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Global Expression Profile of *Bacillus subtilis* Grown in the Presence of Sulfate or Methionine

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DNA arrays were used to investigate the global transcriptional profile of *Bacillus subtilis* grown in the presence of sulfate or methionine as the sole sulfur source. The expression of at least 56 genes differed significantly under the two growth conditions. The expression of several genes belonging to the S-box regulon was repressed in the presence of methionine probably in response to *S*-adenosylmethionine availability. The expression of genes encoding transporters (*yhcL*, *ytmJKLMN*, and *yxeMO*) was high when the sulfur source was methionine or taurine and reduced when it was sulfate.

The pathways involved in the synthesis of sulfur-containing amino acids and the ways in which these pathways are regulated differ in various groups of organisms. The *Bacillus subtilis* pathways leading in the production of cysteine and methionine from inorganic sulfate were recently characterized (Fig. 1). The *cysH* operon encodes a sulfate permease (CysP) and enzymes catalyzing the conversion of sulfate into sulfite (12, 13). Sulfite is then incorporated into cysteine by sulfite reductase and cysteine synthase (27). Two alternative methionine biosynthesis pathways exist in *B. subtilis* (1). The first one requires the sequential action of cystathionine γ-synthase and cystathionine β-lyase with the intermediary formation of cystathionine. The second pathway bypasses cystathionine via direct sulfhydrylation of *O*-acetylhomoserine to homocysteine.

In *Escherichia coli*, regulation of the cysteine and methionine biosynthesis genes involves two LysR-type activators, CysB and MetR, and a repressor, MetJ. Full expression of the cysteine biosynthesis pathway requires the positive regulator CysB, the inducer *N*-acetylserine, and a limited amount of reduced sulfur (8, 17). Repression of methionine biosynthesis in the presence of methionine is mediated by the MetJ repressor. The MetJ–*S*-adenosylmethionine (AdoMet) complex binds to the Met box sequences present in the promoter regions of the *met* genes (20). The MetR activator is required for expression of both the *metE* and *metH* genes, which encode the two methionine synthases (5, 28). In *B. subtilis*, several genes involved in methionine metabolism are regulated by the S-box antitermination mechanism. Grundy and Henkin (6) proposed a model in which the 5′ portion of the leader forms an anti-antiterminator structure that sequesters sequences required for the formation of an antiterminator, which, in turn, sequesters sequences required for the formation of the terminator. The only regulator known to be involved in the response to sulfur availability in *B. subtilis* is the LysR-like YtlI activator, which controls the expression of an operon containing an ABC transport system (3).

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Complete genome sequences and expression profiling experiments provide a powerful tool for global transcriptional pattern analysis and gene function identification. DNA arrays have already been successfully used to study the *B. subtilis* responses to various growth conditions (15, 18, 29, 31).

Comparison of global gene expression profiles of *B. subtilis* grown with sulfate or methionine as the sole sulfur source. The genomic expression profiles of *B. subtilis* 168 grown in minimal medium in the presence of 1 mM sulfate or 1 mM methionine as the sole sulfur source were analyzed by using DNA macroarrays. The cells grew at similar rates on both sulfur sources. Exponentially growing cells were collected and broken by shaking in a Fastprep apparatus (Bio 101). Total RNA was then extracted by Trizol (Gibco-BRL) treatment. cDNAs, which were generated by using 1 μg of total RNA and *B. subtilis* CDS-specific primers, were hybridized to panorama *B. subtilis* gene arrays (Sigma-GenoSys Biotechnologies). The intensity of each dot was quantified with XDOTSREADER software (Cose). To account for unspecific variations, six experiments were carried out with five independent RNA preparations and two different sets of DNA arrays. Comparison of the intensities of the signals from duplicate or independent hybridizations (Fig. 2) showed that the procedures for RNA extraction, reverse transcription, and hybridization were reproducible. The nonparametric Wilcoxon statistical test (STATVIEW 5.0.1 package) allowed us to identify 101 genes, the expression levels of which were significantly different when cells were grown with sulfate or methionine ($P \leq 0.05$). The genes whose expression levels differed by a factor ≥ 1.5 are listed in Table 1. Most of the genes identified during this work were further studied by using *lacZ* reporter fusions (Tables 1 and 2). The DNA array results were consistent with those of the *lacZ* fusion experiments, but the regulation factor was generally higher when *lacZ* fusions were used, suggesting that DNA arrays are less sensitive. We may therefore have missed some genes that are tightly regulated in response to sulfur availability.

Interestingly, most of the genes that were upregulated in the presence of methionine encode proteins containing no cysteine residues or one cysteine residue. This suggests that the growth of *B. subtilis* in the presence of methionine is associated to sulfur limitation conditions.

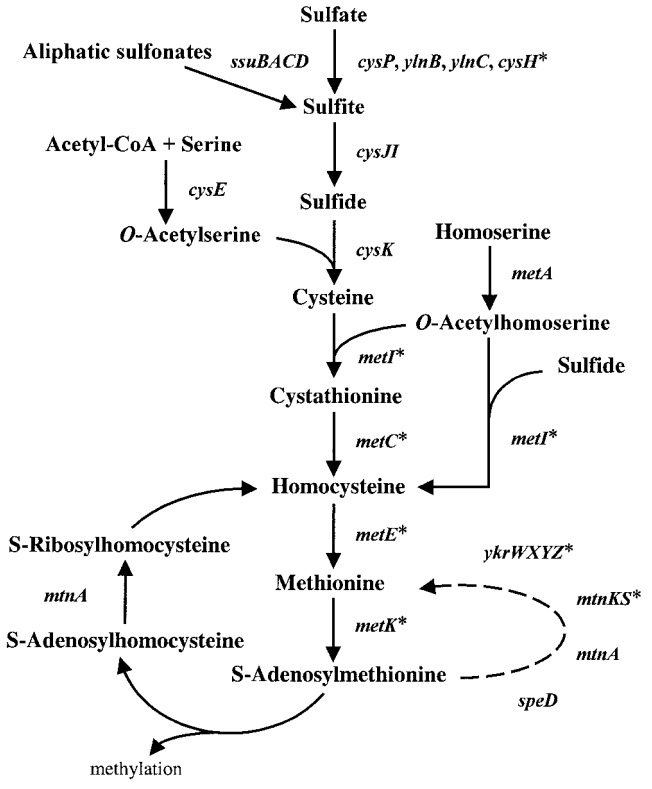

FIG. 1. Cysteine and methionine biosynthesis pathways in *B. subtilis*. The different genes encoding proteins involved in sulfate assimilation, cysteine biosynthesis, and methionine biosynthesis are indicated. The functions of some genes are indicated in Table 1. *ylnB*, ATP sulfurylase; *ylnC*, adenosine phosphosulfate kinase; *cysE*, serine acetyltransferase; *cysH*, 3′-phosphoadenosine 5′-phosphosulfate sulfotransferase; *metA*, homoserine acetyltransferase; *metI*, cystathionine γ-synthase/*O*-acetylhomoserine sulfhydrylase; *metC*, cystathionine β-lyase; *metK*, *S*-adenosylmethionine synthetase; *speD*, *S*-adenosylmethionine decarboxylase; *mtnK*, methylthioribose kinase. The arrow between *S*-adenosylmethionine and methionine represents several enzymatic steps. Asterisks indicate genes that contain an S box in the leader region.

Links with different metabolic pathways. The expression of several genes not directly related to sulfur metabolism was modified, depending on the sulfur source. Some genes involved in histidine biosynthesis (*hisB*, *hisD*, and *hisI*), pyrimidine biosynthesis (*pyrAA* and *pyrE*), one-carbon metabolism (*folD*), nitrogen metabolism (*glnR* and *nrgA*), NAD biosynthesis (*nadA* and *nadB*), and energy production (*atpI*) were upregulated in the presence of sulfate. The synthesis of proteins implicated in the metabolism of one-carbon units and in the biosynthesis of nucleotides has been shown to be repressed under sulfur-limiting conditions (3). This is consistent with our transcriptome data. As one-carbon units are used in the synthesis of methionine, these two metabolic pathways seem to be linked in *B. subtilis*, as observed in *E. coli* (14). Two genes, *accA* and *ydbM*, are related to lipid metabolism (Table 1). The *accA* transcript level was higher in the presence of sulfate, while the expression of the *ydbM* gene, which encodes a polypeptide with similarities to acyl coenzyme A (acyl-CoA) dehydrogenases, was increased in the presence of methionine (Table 1). The expression of *yciA*, *yciB*, and *yciC*, which are adjacent on the chromosome, was increased in the presence of sulfate when both transcriptome and *lacZ* fusions were used (Table 1). The YciC protein is an integral membrane protein that may be part of a low-affinity zinc transport system (4).

Interestingly, the expression of the *katA* gene, which encodes the major catalase in growing cells (10), increased in the presence of methionine. A transcriptional fusion of the *katA* promoter region (from position −261 to position +6 relative to the translational start site) and the promoterless *lacZ* gene was inserted at the *amyE* locus (Table 2). During the exponential growth phase, β-galactosidase activity was 216 U mg of protein^{-1} in methionine-grown cells, 77 U mg of protein^{-1} in sulfate-grown cells, 76 U mg of protein^{-1} in cysteine-grown cells, 82 U mg of protein^{-1} in taurine-grown cells, and 12 U mg of protein^{-1} in methionine-plus-sulfate-grown cells. Therefore, *katA* expression was regulated in response to sulfur availability. We tested the resistance of the wild-type strain to oxidative stress on minimal-medium plates by using a disk impregnated with 2 μl of a 30% hydrogen peroxide solution. The area of growth inhibition was 50% smaller in the presence of methionine than in the presence of sulfate. More catalase activity was also observed in cells grown in the presence of methionine than in cells grown in the presence of sulfate (data not shown). Thus, there is a correlation between (i) the sulfur source used for growth and (ii) catalase activity, which is consistent with the regulation of *katA* gene expression.

Genes of the S-box family. Of the 11 transcriptional units containing an S box in the leader region (6), 5 showed increased transcript levels in the presence of sulfate: *metE*, *yxjH*, *yxjG*, *yoaDCB*, and *ykrWXYZ* (Table 1). The *yoaDCB* operon encodes proteins similar to a phosphoglycerate dehydrogenase, a xylulokinase, and a transporter, respectively. The *ykrWXYZ* operon is involved in the scavenging of methylthioribose to generate methionine (16, 23). The amino acid sequences of YxjG and YxjH are 70% identical to each other. These polypeptides have moderate similarities to a C-terminal portion of the MetE protein (7), which is highly similar to the *E. coli* cobalamin-independent methionine synthase. A mutation in a methionine auxotrophic mutant (strain 1A607; Table 2) mapped to the same region as the *metE* gene. We determined that the transposon is inserted 102 bp downstream of the translational start site of the *metE* gene in this mutant. The *metE* mutant was unable to grow in the presence of sulfate, cysteine, cystathionine, or homocysteine as the sole sulfur source, but it grew similarly to the wild-type strain in the presence of methionine (data not shown). Thus, the *metE* gene appears to encode the unique methionine synthase in *B. subtilis*.

Surprisingly, six genes or operons containing an S-box motif were not downregulated in the presence of methionine in our transcriptome experiments. We further compared the data obtained with the DNA arrays with those obtained with *lacZ* fusions constructed either during the *B. subtilis* functional-analysis project or during this work (Tables 1 and 2). A fragment corresponding to the *metE* promoter region (nucleotides −369 to +56 relative to the translational start site) was inserted into pAC6 (25). This *metE′-lacZ* fusion was then integrated at the *amyE* locus of the wild-type strain. An internal fragment of the *yxjH* gene (nucleotides +7 to +359 relative to the translational start site) was cloned into the integrative

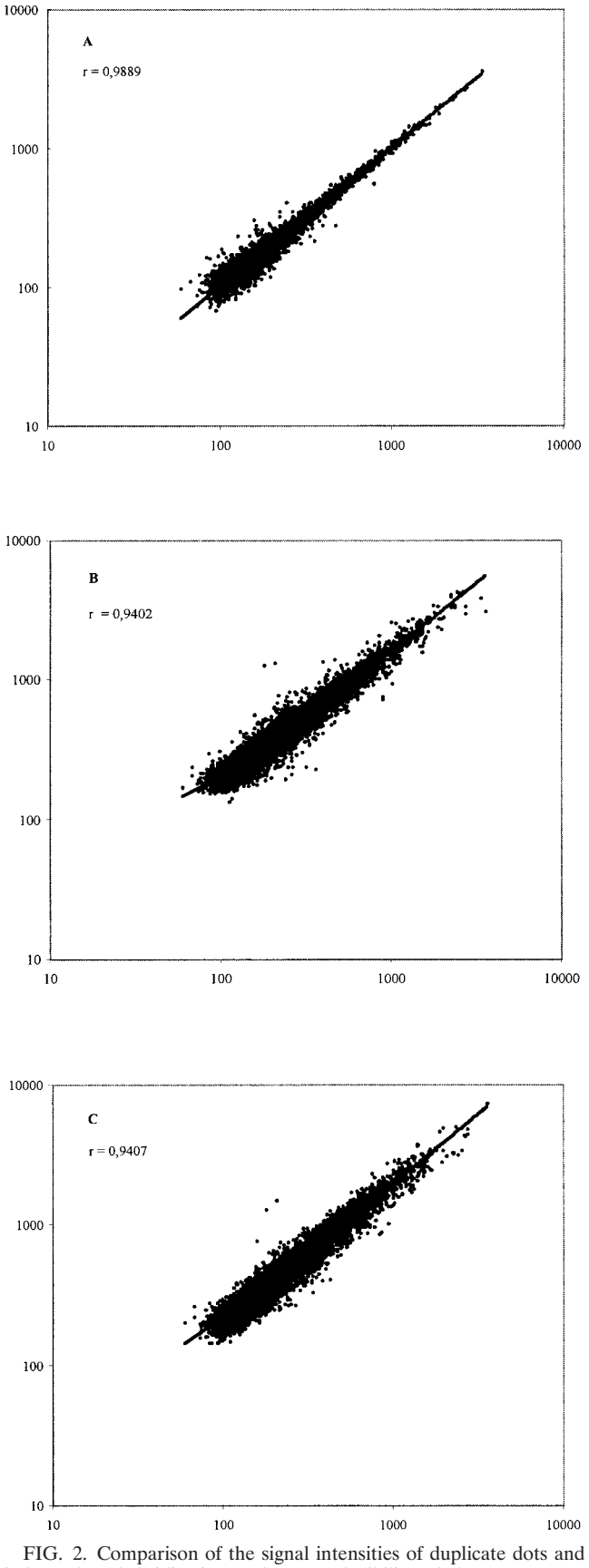

FIG. 2. Comparison of the signal intensities of duplicate dots and independent hybridizations. The reproducibility of the DNA array results obtained with 1 μg of total RNA extracted from *B. subtilis* 168 grown in minimal medium in the presence of sulfate as the sole sulfur source was assessed before subtraction of the background. In all experiments, the results showed a high degree of correlation (>0.94). (A) Comparison of signal intensities of pairs of dots corresponding to each gene. (B) Comparison of signal intensities of each gene in two independent hybridizations obtained from the same RNA sample but reverse transcribed and hybridized independently.(C) Comparison of signal intensities of each gene in two independent hybridizations of RNAs isolated from different cultures grown under the same conditions. Similar results were obtained with *B. subtilis* 168 grown in minimal medium in the presence of methionine as the sole sulfur source.

plasmid pDIA5307 (2). The *yxjH′-lacZ* fusion was integrated into the *yxjH* locus by a Campbell-type mechanism (Table 2).

The expression levels of the *lacZ* fusions with several representative genes from the S-box family were determined after growth in minimal medium plus sulfate or methionine (Table 1 and data not shown). All of the *met* genes implicated in methionine metabolism are members of the S-box family, with the exception of the *metA* gene, which is hardly regulated, depending on the sulfur source (S. Auger, unpublished results). The expression of the *metE* gene was 8.5-fold higher in the presence of sulfate than in the presence of methionine, whereas the expression of the *metI* and *metK* genes was only three- and twofold higher in the presence of sulfate, respectively (1, 30; this work). Thus, the focal point of transcriptional regulation of the methionine biosynthesis pathway corresponds to the *metE* gene.

All members of the S-box family are not tightly regulated by methionine under the growth conditions used. Indeed, the regulation factors observed with transcriptional fusions range from ≤2 for *mtnK* (*ykrT*), *yitJ*, and *cysH* to 15.5 for *yoaD* (11) (Table 1 and data not shown). This is in contrast to the data presented by Grundy and Henkin (6), who found a much higher factor of repression by methionine for the *yitJ* and *mtnK* genes under more drastic methionine starvation conditions. It is possible that different genes from the S-box regulon respond to different levels of methionine starvation (6) and/or are controlled by other regulatory signals (11, 24). A comparison of the $\Delta G°$ values of the terminator and antiterminator of all of the S-box structures did not establish a clear correlation between the stability of the terminator or antiterminator and the efficiency of methionine-dependent repression.

Role of AdoMet in the regulation of the genes controlled by the S box. To determine the mechanisms that are involved in the regulation of the S-box regulon, the role of methionine and AdoMet in this regulation has been further examined. Overexpression of AdoMet synthetase leads to methionine auxotrophy in *B. subtilis*, suggesting that AdoMet is a corepressor of methionine biosynthesis in this organism (30). The expression of the *metE′-lacZ*, *metI′-lacZ*, and *yxjH′-lacZ* transcriptional fusions in response to methionine limitation was then tested in the wild-type strain and the SA29 strain, which overproduces AdoMet synthetase. The *metE′-lacZ*, *metI′-lacZ*, and *yxjH′-lacZ* fusions were 3- to 20-fold less strongly expressed in the SA29 strain than in the wild type, both in the presence and in the absence of methionine (Table 3). The downregulation of *metE* and *metI* gene expression in strain SA29 can explain its methionine auxotrophy. The sixfold increase in AdoMet syn-

TABLE 1. Genes differentially expressed in *B. subtilis* 168 grown in the presence of methionine or sulfate as the sole sulfur source[a]

Gene	Function/similarity	Transcriptome analysis		β-Galactosidase activity (U mg of protein^{-1})[b]		
		Sulfate/methionine expression ratio	*P* value	Sulfate	Methionine	Sulfate/methionine expression ratio
Genes related to sulfur metabolism						
cysJ	Sulfite reductase flavoprotein	1.52	5×10^{-3}			
cysI	Sulfite reductase hemoprotein	1.94	1×10^{-2}			
cysK	*O*-Acetylserine sulfhydrylase	0.66	2×10^{-3}			
mtnA	Methylthioadenosine nucleosidase	0.66	3×10^{-2}			
yrhA	Similar to *O*-acetylserine sulfhydrylase	0.44	2×10^{-3}	28	106	0.26
yrhB	Similar to cystathionine γ-synthase	0.26	1×10^{-2}	51	206	0.25
S-box family						
metE	Probable cobalamin-independent methionine synthase	3.54	5×10^{-3}	110	13	8.5
ykrW	Similar to ribulose bisphosphate carboxylase	1.95	3×10^{-2}			
ykrY	Similar to proteins of unknown function	4.84	1×10^{-2}			
ykrZ	Similar to proteins of unknown function	1.50	2×10^{-3}	151	58	2.6
yoaD	Similar to phosphoglycerate dehydrogenase	2.00	2×10^{-3}	125	8	15.6
yoaC	Similar to xylulokinase	1.80	1×10^{-2}			
yoaB	Similar to permease	1.80	4×10^{-3}			
yxjG	Similar to YxjH	1.50	3×10^{-3}	115	27	4.3
yxjH	Similar to YxjG	2.37	1×10^{-2}	146	32	4.6
Transporters and associated genes						
ssuA	Aliphatic sulfonate ABC transporter (binding lipoprotein)	0.47	2×10^{-3}			
ssuC	Aliphatic sulfonate ABC transporter (permease)	0.52	5×10^{-3}			
ssuD	Aliphatic sulfonate monooxygenase	0.04	2×10^{-3}			
ygaN	Unknown	0.08	2×10^{-3}			
yhcL	Similar to sodium-glutamate symporter	0.29	2×10^{-3}	22	223	0.10
ytmI	Similar to proteins of unknown function	0.55	8×10^{-3}			
ytmJ	Similar to amino acid ABC transporter (binding protein)	0.19	5×10^{-3}			
ytmK	Similar to amino acid ABC transporter (binding protein)	0.17	5×10^{-3}			
ytmL	Similar to amino acid ABC transporter (permease)	0.12	2×10^{-3}			
ytmM	Similar to amino acid ABC transporter (permease)	0.55	2×10^{-3}			
ytmN	Similar to amino acid ABC transporter (ATP-binding protein)	0.31	2×10^{-3}			
ytmO	Similar to proteins of unknown function	0.16	2×10^{-3}			
ytnI	Unknown	0.35	6×10^{-3}			
ribR	Riboflavin kinase	0.29	3×10^{-3}			
ytnL	Similar to aminohydrolase	0.51	3×10^{-3}			
ytnM	Unknown	0.16	2×10^{-3}			
yxeK	Similar to monooxygenase	0.40	3×10^{-2}	55	292	0.19
yxeL	Similar to proteins of unknown function	0.66	1×10^{-2}			
yxeM	Similar to amino acid ABC transporter (binding protein)	0.50	2×10^{-2}	33	353	0.09
yxeO	Similar to amino acid ABC transporter (ATP-binding protein)	0.66	1×10^{-2}	15	150	0.10
yxeQ	Unknown	0.37	2×10^{-3}	20	311	0.06
Regulator, *ytlI*	Similar to transcriptional regulator (LysR family)	0.66	4×10^{-2}	12	216	0.05
Genes with other functions						
accA	Acetyl-CoA carboxylase (alpha subunit)	2.15	2×10^{-2}			
appB	Oligopeptide ABC transporter (permease)	0.52	5×10^{-2}			

Continued on following page

TABLE 1—*Continued*

Gene	Function/similarity	Transcriptome analysis		β-Galactosidase activity (U mg of protein^{-1})[b]		
		Sulfate/methionine expression ratio	*P* value	Sulfate	Methionine	Sulfate/methionine expression ratio
asnB	Asparagine synthetase	1.55	1×10^{-2}			
atpI	ATP synthase (subunit i)	1.80	4×10^{-3}			
folD	Probable methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase	1.90	1×10^{-2}			
glnR	Transcriptional repressor of the glutamine synthetase gene	1.90	3×10^{-2}			
hisB	Imidazoleglycerol-phosphate dehydratase	1.80	1×10^{-2}			
hisD	Histidinol dehydrogenase	1.76	8×10^{-3}			
hisI	Phosphoribosyl-AMP cyclohydrolase/ phosphoribosyl-ATP pyrophosphohydrolase	2.23	2×10^{-3}			
katA	Vegetative catalase 1	0.33	4×10^{-3}	77	216	0.35
nadA	Probable quinolinate synthetase	1.95	3×10^{-3}			
nadC	Probable nicotinate-nucleotide pyrophosphorylase	1.40	2×10^{-3}			
nadB	L-Aspartate oxidase	1.69	3×10^{-3}			
nrgA	Ammonium transporter	1.70	1×10^{-2}			
pyrAA	Carbamoyl-phosphate synthetase (glutaminase subunit)	2.12	8×10^{-3}			
pyrE	Orotate phosphoribosyltransferase	2.93	6×10^{-3}			
yciA	Similar to proteins of unknown function	3.09	1×10^{-2}			
yciB	Similar to proteins of unknown function	1.80	6×10^{-3}	3	0.8	3.70
yciC	Involved in low-affinity zinc transport system	2.44	1×10^{-2}	161	55	3.00
ydbM	Similar to butyryl-CoA dehydrogenase	0.30	3×10^{-3}	7	32	0.21

[a] The results obtained are representative of at least six hybridizations from five independent RNA extractions. The expression intensities of 3,830 genes were above the background level. Only genes with a *P* value of ≤0.05 in a Wilcoxon test and whose expression differed by a factor of ≥1.5 between the two growth conditions are listed. Genes are grouped according to function. Column two indicates protein function according to the SubtiList database (http://genolist.pasteur.fr/SubtiList/).

[b] Cells were grown in minimal medium (6 mM K_2HPO_4, 4.4 mM KH_2PO_4, 0.3 mM trisodium citrate, 5 mM $MgCl_2$, 0.5% glucose, 50 mg of L- tryptophan liter^{-1}, 22 mg of ferric ammonium citrate liter^{-1}, 0.1% L-glutamine) containing 1 mM sulfate or 1 mM methionine as the sulfur source. β-Galactosidase activities were determined in extracts prepared from exponentially growing cells. The values shown are averages from at least three independent experiments.

thetase activity observed in this strain (30) probably decreases the cellular content of methionine and increases the cellular concentration of AdoMet. Methionine depletion was expected to derepress the transcription of the S-box regulon (1, 6). In contrast, we demonstrated that overproduction of AdoMet synthetase led to lower levels of transcription of *yxjH* and of some *met* genes, strongly suggesting that this effect is due to an increase in the concentration of AdoMet.

In the wild-type background, the *metE′-lacZ* and *metI′-lacZ* fusions were induced 19- and 5-fold 2 h after the removal of methionine (Table 3). In strain SA29, these fusions were induced 16- and 3.5-fold 2 h after methionine was removed, respectively. Overproduction of AdoMet synthetase may not be sufficient to completely repress the expression of the *met* gene in the absence of methionine. Under conditions of drastic methionine starvation, the AdoMet concentration of the cell probably remains low even when AdoMet synthetase is overproduced.

We obtained a *metE′-lacZ* fusion containing a substitution in the S box. During the cloning of the *metE* promoter fragment into pAC6, we isolated a plasmid that conferred high-level constitutive expression of the *metE′-lacZ* fusion on *B. subtilis* 168 (Table 3). Sequencing revealed the presence of a G→A substitution at position −273 relative to the translational start site of *metE*. This mutation, located in the region between 5′-half helix 1 and 5′-half helix 2 of the S box, alters a highly conserved residue but does not disrupt the structural features of the S-box region (6). Thus, it may lead to loss of binding of a negative regulatory factor rather than destabilization of the RNA structure. The AdoMet synthetase overproduction in strain SA29 did not modify the expression of the constitutive p(G_{-273}→A)*metE′-lacZ* fusion, while the expression of the wild-type *metE′-lacZ* fusion was decreased 4- to 10-fold in this strain (Table 3). Thus, it appears that expression of the *metE* gene in response to AdoMet synthetase overproduction is mediated via the S box. We therefore propose that AdoMet could act as a corepressor to modulate the binding of a negative regulatory factor to the S-box region. However, we cannot exclude the possibility that AdoMet has an indirect effect. Further information is needed about the nature of the repressor and its ability to interact with the regulatory S-box region to elucidate how AdoMet functions in this system.

The YtlI regulator. In addition to the S-box transcription antitermination system, the YtlI activator was recently shown to be involved in the control of transcription in response to sulfur availability. The *ytlI* gene is transcribed divergently from the *ytmI* operon. The YtlI protein controls the expression of this operon (3). Interestingly, DNA arrays showed that the expression of the *ytlI* gene was 1.5-fold higher in methionine than in sulfate (Table 1). To confirm this result, a transcriptional *ytlI′-lacZ* fusion was constructed by inserting a 213-bp DNA fragment containing the promoter region of the *ytlI* gene

TABLE 2. Bacterial strains used in this study[a]

Strain	Genotype	Source or reference
168	*trpC2*	Laboratory stock
BSIP1142	*trpC2 amyE*::p*metI′-lacZ cat*	1
BSIP1159	*trpC2 amyE*::p*metK′-lacZ cat*	21
BSIP1214	*trpC2 ytlI*::*aphA3*	3
BSIP1215	*trpC2 amyE*::p*ytlI′-lacZ cat*	pDIA5575→168
BSIP1306	*trpC2 amyE*::p*metE′-lacZ cat*	pDIA5626→168
BSIP1307	*trpC2 amyE*::p(G_{-273}→A) *metE′-lacZ cat*	pDIA5627→168
BSIP1324	*trpC2 amyE*::p*katA′-lacZ cat*	pDIA5624→168
BSIP1379	*trpC2 metK1 sacB*::ϕP_{veg}-*metK*$^+$ *amyE*::p*metI′-lacZ cat*	pDIA5510→SA29
BSIP1382	*trpC2 metK1 sacB*::ϕP_{veg}-*metK*$^+$ *amyE*::p*metE′-lacZ cat*	pDIA5626→SA29
BSIP1385	*trpC2 metK1 sacB*::ϕP_{veg}-*metK*$^+$ *amyE*::p(G_{-273}→A) *metE′-lacZ cat*	pDIA5627→SA29
BSIP1386	*trpC2 yxjH′*::*lacZ cat*	pDIA5628→168
BSIP1387	*trpC2 metK1 sacB*::ϕP_{veg}-*metK*$^+$ *yxjH′*::*lacZ cat*	pDIA5628→SA29
BFS1605[b]	*trpC2 yhcL′*::*lacZ erm*	S. Bron
BFS1850[b]	*trpC2 ykrT′*::*lacZ erm*	K. M. Devine
BFS1853[b]	*trpC2 ykrZ′*::*lacZ erm*	K. M. Devine
BFS2048[b]	*trpC2 yoaD′*::*lacZ erm*	W. Schumann
BFS2062[b]	*trpC2 yrhB′*::*lacZ erm*	W. Schumann
BFS2063[b]	*trpC2 yrhA′*::*lacZ erm*	W. Schumann
BFS3022[b]	*trpC2 yitJ′*::*lacZ erm*	S. J. Seror
BFS4035[c]	*trpC2 yxjG′*::*lacZ erm*	Y. Fujita
BFS4069[c]	*trpC2 yxeQ′*::*lacZ erm*	Y. Fujita
BFS4071[c]	*trpC2 yxeO′*::*lacZ erm*	Y. Fujita
BFS4073[c]	*trpC2 yxeM′*::*lacZ erm*	Y. Fujita
BFS4075[c]	*trpC2 yxeK′*::*lacZ erm*	Y. Fujita
BFS4363[c]	*trpC2 yciB′*::*lacZ erm*	K. Yamane
BFS4364[c]	*trpC2 yciC′*::*lacZ erm*	K. Yamane
BFS4451[c]	*trpC2 ydbM′*::*lacZ erm*	F. Kawamura
SA29	62378 with *sacB*::ϕP_{veg}-*metK*$^+$ (formerly *metE*$^+$)	30
1A607[d]	*trpC2 metC85*::Tn*917*	BGSC[e]

[a] Arrows indicate construction by transformation. *cat* is the pC194 chloramphenicol acetyl-transferase gene, *aphA3* is the *Enterococcus faecalis* kanamycin resistance gene, and *erm* is an erythromycin resistance gene.

[b] Strain constructed as part of the EC project for the functional characterization of the *B. subtilis* genome (http://locus.jouy.inra.fr/cgi-bin/genmic/madbase/progs/madbase.operl).

[c] Strain constructed as part of the Japanese project for functional characterization of the *B. subtilis* genome (http://bacillus.genome.ad.jp).

[d] In the *metC85*::Tn*917* mutant, the transposon is inserted in the *metE* (formerly *metC*) gene.

[e] BGSC, *Bacillus* Genetic Stock Center.

(nucleotides −209 to +4 relative to the translational start site) into pAC6 (25). The resulting *ytlI′-lacZ* fusion was integrated at the *amyE* locus of a wild-type strain (Table 2). β-Galactosidase activity was tested after growth in the presence of various sulfur sources. The level of expression of the *ytlI* gene was high in the presence of methionine (215 U mg of protein^{-1}) or taurine (80 U mg of protein^{-1}) and 8- to 30-fold lower in the presence of sulfate (12 U mg of protein^{-1}) or cysteine (7 U mg of protein^{-1}). This suggests that a regulatory cascade controls the expression of the *ytmI* operon.

Regulation of genes involved in the uptake and metabolism of sulfur compounds. Other genes involved in the metabolism of sulfur-containing amino acids were differently expressed in DNA array experiments (Table 1). The expression of the genes ensuring the last two steps of cysteine biosynthesis (Fig. 1) was regulated in the opposite way. Whereas the level of expression of the *cysJI* operon was higher in the presence of sulfate than in the presence of methionine, the expression of the *cysK* gene was downregulated in sulfate-grown cells (Table 1). The *B. subtilis* genome contains two other putative cysteine synthases encoded by the *yrhA* and *ytkP* genes (9). As observed for *cysK*, the amount of *yrhA* mRNA was lower in the presence of sulfate whereas no difference in expression was detected for the *ytkP* gene. Two other genes whose expression was increased in the presence of methionine were *yrhB* and *mtnA*, which encode a putative cystathionine γ-synthase and a methylthioadenosine nucleosidase (22), respectively. The role of YrhA, YrhB, and YtkP polypeptides remains to be determined, but YrhA and YrhB are probably involved in the recycling of methionine to cysteine (7) (Auger, unpublished).

Several sulfur compounds including methionine, homocysteine, cysteine, sulfate, sulfite, and thiosulfate are taken up by *B. subtilis*. The expression of the *ssu* operon, which encodes an ABC permease system for aliphatic sulfonates and an oxygenase, is derepressed in the presence of methionine (Table 1) (3, 26). Several genes encoding transporters (*yhcL*, *ytmJ*, *ytmK*, *ytmL*, *ytmM*, *ytmN*, *yxeM*, and *yxeO*) are also expressed more strongly in the presence of methionine than in the presence of sulfate (Table 1). The YtmJKLMN, YxeMNO, and YhcL polypeptides are good candidates for the transport of sulfur-containing compounds. The *yhcL* gene encodes a membrane protein that shows homology with the sodium-dicarboxylate symporter family. The YtmJKLMN and YxeMNO proteins exhibit substantial sequence similarities to ABC transporters specific for polar amino acids (19). The YtmJKLMN permease belongs to the large *ytmIJKLMNO-ytnIJ-ribR-ytnLM* operon (3). The *yxeMNO* genes are located close to the *yxeK*, *yxeL*, and *yxeQ* genes, which were also found to be upregulated in the presence of methionine (Table 1). The transcriptional fusions between *lacZ* and all of the genes from *yxeK* to *yxeR* were expressed more strongly in the presence of methionine than in the presence of sulfate. These results strongly suggest the existence of a large operon stretching from *yxeK* to *yxeR*.

The transcription level of the *ytmI* gene is high in the presence of methionine, taurine, or glutathione and very low in the presence of sulfate, thiosulfate, and cysteine (3). To determine whether the *yhcL* gene and the *yxeK* operon are regulated together with the *ytmI* operon, the expression of *yhcL′-lacZ* and *yxeK′-lacZ* transcriptional fusions was measured after growth in the presence of taurine, methionine, or sulfate. The β-galactosidase activities of these fusions were 10- and 8-fold higher in the presence of methionine than in the presence of sulfate, respectively (Table 1). These results confirm the data obtained by transcriptome analysis. The expression of these fusions was also high in the presence of taurine (data not shown). The expression of the *yhcL* gene and that of the *yxeK* and *ytmI* operons appear, therefore, to be coordinately regulated. The *ssu* operon is expressed under the same conditions (26). However, the expression level of the *ytmI* and *ssu* operons is about 200- to 1,000-fold higher in the presence of methionine than in the presence of sulfate while the *yxeK* and *yhcL* genes are only 5- to 10-fold more strongly expressed in the presence of methionine (3, 26) (this study). It is noteworthy that the *ssu* operon is regulated at two different levels (initiation and termination of transcription) (26), whereas a cascade of regulation modulates the expression of the *ytmI* operon. Whether these genes are all controlled by one or several reg-

TABLE 3. Effect of AdoMet synthetase overproduction on expression of *metE′-lacZ, metI′-lacZ,* and *yxjH′*::*lacZ* transcriptional fusions[a]

Strain	Relevant genotype	β-Galactosidase activity (U mg of protein^{-1})				
		t_0	t_1 + methionine	t_1 − methionine	t_2 + methionine	t_2 − methionine
BSIP1306	*amyE*::p*metE′-lacZ*	10	9.5	121	9	172
BSIP1382	*metK1 sacB*::ϕP_{veg}-*metK*$^+$ *amyE*::p*metE′-lacZ*	2	2.5	11	2	32
BSIP1307	*amyE*::p(G_{-273}→A) *metE′-lacZ*	710	721	634	1,185	905
BFS1385	*metK1 sacB*::ϕP_{veg}-*metK*$^+$ *amyE*::p(G_{-273}→A) *metE′-lacZ*	661	734	633	961	993
BSIP1142	*amyE*::p*metI′-lacZ*	91	89	452	107	510
BSIP1379	*metK1 sacB*::ϕP_{veg}-*metK*$^+$ *amyE*::p*metI′-lacZ*	22	15	23	16	56
BSIP1386	*yxjH′*::*lacZ*	30	26	139	30	149
BSIP1387	*metK1 sacB*::ϕP_{veg}-*metK*$^+$ *yxjH′*::*lacZ*	10	10.5	35	6	29

[a] Cells were grown in minimal medium (6 mM K_2HPO_4, 4.4 mM KH_2PO_4, 0.3 mM trisodium citrate, 5 mM $MgCl_2$, 0.5% glucose, 50 mg of L-tryptophan liter^{-1}, 22 mg of ferric ammonium citrate liter^{-1}, 0.1% L-glutamine) supplemented with 1 mM methionine. The cells were collected by centrifugation and resuspended in minimal medium in the presence or absence of methionine. Samples were taken before resuspension (t_0), 1 h after resuspension (t_1), and 2 h after resuspension (t_2). To overproduce AdoMet synthetase in the SA29 strain, the *metK* gene was placed under the control of a strong constitutive promoter, P_{veg} (30).

ulatory systems remains to be determined. However, we failed to identify a target sequence common to the promoter regions of *ssuB, ytmI*, *ytlI, yxeK*, and *yhcL*. The identification of other regulators and the systematic characterization of *cis*-acting targets in the promoter regions of these genes will help to define the sulfur regulatory network in *B. subtilis*.

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