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The Blueprint of a Minimal Cell: *MiniBacillus*

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SUMMARY

Bacillus subtilis is one of the best-studied organisms. Due to the broad knowledge and annotation and the well-developed genetic system, this bacterium is an excellent starting point for genome minimization with the aim of constructing a minimal cell. We have analyzed the genome of *B. subtilis* and selected all genes that are required to allow life in complex medium at 37°C. This selection is based on the known information on essential genes and functions as well as on gene and protein expression data and gene conservation. The list presented here includes 523 and 119 genes coding for proteins and RNAs, respectively. These proteins and RNAs are required for the basic functions of life in information processing (replication and chromosome maintenance, transcription, translation, protein folding, and secretion), metabolism, cell division, and the integrity of the minimal cell. The completeness of the selected metabolic pathways, reactions, and enzymes was verified by the development of a model of metabolism of the minimal cell. A comparison of the *MiniBacillus* genome to the recently reported designed minimal genome of *Mycoplasma mycoides* JCVI-syn3.0 indicates excellent agreement in the information-processing pathways, whereas each species has a metabolism that reflects specific evolution and adaptation. The blueprint of *MiniBacillus* presented here serves as the starting point for a successive reduction of the *B. subtilis* genome.

INTRODUCTION

Three technological revolutions dramatically changed our view of biology. The genomic revolution gives us access to genome sequences of any organism of interest at low cost. The analytical revolution, especially with respect to mass spectrometry, allows us not only to detect the presence and the fluxes of any molecule in the cell but also to study its precise concentration under any desired condition. Last but not least, the informatics revolution paves the way for the evaluation of the tremendous data sets and for the generation of meaningful models and predictions of cellu-

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lar behavior. However, even knowledge of all components of a cell and of their precise concentrations does not give us a complete understanding of a living cell. For this, we have to consider all the functional interactions between different biological molecules and the dynamics of both the molecules and their interactions.

The complexity of all naturally existing organisms still precludes a deep understanding of the functions of all components of a cell and their interactions. Even small organisms such as bacteria are too complex to understand all processes in their cells. This is even the case for bacteria with naturally minimal genomes, as found in the genus *Mycoplasma*. These bacteria may have as few as 482 protein-coding genes and are still capable of independent life in the absence of any host cells. However, the functions of many *Mycoplasma* genes are so far unknown, and not the full gene set is essential, indicating that we are far from a full picture of these bacteria despite tremendous efforts in their analysis (1–8).

These limitations in our understanding of natural organisms call for a reduction of complexity: the creation of cells with a defined set of genes. Such cells can be obtained by applying either bottom-up or top-down approaches. The former approach has so far been pursued with the chemical synthesis of a bacterial genome and its application to create a semiartificial cell (9, 10). In this case, a known genome was reduced and transplanted into a closely related host cell. With only 473 protein-coding genes, the recently achieved *Mycoplasma mycoides* JCVI-syn3.0 minigenome is so far the smallest semiartificially designed organism. Importantly, about one-third of the proteins in this minimal cell are of unknown function (10). Moreover, there have been attempts to create so-called protocells, which are lipid vesicles that contain genetic material and/or enzymes (11–14). Even though protocells do not allow recapitulation of the evolutionary emergence of life, they are well-suited systems to study the physical and biochemical properties of basic cellular processes such as self-reproduction, permeability, enzymatic replication, and Darwinian evolution (15–17). Reduction of complexity can also be achieved by a top-down approach that starts with existing bacteria and aims at consecutively reducing their complexity. This approach is, of course, very time-consuming; on the other hand, it allows advancing from step to step. Moreover, this iterative process of genome reduction allows the immediate discovery of possible problems and, thus, finding appropriate solutions. Genome reduction is a common theme in synthetic biology, not only for pure scientific curiosity but also from an industrial point of view to create workhorses for biotechnology. Ongoing projects of genome reduction have been reported for several intensively studied bacteria such as *Bacillus subtilis*, *Corynebacterium glutamicum*, *Escherichia coli*, *Pseudomonas putida*, and *Streptomyces avermitilis* (18–27; for a review, see reference 28) as well as for yeast (29). All these projects are still far from the final goal, the minimal cell.

With the progress of genome reduction, it is necessary to define the set of genes that should be part of the final minimal genome. It is obvious that such a set of genes is determined by several factors, including the intended lifestyle of the final minimal cell, but also by the general biology of the organism that is to be reduced. Conceivably, a eukaryotic yeast cell will still contain a nucleus even at a late genome reduction stage. Similarly, the bacteria mentioned above differ strongly in their cellular organizations. For example, *M. mycoides* does not possess a cell wall, while the cell wall is differently structured yet essential in *B. subtilis*, *C. glutamicum*, and *E. coli*. In this work, we aim at defining the set of genes that is required for the life of a minimal cell based on *B. subtilis*. For several reasons, this bacterium is particularly well suited for genome minimization approaches. First, *B. subtilis* is one of the most intensively studied organisms, with extensive genome annotation and excellent knowledge of the major cellular processes. Second, the elaborated genetic system for *B. subtilis* makes all kinds of genetic manipulations very easy (see below). Finally, *B. subtilis* is one of the major organisms in biotechnology, suggesting that genome-reduced strains may also serve as a chassis for novel applications.

CONSIDERATIONS FOR THE DEFINITION OF THE GENE SET FOR A MINIMAL CELL

Several independent sets of information serve as the basis to define which genes are required for a viable minimal cell. First of all, this is the set of essential genes. These genes were identified for *B. subtilis* in 2003 (30). In addition, large dispensable regions of the chromosome have been studied, resulting in the identification of novel essential and coessential genes (31). A recent reevaluation of the essential genes of *B. subtilis* revealed that several metabolic genes involved in glycolysis and the tricarboxylic acid cycle originally listed as essential could be removed from the list. With the exception of the *ylaN* gene, all other genes of unknown function could also be removed from the list (32, 33). Moreover, recent studies indicated that the *ycgG* and *yfkN* genes as well as the *rny* gene, encoding RNase Y, are also dispensable (28, 34; our unpublished data). The current list of 251 essential protein-coding genes is available in the *Subti*Wiki database (http://subtiwiki.uni-goettingen.de/wiki/index.php/Essential_genes) (33).

The essential genes are by definition only those genes that cannot be deleted as single genes under defined optimal growth conditions (for *B. subtilis*, lysogeny broth [LB] with glucose at 37°C). Moreover, a recent knockdown study of essential *B. subtilis* genes showed that the encoded proteins are also very important for outgrowth from stationary phase, adding another level of relevance (35). However, many genes are redundant, and cellular functions can be achieved in completely different ways. The former is the case for DNA polymerase I (PolA) and its paralog YpcP or the diadenylate cyclase CdaA and one of the paralogs DisA and CdaS (36, 37). Moreover, the same function may even be fulfilled by unrelated proteins, as observed for the membrane anchors for the Z-ring protein for cell division, FtsZ. In *E. coli*, the essential FtsA protein serves as a membrane anchor for FtsZ. Why FtsA is nonessential in *B. subtilis* has been enigmatic for a long time. Only the discovery of the unrelated alternative membrane anchor SepF provided the answer (38). Finally, alternative pathways may lead to the same results. This is obvious in the acquisition of cellular building blocks such as amino acids and nucleotides. These metabolites can be either synthesized in the cell or taken up from the medium. In any case, none of the involved genes would be classified as being essential. In all these cases, a decision has to be made regarding which of the possible alternatives will be included in the minimal gene set. Accordingly, the gene complement of a minimal organism has to be designed according to essential functions.

If *B. subtilis* possesses multiple genes for the same function, one of them has to be selected. The criteria for the selection applied in this study are as follows. (i) The final number of genes should be as

small as possible. Therefore, it seems reasonable to include transporters rather than biosynthetic genes for the acquisition of building blocks whenever possible. (ii) In some functional categories, such as cell division, the deletion of a gene may have only a mild effect; however, combination with the deletion of a second, sometimes functionally unrelated gene may be lethal (see "Cell Division," below, for details). Therefore, such synthetic lethalities have to be considered. (iii) Prior decisions will have an impact on later selections. This is the case, for example, for cell wall-biosynthetic proteins (see below). (iv) Both gene expression and protein levels have been extensively studied in *B. subtilis*, and all these data are accessible in the *Subti*Wiki database (33, 39–41). In case of doubt, the most strongly expressed protein has been chosen. (v) Finally, conservation of genes served as a criterion. More strongly conserved genes were preferred over less conserved genes. In this respect, gene conservation and essentiality in genome-reduced *Mycoplasma* and other mollicutes species and the inclusion of genes in the genome of *M. mycoides* JCVI-syn3.0 had a high priority (8, 10, 42).

In many cases, it is not known whether a gene is truly required in the context of a minimal cell. In particular, this is the case for functions involved in RNA modification. In these cases, expression levels and gene conservation were valuable clues for deciding whether a gene should be included in the minimal gene list or not. Based on the list of the most abundant proteins (see http://subtiwiki.uni-goettingen.de/wiki/index.php/Most_abundant_proteins) (33, 43), we have decided whether there is a good reason to keep the corresponding genes or not. As an example, highly abundant enzymes required for amino acid biosynthesis were selected for deletion, whereas RNA chaperones were added. Similarly, genes conserved both in all mollicutes and in *B. subtilis* were regarded as being highly relevant for a minimal organism based on *B. subtilis*.

THE GENETIC COMPLEMENT OF A MINIMAL CELL

Based on the considerations explained above, we have selected 523 and 119 protein- and RNA-coding genes, respectively, as being important for a minimal organism that is capable of growing in LB medium supplemented with glucose at 37°C. Moreover, the growth and physiology of the minimal cell should be comparable to those of *B. subtilis* wild-type cells. *B. subtilis* has a generation time of about 20 min, whereas natural minimal organisms like *M. mycoides* and *Mycoplasma pneumoniae* divide in about 1 and 30 h, respectively. The minimal organism *M. mycoides* JCVI-syn3.0 has a generation time of 180 min (10, 44). This slow growth of *Mycoplasma* cells is another reason to rely on *B. subtilis* as the basis for a minimal cell. Most likely, a reduction of the growth rate has to be expected; however, the set of genes suggested in this study should allow generation times of <1 h. As a consequence, the number of genes included in the list is much larger than that of essential genes in *B. subtilis* 168. Moreover, not all essential genes are required for a minimal cell since several essential genes fulfill protective functions that are dispensable if, e.g., prophages have been deleted (32).

The genes of this minimal set satisfy all essential functions of the cell, such as information processing (DNA replication, transcription, and translation), metabolic pathways (metabolism of building blocks and cofactors and acquisition of ions, etc.), as well as cell division and integrity. Interestingly, there is a very good match between the genes in the *MiniBacillus* minimal gene set and those of *M. mycoides* JCVI-syn3.0 as far as information processing is concerned. In contrast, the two lists show only little overlap of genes required for metabolism, cell division, and protective functions. An overview of the set of genes required for *MiniBacillus* is provided in Table 1. A model of the metabolism of the minimal cell is outlined in Fig. 1, and details that include all metabolic pathways, reactions, and enzymes are provided in Fig. 2 to 11. Detailed information on each individual gene can be found in Tables 2 and 3 and Table S1 in the supplemental material. Table 2 also shows whether the components proposed to be important in the frame of a minimal *B. subtilis* genome are also present in the recently published minimal strain *M. mycoides* JCVI-syn3.0.

DNA Replication and Chromosome Segregation/Maintenance

A set of 18 genes was selected as being important for DNA replication. Among these 18 genes are 15 essential genes, which are absolutely required for growth of *B. subtilis*. With the exception of the essential NAD-dependent DNA ligase LigA, all these essential proteins are members of one or both of the protein complexes that catalyze DNA replication, i.e., the primosome and the replisome (45, 46). In addition to these essential proteins, we have added the replication termination protein Rtp, the inhibitor of DnaA oligomerization YabA, and the DNA polymerase I PolA to the list. Rtp is required for the correct termination of DNA replication and the subsequent segregation of daughter chromosomes (47). YabA, on the other hand, controls replication initiation by inhibiting the oligomerization of the initiator protein DnaA (48). Finally, DNA polymerase I fulfills an essential function, the removal of RNA primers that initiate the synthesis of Okazaki fragments. This function can be taken over by the paralog YpcP, and one of the two proteins has to be present for viability of *B. subtilis*. While YpcP has only the 5′-to-3′ exonuclease domain required for the removal of RNA primers, polymerase I is also capable of filling the resulting gaps (49). This additional property as well as the genetic linkage with the *mutM* gene, which is necessary for DNA integrity (see below), suggested that PolA should be kept in the minimal genome. Interestingly, *B. subtilis* needs either PolA or YpcP, whereas the *M. mycoides* minimal strain JCVI-syn3.0 encodes both proteins.

After DNA replication, the daughter chromosomes have to be efficiently distributed to the daughter cells. Moreover, correct chromosome condensation is essential for all biological processes that involve DNA. Chromosome segregation and condensation are highly overlapping functions; i.e., several of the proteins mentioned below are involved in both activities (for a review, see reference 50). This functional group encompasses 13 proteins, 9 of which are essential. Seven of the corresponding essential genes, but none of the nonessential genes, are present in the genome of *M. mycoides* JCVI-syn3.0. After the initiation of DNA replication, the newly formed origin regions are bound and separated by the condensin protein complex. The condensin complex is composed of two subunits of the Smc protein and monomers of ScpA and ScpB (51, 52). The essential topoisomerase IV formed by ParC and ParE is required for both DNA condensation and segregation (53). For the separation and segregation of the chromosome terminus, the DNA translocases SpoIIIE and SftA and the site-specific recombinases CodV and RipX are necessary. In principle, SpoIIIE and SftA as well as CodV and RipX are paralogous proteins, and one protein might be sufficient for each function. However, as we

TABLE 1 Overview of the genetic complement of a minimal *B. subtilis* cell

Function	No. of proteins (no. of essential proteins)[a]	No. of RNA genes (no. of essential genes)[a]	Figure(s)
Information processing	197 (125)	119 (2)	
DNA replication	18 (15)		
Chromosome maintenance	13 (9)		
Transcription	8 (5)		
RNA folding and degradation	6 (1)		
Aminoacyl-tRNA synthetases	24 (23)		4
Ribosomal proteins	53 (35)		
rRNA and tRNA		116 (0)	
rRNA/tRNA maturation and modification	31 (13)	1 (1)	
Ribosome maturation and assembly	9 (6)		
Translation factors	11 (9)		
Translation/other	5 (2)	1 (0)	
Protein secretion	12 (5)	1 (1)	2
Proteolysis, protein quality control, chaperones	7 (2)		
Metabolism	218 (59)		
Central carbon metabolism	26 (4)		3
Respiration/energy	16 (2)		3
Amino acids	30 (1)[b]		3
Nucleotides/phosphate	36 (11)		2, 5
Lipids	19 (17)		6
Cofactors	62 (14)[b]		
General components of ECF transporters	3 (0)[b]		4, 7
NAD	5 (4)		7
FAD	2 (1)[b]		7
Pyridoxal phosphate	2 (0)		7
Biotin	1 (0)[b]		7
Thiamine	2 (0)[b]		7
Lipoate	4 (1)		7
Coenzyme A	9 (1)		7
S-Adenosylmethionine	1 (1)		7
Folate	13 (1)		8
Heme	12 (0)		8
Menaquinone	8 (5)		9
Metals/iron-sulfur clusters	29 (10)		2, 7
Cell division	81 (52)		
Cell wall synthesis	55 (41)		
Amino acid precursor	11 (10)		10
Undecaprenyl phosphate	13 (10)		10
Lipid II biosynthesis	12 (11)		10
Peptidoglycan polymerization	5 (1)		10
Wall teichoic acid	9 (8)		11
Lipoteichoic acid	5 (1)		6
Coordination	22 (9)		
Signaling	4 (2)		2
Integrity of the cell	16 (5)		
Protection	8 (4)		2
Repair/genome integrity	8 (1)		
Other/unknown	11 (2)		
Total minimal genome	523 (243)	119 (2)	

[a] The numbers of proteins and RNAs required for each function are listed. Numbers in parentheses indicate the numbers of proteins and RNAs that are essential in the context of *B. subtilis* 168.

[b] Tryptophan, riboflavin, biotin, and thiamine are transported by transporters of the ECF (energy-coupling factor) family. The three general components are shared among all these transporters. They are listed separately with the cofactors.

are aiming for a well-growing strain that is not significantly compromised in its major cellular functions, and as synergistic activities of these proteins were recently shown (54–56), we decided to keep all four genes. DNA topology is maintained by the interplay of gyrase and topoisomerase I activities, encoded by the essential *gyrAB* and *topA* genes, respectively (28, 57). Moreover, the histone-like protein HBsu nonspecifically binds the DNA and is involved in DNA packaging (58).

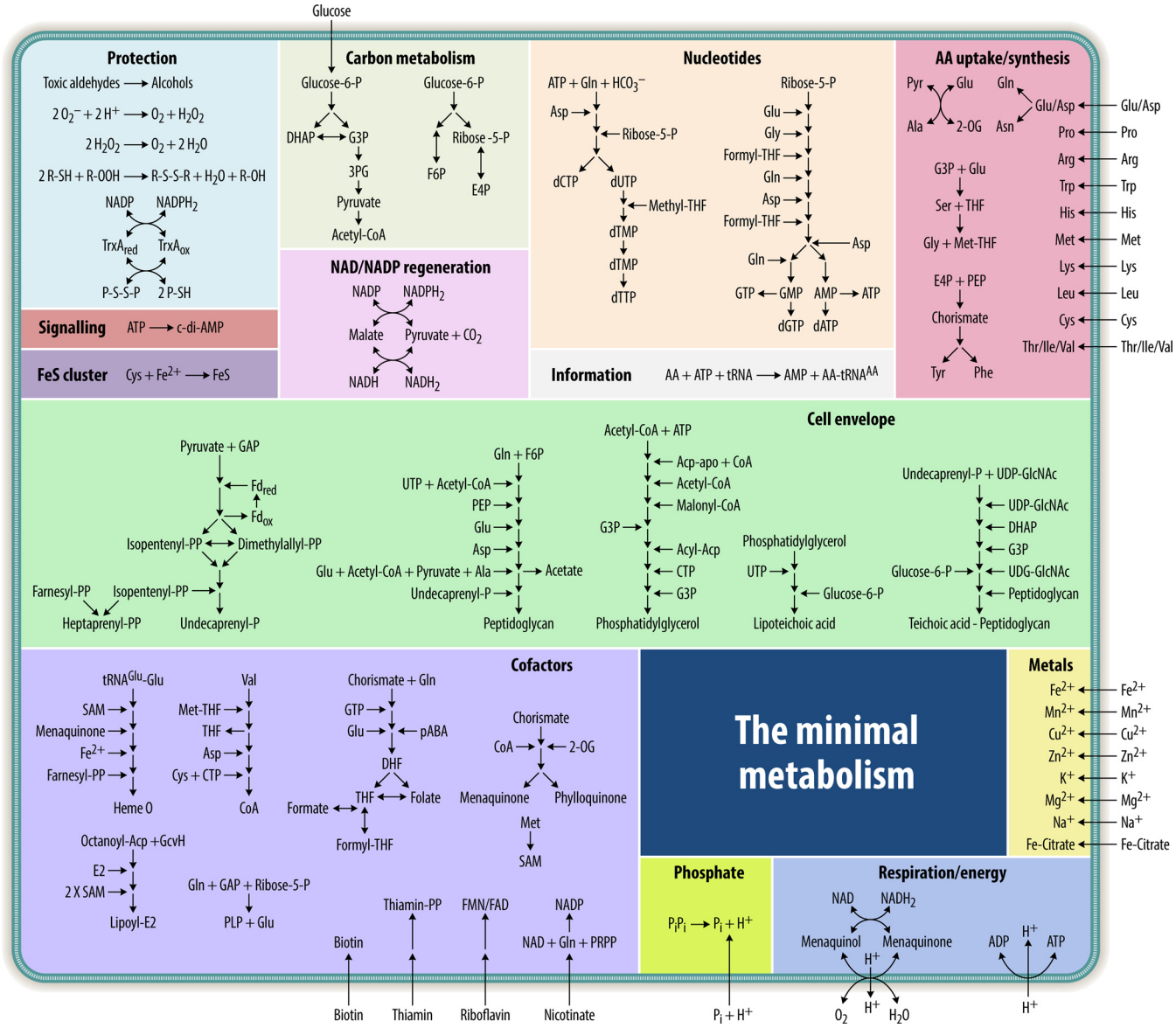

FIG 1 Outline of the metabolic model of the minimal cell. The model gives an overview of the metabolic pathways of the intended minimal organism. Functionally related pathways are grouped in boxes. Details on all reactions and enzymes are provided in Fig. 2 to 11. DHAP, dihydroxyacetone phosphate; G3P, glycerol-3-phosphate; AA, amino acid; THF, tetrahydrofolate; 2-OG, 2-oxoglutarate; GAP, glyceraldehyde-3-phosphate; PEP, phosphoenolpyruvate; PLP, pyridoxal phosphate; DHF, 7,8-dihydrofolate; pABA, 4-aminobenzoate; FMN, flavin mononucleotide; PRPP, phosphoribosyl pyrophosphate; E4P, erythrose-4-phosphate; 3PG, 3-phosphoglycerate; PP, pyrophosphate; F6P, fructose-6-phosphate.

Transcription

Eight proteins, among them five essential proteins, are required for transcription. Importantly, this activity requires the RNA polymerase, which consists of the essential core subunits (RpoA, RpoB, and RpoC) and sigma factor A (SigA) for promoter binding and recognition. Moreover, we have included the RNA polymerase-interacting protein HelD and the nonessential delta subunit (RpoE). HelD binding stimulates transcription in an RpoE-dependent manner, suggesting that these two accessory proteins are important to allow rapid growth (59, 60). Interestingly, both proteins are absent from the rather slowly growing *M. mycoides* strain JCVI-syn3.0. Finally, GreA and the essential NusA protein are required for transcription elongation and termination, respectively (61, 62). In contrast, the transcription termination protein Rho does not affect the growth of *B. subtilis* in rich medium (63) and therefore has not been included in the list.

mRNA Folding and Degradation

Once the RNA has been formed by the RNA polymerase, it has to adopt a structure that is compatible with its function. The rRNAs and tRNAs adopt complex three-dimensional structures, whereas the mRNAs have to be unstructured to allow access to the ribosomes. Bacterial cells have to adapt very rapidly to changes of external conditions. A major component of this rapid adaptation is the rapid degradation of bacterial mRNAs, which have average half-lives in the minute range. RNA degradation is accomplished

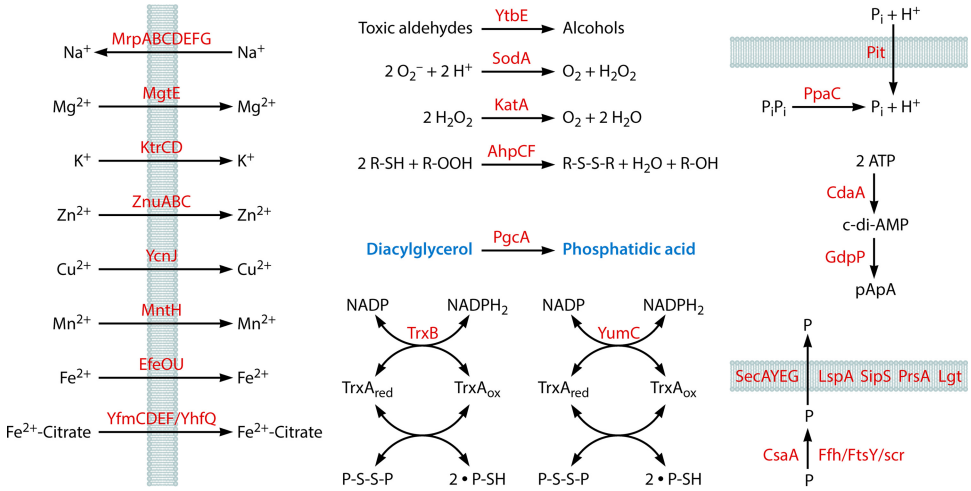

FIG 2 Miscellaneous pathways. The model shows the uptake of metal ions and inorganic phosphate (P_i) and reactions for protective functions, for the generation of phosphatidic acid, and for the synthesis and degradation of c-di-AMP. Finally, protein secretion is included. The metabolic intermediates diacylglycerol (Fig. 6) and phosphatidic acid (Fig. 6) that occur in other pathways are labeled in blue. P, protein.

by a set of RNases (64). In total, six proteins are required for the proper folding and degradation of RNA. To keep RNA molecules unstructured, the cell possesses so-called RNA chaperones. Two of these RNA chaperones, CspB and CspD, belong to the most abundant proteins in *B. subtilis* during growth in LB medium with glucose at 37°C (43, 65). Moreover, *cspD* is also one of the most highly expressed genes of *B. subtilis* under more than 100 different conditions (39). These data suggest that these RNA chaperones should be included in the minimal cell. In contrast, we decided to exclude all DEAD-box RNA helicases since *B. subtilis* has no growth defect even in the absence of all four helicases at 37°C (66). Conflicting reports are available for RNases in *B. subtilis*. The endoribonuclease RNase Y and the 5′-to-3′ exonuclease RNase J1 have long been considered to be essential, whereas a recent report suggests that these proteins are dispensable (34, 67–69). However, the strong expression of the corresponding genes suggests that they should be part of a minimal cell. The same is true for the 3′-to-5′ exoribonuclease PnpA, which also belongs to the most abundant proteins. Moreover, PnpA has also been implicated in DNA repair (70). Finally, we decided to include the nano-RNase NrnA, which degrades and thus recycles bi- and oligoribonucleotides (71). This enzyme is conserved in all groups of mollicutes despite their significant genome reduction (42). This strong conservation is suggestive of an important function and justifies the inclusion of this protein in the minimal cell. Moreover, RNases J1 and Y as well as the nano-RNase NrnA are also encoded by *M. mycoides* JCVI-syn3.0.

Translation

A large set of 133 proteins and 118 RNAs is required for translation. This includes aminoacyl-tRNA synthetases, ribosomal proteins, rRNAs, tRNAs, proteins involved in rRNA and tRNA modification and maturation, ribosome biogenesis factors, and translation factors.

Twenty-four proteins constitute the set of 20 aminoacyl-tRNA synthetases (72). With the exception of ThrS, the threonyl-tRNA synthetase, all of these enzymes are essential in *B. subtilis*. ThrS has a weakly expressed paralog, and one of these proteins is required for the viability of *B. subtilis* (36). Similarly, the enzyme for tyrosine, TyrS, has a paralog. However, this paralog is not expressed under the most common conditions, rendering TyrS essential (73). It should be noted that the aminoacyl-tRNA synthetases for glycine and phenylalanine are composed of two subunits, and the transamidosome for the formation of glutamine-tRNA is composed of the three subunits (GatA, GatB, and GatC) of the glutamyl-tRNA(Gln) amidotransferase, the glutamyl-tRNA synthetase GltX, and glutamine-specific tRNA (74). In the artificial minimal organism *M. mycoides* JCVI-syn3.0, there are no paralogs of the genes encoding the aminoacyl-tRNA synthetases for glycine and proline. It is tempting to speculate that these functions are fulfilled by some of the 149 unknown proteins encoded by this genome.

The *B. subtilis* ribosome consists of 53 proteins under standard conditions, among them 33 and 20 in the large and small subunits, respectively. Several of these proteins are nonessential; however, this has been tested only with single-gene inactivation (75). It is therefore conceivable that the simultaneous lack of multiple ribosomal proteins would have a significant impact on viability. In addition, *B. subtilis* encodes auxiliary ribosomal proteins that are synthesized only under stress conditions or that replace zinc-containing proteins during zinc limitation (76–78). All these proteins were excluded from the list (Table 2). The genome of the artificial organism *M. mycoides* JCVI-syn3.0 lacks genes for the essential protein L30 (RpmD) as well as those for 10 nonessential ribosomal proteins, mainly of the large ribosomal subunit.

B. subtilis has 10 operons for rRNA. All these operons also contain genes for tRNAs. Moreover, several tRNAs are encoded by scattered genes. It is well established that the redundancy of rRNAs and tRNAs is an important determinant of the growth rate (79); therefore, we included all 30 and 86 genes encoding rRNAs and tRNAs, respectively, in the minimal genome. However, reduction of the copy numbers of rRNA and tRNA genes remains to be explored to achieve a robust minimal translation machinery.

To be functional, rRNAs and tRNAs have to be processed and

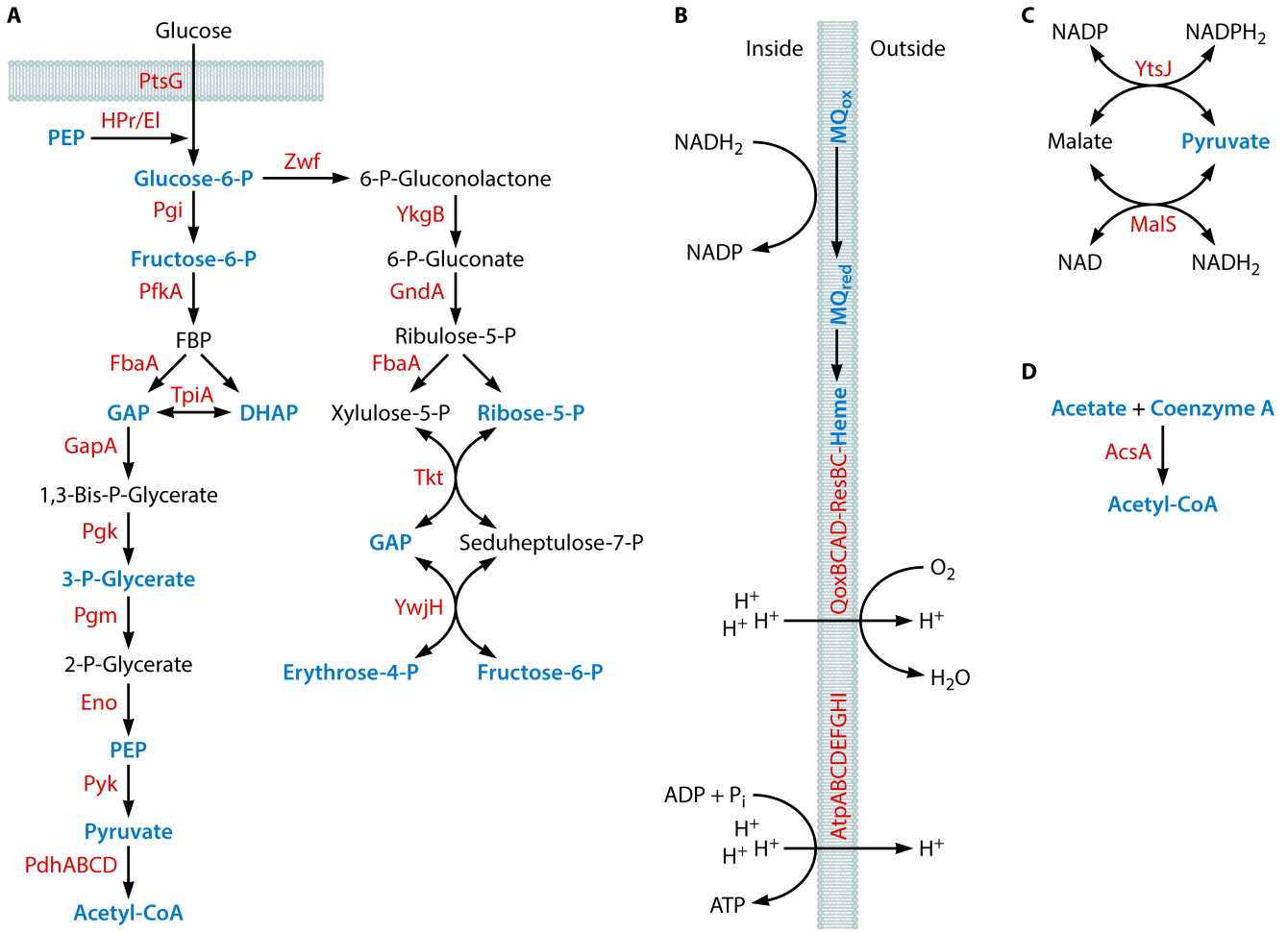

FIG 3 Central carbon metabolism and energy conservation. (A) Glycolytic and pentose phosphate pathways. (B) The respiratory chain and ATPase. (C) The transhydrogenase cycle for balancing $NADPH_2$. (D) Recycling of acetate derived from cell wall metabolism (Fig. 10). The following metabolic intermediates that occur in other pathways are labeled in blue: phosphoenolpyruvate (PEP) (Fig. 4 and 10), glucose-6-phosphate (Glucose-6-P) (Fig. 6), fructose-6-phosphate (Fig. 10), glyceraldehyde-3-phosphate (GAP) (Fig. 7 and 10), dihydroxyacetone phosphate (DHAP) (Fig. 6), 3-P-Glycerate (Fig. 4), pyruvate (Fig. 4 and 8 to 10), acetyl-CoA (Fig. 6 and 10), ribose-5-phosphate (Fig. 5 and 7), erythrose-4-phosphate (Fig. 4), menaquinone/menaquinol (MQ) (Fig. 9), heme (Fig. 8), acetate (Fig. 10), and coenzyme A (Fig. 6 and 10). FBP, fructose 1,6-bisphosphate.

modified. In the minimal gene set, we include 31 proteins and 1 RNA for rRNA and tRNA maturation/modification, respectively. Since the functional RNAs are usually expressed as parts of large operons, proper processing is the first important step in their maturation. It should be noted that RNases Y and J1 (see above) participate in the processing and maturation of these functional RNA molecules (80, 81). Moreover, RNases P, PH, and Z are involved in rRNA and tRNA processing. Of these, RNase P (composed of a protein and the catalytically active RNA component) and RNase Z are essential (82, 83). In addition, RimM and RbfA are important for ribonucleolytic 16S rRNA maturation (84, 85). rRNAs and tRNAs are subject to multiple and highly diverse modifications, including methylation, thiouridylation, and pseudouridylation. The modification of tRNAs is one of the functions that still needs substantial research effort. The proteins important for these activities have been derived from the *Subti*Wiki database and from a recent study on the minimal translation apparatus (33, 42). As shown in Table 2, there is a good match with the corresponding set of genes in *M. mycoides* JCVI-syn3.0 (10).

Nine proteins, including six essential proteins, are involved in ribosome maturation and assembly. The three nonessential proteins process or modify ribosomal proteins. The six essential proteins are all GTPases that participate in different aspects of ribosome assembly (86, 87). Four of the genes encoding these essential GTPases are also part of the genome of *M. mycoides* JCVI-syn3.0.

Eleven proteins function as translation factors in different steps of translation, i.e., initiation, elongation, peptide chain release, and ribosome recycling. Of these proteins, nine are essential. Of the nonessential proteins, Efp is important for the efficient translation of proteins containing multiple consecutive proline residues, among them three essential proteins (Fmt, TopA, and ValS) (see http://subtiwiki.uni-goettingen.de/wiki/index.php/Efp-dependent_proteins) (33, 88, 89). With the exception of peptide chain release factor 2 (PrfB), but including elongation factor P (Efp), all of these proteins are encoded by the genome of *M. mycoides* JCVI-syn3.0.

In addition, five proteins and one RNA are important for translation. These include the essential methionine aminopep-

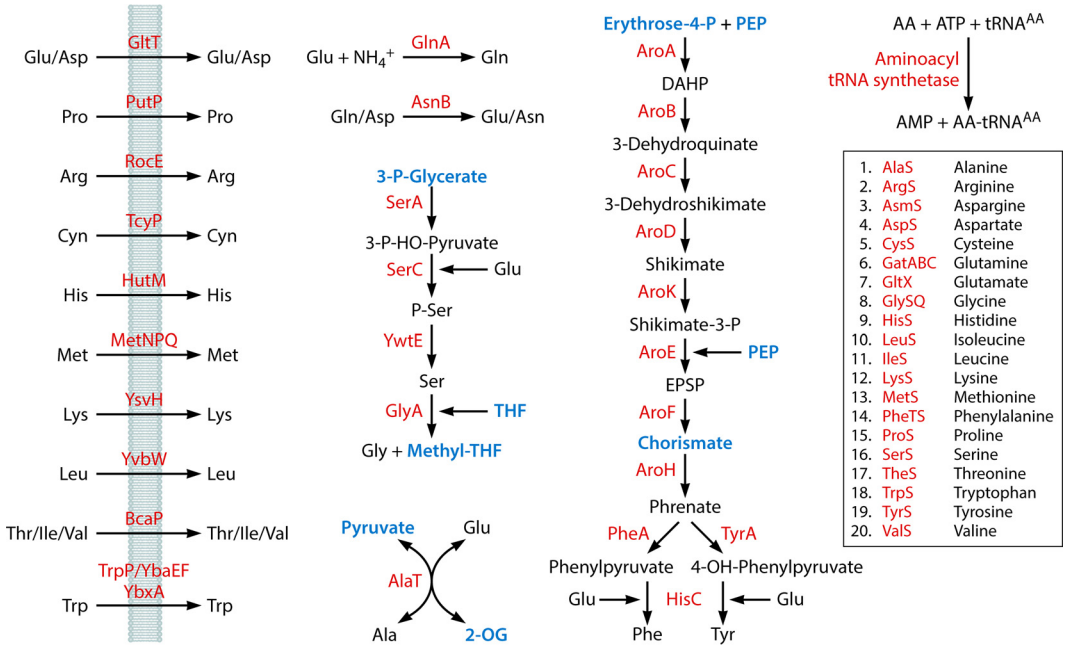

FIG 4 Acquisition of amino acids and charging of tRNAs. The following metabolic intermediates that occur in other pathways are labeled in blue: 3-P-Glycerate (Fig. 3), tetrahydrofolate (THF) (Fig. 7 and 8), methyltetrahydrofolate (Methyl-THF) (Fig. 5 and 7), pyruvate (Fig. 3 and 8 to 10), 2-oxoglutarate (2-OG) (Fig. 9), erythrose-4-phosphate (Fig. 3), phosphoenolpyruvate (PEP) (Fig. 3 and 10), and chorismate (Fig. 8 and 9). DAHP, 3-deoxy-D-arabino-hept-2-ulosonate 7-phosphate; EPSP, 5-*O*-(1-carboxyvinyl)-3-phosphoshikimate; AA, amino acid.

tidase, which removes the N-terminal methionine from nascent proteins (90). In addition, transfer-messenger RNA (tmRNA) and its binding protein rescue stalled ribosomes, whereas the essential peptidyl-tRNA hydrolase SpoVC recycles tRNA molecules sequestered as peptidyl-tRNA as a result of premature dissociation from the ribosome (91–93). The precise functions of YwkE and YbxF still have to be discovered. YbxF binds to turns in RNA molecules and is very strongly expressed under most conditions (33, 39, 94).

Protein Secretion

Several proteins of the minimal cell have to be targeted either to the membrane or for secretion into the medium. For this activity, the minimal cell needs a set of 12 proteins (5 of them essential) and 1 essential RNA for protein secretion (Fig. 2). The first component in cotranslational targeting of the membrane and secreted proteins is the universally conserved signal recognition particle composed of the essential RNA *scr*, the essential protein Ffh, and FtsY (95–99). The next step in protein translocation is performed by the chaperone CsaA and by the translocation motor SecA, which provides the energy for the export of unfolded secretory precursor proteins through the channel formed by SecYEG (100–102). Alternatively, YidC2 can translocate the preproteins through the membrane. In *B. subtilis*, YidC2 has a paralog, SpoIIIJ, and one of the two proteins is required for viability (36, 103, 104). We included YidC2 since this protein is also important for genetic competence (105) (see below). For secreted proteins, the signal peptide is then cleaved off by signal peptidase I (SipS) (106, 107). In the case of lipoproteins, the protein is diacylglyceryl modified by Lgt for attachment to the membrane, and signal peptide cleavage is subsequently performed by LspA (108–110). Proper folding of extracellular proteins is assisted by the posttranslocation chaperone PrsA, a lipoprotein attached to the outer surface of the cytoplasmic membrane (111, 112). *B. subtilis* also possesses the TAT (twin arginine translocation) and type VII protein secretion systems. However, these systems are not essential in a minimal cell (113–115). Interestingly, the reduced genome of *M. mycoides* JCVI-syn3.0 encodes only the signal recognition particle, the translocation motor SecA, and an incomplete Sec channel. Importantly, this minimal organism seems to lack enzymes for the export and membrane attachment of lipoproteins. It is tempting to speculate that these functions are encoded by some of the unknown genes, since lipoprotein-dependent metabolite uptake is crucial for *Mycoplasma* bacteria with their strongly reduced metabolism. This is the case not only for artificially genome-reduced bacteria but also for natural *Mycoplasma* species. In *M. pneumoniae*, lipoproteins account for about 10% of all encoded proteins (116, 117). Moreover, many of the 149 unknown genes in the minimal genome of *M. mycoides* JCVI-syn3.0 encode lipoproteins (10).

Intracellular Chaperones, Protein Quality Control, and Proteolysis

To be active, proteins have to be properly folded, and misfolded proteins need to be detected and then be refolded or degraded. These activities can be performed by a set of seven proteins, including two essential proteins. The universally conserved chaperone DnaK with its cochaperones DnaJ and GrpE, the trigger factor Tig, as well as the universally conserved essential chaperonin GroES/GroEL are important for the folding of intracellular proteins (118, 119). It should be noted that DnaK, its partners, and Tig are nonessential in *B. subtilis*; however, the corresponding genes are highly expressed, and they are conserved among the genome-reduced mollicutes. Of note, the nonessential DnaK/

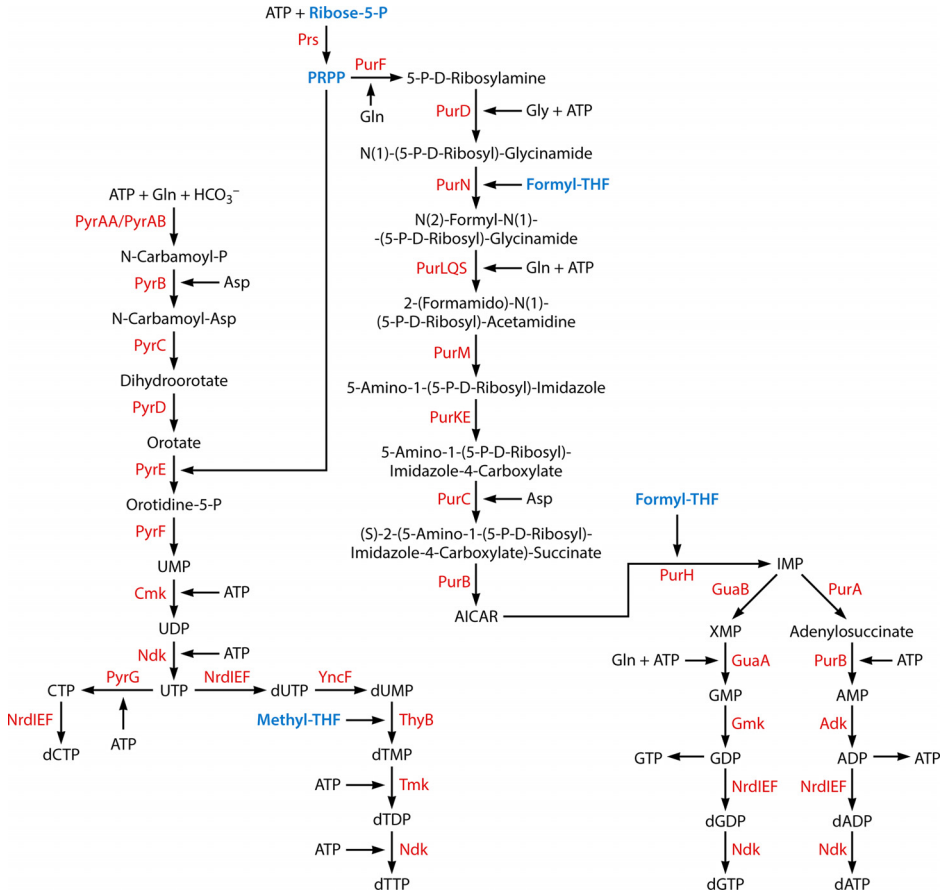

FIG 5 Acquisition of nucleotides. The following metabolic intermediates that occur in other pathways are labeled in blue: methyltetrahydrofolate (Methyl-THF) (Fig. 4 and 7), ribose-5-phosphate (Fig. 3 and 7), phosphoribosyl pyrophosphate (PRPP) (Fig. 7), and formyltetrahydrofolate (Formyl-THF) (Fig. 8). AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide.

DnaJ chaperone is also present in *M. mycoides* JCVI-syn3.0, whereas the universally conserved essential GroES/GroEL chaperonin is not (10). In addition, viability of *B. subtilis* requires the presence of one intramembrane quality control protease, and we selected HtrB for this activity (120, 121).

Central Carbon Metabolism

Central carbon metabolism is at the heart of any cellular metabolism. We have selected 26 proteins (among them 4 essential proteins) for this function. Glycolysis provides the cell with precursors for further metabolic pathways, ATP for substrate-level phosphorylation, and reducing power to drive respiration (see Fig. 1 and 3 for details). Moreover, the pentose phosphate pathway generates reducing power for anabolic reactions, erythrose-4-phosphate as a precursor for chorismate synthesis (Fig. 3), and ribose-5-phosphate for the synthesis of nucleic acids. To generate acetyl coenzyme A (acetyl-CoA) for lipid biosynthesis, pyruvate dehydrogenase is required (122, 123). Moreover, the acetyl-CoA synthetase recycles the acetate derived from cell wall biosynthesis (124) (Fig. 3 and 10) (see below). Both glycolysis and the pentose phosphate pathway generate a large amount of reducing power. NAD^+ can be regenerated in respiration, and the YtsJ/MalS transhydrogenation cycle is used for balancing $NADPH^+$ levels (Fig. 3) (125). The minimal cell does not need the citric acid cycle. This pathway can be completely deleted in *B. subtilis* (our unpublished results). The minimal organism *M. mycoides* JCVI-syn3.0 encodes the enzymes for glycolysis but only a strongly reduced set (three out of seven) enzymes of the pentose phosphate pathway. Moreover, these cells lack the citric acid cycle. The absence of a full pentose phosphate pathway and of the citric acid cycle is characteristic of the reduced metabolism of *Mycoplasma* species and reflects their reliance on the uptake of nutrients from the environment (117). Interestingly, of the glycolytic enzymes, the classical fructose 1,6-bisphosphate aldolase is missing in *M. mycoides* JCVI-syn3.0. The cleavage of fructose 1,6-bisphosphate may be catalyzed by the paralogous IolJ aldolase in these bacteria. It is worth noting that the nearly complete match of the gene sets for glycolysis in the suggested *MiniBacillus* genome and in *M. mycoides* JCVI-syn3.0 is exceptional among all metabolic pathways.

Respiration/Energy

ATP production is achieved efficiently by the generation of a proton motive force in respiration and its subsequent use to drive ATP synthesis. A total of 16 proteins are involved in these processes, among them 2 essential proteins (Fig. 3). $NADH_2$ from glycolysis is reoxidized by the respiration chain consisting of NADH dehydrogenase, menaquinone, and the terminal heme-copper cytochrome aa_3 oxidase. It is important to note

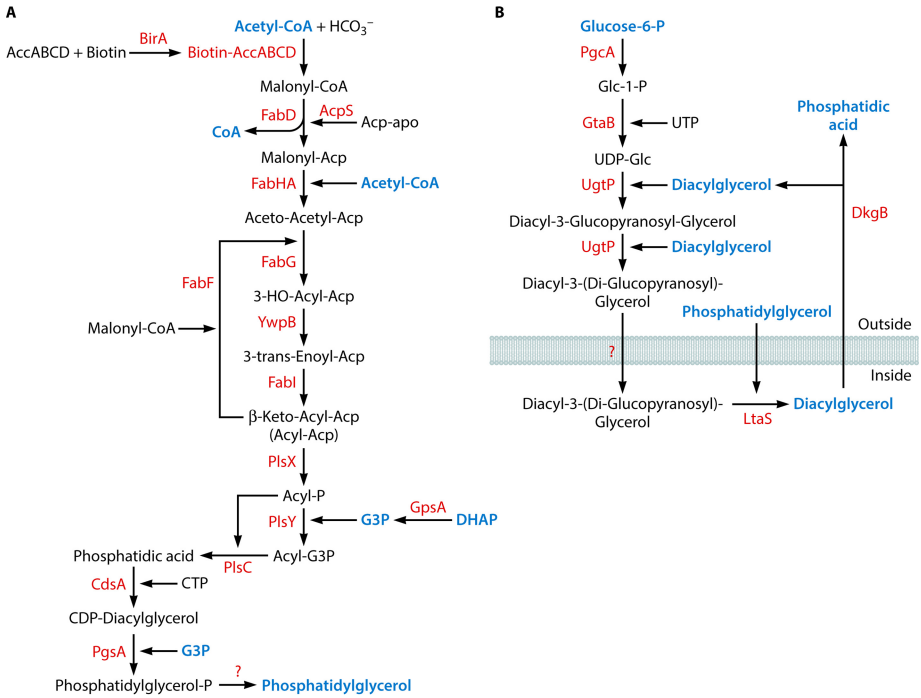

FIG 6 (A) Biosynthesis of lipids. The enzyme required for the conversion of phosphatidylglycerol phosphate to phosphatidylglycerol is unknown. (B) Biosynthesis of lipoteichoic acids. The enzyme required for the export of diacyl-3-(diglucopyranosyl)-glycerol is unknown. The following metabolic intermediates that occur in other pathways are labeled in blue: acetyl-CoA (Fig. 3 and 10), CoA (Fig. 3, 7, and 9), glycerol-3-phosphate (G3P) (Fig. 11), dihydroxyacetone phosphate (DHAP) (Fig. 3), phosphatidylglycerol (this figure), glucose-6-phosphate (Fig. 3), diacylglycerol (Fig. 2), and phosphatidic acid (Fig. 2).

that *B. subtilis* is unable to live under aerobic conditions in the absence of both terminal quinol oxidases (126). We have selected this respiration chain because it is a minimal chain and because the selected terminal oxidase is capable to pumping protons to energize the membrane. Moreover, loss of the heme-copper cytochrome aa_3 oxidase results in impaired growth (126). Next, the multisubunit ATPase uses the proton gradient to provide the cell with ATP. Interestingly, several subunits of the ATPase in *M. my-*

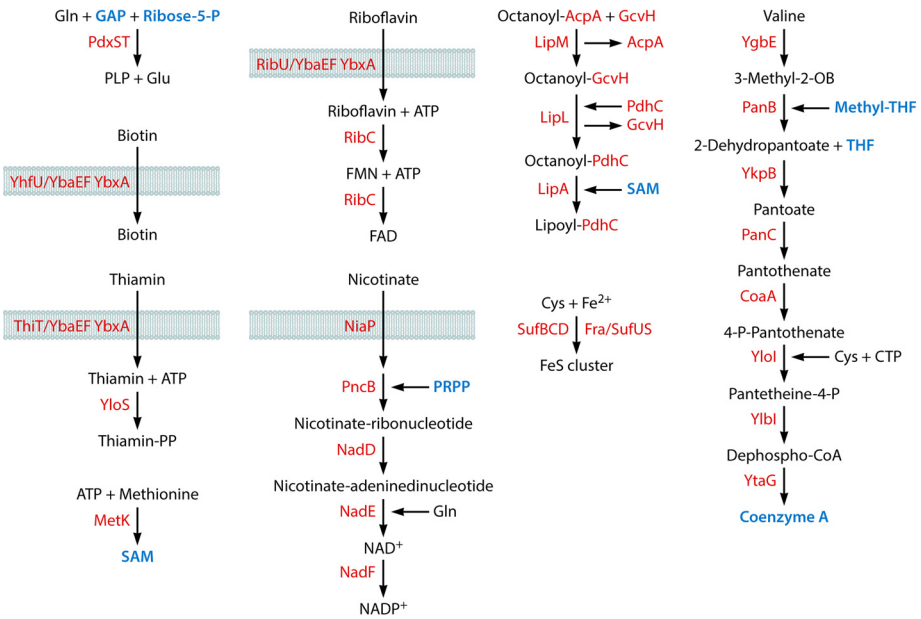

FIG 7 Acquisition of cofactors and biosynthesis of iron-sulfur clusters. The following metabolic intermediates that occur in other pathways are labeled in blue: glyceraldehyde-3-phosphate (GAP) (Fig. 3 and 10), ribose-5-phosphate (Fig. 3 and 5), *S*-adenosylmethionine (SAM) (this figure), phosphoribosyl pyrophosphate (PRPP) (Fig. 5), methyltetrahydrofolate (Methyl-THF) (Fig. 4 and 5), tetrahydrofolate (THF) (Fig. 4 and 8), and coenzyme A (CoA) (Fig. 3, 6, and 9). PLP, pyridoxal phosphate; FMN, flavin mononucleotide; FeS, iron-sulfur cluster; 3-Methyl-2-OB, 3-methyl-2-oxobutanoate.

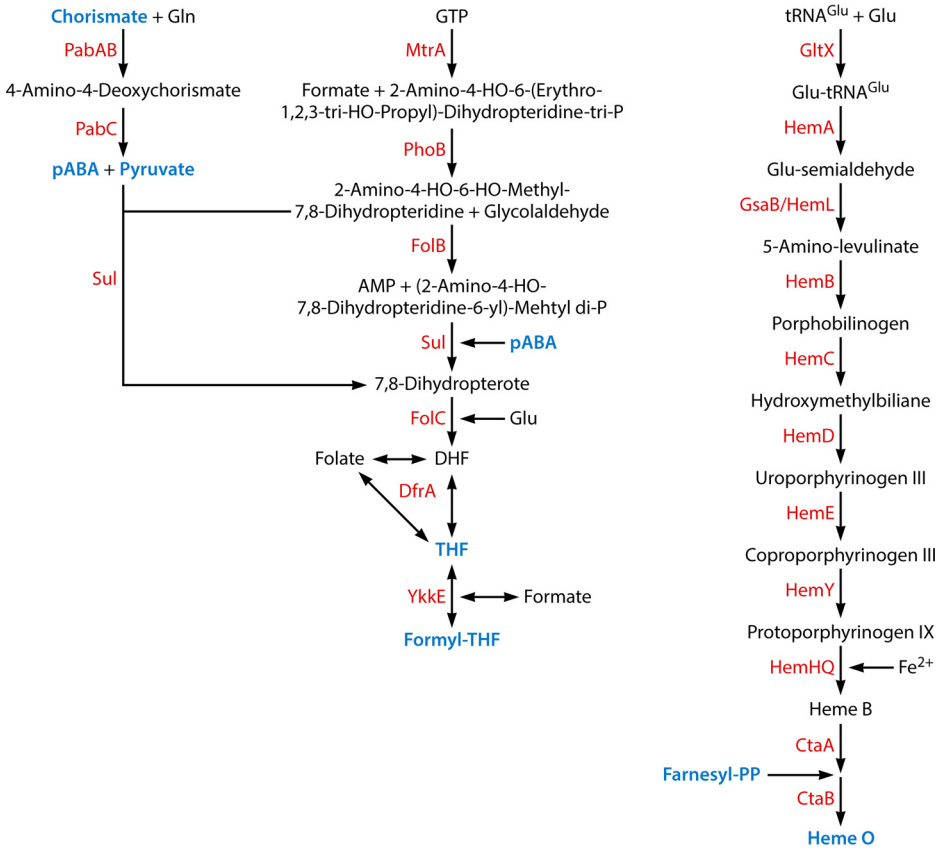

FIG 8 Acquisition of cofactors. The following metabolic intermediates that occur in other pathways are labeled in blue: chorismate (Fig. 4 and 9), 4-amino-benzoate (pABA) (this figure), pyruvate (Fig. 3, 4, 9, and 10), tetrahydrofolate (THF) (Fig. 4 and 7), formyltetrahydrofolate (Formyl-THF) (Fig. 5), farnesyl pyrophosphate (farnesyl-PP) (Fig. 10), and heme O (Fig. 3). DHF, 7,8-dihydrofolate.

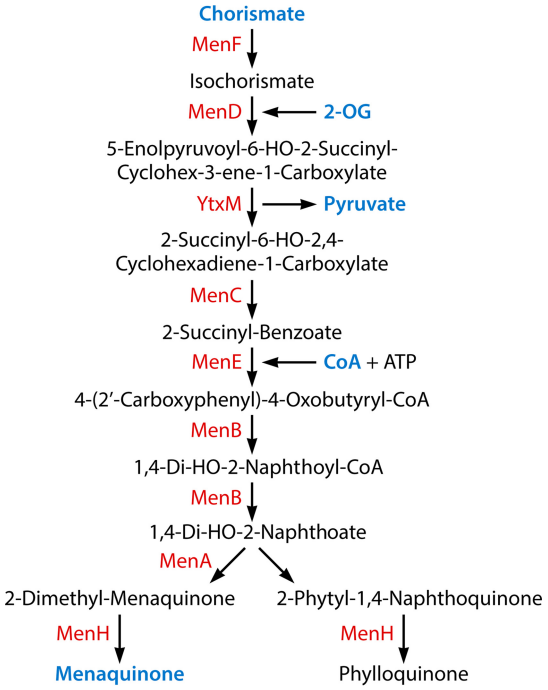

FIG 9 Acquisition of cofactors. The following metabolic intermediates that occur in other pathways are labeled in blue: chorismate (Fig. 4 and 8), 2-oxo-glutarate (2-OG) (Fig. 4), pyruvate (Fig. 3, 4, 8, and 10), coenzyme A (CoA) (Fig. 3, 6, and 7), and menaquinone (Fig. 3).

coides JCVI-syn3.0 seem to be too poorly conserved with those of *B. subtilis* to allow detection by sequence comparison.

Amino Acids

Acquisition of amino acids is one of the major activities of any living cell. This can be achieved by either the uptake of external amino acids, the uptake and subsequent degradation of peptides, or the biosynthesis of amino acids. As biosynthesis usually involves significantly more proteins than does uptake, we included mainly transport systems for amino acids in the list (see also Fig. 1 and 4 for details). Exceptions are the biosyntheses of alanine, glycine, serine, phenylalanine, tyrosine, asparagine, and glutamine. The single glycine biosynthetic enzyme (serine hydroxymethyltransferase [GlyA]) is essential, suggesting that *B. subtilis* has to synthesize this amino acid. For alanine, serine, asparagine, and the aromatic amino acids, no transporter is known. For glutamine, biosynthesis requires only one enzyme (glutamine synthetase [GlnA]), and the only known glutamine transporter is composed of several subunits and is expressed only during sporulation. Among the 30 proteins that are required for amino acid acquisition in the frame of the *MiniBacillus* genome, only 2 (the essential GlyA protein and a cysteine transporter) are also present in *M. mycoides* JCVI-syn3.0, suggesting that this organism acquires amino acids in a completely different way. Likely, the latter organism relies on the transport and intracellular degradation of oligopeptides to obtain amino acids (10).

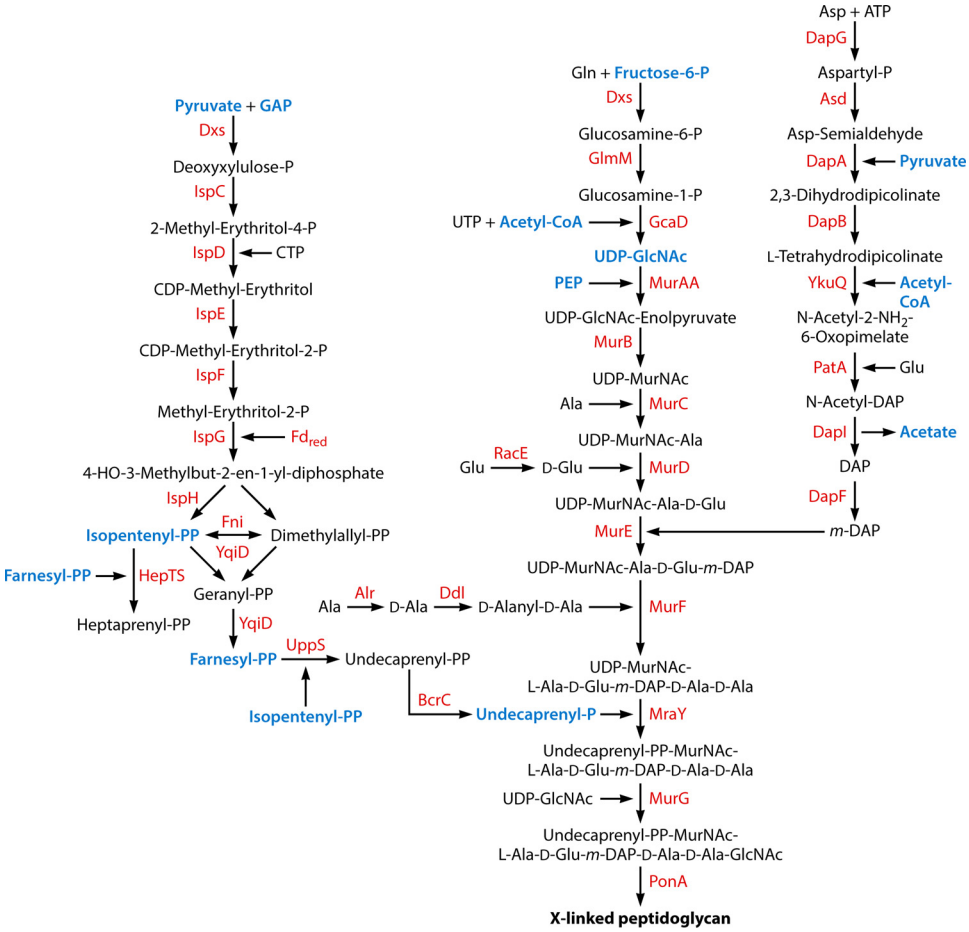

FIG 10 Biosynthesis of the cell wall. The following metabolic intermediates that occur in other pathways are labeled in blue: pyruvate (Fig. 3, 4, 8, and 9), glyceraldehyde-3-phosphate (GAP) (Fig. 3 and 7), isopentenyl pyrophosphate (isopentenyl-PP) (this figure), farnesyl pyrophosphate (farnesyl-PP) (Fig. 8), undecaprenyl phosphate (Fig. 11), fructose-6-phosphate (Fig. 3), acetyl-CoA (Fig. 3 and 6), UDP-*N*-acetylglucosamine (UDP-GlcNAc) (Fig. 11), phosphoenol pyrophosphate (PEP) (Fig. 3 and 4), and acetate (Fig. 3). UDP-MurNAc, UDP-*N*-acetylmuramic acid; DAP, diaminopimelate.

Nucleotides/Phosphate

Complex media like LB cannot meet the demand of *B. subtilis* for nucleotides (R. Switzer, personal communication). Therefore, the minimal cell has to carry 35 genes for nucleotide *de novo* synthesis. Of these genes, 11 are essential. Both purine and pyrimidine biosyntheses are initiated by the synthesis of phosphoribosylpyrophosphate from ribose-5-phosphate (catalyzed by the essential and universally conserved phosphoribosylpyrophosphate synthetase Prs). The pathways of nucleotide biosynthesis are schematically shown in Fig. 1 (for more details, see Fig. 5). The pyrimidine and purine nucleotide biosynthetic pathways converge with the reduction of ribonucleotides by the NrdEIF complex and the final formation of nucleoside triphosphates by the nucleoside diphosphate kinase Ndk (127). For one of the essential proteins implicated in nucleotide biosynthesis, HprT, the reason for its essentiality is unclear (128).

One protein is required for phosphate acquisition. Pit is a constitutively expressed low-affinity phosphate transporter (Fig. 2).

Lipids

Lipid biosynthesis is an essential function in most bacterial cells. The pathway starts with the carboxylation of acetyl-CoA derived from glycolysis (Fig. 1) and ends with the addition of a so-called head group to phosphatidic acid. Fatty acid biosynthesis is an essential process in nearly all bacteria, but this pathway is lacking in *Mycoplasma* species (129). Accordingly, fatty acid biosynthetic genes are absent from the minimal genome of *M. mycoides* JCVI-syn3.0. These organisms rely on fatty acid acquisition from their hosts or the provided complex medium (10). In total, this pathway requires 19 genes (Fig. 6). With the exception of *fabHA* and *fabI*, all of these genes are essential in *B. subtilis*. The two nonessential genes in this pathway have functional paralogs, and it is known that the encoded functions are essential; i.e., one of the paralogs has to be present (36). The pathway is initiated by the synthesis of malonyl-CoA from acetyl-CoA by the four-subunit enzyme complex acetyl-CoA carboxylase. It should be noted that AccB, one of the proteins of this complex, needs to be biotinylated by the essential biotin protein ligase BirA (130). After the formation of acetoacetyl-acyl carrier protein (ACP), the cycle of consecutive fatty acid elongation involves FabG, YwpB, FabI, and FabF. The formation of phospholipids is initiated by the replacement of the acyl carrier protein by a phosphate group and the subsequent addition of glycerol-3-phosphate. After the addition of the second fatty acid, the head group is attached to form phosphatidylglyc-

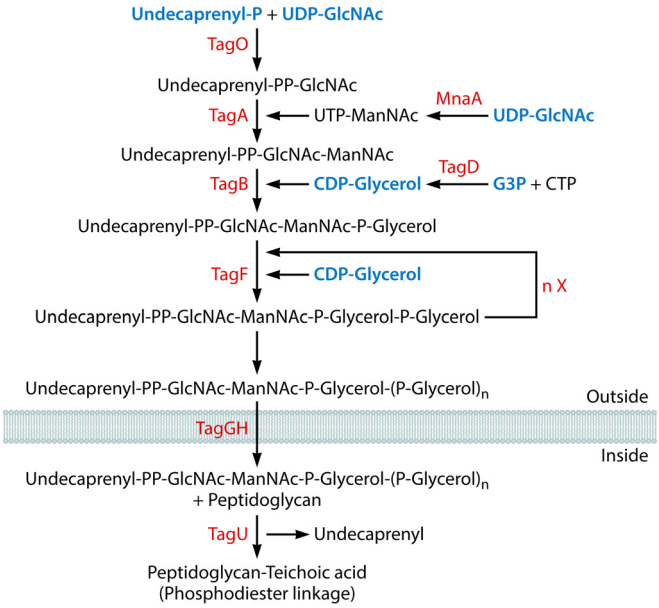

FIG 11 Biosynthesis of wall teichoic acids. The following metabolic intermediates that occur in other pathways are labeled in blue: undecaprenyl phosphate (Fig. 10), UDP *N*-acetylglucosamine (UDP-GlcNAc) (Fig. 10), CDP-glycerol (this figure), and glycerol-3-phosphate (G3P) (Fig. 6). UDP-ManNAc, UDP-*N*-acetylmannosamine.

erol. It should be noted that the enzyme catalyzing the final reaction of the pathway, the dephosphorylation of phosphatidylglycerol phosphate, has not yet been identified in *B. subtilis*. The lipids of *B. subtilis* 168 contain up to five different head groups (131). However, it has been established that membranes consisting of only phosphatidylglycerol do not confer any growth disadvantages (131). Therefore, we decided to keep only this head group.

Cofactors

B. subtilis needs 11 distinct cofactors, i.e., NAD, flavin adenine dinucleotide (FAD), pyridoxal phosphate, biotin, thiamine, lipoic acid, coenzyme A, *S*-adenosylmethionine, folate, heme, and menaquinone. For some of these molecules, their acquisition is possible by uptake and biosynthesis. For the minimal cell, we have chosen to include uptake whenever possible (nicotinate and riboflavin as precursors for NAD and FAD, respectively; biotin; and thiamine) (see Fig. 1 and 7 to 9 for details). As a result, 62 proteins are required for the acquisition of cofactors. Of these proteins, those necessary for the synthesis of NAD from nicotinate, the two subunits of the pyridoxal-5-phosphate synthase, the lipoic acid synthase, the enzyme for the final step of CoA synthesis, the *S*-adenosylmethionine synthetase, the dihydrofolate reductase, and the enzymes for menaquinone synthesis are essential (a total of 14 proteins). It is worth noting that MenH, an enzyme of the menachinone biosynthetic pathway, was recently shown to be dispensable (132, 133). While none of the cofactor biosynthetic pathways are present in *Mycoplasma* species, and thus are also lacking in *M. mycoides* JCVI-syn3.0, this minimal genome possesses all three general components of the so-called ECF (energy-coupling factor) transporters that are capable of transporting several cofactors (10). The substrate-specific S proteins for these transporters are generally poorly conserved and barely detectable in sequence comparisons (134, 135).

Metals and Iron-Sulfur Clusters

Many enzymes and proteins need metal ions for activity. Moreover, potassium and sodium are important for the osmotic stability of the cell. The minimal *B. subtilis* cell has to transport sodium, potassium, iron, magnesium, manganese, zinc, and copper ions. With the exception of iron, we have included one transporter (usually of low affinity) for each ion. For iron uptake, the minimal cell should possess the EfeUO system for elemental iron uptake and the iron-citrate ABC transporter YhfQ-YfmCDEF (136, 137). Sodium is imported by amino acid-sodium symporters. It has to be exported by the Mrp complex (138) (Fig. 2). Surprisingly, in the genome of *M. mycoides* JCVI-syn3.0, only a potassium transporter and two putative transporters for magnesium have been annotated (10). The identities of other metal transporters in the minimal cell thus remain unclear.

Many proteins involved in redox reactions need iron-sulfur clusters for their activity. The synthesis of these clusters from cysteine and ferrous iron and their attachment to proteins involve seven proteins, five of which are essential and are therefore included (Fig. 7) (139).

Cell Wall Biosynthesis

Cell wall biosynthesis is intimately linked to cell division. First, we briefly discuss the set of enzymes that is involved in catalytic activities for the cell wall components, the peptidoglycan, and the teichoic and lipoteichoic acids. Fifty-five proteins (43 essential proteins) are required for these pathways.

Peptidoglycan synthesis starts with the synthesis of glucosamine-6-phosphate from glutamine and fructose-6-phosphate (140). The next important intermediate is UDP-*N*-acetylglucosamine (UDP-GlcNAc). This compound is converted to UDP-*N*-acetylmuramic acid, to which the 5 amino acids of the peptide are subsequently attached. The peptide contains three unusual amino acids, D-glutamate, *meso*-diaminopimelic acid, and D-alanine, which have to be provided by the action of racemases (D-Glu and D-Ala) or by a biosynthetic pathway starting from aspartate. The enzyme MraY replaces the activating UDP moiety by undecaprenyl phosphate, which has to be synthesized starting from pyruvate and glyceraldehyde-3-phosphate (see Fig. 1 and 10 for details). *N*-Acetylglucosamine is added to give rise to a molecule called lipid II (141). Owing to the undecaprenyl phosphate moiety, this molecule can integrate into the membrane and is then flipped to the outer side by the action of a flippase, MurJ. Most of the enzymes participating in the pathways mentioned above are essential. MurJ is an exception, as *B. subtilis* also possesses the weakly expressed functional paralog Amj (142).

Lipid II is the functional unit of peptidoglycan polymerization driven by the penicillin-binding proteins (141). These proteins catalyze the consecutive elongation of the peptidoglycan chain as well as the introduction of cross-links between the peptides. Moreover, autolysins are necessary to introduce breaks in the molecule that serve as targets for the introduction of new material. Penicillin-binding proteins and autolysins are present with several paralogs in *B. subtilis*. For the minimal cell, we have selected penicillin-binding proteins 1 (PonA), 2B (PbpB), and 2A (PbpA) and the autolysins LytE and LytF (143–147). As outlined above, this selection was made according to their expression profiles and the dependence on other proteins. As an example, there is a functional paralog of LytE, CwlO (148). For the activity of CwlO, *B. subtilis*

TABLE 2 The complete gene set of *MiniBacillus*[e]

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
Information							
DNA replication							
dnaA	BSU00010	Yes	Yes		2Z4R	*Thermotoga maritima*	Replication initiation protein
dnaB	BSU28990	Yes					Initiation of chromosome replication
dnaC	BSU40440	Yes	Yes	3.6.4.12	2VYE	*Geobacillus kaustophilus*	Replicative DNA helicase
dnaD	BSU22350	Yes			2V79	*B. subtilis*	Initiation of chromosome replication
dnaE	BSU29230	Yes	Yes	2.7.7.7	3E0D	*Thermus aquaticus*	DNA polymerase III (alpha subunit)
dnaG	BSU25210	Yes	Yes	2.7.7.-	4E2K	*S. aureus*	DNA primase
dnaI	BSU28980	Yes	Yes		4M4W	*B. subtilis*	Primosome component (helicase loader)
dnaN	BSU00020	Yes	Yes	2.7.7.7	4TR6	*B. subtilis*	DNA polymerase III (beta subunit), beta clamp
dnaX	BSU00190	Yes	Yes	2.7.7.7	1JR3	*E. coli*	DNA polymerase III (gamma and tau subunits)
holA	BSU25560	Yes	Yes	2.7.7.7	3ZH9	*B. subtilis*	DNA polymerase III, delta subunit
holB	BSU00310	Yes		2.7.7.7	1NJG	*E. coli*	DNA polymerase III (delta subunit)
ligA	BSU06620	Yes	Yes	6.5.1.2	2OWO	*E. coli*	DNA ligase (NAD dependent)
priA	BSU15710	Yes		3.6.4.-	4NL4	*Klebsiella pneumoniae*	Primosomal replication factor Y
polC	BSU16580	Yes	Yes	2.7.7.7	3F2B	*G. kaustophilus*	DNA polymerase III (alpha subunit)
rtp	BSU18490	No			1BM9	*B. subtilis*	Replication terminator protein
ssbA	BSU40900	Yes	Yes		3VDY	*B. subtilis*	Single-strand DNA-binding protein
yabA	BSU00330	No			5DOL	*B. subtilis*	Inhibitor of DnaA oligomerization
polA	BSU29090	No	Yes	2.7.7.7	1BGX	*T. aquaticus*	DNA polymerase I
Chromosome maintenance							
scpA	BSU23220	Yes			3ZGX	*B. subtilis*	DNA segregation/condensation protein
scpB	BSU23210	Yes	Yes		3W6J	*Geobacillus stearothermophilus*	DNA segregation/condensation protein
smc	BSU15940	Yes	Yes		3ZGX	*B. subtilis*	SMC protein
parE	BSU18090	Yes	Yes	5.99.1.-	4I3H	*Streptococcus pneumoniae*	Subunit of DNA topoisomerase IV
parC	BSU18100	Yes	Yes	5.99.1.-	2INR	*S. aureus*	Subunit of DNA topoisomerase IV
spoIIIE	BSU16800	No			2IUT	*Pseudomonas aeruginosa*	ATP-dependent DNA translocase
sftA	BSU29805	No			2IUT	*P. aeruginosa*	DNA translocase
codV	BSU16140	No			1A0P	*E. coli*	Site-specific integrase/recombinase
ripX	BSU23510	No			1A0P	*E. coli*	Site-specific integrase/recombinase
gyrB	BSU00060	Yes	Yes	5.99.1.3	4I3H	*S. pneumoniae*	DNA gyrase (subunit B)
gyrA	BSU00070	Yes	Yes	5.99.1.3	4DDQ	*B. subtilis*	DNA gyrase (subunit A)
topA	BSU16120	Yes	Yes	5.99.1.2	4RUL	*E. coli*	DNA topoisomerase I
hbs	BSU22790	Yes			1HUE	*G. stearothermophilus*	Nonspecific DNA-binding protein HBsu
Transcription							
rpoA	BSU01430	Yes	Yes	2.7.7.6	3IYD	*E. coli*	RNA polymerase alpha subunit
rpoB	BSU01070	Yes	Yes	2.7.7.6	3IYD	*E. coli*	RNA polymerase beta subunit
rpoC	BSU01080	Yes	Yes	2.7.7.6	3IYD	*E. coli*	RNA polymerase beta′ subunit
sigA	BSU25200	Yes	Yes		3IYD	*E. coli*	RNA polymerase sigma factor SigA
rpoE	BSU37160	No		2.7.7.6	2KRC	*B. subtilis*	RNA polymerase delta subunit
helD	BSU33450	No		3.6.4.12			DNA 3′–5′ helicase IV
greA	BSU27320	No	Yes		1GRJ	*E. coli*	Transcription elongation factor
nusA	BSU16600	Yes	Yes		1HH2	*T. maritima*	Transcription termination factor
RNA folding and degradation							
cspD	BSU21930	No			1C9O	*Bacillus caldolyticus*	Cold shock protein
cspB	BSU09100	No			2ES2	*B. subtilis*	Major cold shock protein
rny	BSU16960	No	Yes	3.1.4.16			RNase Y
rnjA	BSU14530	Yes	Yes		3ZQ4	*B. subtilis*	RNase J1
pnpA	BSU16690	No		2.7.7.8	3CDI	*E. coli*	Polynucleotide phosphorylase
nrnA	BSU29250	No	Yes	3.1.3.7	3DEV	*Staphylococcus haemolyticus*	Oligoribonuclease (nano-RNase)
Aminoacyl tRNA synthetases							
alaS	BSU27410	Yes	Yes	6.1.1.7	3HTZ	*E. coli*	Alanine-tRNA synthetase
argS	BSU37330	Yes	Yes	6.1.1.19	3FNR	*Campylobacter jejuni*	Arginyl-tRNA synthetase, universally conserved protein
asnS	BSU22360	Yes	Yes	6.1.1.22	1X54	*Pyrococcus horikoshii*	Asparagyl-tRNA synthetase
aspS	BSU27550	Yes	Yes	6.1.1.12	1EQR	*E. coli*	Aspartyl-tRNA synthetase
cysS	BSU00940	Yes	Yes	6.1.1.16	3TQO	*Coxiella burnetii*	Cysteine-tRNA synthetase
gatC	BSU06670	Yes		6.3.5.7	2DF4	*S. aureus*	Production of glutamyl-tRNA^{Gln}
gatA	BSU06680	Yes	Yes	6.3.5.7	2DF4	*S. aureus*	Production of glutamyl-tRNA^{Gln}
gatB	BSU06690	Yes	Yes	6.3.5.7	2DF4	*S. aureus*	Production of glutamyl-tRNA^{Gln}
gltX	BSU00920	Yes	Yes	6.1.1.17	2O5R	*T. maritima*	Glutamyl-tRNA synthetase, universally conserved protein
glyS	BSU25260	Yes		6.1.1.14			Glycyl-tRNA synthetase (beta subunit)
glyQ	BSU25270	Yes		6.1.1.14	1J5W	*T. maritima*	Glycyl-tRNA synthetase (alpha subunit)
hisS	BSU27560	Yes	Yes	6.1.1.21	1QE0	*S. aureus*	Histidyl-tRNA synthetase
ileS	BSU15430	Yes	Yes	6.1.1.5	1QU2	*S. aureus*	Isoleucyl-tRNA synthetase

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
leuS	BSU30320	Yes	Yes	6.1.1.4	1OBH	*Thermus thermophilus*	Leucyl-tRNA synthetase
lysS	BSU00820	Yes	Yes	6.1.1.6	3E9H	*G. stearothermophilus*	Lysyl-tRNA synthetase
metS	BSU00380	Yes	Yes	6.1.1.10	4QRD	*S. aureus*	Methionyl-tRNA synthetase
pheT	BSU28630	Yes	Yes	6.1.1.20	2RHS	*S. haemolyticus*	Phenylalanyl-tRNA synthetase (beta subunit)
pheS	BSU28640	Yes	Yes	6.1.1.20	2RHQ	*S. haemolyticus*	Phenylalanyl-tRNA synthetase (alpha subunit), universally conserved protein
proS	BSU16570	Yes		6.1.1.15	2J3L	*Enterococcus faecalis*	Prolyl-tRNA synthetase
serS	BSU00130	Yes	Yes	6.1.1.11	2DQ3	*Aquifex aeolicus*	Seryl-tRNA synthetase
thrS	BSU28950	No	Yes	6.1.1.3	1NYQ	*S. aureus*	Threonyl-tRNA synthetase (major)
trpS	BSU11420	Yes	Yes	6.1.1.2	3PRH	*B. subtilis*	Tryptophanyl-tRNA synthetase
tyrS	BSU29670	Yes	Yes	6.1.1.1	2TS1	*G. stearothermophilus*	Tyrosyl-tRNA synthetase (major)
valS	BSU28090	Yes	Yes	6.1.1.9	1GAX	*T. thermophilus*	Valyl-tRNA synthetase
Ribosomal proteins							
rplA	BSU01030	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L1
rplB	BSU01190	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L2
rplC	BSU01160	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L3
rplD	BSU01170	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L4
rplE	BSU01280	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L5
rplF	BSU01310	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L6
rplI	BSU40500	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L9
rplJ	BSU01040	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L10
rplK	BSU01020	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L11
rplL	BSU01050	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L12
rplM	BSU01490	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L13
rplN	BSU01260	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L14
rplO	BSU01350	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L15
rplP	BSU01230	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L16
rplQ	BSU01440	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L17
rplR	BSU01320	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L18
rplS	BSU16040	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L19
rplT	BSU28850	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L20
rplU	BSU27960	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L21
rplV	BSU01210	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L22
rplW	BSU01180	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L23
rplX	BSU01270	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L24
rpmA	BSU27940	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein L27
rpmB	BSU15820	No			3J9W	*B. subtilis*	Ribosomal protein L28
rpmC	BSU01240	No			3J9W	*B. subtilis*	Ribosomal protein L29
rpmD	BSU01340	Yes			3J9W	*B. subtilis*	Ribosomal protein L30
rpmE	BSU37070	No			3J9W	*B. subtilis*	Ribosomal protein L31
rpmF	BSU15080	No			3J9W	*B. subtilis*	Ribosomal protein L32
rpmGA	BSU24900	No			3J9W	*B. subtilis*	Ribosomal protein L33a
rpmGB	BSU00990	No			3J9W	*B. subtilis*	Ribosomal protein L33b
rpmH	BSU41060	No			3J9W	*B. subtilis*	Ribosomal protein L34
rpmI	BSU28860	No			3J9W	*B. subtilis*	Ribosomal protein L35
rpmJ	BSU01400	No	Yes		3J9W	*B. subtilis*	Ribosomal protein L36
rpsB	BSU16490	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S2
rpsC	BSU01220	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S3
rpsD	BSU29660	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S4
rpsE	BSU01330	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S5
rpsF	BSU40910	No	Yes		3J9W	*B. subtilis*	Ribosomal protein S6
rpsG	BSU01110	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S7
rpsH	BSU01300	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S8
rpsI	BSU01500	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S9
rpsJ	BSU01150	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S10
rpsK	BSU01420	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S11
rpsL	BSU01100	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S12
rpsM	BSU01410	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S13
rpsN	BSU01290	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S14
rpsO	BSU16680	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S15
rpsP	BSU15990	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S16
rpsQ	BSU01250	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S17
rpsR	BSU40890	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S18
rpsS	BSU01200	Yes	Yes		3J9W	*B. subtilis*	Ribosomal protein S19
rpsT	BSU25550	No			3J9W	*B. subtilis*	Ribosomal protein S20
rpsU	BSU25410	No			3J9W	*B. subtilis*	Ribosomal protein S21

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
rRNA/tRNA maturation and modification							
rnpA	BSU41050	Yes	Yes	3.1.26.5	3Q1R	*T. maritima*	Protein component of RNase P
rnpB	BSU_misc_RNA_35	Yes			3Q1R	*T. maritima*	RNA component of RNase P
rnz	BSU23840	Yes		3.1.26.11	4GCW	*B. subtilis*	RNase Z
rph	BSU28370	No		2.7.7.56	1OYP	*B. subtilis*	RNase PH
rbfA	BSU16650	No			1JOS	*Haemophilus influenzae*	Ribosome-binding factor A
rimM	BSU16020	No			3H9N	*H. influenzae*	16S rRNA-processing protein, RNase
cca	BSU22450	Yes		2.7.7.25	1MIY	*G. stearothermophilus*	tRNA nucleotidyltransferase
fmt	BSU15730	Yes	Yes	2.1.2.9	4IQF	*Bacillus anthracis*	Methionyl-tRNA formyltransferase
folD	BSU24310	Yes	Yes	1.5.1.5	1B0A	*E. coli*	Methylenetetrahydrofolate dehydrogenase
rlmCD	BSU06730	No		2.1.1.190	2BH2	*E. coli*	rRNA methyltransferase
ysgA	BSU28650	Yes	Yes	2.1.1.-	4X3M	*T. thermophilus*	Similar to rRNA methylase
mraW	BSU15140	No	Yes	2.1.1.199	1WG8	*T. thermophilus*	SAM-dependent methyltransferase
cspR	BSU08930	Yes		2.1.1.-	4PZK	*B. anthracis*	Similar to tRNA(Um34/Cm34) methyltransferase
trmD	BSU16030	Yes	Yes	2.1.1.31	3KY7	*S. aureus*	tRNA methyltransferase
trmU	BSU27500	Yes	Yes	2.8.1.-	2HMA	*S. pneumoniae*	tRNA(5-methylaminomethyl-2-thiouridylate) methyltransferase
yrvO	BSU27510	Yes		2.8.1.7	1P3W	*E. coli*	Cysteine desulfurase
yacO	BSU00960	No	Yes	2.1.1.-	1GZ0	*E. coli*	Putative 23S rRNA methyltransferase
ksgA	BSU00420	No	Yes	2.1.1.-	3FUU	*T. thermophilus*	rRNA adenine dimethyltransferase
rluB	BSU23160	No	Yes	5.4.99.22	4LAB	*E. coli*	Pseudouridine synthase
ypuI	BSU23200	No					rRNA pseudouridine 2633 synthase
tilS	BSU00670	Yes	Yes	6.3.4.19	3A2K	*G. kaustophilus*	$tRNA^{Ile}$ lysidine synthetase
tsaB	BSU05920	Yes			2A6A	*T. maritima*	Threonyl carbamoyl adenosine (t6A) modification of tRNAs that pair with ANN codons in mRNA
tsaD	BSU05940	Yes	Yes	2.3.1.234	3ZET	*Salmonella enterica* serovar Typhimurium	Threonyl carbamoyl adenosine (t6A) modification of tRNAs that pair with ANN codons in mRNA, universally conserved protein
tsaC	BSU36950	No		2.7.7.87	3AJE	*Sulfolobus tokodaii*	L-Threonyl carbamoyl AMP synthase, biosynthesis of the hypermodified base threonyl carbamoyl adenosine [t(6)A]
gidA	BSU41010	No	Yes		3CP2	*E. coli*	tRNA uridine 5-carboxymethyl-aminomethyl modification enzyme
thdF	BSU41020	No	Yes		1XZP	*T. maritima*	GTP-binding protein, putative tRNA modification GTPase
truA	BSU01480	No	Yes	5.4.99.12	1VS3	*T. thermophilus*	Pseudouridylate synthase I, universally conserved protein
tsaE	BSU05910	No	Yes		1HTW	*H. influenzae*	P-loop ATPase
trmFO	BSU16130	No	Yes	2.1.1.74	3G5Q	*T. thermophilus*	tRNA:m(5)U-54 methyltransferase
miaA	BSU17330	No		2.5.1.8	2QGN	*Bacillus halodurans*	tRNA isopentenylpyrophosphate transferase
yaaJ	BSU00180	No			2B3J	*S. aureus*	tRNA-specific adenosine deaminase
ylyB	BSU15460	No	Yes	5.4.99.23	1V9F	*E. coli*	Similar to pseudouridylate synthase
Ribosome maturation/assembly							
ydiD	BSU05930	No		2.3.1.128	2CNM	*S. enterica*	Similar to ribosomal protein alanine *N*-acetyltransferase
ylxS	BSU16590	No			1IB8	*S. pneumoniae*	Similar to 30S ribosomal subunit maturation protein
prp	BSU27950	No			4PEO	*S. aureus*	Maturation of L27
engA	BSU22840	Yes			2HJG	*B. subtilis*	GTPase, ribosome 50S subunit assembly
era	BSU25290	Yes	Yes		3R9W	*A. aeolicus*	GTP-binding protein
obg	BSU27920	Yes	Yes	3.6.5.-	1LNZ	*B. subtilis*	GTP-binding protein
rbgA	BSU16050	Yes	Yes		1PUJ	*B. subtilis*	Assembly of the 50S subunit of the ribosome
yqeH	BSU25670	Yes			3H2Y	*B. anthracis*	Assembly/stability of the 30S subunit of the ribosome, assembly of the 70S ribosome
ysxC	BSU28190	Yes	Yes		1SVI	*B. subtilis*	Assembly of the 50S subunit of the ribosome
Translation factors							
efp	BSU24450	No	Yes		1YBY	*Clostridium thermocellum*	Elongation factor P
frr	BSU16520	Yes	Yes		4GFQ	*B. anthracis*	Ribosome recycling factor
fusA	BSU01120	Yes	Yes		2XEX	*S. aureus*	Elongation factor G
infA	BSU01390	Yes	Yes		4QL5	*S. pneumoniae*	Translation initiation factor IF-1
infB	BSU16630	Yes	Yes		1ZO1	*E. coli*	Translation initiation factor IF-2

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
infC	BSU28870	Yes	Yes		1TIG	*G. stearothermophilus*	Translation initiation factor IF-3
prfA	BSU37010	Yes	Yes		1ZBT	*Streptococcus mutans*	Peptide chain release factor 1
prfB	BSU35290	Yes			1MI6	*E. coli*	Peptide chain release factor 2
tsf	BSU16500	Yes	Yes		1EFU	*E. coli*	Elongation factor Ts
tufA	BSU01130	Yes	Yes	3.6.5.3	4R71	*E. coli*	Elongation factor Tu
lepA	BSU25510	No	Yes		4QJT	*T. thermophilus*	Elongation factor 4
Translation/others							
map	BSU01380	Yes	Yes	3.4.11.18	1O0X	*T. maritima*	Methionine aminopeptidase
ywkE	BSU37000	No	Yes	2.1.1.297	2B3T	*E. coli*	Similar to N^5-glutamine methyltransferase that modifies peptide release factors
ybxF	BSU01090	No			3V7E	*B. subtilis*	Similar to ribosomal protein L7 family
spoVC	BSU00530	Yes	Yes	3.1.1.29	4QT4	*S. pyogenes*	Putative peptidyl-tRNA hydrolase
ssrA	BSU_MISC_RNA_55	No			1P6V	*A. aeolicus*	tmRNA
smpB	BSU33600	No	Yes		1P6V	*A. aeolicus*	tmRNA-binding protein
Protein secretion							
scr	BSU_misc_RNA_2	Yes			4UE5	*B. subtilis*	Signal recognition particle RNA
ffh	BSU15980	Yes	Yes		4UE5	*B. subtilis*	Signal recognition particle component
ftsY	BSU15950	No	Yes		2XXA	*E. coli*	Signal recognition particle
yidC2	BSU23890	No			3WO6	*B. halodurans*	Sec-independent membrane protein translocase
secA	BSU35300	Yes	Yes		3DL8	*B. subtilis*	Preprotein translocase subunit (ATPase)
secE	BSU01000	Yes			3DL8	*B. subtilis*	Preprotein translocase subunit
secY	BSU01360	Yes	Yes		3DL8	*B. subtilis*	Preprotein translocase subunit, universally conserved protein
secG	BSU33630	No			3DL8	*B. subtilis*	Preprotein translocase subunit
sipS	BSU23310	No			4NV4	*B. anthracis*	Signal peptidase I
prsA	BSU09950	Yes		5.2.1.8	4WO7	*B. subtilis*	Protein secretion (posttranslocation molecular chaperone)
csaA	BSU19040	No			2NZH	*B. subtilis*	Molecular chaperone involved in protein secretion
lgt	BSU34990	No		2.4.99.-	5AZB	*E. coli*	Prolipoprotein diacylglyceryl transferase
lspA	BSU15450	No			5DIR	*P. aeruginosa*	Signal peptidase II
Proteolysis/quality control/chaperones							
htrB	BSU33000	No			3QO6	*Arabidopsis thaliana*	Serine protease
groES	BSU06020	Yes			1WE3	*T. thermophilus*	Chaperonin, universally conserved protein
groEL	BSU06030	Yes			1WE3	*T. thermophilus*	Chaperonin
dnaJ	BSU25460	No	Yes		3LZ8	*K. pneumoniae*	Activation of DnaK
dnaK	BSU25470	No	Yes		2V7Y	*G. kaustophilus*	Molecular chaperone
grpE	BSU25480	No			4ANI	*G. kaustophilus*	Activation of DnaK
tig	BSU28230	No			2MLX	*E. coli*	Trigger factor (prolyl isomerase)
Metabolism							
Central carbon metabolism							
Glycolysis							
ptsG	BSU13890	No	Yes	2.7.1.69			PTS glucose permease, EIICBA(Glc)
ptsH	BSU13900	No	Yes	2.7.11.-	2FEP	*B. subtilis*	HPr, general component of the PTS
ptsI	BSU13910	No	Yes	2.7.3.9	2WQD	*S. aureus*	Enzyme I, general component of the PTS
pgi	BSU31350	No	Yes	5.3.1.9	3IFS	*B. anthracis*	Glucose-6-phosphate isomerase
pfkA	BSU29190	No	Yes	2.7.1.11	4A3S	*B. subtilis*	Phosphofructokinase
fbaA	BSU37120	No		4.1.2.13	4TO8	*S. aureus*	Fructose 1,6-bisphosphate aldolase
tpi	BSU33920	No	Yes	5.3.1.1	2BTM	*G. stearothermophilus*	Triose phosphate isomerase
gapA	BSU33940	Yes	Yes	1.2.1.12	1GD1	*G. stearothermophilus*	Glyceraldehyde-3-phosphate dehydrogenase
pgk	BSU33930	No	Yes	2.7.2.3	1PHP	*G. stearothermophilus*	Phosphoglycerate kinase, universally conserved protein
pgm	BSU33910	Yes	Yes	5.4.2.1	1EJJ	*G. stearothermophilus*	Phosphoglycerate mutase
eno	BSU33900	Yes	Yes	4.2.1.11	4A3R	*B. subtilis*	Enolase, universally conserved protein
pyk	BSU29180	No	Yes	2.7.1.40	2E28	*G. stearothermophilus*	Pyruvate kinase
pdhA	BSU14580	Yes	Yes	1.2.4.1	3DUF	*G. stearothermophilus*	Pyruvate dehydrogenase (E1 alpha subunit)
pdhB	BSU14590	No	Yes	1.2.4.1	3DUF	*G. stearothermophilus*	Pyruvate dehydrogenase (E1 beta subunit)
pdhC	BSU14600	No	Yes	2.3.1.12	3DUF	*G. stearothermophilus*	Pyruvate dehydrogenase (dihydrolipoamide acetyltransferase E2 subunit)
pdhD	BSU14610	No	Yes	1.8.1.4	1EBD	*G. stearothermophilus*	Dihydrolipoamide dehydrogenase E3 subunit of both pyruvate and 2-oxoglutarate dehydrogenase complexes

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
Transhydrogenation cycle							
ytsJ	BSU29220	No		1.1.1.38	2A9F	*S. pyogenes*	Malic enzyme
malS	BSU29880	No		1.1.1.38	1LLQ	*Ascaris suum*	Malate dehydrogenase (decarboxylating)
Pentose phosphate pathway							
ykgB	BSU13010	No		3.1.1.31	3HFQ	*Lactobacillus plantarum*	6-Phosphogluconolactonase
rpe	BSU15790	No	Yes	5.1.3.1	1TQJ	*Synechocystis* sp.	Ribulose 5-phosphate 3-epimerase
tkt	BSU17890	No	Yes	2.2.1.1	3HYL	*B. anthracis*	Transketolase
zwf	BSU23850	No		1.1.1.49	1DPG	*Leuconostoc mesenteroides*	Glucose-6-phosphate dehydrogenase
gndA	BSU23860	No		1.1.1.44	2W8Z	*G. stearothermophilus*	NADP-dependent phosphogluconate dehydrogenase
ywlF	BSU36920	No	Yes		3HE8	*C. thermocellum*	Ribose-5-phosphate isomerase
ywjH	BSU37110	No		2.2.1.2	3R8R	*B. subtilis*	Transaldolase
Recycling of acetate							
acsA	BSU29680	No		6.2.1.1	2P2F	*S. enterica*	Acetyl-CoA synthetase
Respiration/energy							
ndh	BSU12290	No			4NWZ	*Caldalkalibacillus thermarum*	NADH dehydrogenase
Cytochrome aa_3							
qoxD	BSU38140	No					Cytochrome aa_3 quinol oxidase (subunit IV)
qoxC	BSU38150	No			1FFT	*E. coli*	Cytochrome aa_3 quinol oxidase (subunit III)
qoxB	BSU38160	No			1FFT	*E. coli*	Cytochrome aa_3 quinol oxidase (subunit I)
qoxA	BSU38170	No			1FFT	*E. coli*	Cytochrome aa_3 quinol oxidase (subunit II)
Cytochrome maturation							
resC	BSU23130	Yes					Part of heme translocase, required for cytochrome *c* synthesis
resB	BSU23140	Yes					Part of heme translocase, required for cytochrome *c* synthesis
ATPase							
atpC	BSU36800	No			2E5Y	*Bacillus* sp.	ATP synthase, F_1 (subunit epsilon)
atpD	BSU36810	No	Yes	3.6.3.14	1SKY	*Bacillus* sp.	ATP synthase, F_1 (subunit beta)
atpG	BSU36820	No	Yes		4XD7	*G. kaustophilus*	ATP synthase, F_1 (subunit gamma)
atpA	BSU36830	No	Yes	3.6.3.14	1SKY	*Bacillus* sp.	ATP synthase, F_1 (subunit alpha)
atpH	BSU36840	No					ATP synthase, F_1 (subunit delta)
atpF	BSU36850	No	Yes				ATP synthase, F_o (subunit b)
atpE	BSU36860	No			1WU0	*Bacillus* sp.	ATP synthase, F_o (subunit c)
atpB	BSU36870	No			1C17	*E. coli*	ATP synthase, F_o (subunit a)
atpI	BSU36880	No					ATP synthase (subunit i)
Amino acids							
Asp, Glu							
gltT	BSU10220	No			3V8F	*P. horikoshii*	Major H^+/Na^+-glutamate symport protein
Arg							
rocE	BSU40330	No			3LRB	*E. coli*	Amino acid permease
Pro							
putP	BSU03220	No			2XQ2	*Vibrio parahaemolyticus*	High-affinity proline permease
Trp							
trpP	BSU10010	No					S protein of tryptophan ECF transporter
Met							
metQ	BSU32730	No			4GOT	*B. subtilis*	Methionine ABC transporter (binding lipoprotein)
metP	BSU32740	No			3DHW	*E. coli*	Methionine ABC transporter, permease
metN	BSU32750	No			3DHW	*E. coli*	Methionine ABC transporter (ATP-binding protein)
His							
hutM	BSU39390	No					Histidine permease
Cys							
tcyP	BSU09130	No	Yes		3KBC	*P. horikoshii*	Cystine transporter
Gly							
glyA	BSU36900	Yes	Yes	2.1.2.1	1KKJ	*G. stearothermophilus*	Serine hydroxymethyltransferase
Ile, Val, Thr							
bcaP	BSU09460	No					Branched-chain amino acid transporter
Lys							
yvsH	BSU33330	No			3LRB	*E. coli*	Putative lysine transporter

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
Chorismate for aromatic amino acids, menaquinone, and folate							
aroA	BSU29750	No		2.5.1.54	3NVT	*L. monocytogenes*	3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase/chorismate mutase isozyme 3
aroB	BSU22700	No		4.2.3.4	3CLH	*Helicobacter pylori*	3-Dehydroquinate synthase
aroC	BSU23080	No		4.2.1.10	1QFE	*S. enterica* serovar Typhi	3-Dehydroquinate dehydratase
aroD	BSU25660	No		1.1.1.25	2EGG	*G. kaustophilus*	Shikimate dehydrogenase
aroE	BSU22600	No		2.5.1.19	3RMT	*B. halodurans*	3-Phosphoshikimate 1-carboxyvinyltransferase
aroF	BSU22710	No		4.2.3.5	1Q1L	*A. aeolicus*	Chorismate synthase
aroK	BSU03150	No		2.7.1.71	2PT5	*A. aeolicus*	Shikimate kinase
Phe, Tyr							
pheA	BSU27900	No		4.2.1.51	4LUB	*S. mutans*	Prephenate dehydratase
hisC	BSU22620	No		2.6.1.9	3FFH	*Listeria innocua*	Histidinol-phosphate aminotransferase/tyrosine and phenylalanine aminotransferase
aroH	BSU22690	No			1COM	*B. subtilis*	Chorismate mutase (isozymes 1 and 2)
tyrA	BSU22610	No		1.3.1.12	3DZB	*Streptococcus thermophilus*	Prephenate dehydrogenase
Asn							
asnB	BSU30540	No		6.3.5.4	1CT9	*E. coli*	Asparagine synthase (glutamine hydrolyzing)
Ala, Ser							
alaT	BSU31400	No		2.6.1.-	1DJU	*P. horikoshii*	Alanine aminotransferase
serA	BSU23070	No		1.1.1.95	1YGY	*Mycobacterium tuberculosis*	Phosphoglycerate dehydrogenase
serC	BSU10020	No		2.6.1.52	1W23	*Bacillus alcalophilus*	3-Phosphoserine aminotransferase
ywtE	BSU35850	No			1NRW	*B. subtilis*	Putative phosphatase
Leu							
yvbW	BSU34010	No			3GI9	*Methanocaldococcus jannaschii*	Putative leucine permease
Gln							
glnA	BSU17460	No		6.3.1.2	4S0R	*B. subtilis*	Glutamine synthetase
Nucleotides/phosphate							
PRPP							
prs	BSU00510	Yes	Yes	2.7.6.1	1DKR	*B. subtilis*	Phosphoribosylpyrophosphate synthetase, universally conserved protein
Pyrimidine biosynthesis							
pyrAA	BSU15510	No		6.3.5.5	1JDB	*E. coli*	Carbamoyl-phosphate synthetase (glutaminase subunit)
pyrAB	BSU15520	No		6.3.5.5	1JDB	*E. coli*	Carbamoyl-phosphate synthetase (catalytic subunit)
pyrB	BSU15490	No		2.1.3.2	3R7D	*B. subtilis*	Aspartate carbamoyltransferase
pyrC	BSU15500	No		3.5.2.3	3MPG	*B. anthracis*	Dihydro-orotase
pyrD	BSU15540	No		1.3.3.1	1EP1	*Lactococcus lactis*	Dihydro-orotic acid dehydrogenase (catalytic subunit)
pyrE	BSU15560	No		2.4.2.10	3M3H	*B. anthracis*	Orotate phosphoribosyltransferase
pyrF	BSU15550	No		4.1.1.23	1DBT	*B. subtilis*	Orotidine 5′-phosphate decarboxylase
cmk	BSU22890	Yes	Yes	2.7.4.14	1Q3T	*S. pneumoniae*	Cytidylate kinase (CMP, dCMP)
pyrG	BSU37150	Yes	Yes	6.3.4.2	1S1M	*E. coli*	CTP synthase (NH_3, glutamine)
yncF	BSU17660	No			2XCD	*B. subtilis*	dUTPase
thyB	BSU21820	No		2.1.1.45	3IX6	*Brucella melitensis*	Thymidylate synthase B
tmk	BSU00280	Yes	Yes	2.7.4.9	2CCJ	*S. aureus*	Thymidylate kinase
Purine biosynthesis							
purF	BSU06490	No		2.4.2.14	1GPH	*B. subtilis*	Glutamine phosphoribosyldiphosphate amidotransferase
purD	BSU06530	No		6.3.4.13	2XD4	*B. subtilis*	Phosphoribosylglycinamide synthetase
purN	BSU06510	No		2.1.2.2	3AV3	*G. kaustophilus*	Phosphoribosylglycinamide formyltransferase
purS	BSU06460	No			1TWJ	*B. subtilis*	Phosphoribosylformylglycinamidine synthase
purQ	BSU06470	No		6.3.5.3	3D54	*T. maritima*	Phosphoribosylformylglycinamidine synthase
purL	BSU06480	No		6.3.5.3	3VIU	*T. thermophilus*	Phosphoribosylformylglycinamidine synthase
purM	BSU06500	No		6.3.3.1	2BTU	*B. anthracis*	Phosphoribosylaminoimidazole synthetase
purE	BSU06420	No		4.1.1.21	1XMP	*B. anthracis*	Phosphoribosylaminoimidazole carboxylase (ATP dependent)
purK	BSU06430	No		4.1.1.21	4DLK	*B. anthracis*	Phosphoribosylaminoimidazole carboxylase (ATP dependent)

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
purC	BSU06450	No		6.3.2.6	2YWV	*G. kaustophilus*	Phosphoribosylaminoimidazole succinocarboxamide synthase
purB	BSU06440	No		4.3.2.2	1F1O	*B. subtilis*	Adenylsuccinate lyase
purH	BSU06520	No		2.1.2.3	3ZZM	*M. tuberculosis*	Phosphoribosylaminoimidazole carboxamide formyltransferase
guaB	BSU00090	Yes		1.1.1.205	3TSB	*B. anthracis*	IMP dehydrogenase
guaA	BSU06360	No		6.3.5.2	1GPM	*E. coli*	GMP synthase (glutamine hydrolyzing)
gmk	BSU15680	Yes	Yes	2.7.4.8	3TAU	*L. monocytogenes*	Guanylate kinase (GMP:dATP, dGMP:ATP)
purA	BSU40420	No		6.3.4.4	4M0G	*B. anthracis*	Adenylosuccinate synthetase
adk	BSU01370	Yes	Yes	2.7.4.3	1P3J	*B. subtilis*	Adenylate kinase
Pyrimidine/purine biosynthesis							
nrdE	BSU17380	Yes	Yes		1PEM	*S. enterica*	Ribonucleoside diphosphate reductase (major subunit)
nrdF	BSU17390	Yes	Yes		4DR0	*B. subtilis*	Ribonucleoside diphosphate reductase (major subunit)
nrdI	BSU17370	Yes			1RLJ	*B. subtilis*	Ribonucleoside diphosphate reductase
ndk	BSU22730	No		2.7.4.6	2VU5	*B. anthracis*	Nucleoside diphosphate kinase
hprT	BSU00680	Yes	Yes	2.4.2.8	3H83	*B. anthracis*	Hypoxanthine phosphoribosyltransferase
Phosphate							
pit	BSU12840	No					Low-affinity inorganic phosphate transporter
Lipids							
Malonyl-CoA synthesis							
accC	BSU24340	Yes		6.3.4.14	2VPQ	*S. aureus*	Acetyl-CoA carboxylase (biotin carboxylase subunit)
accB	BSU24350	Yes		6.4.1.2	4HR7	*E. coli*	Acetyl-CoA carboxylase (biotin carboxyl carrier subunit)
accA	BSU29200	Yes		6.4.1.2	2F9I	*S. aureus*	Acetyl-CoA carboxylase (alpha subunit)
accD	BSU29210	Yes		6.4.1.2	2F9I	*S. aureus*	Acetyl-CoA carboxylase (beta subunit)
birA	BSU22440	Yes		6.3.4.15	3RIR	*S. aureus*	Biotin protein ligase
Acyl carrier							
acpS	BSU04620	Yes		2.7.8.7	1F80	*B. subtilis*	Acyl carrier protein synthase, 4′-phosphopantetheine transferase
acpA	BSU15920	Yes			1F80	*B. subtilis*	Acyl carrier protein
Aceto-acyl-Acp synthesis							
fabD	BSU15900	Yes		2.3.1.39	3QAT	*Bartonella henselae*	Malonyl-CoA—acyl carrier protein transacylase
fabHA	BSU11330	No		2.3.1.180	1ZOW	*S. aureus*	Beta-ketoacyl–acyl carrier protein synthase III
β-Ketoacyl-Acp chain elongation							
fabG	BSU15910	Yes		1.1.1.100	2UVD	*B. anthracis*	Beta-ketoacyl–acyl carrier protein reductase
fabF	BSU11340	Yes		2.3.1.179	4LS5	*B. subtilis*	Beta-ketoacyl–acyl carrier protein synthase II
fabI	BSU11720	No		1.3.1.9	3OIF	*B. subtilis*	Enoyl-acyl carrier protein reductase
ywpB	BSU36370	Yes			1U1Z	*P. aeruginosa*	β-Hydroxyacyl (acyl carrier protein) dehydratase
Phosphatidic acid synthesis							
plsC	BSU09540	Yes		2.3.1.51			Acyl-ACP:1-acylglycerolphosphate acyltransferase
plsX	BSU15890	Yes	Yes		1VI1	*B. subtilis*	Acyl-ACP:phosphate acyltransferase
plsY	BSU18070	Yes	Yes				Acylphosphate:glycerol-phosphate acyltransferase
gpsA	BSU22830	Yes		1.1.1.94	1Z82	*T. maritima*	Glycerol-3-phosphate dehydrogenase (NAD)
Phosphatidylglycerol phosphate synthesis							
cdsA	BSU16540	Yes	Yes	2.7.7.41	4Q2G	*T. maritima*	Phosphatidate cytidylyltransferase
pgsA	BSU16920	Yes	Yes	2.7.8.5			Phosphatidylglycerophosphate synthase
Cofactors							
ECF transporter (general component) for riboflavin, biotin, thiamine, tryptophan							
ybxA	BSU01450	No	Yes		4HUQ	*Lactobacillus brevis*	ATP-binding A1 component of ECF transporters
ybaE	BSU01460	No	Yes		4HUQ	*L. brevis*	ATP-binding A2 component of ECF transporters

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
ybaF	BSU01470	No	Yes		4HUQ	*L. brevis*	Transmembrane T component of ECF transporters
NAD							
nadD	BSU25640	Yes		2.7.7.18	1KAM	*B. subtilis*	Nicotinamide-nucleotide adenylyltransferase
nadE	BSU03130	Yes	Yes	6.3.1.5	1NSY	*B. subtilis*	NH_3-dependent NAD^+ synthetase
nadF	BSU11610	Yes	Yes	2.7.1.23	2I1W	*L. monocytogenes*	NAD kinase
niaP	BSU02950	No			4J05	*Piriformospora indica*	Nicotinate transporter
pncB	BSU31750	Yes		2.4.2.11	2F7F	*E. faecalis*	Putative nicotinate phosphoribosyltransferase
Riboflavin/FAD							
ribC	BSU16670	Yes		2.7.1.26	3OP1	*S. pneumoniae*	Riboflavin kinase/FAD synthase
ribU	BSU23050	No			3P5N	*S. aureus*	Riboflavin ECF transporter, S protein
Pyridoxal phosphate							
pdxS	BSU00110	No			2NV2	*B. subtilis*	Pyridoxal-5′-phosphate synthase (synthase domain)
pdxT	BSU00120	No			2NV2	*B. subtilis*	Pyridoxal-5′-phosphate synthase (glutaminase domain)
Biotin							
yhfU	BSU10370	No			4DVE	*L. lactis*	S protein of biotin ECF transporter
Thiamine, TPP							
yloS	BSU15800	No		2.7.6.2	3LM8	*B. subtilis*	Thiamine pyrophosphokinase
thiT	BSU30990	No			4MES	*L. lactis*	S protein of thiamine ECF transporter
Lipoate							
gcvH	BSU32800	No			3IFT	*M. tuberculosis*	Glycine cleavage system protein H, 2-oxo acid dehydrogenase
lipM	BSU24530	No			3A7A	*E. coli*	Octanoyltransferase
lipL	BSU37640	No			2P5I	*B. halodurans*	GcvH:E2 amidotransferase
lipA	BSU32330	Yes		2.8.1.8	4U0O	*T. elongatus*	Lipoic acid synthase
CoA							
ykpB	BSU14440	No			3HN2	*Geobacter metallireducens*	Putative ketopantoate reductase
panD	BSU22410	No		4.1.1.11	2C45	*M. tuberculosis*	Aspartate 1-decarboxylase
panC	BSU22420	No		6.3.2.1	2X3F	*S. aureus*	Pantothenate synthase
panB	BSU22430	No		2.1.2.11	1M3U	*E. coli*	3-Methyl-2-oxobutanoate hydroxymethyltransferase
ybgE	BSU02390	No		2.6.1.42	3HT5	*M. tuberculosis*	Branched-chain amino acid aminotransferase
coaA	BSU23760	No		2.7.1.33	4F7W	*K. pneumoniae*	Probable pantothenate kinase
yloI	BSU15700	No		4.1.1.36	1U7U	*E. coli*	Coenzyme A biosynthesis bifunctional protein CoaBC
ylbI	BSU15020	No		2.7.7.3	1O6B	*B. subtilis*	Pantetheine-phosphate adenylyltransferase
ytaG	BSU29060	Yes		2.7.1.24	4TTP	*Legionella pneumophila*	Dephospho-CoA kinase
SAM							
metK	BSU30550	Yes	Yes	2.5.1.6	1FUG	*E. coli*	*S*-Adenosylmethionine synthetase
Folate							
folE	BSU22780	No		3.5.4.16	4UQF	*L. monocytogenes*	GTP cyclohydrolase I
phoB	BSU05740	No		3.1.3.1	3A52	*Shewanella* sp.	Alkaline phosphatase A
folB	BSU00780	No		4.1.2.25	1RRI	*S. aureus*	Dihydroneopterin aldolase
folK	BSU00790	No		2.7.6.3	4CYU	*S. aureus*	2-Amino-4-hydroxy-6-hydroxymethyl-dihydropteridine diphosphokinase
sul	BSU00770	No		2.5.1.15	1TWS	*B. anthracis*	Dihydropteroate synthase
folC	BSU28080	No	Yes	6.3.2.17	1O5Z	*T. maritima*	Folyl-polyglutamate synthetase
dfrA	BSU21810	Yes		1.5.1.3	1ZDR	*G. stearothermophilus*	Dihydrofolate reductase
pabB	BSU00740	No		2.6.1.85	5CWA	*M. tuberculosis*	*p*-Aminobenzoate synthase (subunit A)
pabA	BSU00750	No		2.6.1.85	1I1Q	*S. enterica* serovar Typhimurium	*p*-Aminobenzoate synthase (subunit B)/ anthranilate synthase (subunit II)
pabC	BSU00760	No		4.1.3.38	4WHX	*Burkholderia pseudomallei*	Aminodeoxychorismate lyase
gsaB	BSU08710	No		5.4.3.8	3L44	*B. anthracis*	Formate dehydrogenase
ykkE	BSU13110	No		3.5.1.10	3W7B	*T. thermophilus*	Formyltetrahydrofolate deformylase
yoaE	BSU18570	No					Formate dehydrogenase
Heme biosynthesis							
hemE	BSU10120	No		4.1.1.37	2INF	*B. subtilis*	Glutamate-1-semialdehyde aminotransferase
hemH	BSU10130	No			1C1H	*B. subtilis*	Uroporphyrinogen decarboxylase (uroporphyrinogen III)
hemY	BSU10140	No		1.3.3.4	3I6D	*B. subtilis*	Ferrochelatase
ctaA	BSU14870	No					Protoporphyrinogen IX oxidase
ctaB	BSU14880	No					Heme A synthase

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
hemL	BSU28120	No		5.4.3.8	3BS8	*B. subtilis*	Heme O synthase (major enzyme)
hemB	BSU28130	No		4.2.1.24	1W5Q	*P. aeruginosa*	Glutamate-1-semialdehyde aminotransferase
hemD	BSU28140	No		4.2.1.75			Porphobilinogen synthase
hemC	BSU28150	No		2.5.1.61	4MLQ	*Bacillus megaterium*	Uroporphyrinogen III synthase
hemX	BSU28160	No					Hydroxymethylbilane synthase
hemA	BSU28170	No		1.2.1.70	4N7R	*A. thaliana*	Glutamyl-tRNA reductase
hemQ	BSU37670	No			1T0T	*G. stearothermophilus*	Heme-binding protein
Menaquinone							
menA	BSU38490	Yes					Probable 1,4-dihydroxy-2-naphthoate octaprenyltransferase
menH	BSU22750	No			4OBW	*Saccharomyces cerevisiae*	Menaquinone biosynthesis methyltransferase
menC	BSU30780	Yes		4.2.1.113	1WUE	*E. faecalis*	*O*-Succinylbenzoate-CoA synthase
menE	BSU30790	Yes		6.2.1.26	5BUQ	*B. subtilis*	*O*-Succinylbenzoate-CoA ligase
menB	BSU30800	Yes		4.1.3.36	2IEX	*G. kaustophilus*	Naphthoate synthase
menD	BSU30820	Yes		2.2.1.9	2X7J	*B. subtilis*	2-Succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase/2-oxoglutarate decarboxylase
ytxM	BSU30810	No			2XMZ	*S. aureus*	Similar to prolyl aminopeptidase
menF	BSU30830	No		5.4.4.2	3HWO	*E. coli*	Menaquinone-specific isochorismate synthase
Metals and iron-sulfur clusters							
Sodium export							
mrpA	BSU31600	Yes			4HE8	*T. thermophilus*	Na^+/H^+ antiporter subunit
mrpB	BSU31610	Yes					Na^+/H^+ antiporter subunit
mrpC	BSU31620	Yes					Na^+/H^+ antiporter subunit
mrpD	BSU31630	Yes			4HE8	*T. thermophilus*	Na^+/H^+ antiporter subunit
mrpE	BSU31640	No					Na^+/H^+ antiporter subunit
mrpF	BSU31650	Yes					Na^+/H^+ antiporter subunit
mrpG	BSU31660	No					Na^+/H^+ antiporter subunit
Potassium							
ktrD	BSU13500	No	Yes		4J7C	*B. subtilis*	Potassium transporter KtrCD
ktrC	BSU14510	No	Yes		4J90	*B. subtilis*	Potassium transporter KtrCD
Iron							
efeO	BSU38270	No			3AT7	*Sphingomonas* sp.	Lipoprotein, elemental iron uptake system (binding protein)
efeU	BSU38280	No					Elemental iron uptake system (permease)
yfmF	BSU07490	No			4G1U	*Yersinia pestis*	Iron/citrate ABC transporter (ATP-binding protein)
yfmE	BSU07500	No			4G1U	*Y. pestis*	Iron/citrate ABC transporter (permease)
yfmD	BSU07510	No			4G1U	*Y. pestis*	Iron/citrate ABC transporter (permease)
yfmC	BSU07520	No			3EIW	*S. aureus*	Iron/citrate ABC transporter (binding protein)
yhfQ	BSU10330	No			3EIW	*S. aureus*	Iron/citrate ABC transporter (solute-binding protein)
Magnesium							
mgtE	BSU13300	No			2YVX	*T. thermophilus*	Primary magnesium transporter
mntH	BSU04360	No			4WGV	*Staphylococcus capitis*	Manganese transporter (proton symport)
Zinc							
znuA	BSU02850	No			2O1E	*B. subtilis*	ABC transporter for zinc (binding protein)
znuC	BSU02860	No			4YMS	*Caldanaerobacter subterraneus*	ABC transporter for zinc (ATP-binding protein)
znuB	BSU02870	No					ABC transporter for zinc (permease)
Copper							
ycnJ	BSU03950	No					Copper transporter
Fe-S cluster							
sufB	BSU32670	Yes			5AWF	*E. coli*	Synthesis of Fe-S clusters
sufU	BSU32680	Yes	Yes		1XJS	*B. subtilis*	Iron-sulfur cluster scaffold protein
sufD	BSU32700	Yes			5AWF	*E. coli*	Synthesis of Fe-S clusters
sufS	BSU32690	Yes	Yes	2.8.1.7	1T3I	*Synechocystis* sp.	Cysteine desulfurase
sufC	BSU32710	Yes			2D2E	*T. thermophilus*	ABC transporter (ATP-binding protein)
fra	BSU05750	No			2OC6	*B. subtilis*	Frataxin-like protein
yutI	BSU32220	No			1XHJ	*Staphylococcus epidermidis*	Putative iron-sulfur scaffold protein
Cell division							
Cell wall synthesis							
Synthesis of D-glutamate							
racE	BSU28390	Yes		5.1.1.3	1ZUW	*B. subtilis*	Glutamate racemase

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TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
Synthesis of D-Ala-D-Ala							
alr	BSU04640	Yes		5.1.1.1	3ZM5	*S. pneumoniae*	Alanine racemase
ddl	BSU04560	Yes		6.3.2.4	2I80	*S. aureus*	D-Alanine-D-alanine ligase
Synthesis of *m*-diaminopimelate							
dapG	BSU16760	No		2.7.2.4	1SFT	*G. stearothermophilus*	Aspartokinase I (alpha and beta subunits)
asd	BSU16750	Yes		1.2.1.11	2GYY	*S. pneumoniae*	Aspartate-semialdehyde dehydrogenase
dapA	BSU16770	Yes		4.2.1.52	1XKY	*B. anthracis*	Dihydrodipicolinate synthase
dapB	BSU22490	Yes		1.3.1.26	5EER	*Corynebacterium glutamicum*	Dihydrodipicolinate reductase (NADPH)
ykuQ	BSU14180	Yes		2.3.1.89	3R8Y	*B. anthracis*	Similar to tetrahydrodipicolinate succinylase
patA	BSU14000	Yes			1GDE	*P. horikoshii*	Aminotransferase
dapI	BSU14190	Yes		3.5.1.47	1YSJ	*B. subtilis*	*N*-Acetyl-diaminopimelate deacetylase
dapF	BSU32170	Yes		5.1.1.7	2OTN	*B. anthracis*	Diaminopimelate epimerase
Isoprenoid biosynthesis							
dxs	BSU24270	Yes		2.2.1.7	2O1S	*E. coli*	1-Deoxyxylulose-5-phosphate synthase
ispC	BSU16550	Yes		1.1.1.267	1R0K	*Zymomonas mobilis*	1-Deoxy-D-xylulose-5-phosphate reductoisomerase
ispD	BSU00900	Yes		2.7.7.60	2YC3	*A. thaliana*	2-*C*-Methyl-D-erythritol 4-phosphate cytidylyltransferase
ispE	BSU00460	Yes		2.7.1.148	3PYD	*M. tuberculosis*	4-Diphosphocytidyl-2-*C*-methyl-D-erythritol kinase
ispF	BSU00910	Yes		4.6.1.12	3GHZ	*S. enterica* serovar Typhimurium	2-*C*-Methyl-D-erythritol-2,4-cyclodiphosphate synthase
ispG	BSU25070	Yes			3NOY	*A. aeolicus*	Similar to peptidoglycan acetylation, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate synthase
ispH	BSU25160	Yes			4N7B	*Plasmodium falciparum*	(*E*)-4-Hydroxy-3-methylbut-2-enyl diphosphate reductase
fni	BSU22870	No		5.3.3.2	1P0K	*B. subtilis*	Isopentenyl diphosphate isomerase
Undecaprenyl phosphate biosynthesis							
yqiD	BSU24280	No		2.5.1.10	1RTR	*S. aureus*	Geranyltransferase
uppS	BSU16530	Yes		2.5.1.31	1F75	*Micrococcus luteus*	Probable undecaprenyl pyrophosphate synthetase
bcrC	BSU36530	No					Undecaprenyl pyrophosphate phosphatase
hepT	BSU22740	Yes		2.5.1.30	3AQB	*M. luteus*	Heptaprenyl diphosphate synthase component II
hepS	BSU22760	Yes		2.5.1.30			Heptaprenyl diphosphate synthase component I
Peptidoglycan biosynthesis							
glmS	BSU01780	Yes		2.6.1.16	4AMV	*E. coli*	Glutamine:fructose-6-phosphate transaminase
glmM	BSU01770	Yes		5.4.2.10	3PDK	*B. anthracis*	Phosphoglucosamine mutase
gcaD	BSU00500	Yes		2.7.7.23	4AAW	*S. pneumoniae*	UDP-*N*-acetylglucosamine pyrophosphorylase
murAA	BSU36760	Yes		2.5.1.7	3SG1	*B. anthracis*	UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase
murB	BSU15230	Yes		1.1.1.158	4PYT	Unidentified	UDP-*N*-acetylenolpyruvoylglucosamine reductase
murC	BSU29790	Yes		6.3.2.8	1GQQ	*H. influenzae*	UDP-*N*-acetylmuramoyl-L-alanine synthetase
murD	BSU15200	Yes		6.3.2.9	3LK7	*Streptococcus agalactiae*	UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate synthetase
murE	BSU15180	Yes		6.3.2.13	4C13	*S. aureus*	UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamyl-*meso*-2,6-diaminopimelate synthetase
murF	BSU04570	Yes		6.3.2.10	1GG4	*E. coli*	UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamyl-*meso*-2,6-diaminopimeloyl-D-alanyl-D-alanine synthetase
mraY	BSU15190	Yes		2.7.8.13	4J72	*A. aeolicus*	Phospho-*N*-acetylmuramoyl-pentapeptide transferase (*meso*-2,6-diaminopimelate)
murG	BSU15220	Yes		2.4.1.227	1F0K	*E. coli*	UDP-*N*-acetylglucosamine-*N*-acetylmuramyl-(pentapeptide)pyrophosphoryl-undecaprenol *N*-acetylglucosamine transferase
murJ	BSU30050	No					Lipid II flippase

(Continued on following page)

TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
Peptidoglycan polymerization							
ponA	BSU22320	No			3DWK	*S. aureus*	Penicillin-binding protein 1A/1B
PG cross-links, cell separation							
pbpB	BSU15160	Yes			1RP5	*S. pneumoniae*	Penicillin-binding protein 2B
pbpA	BSU25000	No			3VSK	*S. aureus*	Penicillin-binding protein 2A
lytE	BSU09420	No			4XCM	*T. thermophilus*	Cell wall hydrolase (major autolysin) for cell elongation and separation
lytF	BSU09370	No			4XCM	*T. thermophilus*	Gamma-D-glutamate-*meso*-diaminopimelate muropeptidase (major autolysin)
Wall teichoic acid							
tagO	BSU35530	Yes			4J72	*A. aeolicus*	Undecaprenyl phosphate-GlcNAc-1-phosphate transferase
mnaA	BSU35660	Yes		5.1.3.14	4FKZ	*B. subtilis*	UDP-*N*-acetylglucosamine 2-epimerase
tagA	BSU35750	Yes		2.4.1.187			UDP-*N*-acetyl-D-mannosamine transferase
tagB	BSU35760	Yes			3L7I	*S. epidermidis*	Putative CDP-glycerol:glycerol phosphate glycerophosphotransferase
tagD	BSU35740	Yes		2.7.7.39	1COZ	*B. subtilis*	Glycerol-3-phosphate cytidylyltransferase
tagF	BSU35720	Yes		2.7.8.12	3L7I	*S. epidermidis*	CDP-glycerol:polyglycerol phosphate glycerophosphotransferase
tagH	BSU35700	Yes		3.6.3.40			ABC transporter for teichoic acid translocation (ATP-binding protein)
tagG	BSU35710	Yes					ABC transporter for teichoic acid translocation (permease)
tagU	BSU35650	No			3OWQ	*L. innocua*	Phosphotransferase, attachment of anionic polymers to peptidoglycan
Lipoteichoic acid							
dgkB	BSU06720	Yes			2QV7	*S. aureus*	Diacylglycerol kinase
pgcA	BSU09310	No	Yes	5.4.2.2	2Z0F	*T. thermophilus*	Alpha-phosphoglucomutase
gtaB	BSU35670	No	Yes	2.7.7.9	2UX8	*Sphingomonas elodea*	UTP-glucose-1-phosphate uridylyltransferase
ltaS	BSU07710	No			2W8D	*B. subtilis*	Lipoteichoic acid synthase
ugtP	BSU21920	No					UDP-glucose diacylglycerol glucosyltransferase
Coordination							
Divisome							
divIC	BSU00620	Yes					Cell division initiation protein (septum formation)
ftsL	BSU15150	Yes					Cell division protein (septum formation)
divIB	BSU15240	Yes			1YR1	*G. stearothermophilus*	Cell division initiation protein (septum formation)
ftsZ	BSU15290	Yes			2VAM	*B. subtilis*	Cell division initiation protein (septum formation)
ftsW	BSU14850	Yes					Cell division protein
ezrA	BSU29610	No			4UXV	*B. subtilis*	Negative regulator of FtsZ ring formation
sepF	BSU15390	No			3ZIH	*B. subtilis*	Part of the divisome
gpsB	BSU22180	No			4UG3	*B. subtilis*	Removal of PBP1 from the cell pole after completion of cell pole maturation
yvcK	BSU34760	No			2HZB	*B. halodurans*	Correct localization of PBP1, essential for growth under gluconeogenic conditions
yvcL	BSU34750	No	Yes		3HYI	*T. maritima*	Involved in Z-ring assembly
Division site selection							
divIVA	BSU15420	No			2WUJ	*B. subtilis*	Cell division initiation protein (septum placement)
minC	BSU28000	No			2M4I	*B. subtilis*	Cell division inhibitor (septum placement)
minD	BSU27990	No			4V03	*A. aeolicus*	Cell division inhibitor (septum placement)
noc	BSU40990	No			1VZ0	*T. thermophilus*	DNA-binding protein, spatial regulator of cell division to protect the nucleoid, coordination of chromosome segregation and cell division
minJ	BSU35220	No					Topological determinant of cell division
Elongasome							
mreD	BSU28010	Yes					Cell shape-determining protein, associated with the MreB cytoskeleton
mreC	BSU28020	Yes			2J5U	*L. monocytogenes*	Cell shape-determining protein, associated with the MreB cytoskeleton

(Continued on following page)

TABLE 2 (Continued)

Gene	BSU no.[a]	Essential[b]	Syn3.0[c]	EC no.	PDB accession no.	Organism[d]	Function(s)
mreB	BSU28030	Yes			4CZE	*Caulobacter vibrioides*	Cell shape-determining protein
rodA	BSU38120	Yes					Control of cell shape and elongation
mreBH	BSU14470	No			1JCF	*T. maritima*	Cell shape-determining protein
rodZ	BSU16910	Maybe					Required for cell shape determination
Coordination of cell division and DNA replication							
walJ	BSU40370	No			4P62	*P. aeruginosa*	Coordination of cell division and DNA replication
Signaling							
walK	BSU40400	Yes			4I5S	*S. mutans*	Two-component sensor kinase
walR	BSU40410	Yes			2ZWM	*B. subtilis*	Two-component response regulator
cdaA	BSU01750	No		2.7.7.85	4RV7	*L. monocytogenes*	Diadenylate cyclase
gdpP	BSU40510	No					c-di-AMP-specific phosphodiesterase
Integrity of the cell							
Protection							
ytbE	BSU29050	Yes			3B3D	*B. subtilis*	Putative aldo/keto reductase
katA	BSU08820	No			1SI8	*E. faecalis*	Vegetative catalase 1
sodA	BSU25020	No			2RCV	*B. subtilis*	Superoxide dismutase
ahpC	BSU40090	No			1WE0	*Amphibacillus xylanus*	Alkyl hydroperoxide reductase (small subunit)
ahpF	BSU40100	No			4O5Q	*E. coli*	Alkyl hydroperoxide reductase (large subunit)/NADH dehydrogenase
trxA	BSU28500	Yes	Yes		2GZY	*B. subtilis*	Antioxidative action by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange
yumC	BSU32110	Yes		1.18.1.2	3LZW	*B. subtilis*	Ferredoxin-NAD(P)$^+$ oxidoreductase
trxB	BSU34790	Yes	Yes	1.8.1.9	4GCM	*S. aureus*	Thioredoxin reductase (NADPH)
Repair/genome integrity							
hlpB	BSU10660	Yes					HNH nuclease-like protein, rescues AddA recombination intermediates
mutY	BSU08630	No			5DPK	*G. stearothermophilus*	A/G-specific adenine glycosylase
polY1	BSU23870	No		2.7.7.7	4IRC	*E. coli*	Translesion synthesis DNA polymerase Y1
mutM	BSU29080	No		3.2.2.23	1L1T	*G. stearothermophilus*	Formamidopyrimidine-DNA glycosidase
mfd	BSU00550	No			2EYQ	*E. coli*	Transcription repair-coupling factor
recD2	BSU27480	No			3E1S	*Deinococcus radiodurans*	5′–3′ DNA helicase replication fork progression
rnhB	BSU16060	No			3O3G	*T. maritima*	RNase HII, endoribonuclease
recA	BSU16940	No			1UBC	*Mycobacterium smegmatis*	Homologous recombination and DNA repair
Other/unknown							
ppaC	BSU40550	Yes		3.6.1.1	1WPM	*B. subtilis*	Inorganic pyrophosphatase
ylaN	BSU14840	Yes			2ODM	*S. aureus*	Unknown
yitI	BSU11000	No			2JDC	*Bacillus licheniformis*	Unknown
yitW	BSU11160	No			3LNO	*B. anthracis*	Unknown
yqhY	BSU24330	No					Unknown
ykwC	BSU13960	No			3WS7	*Pyrobaculum calidifontis*	Putative beta-hydroxy acid dehydrogenase
ylbN	BSU15070	No					Unknown
ypfD	BSU22880	No			4Q7J	*E. coli*	Similar to ribosomal protein S1
yugI	BSU31390	No			2K4K	*B. subtilis*	Similar to polyribonucleotide nucleotidyltransferase
floT	BSU31010	No					Similar to flotillin 1, orchestration of physiological processes in lipid microdomains
yyaF	BSU40920	No	Yes		1JAL	*H. influenzae*	GTP-binding protein/GTPase

[a] The BSU number is the locus tag in the context of the *B. subtilis* 168 genome (GenBank accession no. NC_000964).
[b] The essentiality of a gene refers to wild-type *B. subtilis* 168. By definition, genes that cannot be deleted as individual genes under defined optimal growth conditions (Luria-Bertani broth with glucose at 37°C) are regarded as being essential.
[c] Each gene of the *MiniBacillus* gene set was tested for the presence of a homolog in *Mycoplasma mycoides* JCVI-syn3.0 by using a BLASTP analysis.
[d] The organism refers to the PDB accession number.
[e] PRPP, phosphoribosyl pyrophosphate; TPP, thiamine PP_i; SAM, *S*-adenosylmethionine; PTS, phosphotransferase system; PBP1, penicillin-binding protein 1.

also needs the ABC transporter FtsEX and the small protein Mbl (149). Thus, the choice of LytE allowed a smaller number of genes.

Another essential component of the Gram-positive cell wall is teichoic acids. In *B. subtilis*, these molecules can be attached to either peptidoglycan or the membrane via a lipid anchor. Wall teichoic acid is polyglycerol phosphate attached to the peptidoglycan via a disaccharide anchor (150). The pathway is initiated by the synthesis of the anchor from undecaprenyl phosphate and activated *N*-acetylglucosamine and *N*-acetylmannosamine (UDP-GlcNAc and UDP-*N*-acetylmannosamine [UDP-Man-

TABLE 3 rRNAs and tRNAs

rRNA(s) and/or tRNA(s)
rrnO-16S trnO-Ala trnO-Ile rrnO-23S rrnO-5S
trnSL-Ser1
rrnA-16S trnA-Ile trnA-Ala rrnA-23S rrnA-5S
trnSL-Met1 trnSL-Glu1
rrnJ-16S rrnJ-23S rrnJ-5S trnJ-Ala trnJ-Arg trnJ-Gly trnJ-Leu1 trnJ-Leu2 trnJ-Lys trnJ-Pro trnJ-Thr trnJ-Val rrnW-16S rrnW-23S rrnW-5S
rrnI-16S rrnI-23S rrnI-5S trnI-Ala trnI-Arg trnI-Asn trnI-Gly trnI-Pro trnI-Thr rrnH-16S rrnH-23S rrnH-5S rrnG-16S rrnG-23S rrnG-5S
trnSL-Gln2 trnSL-Glu2 trnSL-Thr1 trnSL-Tyr1 trnSL-Val1
trnS-Asn trnS-Gln trnS-Glu trnS-Leu1 trnS-Leu2 trnS-Lys trnS-Ser
trnE-Arg trnE-Gly rrnE-16S rrnE-23S rrnE-5S trnE-Asp trnE-Met
rrnD-16S rrnD-23S rrnD-5S trnD-Asn trnD-Asp trnD-Cys trnD-Gln trnD-Glu trnD-Gly trnD-His trnD-Leu1 trnD-Leu2 trnD-Met trnD-Phe trnD-Ser trnD-Thr trnD-Trp trnD-Tyr trnD-Val
trnSL-Gly1
trnSL-Val2
trnSL-Arg1
trnSL-Gln1
trnSL-Arg2
rrnB-16S rrnB-23S rrnB-5S trnB-Ala trnB-Arg trnB-Asn trnB-Asp trnB-Glu trnB-Gly1 trnB-Gly2 trnB-His trnB-Ile2 trnB-Leu1 trnB-Leu2 trnB-Lys trnB-Met1 trnB-Met2 trnB-Met3 trnB-Phe trnB-Pro trnB-Ser1 trnB-Ser2 trnB-Thr trnB-Val
trnSL-Ala1
trnQ-Arg
trnY-Asp trnY-Glu trnY-Lys trnY-Phe

NAc]). Glycerol is activated by the synthesis of CDP-glycerol. This compound serves as the substrate for the initial addition of glycerol to the undecaprenyl phosphate-carrying disaccharide and then for consecutive rounds of chain elongation. The polymer is then exported by the TagGH ABC transporter and is finally attached to peptidoglycan by TagU (Fig. 11) (150). Lipoteichoic acid (LTA) is polyglycerol phosphate attached to the membrane via a β-diglucosyl-diacylglycerol anchor. This anchor is synthesized from glucose-6-phosphate and diacylglycerol by the enzyme UgtP (151). This anchor has to be flipped to the outside of the membrane by an as-yet-unknown flippase. The lipoteichoic acid synthase LtaS then processively adds glycerol phosphate moieties using phosphatidylglycerol, the building block of the lipid bilayer, as the substrate. This reaction releases toxic diacylglycerol, which is recycled by the essential diacylglycerol kinase DgkB to phosphatidic acid, which is used for the synthesis of phosphatidylglycerol (see above) (Fig. 6) (32, 152, 153).

Cell Division

Twenty-two proteins are necessary for the functions of cell division and cell shape determination. Of these proteins, nine are essential. Most of these proteins are part of several large protein complexes, i.e., the divisome, which links cell division to cell wall synthesis, and the elongasome, which recruits the cell wall biosynthetic enzyme to the lateral cell wall. Accordingly, cell wall biosynthetic enzymes (see above) are part of these complexes. This is the case for MraY, MurF, MurG, and penicillin-binding proteins 1 and 2B (PonA and PbpB, respectively), which are part of the divisome and/or the elongasome (154, 155). To achieve the correct placement of the division site, DivIVA in concert with the proteins of the Min system prevent the formation of the Z ring close to nascent division sites and cell poles (156, 157). Finally, WalJ coordinates cell division and DNA replication (158). Interestingly, the essential tubulin-like cell division initiation protein FtsZ has long been regarded as being present in all organisms. However, this protein is absent in some *Mycoplasma* species and is nonessential in those *Mycoplasma* species that encode it (159). Accordingly, FtsZ is also not encoded in the genome of *M. mycoides* JCVI-syn3.0 (10). Moreover, FtsZ is dispensable in *B. subtilis* L-form cells that are capable of growing without a cell wall (160, 161). However, as these cells grow very slowly and are fragile, we deemed the FtsZ-dependent cell division pathway essential in the context of the *MiniBacillus* genome.

Signaling in Cell Division

As in other bacteria, most signal transduction systems of *B. subtilis* are dispensable. This is the case for all alternative sigma factors as well as for classical transcription repressors and activators. However, there are two notable exceptions, and they are both involved in cell wall metabolism and cell division. The WalRK two-component regulatory system in *B. subtilis* and related Gram-positive bacteria is the only two-component system known to be essential. This system allows the expression of genes for cell division and the synthesis of wall teichoic acid, such as *lytE*, *mreBH*, *ftsZ*, and the *tag* genes (162, 163). Moreover, cyclic di-AMP (c-di-AMP) is a unique essential second messenger. The precise reason for the essentiality of this nucleotide is unknown, but c-di-AMP has been implicated in cell division and cell wall biosynthesis (164, 165). Interestingly, c-di-AMP is not only essential but also toxic if it accumulates (166). Therefore, CdaA was selected among the three diadenylate cyclases in *B. subtilis*, and GdpP was selected as one of two phosphodiesterases that degrade the second messenger c-di-AMP.

Integrity of the Cell (Protection and Genome Integrity)

All the functions discussed above are essential for the minimal cell. However, a minimal organism also needs activities that are not *per se* essential but that help to keep and protect the integrity of the cell. This involves protection against toxic metabolites and intermediates as well as against damage caused by oxygen (Fig. 2). In addition, the genome of the minimal cell has to be sufficiently stable. For this purpose, proteins involved in DNA repair and genome integrity are important components of any minimal cell that is intended for stable reproduction. In the list of required proteins, we have included eight proteins for protection and genome integrity.

Additional Proteins of the Minimal Cell

There are 11 additional proteins that may be important for a minimal cell. Two of these proteins (PpaC and YlaN) are essential, but their precise function is not known. Moreover, based on our own experimental data and those of colleagues, YitI, YitW, and YqhY are important for viability (P. Dos Santos, personal communication; our unpublished results). Four proteins (YkwC, YlbN, YpfD, and YugI) are very strongly expressed (39) and therefore certainly of vital importance. FloT is involved in the control of membrane fluidity, and the loss of both flotillin-like proteins is known to result in severe defects in cell morphology and transformation (167). Finally, the nonessential GTPase YyaF is highly conserved in Gram-positive bacteria, even among all groups of mollicutes (42). Due to its GTPase activity, it is tempting to speculate that YyaF plays a role in ribosome assembly or in translation.

Open Questions

Even though *B. subtilis* is one of the best-studied and best-understood organisms, there are still gaps in our knowledge. These gaps pose serious challenges to any minimal genome project. Several of these uncertainties are mentioned above. In particular, we have identified gaps in the pathways of phospholipid biosynthesis (the final phosphatase) and lipoteichoic acid synthesis (the flippase for the membrane anchor). Concerning phosphatase, there are several uncharacterized phosphatases encoded in the *B. subtilis* genome, leaving room for further research.

A general uncertainty is the disposal of toxic or harmful compounds. Even though we have included eight proteins for this activity, this list may be far from being complete. Another function with significant gaps in our knowledge is RNA modification. While our list is based on current knowledge, it is possible that not all of these modifications are truly essential. Moreover, some modifications might become important if other modifications are lacking due to the absence of the responsible enzymes.

Finally, only little is known about essential noncoding RNAs and essential structural DNA regions in *B. subtilis*. The importance of these features has been uncovered in a recent saturating-mutagenesis study with *M. pneumoniae* (8).

A MODEL OF *MiniBacillus* METABOLISM

The selection of functions, genes, and pathways presented above is like a bet. To ensure that this set allows the cell to function, we have developed a model of its metabolism. This model is schematically depicted in Fig. 1, and details are provided in Fig. 2 to 11. With this compilation of metabolic pathways, it becomes obvious that all metabolites that are needed as precursors are produced from simple building blocks. These molecules in turn can be obtained from the medium. On the other hand, all compounds that are produced in the pathways and that are not needed in other reactions are disposed of by specific reactions and pathways.

MiniBacillus AS A STARTING POINT TO DISCOVER AND STUDY NOVEL ANTIMICROBIAL DRUG TARGETS

B. subtilis is closely related to many important pathogens, including *Staphylococcus aureus*, *Listeria monocytogenes*, and *Streptococcus pyogenes*. The genetic complement of *MiniBacillus* likely represents a set of highly important genes for all of these organisms, suggesting that this set is an excellent starting point to identify promising novel potential antimicrobial drug targets. Indeed, the conserved essential diadenylate cyclase was recently proposed to be a potential drug target, screening systems have been set up, and a first drug has been discovered (168–170).

For drug targets, knowledge of the protein structure is highly beneficial to facilitate *in silico* screening of virtual compound libraries. Of all 523 proteins that are encoded by our proposed minimal genome, 471 (90.1%) have a known structure. This figure illustrates the huge progress made by structural biologists, especially in the framework of large structural genomic projects (171). The unique combination of high structural coverage and excellent prior knowledge of the protein components of *MiniBacillus* makes the list of proteins presented in Table 2 a good starting point to identify novel potential targets. Moreover, the remaining 10% of the proteins are an important challenge for structural biologists.

Knowing all the structures of the proteins and even protein complexes of *MiniBacillus* not only is important in the context of the search for novel drugs but also is by itself a major scientific goal. With knowledge of the structure of all the components of a minimal cell, we can start making a real image of what is going on in the cell and thus become much closer to the answer of the old question, "What is life?"

EXPERIMENTAL APPROACH TO THE CONSTRUCTION OF *MiniBacillus*

To obtain a minimal cell, *MiniBacillus*, with the described set of genes, the top-down approach seems to be the most appropriate. Such an approach requires efficient systems for markerless gene deletions, which in turn indicates a need for a highly efficient genetic system. *B. subtilis* is known for its natural competence. This system is under the control of a central transcription factor, ComK. In order to be able to perform consecutive deletions, the genes involved in genetic competence have to be kept until the very end of the successive genome reduction process. Therefore, another set of 54 genes has to be considered (see Table S1 in the supplemental material). Optimally, expression of competence genes would occur not just in 10% of the population as in the *B. subtilis* wild-type strain but in all cells of a population. For this purpose, a supercompetent *B. subtilis* strain was recently engineered (172). In this strain, the transcription factor ComK and ComS, a protein which prevents the proteolytic degradation of ComK, are expressed under the control of a strong mannitol-inducible promoter.

Several systems have been proposed for the construction of markerless deletions. The Cre-Lox system is very efficient, and it has been adapted to *B. subtilis* (173) but has the disadvantage of leaving scars behind. Such scars would accumulate if dozens of deletions were performed. Alternative systems rely on the integration of genes, which become toxic under defined conditions with a deletion cassette. Counterselection then allows the rapid and specific loss of the cassette, i.e., the markerless deletion of the desired regions. Several counterselection systems have been implemented for *B. subtilis*. The most popular systems rely on the *mazF* RNase; the *upp* gene, which is counterselected in the presence of 5-fluorouracil; and the mannose transporter ManP, which generates toxic mannose-6-phosphate (19). Using the latter system, a significant reduction of the *B. subtilis* genome was recently achieved (19, 174, 175).

In *B. subtilis*, many important genes that are part of the minimal gene set listed in Table 2 are scattered on the chromosome. Therefore, defragmentation of such regions will facilitate the effective progress of a genome reduction program. For this defragmentation, desired fragments can be assembled and introduced into the chromosome by using, e.g., Gibson assembly or the ligase cycling reaction in combination with the markerless deletion methods mentioned above (176, 177).

FINAL REMARKS

There is not a single solution for the question of a minimal genome, but there are many possible answers. To fully understand the functionality of living cells, minimal organisms based on different model organisms are required. Thus, the construction of *M. mycoides* JCVI-syn3.0 marks an important breakthrough, but with its large number of unknown genes, it cannot provide the full picture (10). It will be essential to continue genome reduction projects with very different bacteria, yeasts, and even archaea to be able to finally draw a conclusion about the essence of life.

(For more information, see http://www.minibacillus.org/.)

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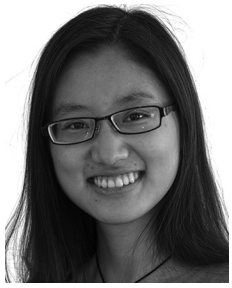
Jan Gundlach studied biology at the University of Göttingen, Germany. During his studies, he was working on protein-protein interactions and second-messenger signaling in *Bacillus subtilis*. He was also part of a group of students that participated in the iGEM competition in 2013, which was organized and held at MIT, Boston, MA. Since 2014, he has been a Ph.D. candidate at the University of Göttingen, Germany. In 2016, his interests are second-messenger signaling and ion homeostasis in Gram-positive bacteria.



Bingyao Zhu finished her bachelor's study in biology at China Agricultural University, China. Later, she pursued a master's degree in microbiology and biochemistry at the University of Göttingen, Germany. She was member of a group of students that participated in the iGEM competition in 2013, which was organized and held at MIT, Boston, MA. After obtaining her master's degree, she became a Ph.D. candidate at the Department of General Microbiology, University of Göttingen. She is currently working on the further development of *Subti*Wiki, an integrated annotation database for *Bacillus subtilis*, as her Ph.D. project. She is interested in developing new tools to collect and organize data, in order to find inner connections.

Jörg Stülke studied biology at Ernst Moritz Arndt University, Greifswald, Germany, and obtained a Ph.D. in microbiology at the University of Greifswald. After postdoctoral work on carbon catabolite repression in *Bacillus subtilis* at the University of Lund, Sweden, and the Institut Pasteur, Paris, France, he became a group leader at the Chair of Microbiology at the University of Erlangen, Germany. He obtained his habilitation on the control of carbon metabolism in *Bacillus subtilis* in 2000. In 2003, he was appointed Full Professor for Microbiology at the Georg August University Göttingen, Germany. He studies RNA-mediated control of gene expression by RNA switches and RNA degradation. Moreover, he is interested in heterogeneous gene expression and in the discovery of the function of the essential second messenger cyclic di-AMP. In the framework of the minimal genome project, he is developing tools for genome annotation and involved in the construction and analysis of genome-reduced strains of *B. subtilis*.