkin, and R. J. Lewis.** 2004. Functional and structural characterization of RsbU, a stress signaling protein phosphatase 2C. J. Biol. Chem. **279:**40927–40937.
5. **de Vries, Y. P., L. M. Hornstra, W. M. de Vos, and T. Abee.** 2004. Growth and sporulation of *Bacillus cereus* ATCC 14579 under defined conditions: temporal expression of genes for key sigma factors. Appl. Environ. Microbiol. **70:**2514–2519.
6. **Drobniewski, F. A.** 1993. *Bacillus cereus* and related species. Clin. Microbiol. Rev. **6:**324–338.
7. **Dufour, A., and W. G. Haldenwang.** 1994. Interactions between a *Bacillus subtilis* anti-sigma factor (RsbW) and its antagonist (RsbV). J. Bacteriol. **176:**1813–1820.
8. **Galperin, M. Y.** 2004. Bacterial signal transduction network in a genomic perspective. Environ. Microbiol. **6:**552–567.
9. **Gaur, A. H., C. C. Patrick, J. A. McCullers, P. M. Flynn, T. A. Pearson, B. I. Razzouk, S. J. Thompson, and J. L. Shenep.** 2001. *Bacillus cereus* bacteremia and meningitis in immunocompromised children. Clin. Infect. Dis. **32:**1456–1462.
10. **Hecker, M., and U. Volker.** 2001. General stress response of *Bacillus subtilis* and other bacteria. Adv. Microb. Physiol. **44:**35–91.
11. **Helmann, J. D., and C. P. Moran, Jr.** 2002. RNA polymerase and sigma factors, p. 289–312. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, D.C.
12. **Hilliard, N. J., R. L. Schelonka, and K. B. Waites.** 2003. *Bacillus cereus* bacteremia in a preterm neonate. J. Clin. Microbiol. **41:**3441–3444.
13. **Jensen, G. B., B. M. Hansen, J. Eilenberg, and J. Mahillon.** 2003. The hidden lifestyles of *Bacillus cereus* and relatives. Environ. Microbiol. **5:**631–640.
14. **Kim, T. J., T. A. Gaidenko, and C. W. Price.** 2004. A multicomponent protein complex mediates environmental stress signaling in *Bacillus subtilis*. J. Mol. Biol. **341:**135–150.
15. **Kotiranta, A., K. Lounatmaa, and M. Haapasalo.** 2000. Epidemiology and pathogenesis of *Bacillus cereus* infections. Microbes Infect. **2:**189–198.
16. **Min, K. T., C. M. Hilditch, B. Diederich, J. Errington, and M. D. Yudkin.** 1993. σ^F, the first compartment-specific transcription factor of *B. subtilis*, is regulated by an anti-sigma factor that is also a protein kinase. Cell **74:**735–742.
17. **Miyazaki, E., J. M. Chen, C. Ko, and W. R. Bishai.** 1999. The *Staphylococcus aureus rsbW* (*orf159*) gene encodes an anti-sigma factor of SigB. J. Bacteriol. **181:**2846–2851.
18. **Namy, O., M. Mock, and A. Fouet.** 1999. Co-existence of *clpB* and *clpC* in the *Bacillaceae*. FEMS Microbiol. Lett. **173:**297–302.
19. **Pfaffl, M. W., G. W. Horgan, and L. Dempfle.** 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. **30:**e36.
20. **Price, C. W.** 2002. General stress response, p. 369–384. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, D.C.
21. **Rasko, D. A., M. R. Altherr, C. S. Han, and J. Ravel.** 2005. Genomics of the *Bacillus cereus* group of organisms. FEMS Microbiol. Rev. **29:**303–329.
22. **Schoeni, J. L., and A. C. Wong.** 2005. *Bacillus cereus* food poisoning and its toxins. J. Food Prot. **68:**636–648.
23. **Smith, K., and P. Youngman.** 1992. Use of a new integrational vector to investigate compartment-specific expression of the *Bacillus subtilis spoIIM* gene. Biochimie **74:**705–711.
24. **Stock, A. M., V. L. Robinson, and P. N. Goudreau.** 2000. Two-component signal transduction. Annu. Rev. Biochem. **69:**183–215.

25. **van Kranenburg, R., J. D. Marugg, I. I. van Swamm, N. J. Willem, and W. M. de Vos.** 1997. Molecular characterization of the plasmid-encoded *eps* gene cluster essential for exopolysaccharide biosynthesis in *Lactococcus lactis*. Mol. Microbiol. **24:**387–397.
26. **Van Schaik, W., M. H. Tempelaars, J. A. Wouters, W. M. De Vos, and T. Abee.** 2004. The alternative sigma factor σ^B of *Bacillus cereus*: response to stress and role in heat adaptation. J. Bacteriol. **186:**316–325.
27. **van Schaik, W., M. H. Zwietering, W. M. de Vos, and T. Abee.** 2004. Identification of σ^B-dependent genes in *Bacillus cereus* by proteome and in vitro transcription analysis. J. Bacteriol. **186:**4100–4109.
28. **van Schaik, W., and T. Abee.** 2005. The role of σ^B in the stress response of gram-positive bacteria—targets for food preservation and safety. Curr. Opin. Biotechnol. **16:**218–224.
29. **Vijay, K., M. S. Brody, E. Fredlund, and C. W. Price.** 2000. A PP2C phosphatase containing a PAS domain is required to convey signals of energy stress to the sigmaB transcription factor of *Bacillus subtilis*. Mol. Microbiol. **35:**180–188.
30. **Volz, K.** 1993. Structural conservation in the CheY superfamily. Biochemistry **32:**11741–11753.
31. **Yang, X., C. M. Kang, M. S. Brody, and C. W. Price.** 1996. Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. Genes Dev. **10:**2265–2275.