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NOTES

Transcriptome and Proteome Analysis of *Bacillus subtilis* Gene Expression Modulated by Amino Acid Availability

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A comprehensive study of *Bacillus subtilis* gene expression patterns in response to amino acid availability was performed by means of proteomics and transcriptomics. The methods of two-dimensional protein gel electrophoresis and DNA macroarray technology were combined to analyze cells exponentially grown in minimal medium with and without 0.2% Casamino Acids (CAA). This approach revealed about 120 genes predominantly involved in amino acid biosynthesis, sporulation, and competence, which were downregulated in CAA-containing medium. Determination of sporulation frequencies confirmed the physiological relevance of the expression data.

The soil bacterium *Bacillus subtilis* is capable of synthesizing all proteinogenic amino acids. Besides their function as building blocks for cellular proteins, amino acids represent precursors in the biosynthesis of nucleotides and other cellular components such as cell wall polymers. Amino acid biosynthetic pathways are regulated on the level of enzyme activity as well as on the level of enzyme synthesis to ensure cellular adaptation to various requirements for amino acids under different growth conditions.

The expression of many amino acid biosynthetic genes in *B. subtilis* is controlled by transcription antitermination mechanisms: the *ilv-leu* operon (15, 27), the *cysES* operon (14), and the *proBA* operon (3) belong to the T-box family, which includes most of the aminoacyl-tRNA synthetase genes (17, 31). These genes are regulated by tRNA-mediated antitermination in response to starvation for a particular amino acid (16). The genes of the S-box regulon are controlled by a transcription antitermination system in response to methionine availability (18). The S-box-specific leader region elements were identified in 11 transcriptional units in the *B. subtilis* genome, whereby the majority of the 26 gene products fulfills functions in sulfate assimilation and methionine biosynthesis (18, 26, 29). Regulation of tryptophan biosynthetic genes by transcription attenuation and translation control mechanisms is mediated by the RNA binding protein TRAP as well as by a T-box-dependent regulatory mechanism (reviewed in reference 1). Expression of the *lysC* gene encoding the lysine feedback-controlled aspartokinase II (30) is regulated by lysine availability via an antitermination system, too (20). In *B. subtilis*, the arginine biosynthetic operons are repressed by the AhrC regulatory protein, which is activated in the presence arginine (6, 37).

In this study we report on the gene expression profile of *B. subtilis* exponentially grown in minimal medium with and without 0.2% Casamino Acids (CAA), thereby providing an insight into the response of *B. subtilis* to different amino acid availabilities. The genes which were differentially expressed under the two growth conditions included those for amino acid biosynthesis, sporulation, and competence development.

Characterization of the proteome under conditions of different amino acid availability. The *B. subtilis* 168 strain was cultivated aerobically at 37°C in a minimal medium (pH 7.5) containing 50 mM Tris, 8 mM $MgSO_4$, 13 mM KCl, 18 mM NaCl, 0.6 mM KH_2PO_4, 2 mM $CaCl_2$, 0.001 mM $FeSO_4$, 0.01 mM $MnSO_4$, 10 mM glutamine, 0.2% (wt/vol) glucose, and 0.8 mM tryptophan. Cells were grown in the presence or absence of 0.2% CAA (vitamin free; Difco, Detroit, Mich.) and harvested in the exponential growth phase after reaching an optical density at 500 nm of 0.5. Compared to the cultures in minimal medium (G = 45 min), shorter generation times (G = 25 min) were observed for the cultures in CAA-containing minimal medium (see Fig. 2). Preparation of protein extracts and two-dimensional protein gel electrophoresis were performed as previously described (2). About 65 protein spots present on the control gel in the pH range of 4 to 7 decreased in intensity or were completely absent when the medium was supplemented with CAA (Fig. 1A). In addition, narrow pH gradient gels were utilized in the pH range of 4.5 to 5.5, which allows for a better resolution of the most overcrowded region of the pH 4 to 7 gels (Fig. 1B). Altogether, 58 protein spots representing 50 different proteins downregulated by CAA (Table 1) were identified by means of matrix-assisted laser desorption ionization-time of flight mass spectrometry as previously described (2).

Of these 50 proteins, 35 represented amino acid biosynthetic

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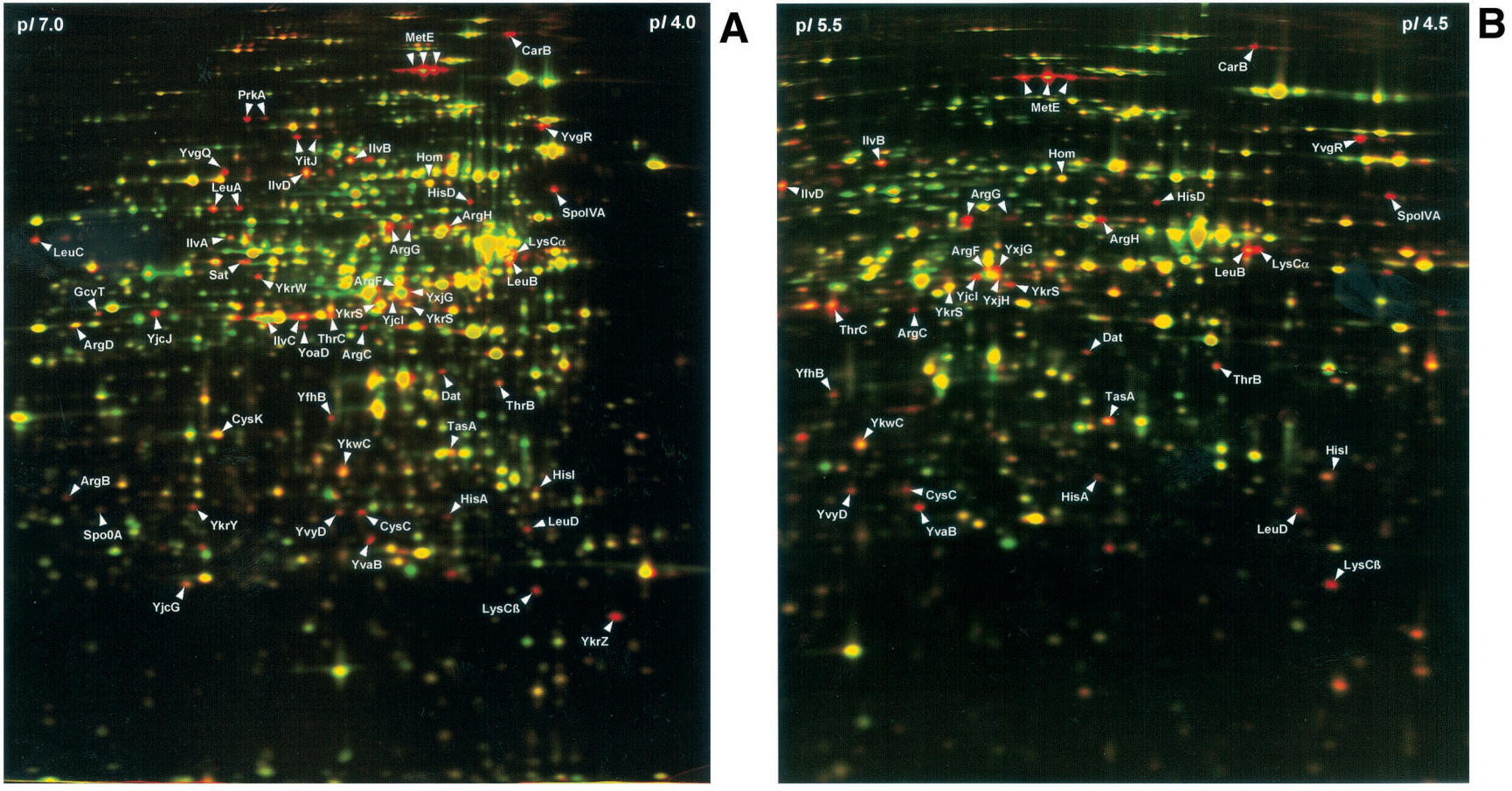

FIG. 1. Dual-channel image analysis of two-dimensional protein patterns of *B. subtilis* 168 exponentially grown in minimal medium and minimal medium supplemented with 0.2% CAA. Cytosolic protein extracts were separated by two-dimensional gel electrophoresis in the pH gradient of 4 to 7 (A) and in the pH gradient of 4.5 to 5.5 (B). Dual-channel images of the silver-stained gels were created by computer-aided transformation of the gel images by using the software DECODON Delta2D (DECODON GmbH, Greifswald, Germany). Red spots represent proteins whose synthesis was decreased in the presence of CAA.

TABLE 1. Genes with significantly higher expression during exponential growth of *B. subtilis* in minimal medium without CAA as revealed by transcriptome and proteome analyses[a]

Gene and category	Function	Induction (expt 1, expt 2)	Transcriptional organization
Amino acid biosynthesis			
argC	*N*-acetylglutamate gamma-semialdehyde dehydrogenase	6.0, 3.8	***argC-argJ-argB-argD-carA-carB-argF***
argJ	Ornithine acetyltransferase; amino acid acetyltransferase	20.5, 11.6	***argC-argJ-argB-argD-carA-carB-argF***
argB	*N*-acetylglutamate 5-phosphotransferase	29.3, 34.2	***argC-argJ-argB-argD-carA-carB-argF***
argD	*N*-acetylornithine aminotransferase	4.7, 8.6	***argC-argJ-argB-argD-carA-carB-argF***
carA	Carbamoyl-phosphate transferase (subunit A)	30.0, 16.2	***argC-argJ-argB-argD-carA-carB-argF***
carB	Carbamoyl-phosphate transferase (subunit B)	4.6, 5.1	***argC-argJ-argB-argD-carA-carB-argF***
argF	Ornithine carbamyoltransferase	5.7, 9.6	***argC-argJ-argB-argD-carA-carB-argF***
argG	Argininosuccinate synthase	17.3, 23.9	***argG-argH****-ytzD*
argH	Argininosuccinate lyase	21.4, 27.5	***argG-argH****-ytzD*
ytzD	Unknown function	—, —	***argG-argH****-ytzD*
cysH	Phosphoadenosine phosphosulfate sulfotransferase	3.3, 3.8	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
cysP	Sulfate permease	5.5, 3.3	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
sat	Sulfate adenylyltransferase	4.7, 4.6	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
cysC	Adenylylsulfate kinase	2.7, 4.7	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
ylnD	Similar to uroporphyrine-III C-methyltransferase	5.7, 5.2	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
ylnE	Unknown function	4.3, 3.1	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
ylnF	Similar to uroporphyrine-III C-methyltransferase	4.0, 3.6	***cysH-cysP-sat-cysC-ylnD-ylnE-ylnF***
cysK	Cysteine synthase A	2.0, 2.2	***cysK***
hisZ	Histidyl-tRNA synthetase	—, —	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisG	ATP phosphoribosyltransferase	—, 1.1	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisD	Histidinol dehydrogenase	4.5, 7.2	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisB	Imidazoleglycerol-phosphate dehydratase	5.7, 5.6	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisH	Amidotransferase	5.2, 5.1	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisA	Phosphoribosylformimino-5-aminoimidazole carboxamideribotide isomerase	5.5, 7.3	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisF	Cyclase-like protein (synthesis of imidazole glycerol phosphate)	4.2, 6.6	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hisI	Phosphoribosyl-ATP pyrophosphohydrolase; phosphribosyl-AMP cyclohydrolase	8.5, 10.4	*hisZ-hisG****-hisD-hisB-hisH-hisA-hisF-hisI***
hom	Homoserine dehydrogenase	2.2, 2.1	***hom-thrC-thrB***
thrC	Threonine synthase	2.0, 2.2	***hom-thrC-thrB***
thrB	Homoserine kinase	3.2, 3.5	***hom-thrC-thrB***
ilyA	Threonine dehydratase	3.9, 3.6	***ilvA****-ypmP*
ypmP	Unknown function	—, —	***ilvA****-ypmP*
ilvB	Acetolactate synthase (large subunit)	7.1, 7.9	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
ilvH	Acetolactate synthase (small subunit)	11.0, 7.0	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
ilvC	Ketol-acid reductoisomerase	9.9, 10.1	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
leuA	2-Isopropylmalate synthase	9.5, 9.7	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
leuB	3-Isopropylmalate dehydrogenase	9.6, 10.6	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
leuC	3-Isopropylmalate dehydratase (large subunit)	10.0, 10.9	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
leuD	3-Isopropylmalate dehydratase (small subunit)	7.0, 10.5	***ilvB-ilvH-ilvC-leuA-leuB-leuC-leuD***
ilvD	Dihydroxyacid dehydratase	4.6, 4.4	***ilvD***
lysC	Aspartokinase II (alpha and beta subunit)	8.5, 13.4	***lysC***
metE	Cobalamin-independent methionine synthase	56.8, 59.9	***metC***
ybgE	Similar to branched chain amino acid aminotransferase	3.0, 4.1	***ybgE***
yitJ	Similar to methionine synthase	4.7, —	***yitJ***
yjcI	Similar to cystathionine gamma-synthase	9.9, 6.9	***yjcI-yjcJ***
yjcJ	Similar to cystathionine beta-lyase	14.0, 9.9	***yjcI-yjcJ***
yoaD	Similar to phosphoglycerate dehydrogenase	3.1, —	***yoaD-yoaC-yoaB***
yoaC	Similar to xylulokinase	2.5, 3.2	***yoaD-yoaC-yoaB***
yoaB	Unknown function	14.5, 9.2	***yoaD-yoaC-yoaB***
yvgR	Similar to sulfite reductase	2.1, 2.9	***yvgR-yvgQ***
yvgQ	Similar to sulfite reductase	5.0, 8.6	***yvgR-yvgQ***
yxjG	Similar to methionine synthase	10.5, 7.6	***yxjG***
yxjH	Similar to methionine synthase	—, —	*yxjH*
Competence			
comER	Nonessential gene for competence	3.2, 3.4	***comER***
comGA	Required for exogenous DNA binding	2.0, 5.3	***comGA-comGB-comGC-comGD-****comGE-comGF-comGG*
comGB	Required for exogenous DNA binding	2.2, 4.2	***comGA-comGB-comGC-comGD-****comGE-comGF-comGG*

Continued on following page

TABLE 1—*Continued*

Gene and category	Function	Induction (expt 1, expt 2)	Transcriptional organization
comGC	Required for exogenous DNA binding	3.2, 7.1	***comGA-comGB-comGC-comGD-****comGE-****comGF-comGG***
comGD	Required for exogenous DNA binding	3.0, 5.6	***comGA-comGB-comGC-comGD-****comGE-****comGF-comGG***
comGE	Required for exogenous DNA binding	—, —	***comGA-comGB-comGC-comGD-****comGE-****comGF-comGG***
comGF	Required for exogenous DNA binding	4.2, 4.2	***comGA-comGB-comGC-comGD-****comGE-****comGF-comGG***
comGG	Required for exogenous DNA binding	2.4, 4.3	***comGA-comGB-comGC-comGD-****comGE-****comGF-comGG***
comK	Competence transcription factor	3.1, 4.8	***comK***
Transition state functions and sporulation			
appD	Oligopeptide ABC transporter (ATP-binding protein)	—, —	*appD-appF-****appA-appB-****appC*
appF	Oligopeptide ABC transporter (ATP-binding protein)	0.7, 0.9	*appD-appF-****appA-appB-****appC*
appA	Oligopeptide ABC transporter (peptide-binding protein)	21.9, 22.5	*appD-appF-****appA-appB-****appC*
appB	Oligopeptide ABC transporter (permease)	—, 5.7	*appD-appF-****appA-appB-****appC*
appC	Oligopeptide ABC transporter (permease)	—, —	*appD-appF-****appA-appB-****appC*
cotE	Spore coat protein	13.5, 5.8	***cotE***
cotV	Spore coat protein	6.1, —	***cotV-cotW-cotX***
cotW	Spore coat protein	3.5, 3.5	***cotV-cotW-cotX***
cotX	Spore coat protein	7.5, —	***cotV-cotW-cotX***
dacF	Penicillin binding protein; required for spore cortex synthesis	4.6, 2.4	***dacF-****spoIIAA-spoIIAB-****sigF***
spoIIAA	Anti-anti-sigma factor (antagonist of SpoIIAB)	0.9, 0.9	***dacF-****spoIIAA-spoIIAB-****sigF***
spoIIAB	Anti-sigma factor (antagonist of sigma F); serine kinase	—, —	***dacF-****spoIIAA-spoIIAB-****sigF***
sigF	RNA polymerase sporulation-specific sigma factor	5.9, 5.3	***dacF-****spoIIAA-spoIIAB-****sigF***
gerM	Germination (cortex hydrolysis) and sporulation (putative role in peptidoglycan synthesis)	3.3, 3.8	***gerM***
prkA	Serine protein kinase	2.5, 2.5	***prkA***
qcrA	Menaquinol:cytochrome *c* oxidoreductase (iron-sulfur subunit)	4.1, 4.0	***qcrA-****qcrB-****qcrC***
qcrB	Menaquinol:cytochrome *c* oxidoreductase (cytochrome *b* subunit)	—, —	***qcrA-****qcrB-****qcrC***
qcrC	Menaquinol:cytochrome *c* oxidoreductase (cytochrome *c* subunit)	3.1, 5.3	***qcrA-****qcrB-****qcrC***
rapA	Aspartyl phosphate phosphatase	3.2, 3.4	***rapA-phrA***
phrA	Inhibitor of the activity of phosphatase RapA	4.1, 1.8	***rapA-phrA***
rsfA	Probable transcriptional regulator of sigma F-dependent genes	4.5, 4.2	***rsfA***
sp0A	Two-component response regulator	2.9, 2.5	***spo0A***
spoIIB	Required for endospore development	6.4, 3.7	***spoIIB***
spoIIIAG	Mutants block sporulation after engulfment	5.5, 4.3	***spoIIIAG-spoIIIAH***
spoIIIAH	Mutants block sporulation after engulfment	7.8, 4.3	***spoIIIAG-spoIIIAH***
usd	Required for translation of *spoIIID*	—, —	*usd-****spoIIID***
spoIIID	Transcriptional regulator of sigma E- and sigma K-dependent genes	10.2, 4.7	*usd-****spoIIID***
spoIVA	Required for proper spore cortex formation and coat assembly	2.4, 2.5	***spoIVA***
spoVID	Required for assembly of the spore coat	3.2, 3.5	***spoVID-****yxsE*
yxsE	Unknown function	—, —	***spoVID-****yxsE*
sspE	Small acid-soluble spore protein	4.3, 2.5	***sspE***
yjbX	Unknown function; glutamic acid-rich protein	7.2, 6.2	***yjbX***
ylaK	Similar to phosphate starvation inducible protein	6.5, 4.7	***ylaK***
ylbO	Unknown function	6.8, 3.0	***ylbO***
yqxM	Unknown function	0.9, 1.1	*yqxM-sipW-****tasA***
sipW	Type I signal peptidase	0.9, 1.3	*yqxM-sipW-****tasA***
tasA	Spore-associated antimicrobial protein required for spore coat assembly	2.0, 3.1	*yqxM-sipW-****tasA***
ytfI	Unknown function	—, —	*ytfI-****ytfJ***
ytfJ	Unknown function	3.7, 3.8	*ytfI-****ytfJ***

Continued on following page

TABLE 1—*Continued*

Gene and category	Function	Induction (expt 1, expt 2)	Transcriptional organization
yuiC	Unknown function	3.1, 3.0	***yuiC***
ywcI	Unknown function	10.1, 7.3	***ywcI-sacT***
sacT	Transcriptional antiterminator (regulation of the *sacP* operon)	9.9, 6.4	***ywcI-sacT***
yvyD	Similar to a sigma 54 modulating factor	2.4, 3.4	***yvyD***
Other functions			
dat	Probable D-alanine aminotransferase	1.5, 1.7	*dat*
gcvT	Probable aminomethyltransferase	1.9, 1.9	*gcvT-gcvPA-gcvPB*
gcvPA	Probable glycine decarboxylase (subunit 1)	2.8, 2.6	*gcvT-gcvPA-gcvPB*
gcvPB	Probable glycine decarboxylase (subunit 2)	2.4, 2.6	*gcvT-gcvPA-gcvPB*
mpr	Extracellular metalloprotease	6.6, 3.2	***mpr-****ybfJ*
ybfJ	Unknown function	—, —	***mpr-****ybfJ*
yfhK	Similar to cell division inhibitor	3.2, 6.5	***yfhK***
yhgB	Unknown function	—, —	*yhgB-yhfA-****yhaA***
yhfA	Unknown function	—, —	*yhgB-yhfA-****yhaA***
yhaA	Similar to aminoacylase	4.2, 4.5	*yhgB-yhfA-****yhaA***
ykrT	Unknown function	1.6, 1.7	*ykrT-****ykrS***
ykrS	Similar to initiation factor eIF-2B (alpha subunit)	2.5, 3.5	*ykrT-****ykrS***
ykrW	Similar to ribulose-bisphosphate carboxylase	2.7, 3.3	***ykrW-ykrX-ykrY-ykrZ***
ykrX	Unknown function	3.9, 4.9	***ykrW-ykrX-ykrY-ykrZ***
ykrY	Unknown function	6.8, 4.8	***ykrW-ykrX-ykrY-ykrZ***
ykrZ	Unknown function	3.1, 2.1	***ykrW-ykrX-ykrY-ykrZ***
ykuN	Similar to flavodoxin	—, —	*ykuN-****ykuO-ykuP-****ykuQ*
ykuO	Unknown function	2.8, 2.9	*ykuN-****ykuO-ykuP-****ykuQ*
ykuP	Similar to flavodoxin	4.7, 3.5	*ykuN-****ykuO-ykuP-****ykuQ*
ykuQ	Similar to tetrahydrodipicolinate succinylase	0.9, 1.3	*ykuN-****ykuO-ykuP-****ykuQ*
ykwC	Similar to 3-hydroxyisobutyrate dehydrogenase	1.7, 1.9	*ykwC*
yodF	Similar to proline permease	4.5, 4.4	***yodF***
yojA	Similar to gluconate permease	—, —	*yojA-****yojB-****yojC*
yojB	Unknown function	3.8, 3.9	*yojA-****yojB-****yojC*
yojC	Unknown function	—, —	*yojA-****yojB-****yojC*
yqiX	Similar to amino acid ABC transporter (binding protein)	7.3, 10.3	***yqiX-yqiY-yqiZ***
yqiY	Similar to amino acid ABC transporter (permease)	5.3, 7.1	***yqiX-yqiY-yqiZ***
yqiZ	Similar to amino acid ABC transporter (ATP binding)	7.1, 16.0	***yqiX-yqiY-yqiZ***
yuaF	Unknown function	—, —	*yuaF-****yuaG-****yuaI*
yuaG	Similar to epidermal surface antigen	3.4, 3.1	*yuaF-****yuaG-****yuaI*
yuaI	Unknown function	—, —	*yuaF-****yuaG-****yuaI*
yvaC	Unknown function	—, —	*yvaC-yvaB*
yvaB	Similar to NAD(P)H dehydrogenase (quinone)	2.3, 1.7	*yvaC-yvaB*
ywfH	Similar to 3-oxoacyl-acyl-carrier protein reductase	3.8, 3.2	***ywfH***
Unknown functions			
ybdO	Unknown function	3.2, 7.2	***ybdO***
yfhB	Unknown function	3.7, 3.0	***yfhB***
yfmA	Unknown function	—, —	*yfmA-****yflT***
yflT	Unknown function	4.6, 40.9	*yfmA-****yflT***
yjcE	Unknown function	3.8, 3.6	***yjcE-****yjcD*
yjcD	Similar to ATP-dependent DNA helicase	0.7, 1.4	***yjcE-****yjcD*
yjcH	Unknown function	1.7, 3.1	*yjcH-****yjcG-****yjcF*
yjcG	Unknown function	3.7, 3.6	*yjcH-****yjcG-****yjcF*
yjcF	Unknown function	2.4, 1.8	*yjcH-****yjcG-****yjcF*
ykvR	Unknown function	3.2, 3.3	***ykvR***
ylaJ	Unknown function	3.1, 3.0	***ylaJ***
ylqB	Unknown function	3.3, 3.8	***ylqB***
yqgZ	Unknown function	4.6, 3.4	***yqgZ***
yvaW	Unknown function	—, —	*yvaW-yvaX-****yvaY***
yvaX	Unknown function	—, —	*yvaW-yvaX-****yvaY***
yvaY	Unknown function	10.6, 12.2	*yvaW-yvaX-****yvaY***

[a] Significantly regulated genes are given in bold face. Significant regulation was defined as at least threefold changes in the mRNA levels in both macroarray experiments. Genes were also regarded as significantly regulated when at least twofold changes in the mRNA levels were confirmed by the proteome analysis or the operon structure. Underlined gene names indicate higher expression in minimal medium without CAA as revealed by the proteome analysis. The calculated expression level ratios are shown for both independent macroarray experiments in the column "induction," whereby dashes indicate that specific signals for these genes were below the significance threshold. The putative functions of the y-gene-encoded proteins were obtained from the SubtiList database.

enzymes involved in the synthesis of lysine, methionine, threonine, arginine, cysteine, histidine, leucine, isoleucine, and valine. Furthermore, the sporulation proteins SpoIVA, SpoOA, and TasA, the serine protein kinase PrkA, the proteins Dat and GcvT involved in metabolism of amino acids, and 9 proteins with still-unknown functions (Y-proteins) were identified to be downregulated by addition of CAA.

Analysis of the transcriptome under conditions of differentamino acid availability. *B. subtilis* strain 168 was cultivated in the described minimal medium with and without 0.2% CAA. Total RNA was isolated from exponentially growing cells (optical density at 500 nm of 0.5) and was checked by Northern blot analysis (data not shown). Cell harvesting, preparation of RNA, and macroarray analysis with Panorama *B. subtilis* gene arrays and specific cDNA labeling primers (Sigma-Genosys, The Woodlands, Tex.) were performed as described by Eymann et al. (10). Two macroarray experiments were carried out by using independently isolated RNA preparations and different array batches. Quantification of hybridization signals, background subtraction, and calculation of normalized intensity values of the individual spots were performed with the ArrayVision software (version 5.1; Imaging Research, St. Catherines, Ontario, Canada) as described by Eymann et al. (10). Expression level ratios of three or more in both independent experiments were considered significant. Final evaluation of the macroarray data included the consideration of putative operons derived from the genome sequence, using the SubtiList database (http://genolist.pasteur.fr/SubtiList/) as well as previously known transcriptional units.

Scatter plots comparing the normalized intensity values revealed that mRNA levels of the majority of genes did not differ significantly between both growth conditions, whereas about 100 genes were expressed at a level more than threefold higher in minimal medium without CAA (data not shown). According to the criteria specified in the footnote to Table 1, altogether 114 genes showed significantly elevated expression levels. Of these genes, most encode proteins with functions in amino acid biosynthesis (42 genes), transition state processes and sporulation (32 genes), and competence (8 genes). The patterns of CAA-regulated genes found by the proteomic and transcriptomic approaches were similar, whereby about 50% of the differentially expressed genes could be detected in the proteome analysis. Interestingly, only three genes (*guaC*, *purK*, and *yxjA*) involved in nucleotide metabolism and transport were expressed at a significantly higher level during growth in CAA-containing medium.

At the mRNA level, 16 transcriptional units involved in amino acid biosynthesis were identified to be significantly downregulated by CAA (Table 1). Previous studies suggested regulation by amino acid availability for the operons *argCJBD–carAB-argF*, *argGH* (37), *hom-thrCB* (40), and *ilvBHC-leuABCD* (15), the *lysC* gene (20), and also the S-box-regulated transcriptional units *cysHP-sat-cysC-ylnDEF*, *yjcIJ*, *yoaDCB*, *ykrWXYZ*, *ykrTS*, *metE*, *yitJ*, and *yxjG*. In this study, of the 11 transcriptional units potentially belonging to the S box regulon (18) the 8 mentioned above were expressed at a significantly lower level in CAA-containing medium. Of the approximately 50 *B. subtilis* genes involved in amino acid transport, only the *yqiXYZ* operon encoding an amino acid ABC transport system (33) and the monocistronic-transcribed *yodF* gene encoding a putative proline permease showed significantly different expression levels in response to amino acid availability. Like the *argCJBD–carAB-argF* and *argGH* operons, the *yqiXYZ* operon is preceded by an AhrC recognition site (25). As a further result of the transcriptome study, addition of 0.2% CAA did not affect expression of glycine, serine, proline, tyrosine, and phenylalanine biosynthetic genes. Glutamine and tryptophan biosynthesis was not expected to be regulated under the conditions compared in this study because of the presence of these amino acids in both cultivation media.

A second group of genes expressed at a significantly higher level during growth in the absence of CAA encodes proteins with functions in competence development. This group includes the *comK* gene encoding the competence transcription factor that activates the genes involved in DNA binding and uptake (reviewed in reference 8). Furthermore, nearly the complete *comG* operon (4) shared the same expression pattern. The *srfA* operon also involved in competence regulation exhibited an approximately twofold increased mRNA level. These results are in agreement with previous observations that competence is repressed by the addition of CAA during exponential growth of *B. subtilis* in minimal medium (35).

As shown in Table 1, many *B. subtilis* genes that encode products with functions in transient-phase adaptation and sporulation exhibited significantly higher mRNA levels in minimal medium without CAA. Among these genes were the Spo0A dependently regulated operons *appDFABC* (21, 22), *qcrABC* (41), and *ywcI-sacT* (11). The phosphorylated response regulator Spo0A activates transcription of many sporulation genes and negatively regulates genes preventing sporulation, such as *abrB* (19). The *abrB* gene encodes a repressor of early-stationary-phase and sporulation genes, including the *sigH* gene. Thus, by repressing *abrB* transcription Spo0A-P stimulates synthesis of σ^H and thereby enhances its own transcription. Besides *spo0A* itself, the σ^H-dependently transcribed genes *tasA* (34, 38), *spoVG*, and *yvyD* were upregulated in the absence of CAA. Most of the sporulation genes significantly upregulated in the absence of CAA belong to the σ^E regulon (U. Völker, personal communication), which comprises early-mother-cell-specific genes.

Sporulation frequency in response to amino acid availability. Sporulation genes were shown to be regulated in response to amino acid availability during exponential growth of *B. subtilis*. To verify the physiological relevance of the proteome and transcriptome data, sporulation frequencies were determined at several time points during growth of *B. subtilis* in the described minimal medium with and without CAA. The cultures grown under the two different conditions were inoculated with the same preculture. As shown in Fig. 2, the sporulation frequency of the minimal medium culture without CAA increased continuously up to 14 h after inoculation, whereas addition of CAA almost completely prevented the appearance of spores at least up to 14 h after inoculation. These data confirmed that a significantly lower amount of the population enters the sporulation process during exponential growth in the presence of CAA.

Concluding remarks. Several adaptive processes are involved in the response of *B. subtilis* to growth-limiting levels of nutrients. As revealed by this study, expression of sporulation and competence genes is affected by amino acid availability

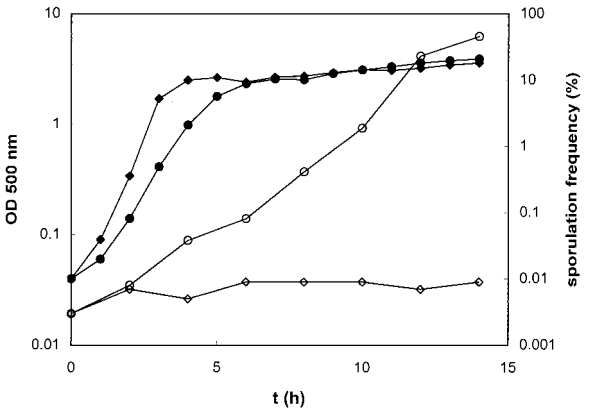

FIG. 2. Growth curve (closed symbols) and sporulation frequencies (open symbols) of *B. subtilis* 168 cultivated in minimal medium (circles) and in minimal medium supplemented with 0.2% CAA (diamonds). The number of spores per milliliter of culture was determined as the number of heat-resistant (80°C for 30 min) CFU on Luria-Bertani plates, and the number of viable cells was determined as the total number of CFU (before heat treatment). Sporulation frequencies were defined as the percentage of heat-resistant CFU. Four independent experiments,which gave comparable results, were carried out.

during exponential growth of *B. subtilis*. Due to the fact that a relatively small portion of the culture enters sporulation in the exponential growth phase, only strongly expressed sporulation genes caused significant signals in the transcriptome analysis.

In agreement with these results, Cosby and Zuber (5) described a negative effect of amino acids on expression of early-stage sporulation genes. They reported that addition of CAA to minimal medium affects *sigH* expression as well as σ^H-dependent transcription. The alternative σ factor σ^H is required for the transcription of early-stationary-phase and sporulation genes and represents the first σ factor in a gene expression cascade resulting in spore formation (23). As reported by Eymann et al. (9), a few σ^H-dependent genes (*spo0A*, *spoVG, yvyD*, and *ytxGHI*) are induced in response to amino acid starvation in a RelA-dependent manner. It was shown earlier that a *relA* mutant sporulates less effectively than the wild type after a shift down from CAA-containing medium to medium without CAA (24). The ribosome-bound ppGpp synthetase RelA is activated by uncharged tRNAs or by glucose starvation. Increased ppGpp levels mediate the stringent control which allows adaptation of cell growth to the present nutrient conditions. Cells growing in minimal medium might be partially starved for amino acids, which possibly elevates the basal ppGpp level. In this study, higher expression of the σ^H-dependent genes *yvyD*, *spo0A*, *spoVG*, and *tasA* in minimal medium without CAA was shown during exponential growth. It is interesting that almost the same σ^H-dependent genes which are induced in a RelA-dependent manner in amino-acid-starved cells were expressed at significantly higher levels during growth in minimal medium without CAA. In *B. subtilis* the CodY regulator mediates amino acid repression of several genes involved in nitrogen metabolism (7, 12, 13, 36, 39) as well as competence development (35), motility (28), and sporulation. Recently, Ratnayake-Lecamwasam et al. (32) reported that CodY represents a GTP-sensing protein and functions as a repressor under conditions of high GTP levels. They suggested that the stringent response might be involved in the inactivation of the CodY regulator by decreasing the cellular GTP pool. As *spo0A* is repressed by CodY (32), a decrease in the GTP level might result in enhanced *sigH* transcription. In the present study, expression of the genes *spo0A* and *comK* as well as the *srfA* operon was shown to be downregulated in CAA-containing medium, whereas most of the other known CodY-dependent genes did not share the same regulatory pattern, indicating the multiple regulation of CodY controlled genes.

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