A Survey of Pyridoxal 5’-Phosphate-Dependent Proteins in the Gram-Positive Model Bacterium *Bacillus subtilis*

Björn Richts, Jonathan Rosenberg and Fabian M. Commichau*

Department of General Microbiology, University of Goettingen, Göttingen, Germany

The B6 vitamer pyridoxal 5’-phosphate (PLP) is a co-factor for proteins and enzymes that are involved in diverse cellular processes. Therefore, PLP is essential for organisms from all kingdoms of life. Here we provide an overview about the PLP-dependent proteins from the Gram-positive soil bacterium *Bacillus subtilis*. Since *B. subtilis* serves as a model system in basic research and as a production host in industry, knowledge about the PLP-dependent proteins could facilitate engineering the bacteria for biotechnological applications. The survey revealed that the majority of the PLP-dependent proteins are involved in metabolic pathways like amino acid biosynthesis and degradation, biosynthesis of antibacterial compounds, utilization of nucleotides as well as in iron and carbon metabolism. Many PLP-dependent proteins participate in *de novo* synthesis of the co-factors biotin, folate, heme, and NAD+ as well as in cell wall metabolism, tRNA modification, regulation of gene expression, sporulation, and biofilm formation. A surprisingly large group of PLP-dependent proteins (29%) belong to the group of poorly characterized proteins. This review underpins the need to characterize the PLP-dependent proteins of unknown function to fully understand the “PLP-ome” of *B. subtilis*.

Keywords: vitamin B6, PLP-ome, amino transferase, metabolic engineering, toxicity

INTRODUCTION

The term “vitamin B6” collectively designates the vitamers pyridoxal (PL), pyridoxine (PN), and pyridoxamine (PM), and the respective phosphate esters pyridoxal 5’-phosphate (PLP), pyridoxine 5’-phosphate (PNP), and pyridoxamine 5’-phosphate (PMP) (György, 1956; Rosenberg, 2012) (Figure 1A). Since vitamin B6 is an essential micronutrient component in the diet of mammals, it is of commercial interest for the pharmaceutical and the food industry (Domke et al., 2005; Fitzpatrick et al., 2007, 2010; Eggersdorfer et al., 2012; Kraemer et al., 2012; Rosenberg et al., 2017; Acevedo-Rocha et al., 2019). As yet, the B6 vitamers are chemically synthesized via different routes (Pauling and Weimann, 1996; Kleemann et al., 2008; Eggersdorfer et al., 2012). Since chemical synthesis requires the usage of expensive and/or toxic chemicals, the shift from chemical synthesis to sustainable fermentation technologies using microorganisms is of great interest (Rosenberg et al., 2017; Acevedo-Rocha et al., 2019). So far, the microbial vitamin B6 production processes could not replace chemical production processes (Commichau et al., 2014, 2015; Rosenberg et al., 2017, 2018; Acevedo-Rocha et al., 2019).
PLP is a co-factor for many proteins and enzymes (Jansonius, 1998; Christen and Mehta, 2001; Eliot and Kirsch, 2004; Phillips, 2015). About 1.5% of the genes of free-living prokaryotes encode PLP-dependent proteins and over 160 enzymes with different catalytic activities require vitamin B6 as a co-factor (about 4% of all described catalytic activities) (Percudani and Peracchi, 2003, 2009). Certainly, novel PLP-dependent proteins and enzymes will be identified and characterized in the future because the number of sequenced genomes is increasing (https://www.ncbi.nlm.nih.gov/genome/browse/#!/overview). The majority of the PLP-dependent enzymes are involved in amino acid metabolism (John, 1995; Eliot and Kirsch, 2004). Some enzymes catalyzing decarboxylation and racemization reactions, cleavage of Cα-Cβ bonds, α-elimination and replacement as well as β- and γ-elimination or replacement reactions also require PLP as a co-factor. Moreover, PMP and PM serves as co-factors for enzymes of deoxysugar and amino acid biosynthetic pathways, respectively (Burns et al., 1996; Mehta and Christen, 2000; Yoshikane et al., 2006; Romo and Liu, 2011). PLP also modulates the activity of DNA-binding transcription factors in eukaryotes and prokaryotes (Oka et al., 2001; Belitsky, 2004a, 2014; Huq et al., 2007; El Qaidi et al., 2013; Tramonti et al., 2015, 2017; Suvorova and Rodionov, 2016). Moreover, vitamin B6 is implicated in oxidative stress responses (Bilski et al., 2000; Mooney et al., 2009; Mooney and Hellmann, 2010; Vanderschuren et al., 2013; Mocand et al., 2014). Thus, vitamin B6 fulfills a variety of vital functions in different cellular processes (Parra et al., 2018).

**DE NOVO SYNTHESIS OF VITAMIN B6**

Two pathways for *de novo* PLP synthesis are currently known (Figure 1B) (Mittenhuber, 2001; Tanaka et al., 2005; Fitzpatrick et al., 2007, 2010; Rosenberg et al., 2017). The deoxyxylulose-5-phosphate (DXP)-dependent vitamin B6 biosynthesis pathway was identified in the Gram-negative model bacterium *Escherichia coli* and consists of two branches and seven enzymatic steps. The first three enzymes Epd, PdxB, and SerC of the longer branch convert a pentose phosphate pathway intermediate to 4-phosphohydroxy-L-threonine (4HTP) (Figure 1B) (Zhao et al., 1995; Drewke et al., 1996; Boschi-Muller et al., 1997; Tazoe et al., 2006; Rudolph et al., 2010). Next, PdxA converts 4HTP to 2-amino-3-oxo-4-(phosphohydroxy)butyric acid, which undergoes spontaneous decarboxylation to 3-phosphohydroxy-1-aminoacetone (Cane et al., 1998; Laber et al., 1999; Sivaraman et al., 2003). The PNP synthase PdxJ produces the B6 vitamer PNP from 3-phosphohydroxy-1-aminoacetone and DXP, of which the latter substrate is generated by the DXP synthase Dxs from glyceraldehyde 3-phosphate and pyruvate in the short branch of the DXP-dependent vitamin B6 pathway (Figure 1B) (Takiff et al., 1992; Sprenger et al., 1997; Cane et al., 1998; Laber et al., 1999). The PNP oxidase PdxH catalyzes the final step yielding in the biologically most-relevant B6 vitamer PLP (Zhao and Winkler, 1995). The DXP-dependent vitamin B6 pathway is present in α- and γ-proteobacteria (Mittenhuber, 2001; Tanaka et al., 2005). Recently, it has been shown that bacteria possess promiscuous enzymes that may feed into the DXP-dependent pathway and bypass a block in pyridoxal-5′-phosphate synthesis (Figure 1B) (Kim J. et al., 2010; Kim and Copley, 2012; Smirnov et al., 2012; Oberhardt et al., 2016; Thiaville et al., 2016; Zhang et al., 2016; Rosenberg et al., 2018). The hybrid pathways consisting of enzymes of native and non-native vitamin B6 pathways and of promiscuous enzymes may be improved by metabolic engineering to enhance production of B6 vitamers (Rosenberg and Commichau, 2019).

The DXP-independent vitamin B6 biosynthetic pathway involves only the PdxST enzyme complex (Ehrenshafschaft and Daub, 2001; Belitsky, 2004b; Burns et al., 2005; Raschle et al., 2005; Strohmeier et al., 2006). PdxT is a glutaminase that hydrolyses glutamine into glutamate and ammonium, of which the latter serves as a substrate to the PLP synthase PdxS (Belitsky, 2004b). The PdxS subunit generates PLP from ammonium together with either ribulose 5-phosphate or ribose 5-phosphate and with either G3P or dihydroxyacetone phosphate (Figure 1B). Many organisms possess a salvage pathway for the interconversion of the B6 vitamers (Figure 1B) (Fitzpatrick et al., 2007; di Salvo et al., 2011). For instance, specific B6-vitamer kinases can phosphorylate PN, PM and PL into their respective phosphate esters (White and Dempsey, 1970; Yang et al., 1996, 1998; di Salvo et al., 2004; Park et al., 2004). Those organisms carrying only salvage pathways have to take up B6 vitamers. So far, only few vitamin B6 transporters have been in described in eukaryotes (Stolz and Vielreicher, 2003; Szyszkowski et al., 2013). The accumulation of PLP to toxic levels can be prevented by dephosphorylation and export of PL. Indeed, bacteria like *E. coli* and *Sinorhizobium meliloti* synthesize a phosphatase for dephosphorylation of PNP and PLP (Tazoe et al., 2005; Nagahashi et al., 2008; Sugimoto et al., 2017).

**VITAMIN B6 METABOLISM IN BACILLUS SUBTILIS**

The Gram-positive model bacterium *Bacillus subtilis* relies on the PdxST enzyme complex to synthesize the B6 vitamer PLP (Pflug and Lingens, 1978; Sakai et al., 2002b; Belitsky, 2004b; Burns et al., 2005). The *B. subtilis* PdxST enzyme complex has been biochemically and structurally studied (Raschle et al., 2005; Zhu et al., 2005; Strohmeier et al., 2006; Wallner et al., 2009; Smith et al., 2015). In many Gram-positive bacteria, the expression of the *pdxST* genes and the genes encoding kinases that phosphorylate the B6 vitamers PM, PN, and PL, are regulated by MocR-like DNA-binding transcription factors (Jochmann et al., 2011; El Qaidi et al., 2013; Belitsky, 2014; Liao et al., 2015; Tramonti et al., 2015; Suvorova and Rodionov, 2016). In *B. subtilis* the *pdxST* genes are not subject to transcriptional regulation (Nicolas et al., 2012).

*B. subtilis* must possess an uptake system and a kinase for the B6 vitamer PL because exogenously supplied PL relieves PLP auxotrophy of a *pdxST* mutant (Belitsky, 2004b; Commichau et al., 2014). The uptake system and the kinase are not specific for PL because the overexpression of the *E. coli* pdxH PN(P) oxidase gene in a *B. subtilis* *pdxST* mutant enabled the
bacteria to synthesize PLP from exogenous PN (Commichau et al., 2014). While the PL and PN uptake system remains to be identified in *B. subtilis*, the kinase phosphorylating the B6 vitamers PL, PM, and PN is known (Park et al., 2004; Newman et al., 2006a,b). It is interesting to note that the *B. subtilis* PL kinase PdxK is phylogenetically related to HMPP ribokinases converting 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) to 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate (HMPP) and HMPP to HMPP phosphate, a precursor of thiamine biosynthesis (Mizote et al., 1999; Newman et al., 2006b). In the future, it will be interesting to elucidate whether exogenously supplied PL controls the activity of the PdxST enzyme complex in *B. subtilis* to prevent the accumulation of PLP to toxic levels (see below).

In contrast to the enzymes of the DXP-dependent vitamin B6 pathway from *E. coli*, the PdxST enzyme complex from *B. subtilis* is rather slow (Rosenberg et al., 2017). Therefore, a heterologous DXP-dependent vitamin B6 pathway has been introduced into *B. subtilis* for producing the B6 vitamer PN (Commichau et al., 2014, 2015). The fact that the engineered *B. subtilis* strains synthesized significant amounts of PN, which was detectable in the culture supernatant, suggests that the bacteria might possess a PNP phosphatase and an export system for PN. Recently, the PLP phosphatase YbhA has been identified in *E. coli* (Sugimoto et al., 2017). YbhA shows about 31% overall sequence identity with the YitU protein from *B. subtilis*. Even though it has been shown that YitU is a HAD phosphatase having a minor activity in dephosphorylating the riboflavin precursor 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione 5′-phosphate (Sarge et al., 2015), it will be interesting to test whether the protein may act as a PNP/PLP phosphatase.

**TOXICITY OF VITAMIN B6 AND PATHWAY INTERMEDIATES**

PLP can be toxic for the cell because the reactive 4′-aldehyde moiety of the B6 vitamer forms covalent adducts with other compounds and PLP-independent proteins containing thiol or amino groups. For instance, PLP was shown to inhibit enzymes that are involved in DNA metabolism and in central carbon metabolism in eukaryotes (Mizushina et al., 2003; Vermeersch et al., 2004; Lee et al., 2005). Moreover, the modification of the *E. coli* initiation factor 3, the adenylsuccinate synthetase and the PL kinase by PLP results in activity loss (Ohsawa and Gualerzi, 1981; Dong and Fromm, 1990; Ghatge et al., 2012). Recently, it has been shown that the addition of vitamin B6 to the *E. coli* wild type strain BW25113 and an *E. coli* mutant strain lacking the ZipA cell division protein affects multiple metabolic pathways, which are involved in amino acid biosynthesis (Vega and Margolin, 2017). Thus, excess of PLP affects different cellular processes. PLP is also prone to damage either due to side reactions that are catalyzed by promiscuous enzymes or due to spontaneous chemical reactions (Linster et al., 2013). In fact, the B6 vitamers PLP and PMP were identified as members of the 30 most damage-prone metabolites (Lerma-Ortiz et al., 2016). However, given the fact that PLP is
required for optimal growth in little amounts, the essential cofactor can be synthesized at a minimal necessary rate (Hartl et al., 2017). The low requirement of PLP and its low cellular concentration prevent perturbation of other essential processes in the cell.

In the past years, several attempts have been made to engineer bacteria for the overproduction of the B6 vitamins PL and PN (Rosenberg et al., 2017). In contrast to PL(P), PN(P) is less toxic for living cells (see above; Commichau et al., 2014). Therefore, the DXP-dependent pathway for vitamin B6 overproduction. However, it has also been shown that intermediates of the DXP-dependent pathway can be highly toxic for bacteria. For instance, the erythrose-4-phosphate dehydrogenase Epd generates the DXP-dependent vitamin B6 pathway intermediate 4-phosphoerythronate (4PE), which is required in low amounts for PLP biosynthesis, is toxic for the cells when overproduced (Sachla and Helmann, 2019). Eukaryotic cells do have a phosphatase that hydrolyzes and detoxifies 4PE that is also mistakenly generated by the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Beaudoin and Hanson, 2016; Collard et al., 2016). 4PE inhibits the 6-phosphogluconate dehydrogenase from the pentosephosphate pathway (Collard et al., 2016). Recently, it has been demonstrated that 4PE also inhibits the B. subtilis 6-phosphogluconate dehydrogenase GndA (Sachla and Helmann, 2019). In this organism, 4PE is detoxified by the GTase CpgA, which is a checkpoint protein known to be involved in ribosome assembly (Campbell et al., 2005). It will be interesting to assess whether 4PE also inhibits the 6-phosphogluconate dehydrogenase in E. coli because the bacterium does not possess a CpgA homolog. However, the accumulation of 4PE to toxic levels does not seem to be problematic in E. coli because 4PE can be produced in only small amounts that are sufficient for de novo synthesis of PLP. The intermediate 4HTP from the DXP-dependent vitamin B6 pathway is also inhibits bacterial growth. 4HTP interferes with biosynthesis of threonine and isoleucine in E. coli and B. subtilis (Drewke et al., 1993; Farrington et al., 1993; Commichau et al., 2014, 2015; Rosenberg et al., 2016). The toxicity of the pathway intermediates may explain why it is difficult to engineer bacteria that stably express the genes of the DXP-dependent vitamin B6 pathway (Commichau et al., 2014). The understanding of the metabolite toxicity is crucial for the rational design and engineering of bacteria overproducing PN at commercially attractive levels. Moreover, the knowledge about the functions of target proteins of PLP is very important to understand how the B6 vitamer affects cellular metabolism upon overproduction.

**PLP-DEPENDENT PROTEINS AND ENZYMES INVOLVED IN VITAMIN B6 METABOLISM IN BACILLUS SUBTILIS**

To identify the B. subtilis proteins and enzymes that require PLP for activity and are involved in vitamin B6 metabolism, we compared the Enzyme Commission (E.C.) numbers of the proteins from the B. subtilis 168 laboratory strain found in the SubtiWiki database (http://subtiwiki.uni-goettingen.de/v3/) (Zhu and Stülke, 2017) with the E.C. numbers that are deposited in the B6 database (Table 1) (http://bioinformatics.uniprot.it/cgi-bin/bioinformatics/B6db/home.pl) (Percudani and Peracchi, 2009). We also describe proteins from the SubtiWiki database that are specific for B. subtilis and are therefore not present in the B6 database. Publications describing proteins involved in vitamin B6 metabolism in B. subtilis were also added to the Table. A recent mass spectrometry approach in combination with modified pyridoxal analogs identified proteins in the Gram-positive pathogen Staphylococcus aureus that probably depend on the B6 vitamer PLP (Hoegl et al., 2018). The study confirmed the binding of PLP to proteins of known and unknown function and identified 4 additional PLP-binding proteins (HemH, HemQ, YtoP, and YwlG) (see below). In total we ended up with 65 PLP-dependent proteins in B. subtilis, of which 61 proteins are bona fide PLP-dependent proteins. The PLP-dependency of four proteins remains to be experimentally validated. Table 1 also contains the PDB identifiers of structures that are available in the PDB database for the B. subtilis proteins. In case the structural information was not available, we have added the PDB identifiers from PLP-dependent homologs showing more than 27% overall sequence identity. We have also included information about the physiological functions of the proteins and their paralogs, the transcription factors that are involved in synthesis of the proteins and information about the sequence similarities with other proteins from the UniProt database (https://www.uniprot.org). The list of proteins involved in vitamin B6 metabolism in B. subtilis will certainly be extended in the future because PLP-dependent enzymes are ubiquitous and evolutionary diverse, making their classification based on sequence homology difficult.

**FUNCTIONAL ASSIGNMENT OF KNOWN PLP-DEPENDENT PROTEINS IN B. SUBTILIS**

Most of the proteins that require PLP in B. subtilis are metabolic enzymes, of which the majority is involved in anabolism and catabolism of proteinogenic and non-proteinogenic amino acids (Table 1; Figure 2). The enzymes can be assigned to known protein families of PLP-dependent enzymes and for most of them it has been shown that they are indeed active in amino acid metabolism (Mehta et al., 1993; Mehta and Christen, 2000) (Table 1). B. subtilis also possesses a PLP-dependent 2-amino-3-ketobutyrate CoA ligase (Kbl), which could be involved in threonine utilization together with the L-threonine dehydrogenase Tdh (Schmidt et al., 2001; Reitzer, 2005). Both enzymes are encoded in the bicistronic tdh-kbl operon (Nicolas et al., 2012). The regulation of the tdh and kbl genes and the catalytic activities of the Tdh and Kbl enzymes remain to be studied. Two PLP-dependent enzymes BacF and NtdA are involved in the synthesis of bacilysin and kanosamine in B. subtilis. Bacilysin is a non-ribosomally synthesized peptide that is active against various bacteria and some fungi (Inaoka et al., 2003, 2009; Karatas et al., 2003; Köröglu et al., 2011). Kanosamine is an antibiotic, which is produced by Bacillus
| Protein | BSU no. | Essential | E.C. no. | PDB no. | Function | Pathway | Regulation | Paralogs/Protein family | References |
|---------|---------|-----------|----------|---------|----------|---------|------------|------------------------|------------|
| ArgD    | BSU11220| No        | 2.6.1.11| 2EH6    | Acetylmethine  | Arginine biosynthesis   | SigA, AhrC, CodX, YnrX | PLP AAT class III family AAT, ArgD subfamily | Czaplewski et al., 1992; Brinsmade et al., 2014; Ogura and Kanesaki, 2018 |
| AspB    | BSU22370| No        | 2.6.1.1| 1J32    | Aspartate aminotransferase | Aspartate biosynthesis | Unknown | AlaT, PatA/PLP AAT class I family | Zhao et al., 2018 |
| CysK    | BSU00730| No        | 2.5.1.47| 1Y7L    | Cysteine synthase | Cysteine biosynthesis   | SigA, SigM, Spx, YtkP, MccA/PLP AAT class II family | Tanous et al., 2008 |
| GlyA    | BSU06900| No        | 2.1.2.1| 2V8     | Serine hydroxymethyltransferase | Glycine biosynthesis   | SigA, T-Box, PurR | SHMT family | Gutiérrez-Preciado et al., 2009 |
| HisC    | BSU22620| No        | 2.6.1.9| 3FFH    | Histidinol-phosphate aminotransferase | Aromatic amino acids | MtrB | PLP AAT class II family | Nester and Montoya, 1976; Babbitt et al., 1992 |
| IlvA    | BSU17770| No        | 4.3.1.19| 1TDU    | Threonine dehydratase | Branched-chain amino acid biosynthesis | CodY | Ser/Thr dehydratase family | Molle et al., 2003a; Rosenberg et al., 2016 |
| LysA    | BSU23380| No        | 4.1.1.20| 1HKW    | Diaminopimelate decarboxylase | Lysine biosynthesis | SigG, SpoVT | Om/Lys/Arg decarboxylase class II family | Kalcheva et al., 1997; Stell et al., 2005 |
| MccA    | BSU27260| No        | –       | 4QL4    | O-Acetylsery-thiol-lyase | Methionine/cysteine conversion | SigA, Spx, CymR | PLP AAT class II family | Nakano et al., 2003; Choi et al., 2006; Even et al., 2006 |
| MccB    | BSU27250| No        | 4.4.1.1| 4L00    | Cystathionine lyase/homocysteine Y-lyase | Methionine/cysteine conversion | SigA, CmpM, CmpR | PLP AAT class II family | Nakano et al., 2003; Choi et al., 2006; Even et al., 2006 |
| MetC    | BSU11880| No        | 4.4.1.8| 4L00    | Cystathionine β-lyase | Methionine biosynthesis | SigA, S-box | PLP AAT class II family | Grundy and Henkin, 1998; Auger et al., 2002; Tomsic et al., 2008 |
| MetI    | BSU11870| No        | –       | 4L00    | O-Succinyl-homoserine lyase | Methionine biosynthesis | SigA, S-box | PLP AAT class II family | Grundy and Henkin, 1998; Auger et al., 2002; Tomsic et al., 2008 |
| MhrE    | BSU13580| No        | –       | 2O1B    | Glutamine transaminase | Methionine salvage | SigA, unknown | PLP AAT class I family | Sekowska and Danchin, 2002; Berger et al., 2003 |
| PatB    | BSU31440| No        | 4.4.1.8| 3TS2    | Cystathionine β-lyase | Methionine biosynthesis | Unknown | PLP AAT class II family | Auger et al., 2005 |
| SerC    | BSU10020| No        | 2.6.1.52| 1W23    | 3-Phosphoserine aminotransferase | Serine biosynthesis | Unknown | PLP AAT class V family | Sakai et al., 2002a |

(Continued)
| Protein | BSU no. | Essential | E.C. no. | PDB no. | Function | Pathway | Regulation | Paralogs/Protein family | References |
|---------|---------|-----------|----------|---------|----------|---------|------------|------------------------|------------|
| ThrC    | BSU32250| No        | 4.2.3.1  | 1UIN    | Threonine synthase | Threonine biosynthesis | CodY (–), TrnA (–), ThrR (–) | Thr synthase family | Nicolas et al., 2012; Kriel et al., 2014; Mroue et al., 2015; Rosenberg et al., 2016 |
| TrpB    | BSU22640| No        | 4.2.1.20 | 4NEG    | Tryptophan synthase β-subunit | Tryptophan biosynthesis | MtrB (–) | TrpB family | Shimosu et al., 1986; Babitzke et al., 1992 |
| YbgE    | BSU02390| No        | 2.6.1.42 | 3HT5    | Branched-chain amino acid aminotransferase | Branched-chain amino acid biosynthesis | CodY (–) | YwaA (60%)/PLP AAT class IV family | Mole et al., 2003a; Belitsky and Sonenshein, 2008, 2011 |
| YwaA    | BSU38550| No        | 2.6.1.42 | 3HT5    | Branched-chain amino acid aminotransferase | Branched-chain amino acid biosynthesis | CodY (–) | YbgE (60%)/PLP AAT class IV family | Kriel et al., 2014 |
| GabT    | BSU03900| No        | 2.6.1.19 | 1SF2    | 4-Aminobutyrate aminotransferase | 4-Aminobutyrate utilization | SigA, GabR (+) | YwaA (60%)/PLP AAT class III family | Belitsky and Sonenshein, 2002 |
| GcvPA   | BSU24560| No        | 1.4.4.2  | 1WYT    | Glycine decarboxylase subunit 1 | Glycine utilization | Gly-box | GcvP family, N-terminal subunit family | Mandal et al., 2004 |
| GcvPB   | BSU24550| No        | 1.4.4.2  | 1WYT    | Glycine decarboxylase subunit 1 | Glycine utilization | Gly-box | GcvP family, C-terminal subunit family | Mandal et al., 2004 |
| Kbl     | BSU17000| No        | 2.3.1.29 | 1FC4    | 2-Amino-3-ketobutyrate CoA ligase | Theonine utilization | Unknown | BioF (45%)/ PLP AAT class III family | Nicolas et al., 2012 |
| RocD    | BSU40340| No        | 2.6.1.13 | 3PUY    | Ornithine transaminase | Ornithine, ornithine and citrulline utilization | SigL, SpoOA (–), CodY (–), AhrC (–), RocR (+) | PLP AAT class III family, OAT subfamily | Gardan et al., 1995; Mole et al., 2003a,b |
| BacF    | BSU36790| No        | 2.6.1.1  | 201B    | Aminotransferase | Bacilysin biosynthesis | AtrB (–), CodY (–), ScoC (–) | MtnE (60%)/ PLP AAT class I family | Inacka et al., 2003, 2009; Karatas et al., 2003; Kioegli et al., 2011 |
| NtdA    | BSU10550| No        | –        | 4K2I    | 3-Oxo-glucose-6-phosphate-glutamate aminotransferase | Kanosamine biosynthesis | NtdR (+) | DegT/DnrJ/EnyC1 family | Inacka et al., 2004; Inacka and Ochi, 2011 |
| SufS    | BSU32690| Yes       | 2.8.1.7  | 58BQ    | Cysteine desulfurase | Iron-sulfur cluster formation | SigA | PLP AAT class V family, Cid subfamily | Albrecht et al., 2010; Nicolas et al., 2012; Black and Dos Santos, 2015 |
| GlgP    | BSU00940| No        | 2.4.1.1  | 1PYG    | Glycogen phosphorylase | Glycogen biosynthesis | SigE | Glycogen phosphorylase family | Kriel et al., 1994 |

(Continued)
| Protein | BSU no.  | Essential<sup>a</sup> | E.C. no. | PDB no.<sup>b</sup> | Function | Pathway | Regulation | Paralogs/Protein family<sup>d</sup> | References |
|---------|----------|------------------------|----------|----------------------|----------|---------|------------|--------------------------------|------------|
| **Nucleotide utilization** | | | | | | | | |
| PucG    | BSU32520 | No                     | –        | 3ISL                 | S-Ureidoglycine-glyoxylate aminotransferase | Purine utilization | SigA, PucR (+) | PLP AAT class V family | Schultz et al., 2001; Beier et al., 2002; Ramazzina et al., 2010 |
| **COFACTORS** | | | | | | | | |
| BioA    | BSU30230 | No                     | 2.6.1.62 | 3DRD                | Lysine-8-amino-7-oxononanoate aminotransferase | Biotin biosynthesis | BirA (-) | YhxA (33%)/ PLP AAT class III family | Bower et al., 1996; Perkins et al., 1996 |
| BioF    | BSU30220 | No                     | 2.3.1.47 | 3A2B                | 8-Amino-7-oxononanoate synthase | Biotin biosynthesis | BirA (-) | Kdb (45%)/ PLP AAT class II family | Bower et al., 1996; Perkins et al., 1996 |
| **Folate** | | | | | | | | |
| PabC    | BSU00760 | No                     | 4.1.3.38 | 4WHX (Burkholderia pseudomallei, 27%) | Aminodeoxy-chorismate lyase | Biosynthesis of folate | SigA, MtbR (-) | PLP AAT class IV family | de Saizieu et al., 1997 |
| **Heme** | | | | | | | | |
| GsaB    | BSU06710 | No                     | 5.4.3.8  | 3BS6 (B. subtilis, 48%) | Glutamate-1-semialdehyde aminotransferase | Heme biosynthesis | Unknown | HemL (48%)/ PLP AAT class III family, HemL subfamily | Dailey et al., 2017 |
| HemH<sup>d</sup> | BSU10130 | No                     | 4.99.1.1 | 2HK6                | Coproporphyrin ferrochelatase | Heme biosynthesis | Unknown | Ferrochelatase family | Dailey et al., 2017 |
| HemL    | BSU28120 | No                     | 5.4.3.8  | 3BS8                | Glutamate-1-semialdehyde aminotransferase | Heme biosynthesis | SigA, PerR (-) | GsaB (48%)/ PLP AAT class III family, HemL subfamily | Hansen et al., 1991; Herbig and Helmann, 2001; Ge et al., 2010 |
| HemQ<sup>d</sup> | BSU37670 | No                     | 1.11.1-  | 5T2K (Geobacillus stearothermophilus, 67%) | Copropoerheme decaerboxylase | Heme biosynthesis | Unknown | UPF0447 family | Dailey et al., 2017 |
| **NAD** | | | | | | | | |
| NifS    | BSU27880 | No                     | 2.8.1.7  | 4RSF (Archeoglobus fulgidus, 34%) | Cysteine desulfurase | NAD biosynthesis | SigA, NifR (-) | YntO (35%), NifZ (32%)/ PLP AAT class V family, NifS tacS subfamily | Sun and Setlow, 1993; Rossolillo et al., 2005 |
| **CELLULAR PROCESSES** | | | | | | | | |
| **Cell wall metabolism** | | | | | | | | |
| Air     | BSU04640 | Yes                    | 5.1.1.1  | 1L6G (Geobacillus stearothermophilus, 56%) | Alanine racemase | Peptidoglycan biosynthesis | Unknown | YncD (41%)/ Ala racemase family | Heaton et al., 1988; Nicolas et al., 2012 |
| Dat     | BSU09670 | No                     | 2.6.1.21 | 1G2W (G stearothermophilus, 43%) | D-Alanine aminotransferase | Peptidoglycan biosynthesis | AabB (-), CodY (-), ScoC (-) | PLP AAT class IV family | Nicolas et al., 2012 |
| PaA     | BSU14000 | Yes                    | 2.6.1-   | 1GDE (Pyrococcus sp., 42%) | N-Acetyl-L,L-diaminopimelate aminotransferase | Biosynthesis of fufosine and peptidoglycan | Unknown | AaiT (41%), AspB (41%)/ PLP AAT class I family | Nicolas et al., 2012; Rueff et al., 2014 |

(Continued)
| Protein | BSU no. | Essential | E.C. no. | PDB no. | Function | Pathway | Regulation | Paralogs/Protein family | References |
|---------|---------|-----------|----------|---------|----------|---------|------------|------------------------|------------|
| **INFORMATION PROCESSING** | | | | | | | | | |
| tRNA modification | | | | | | | | | |
| NifZ | BSU29590 | No | – | 1EG5 ( *Thermotoga maritima*, 35%) | Cysteine desulfurase | 4-Thiouridine in tRNA biosynthesis | Unknown | YnoO (42%), NifS (32%)/PLP AAT class V family, NifS/fscS subfamily | Nicolas et al., 2012; Rajakovich et al., 2012 |
| YrvO | BSU27510 | Yes | 2.8.1.7 | 1EG5 ( *T. maritima*, 35%) | Cysteine desulfurase | tRNA modification | Unknown | NIF (42%), NifS (35%)/PLP AAT class V family, NifS/fscS subfamily | Nicolas et al., 2012; Black and Dos Santos, 2015 |
| **Regulation of gene expression** | | | | | | | | | |
| GabR | BSU03890 | No | – | 4MGR | Regulator of gabTD and gabR genes | Y-Am inobutyrate utilization | SigA, GabR (–) | MocR/GabR family; PLP AAT class I family (C-terminal section) | Belitsky and Sonenshein, 2002; Bramucci et al., 2011 |
| **LIFESTYLES** | | | | | | | | | |
| Sporulation | | | | | | | | | |
| YncD | BSU17640 | No | 5.1.1.1 | 1L6G ( *Geobacillus stearothermophilus*, 42%) | Alanine racemase | Spore protection | SigE | Air (41%)/Ala racemase family | Pierce et al., 2008 |
| **Biofilm formation** | | | | | | | | | |
| EpsN | BSU34230 | No | – | 1O61 ( *Campylobacter jejuni*, 46%) | UDP-2,6-Dideoxy-2-acetamido 4-keto glucose aminotransferase | | | DegT/Dnr/J/EryC1 family | Kearns et al., 2005; Imrov and Winkler, 2010; Marvasi et al., 2010; Chumsakul et al., 2011; Winkelman et al., 2013 |
| SpeA | BSU14630 | No | 4.1.1.19 | 2X3L ( *S. aureus*, 28%) | Arginine decarboxylase | Spermidine, polyamine biosynthesis | Unknown | YaaO (34%)/Orn/Lys/Arg decarboxylase class I family | Sekowska et al., 1998; Burrell et al., 2010; Nicolas et al., 2012 |
| **POORLY CHARACTERIZED PROTEINS** | | | | | | | | | |
| Regulation of gene expression | | | | | | | | | |
| YoxD | BSU03560 | No | – | 4MGR (GabR, 27%) | Unknown | Unknown | Unknown | MocR/GabR family; PLP AAT class I family (C-terminal section) | Bramucci et al., 2011 |
| YdeF | BSU05180 | No | – | 4MGR (GabR, 23%) | Unknown | Unknown | Unknown | MocR/GabR family; PLP AAT class I family (C-terminal section) | Bramucci et al., 2011 |
| YdeL | BSU05240 | No | – | 4MGR (GabR, 23%) | Unknown | Unknown | Unknown | MocR/GabR family; PLP AAT class I family (C-terminal section) | Bramucci et al., 2011 |
| YdD | BSU05370 | No | – | 1WST ( *Thermococcus profundus*, 30%) | Unknown | Unknown | Unknown | MocR/GabR family; PLP AAT class I family (C-terminal section) | Bramucci et al., 2011 |
| YhdI | BSU09480 | No | – | 4MGR (GabR, 40%) | Unknown | Unknown | Unknown | MocR/GabR family; PLP AAT class I family (C-terminal section) | Bramucci et al., 2011 |
| Protein | BSU no. | Essential | E.C. no. | PDB no. | Function | Pathway | Regulation | Paralogs/Protein family | References |
|---------|---------|-----------|----------|---------|----------|---------|------------|------------------------|------------|
| YovV    | BSU10880| No        | –        | 1WST    | (Thermococcus profundus, 29%) | Unknown | Unknown   | MocR/GabR family; PLP AAT class I family (C-terminal section) | Bramucci et al., 2011 |
| DsdA    | BSU23770| No        | 4.3.1.18 | 3SS7    | (E. coli, 58%) | D-Serine deaminase | Unknown | Ser/Thr dehydratase family, DsdA subfamily | McFall, 1964; Nicolas et al., 2012; Urusova et al., 2012 |
| KamA    | BSU19690| No        | 5.4.3.2 | 2ASH    | (Clostridium subterminale Sb4, 60%) | Lysine 2,3-aminomutase | Unknown | SigE, Radical SAM superfamily, KamA family | Chen et al., 2000; Feucht et al., 2003 |
| YaaO    | BSU00270| No        | 4.1.1.19 | 2XL    | (S. aureus, 36%) | Putative arginine decarboxylase | Unknown | SigK, SigW, SpeA (34%)/Orn/Lys/Arg decarboxylase class I family | Huang et al., 1999; Burrell et al., 2010; Nicolas et al., 2012; Witzky et al., 2018 |
| YdbU    | BSU02660| No        | –        | –       | Putative cysteine desulphurase | Unknown | Unknown | PLP AAT class V family | Nicolas et al., 2012 |
| YhdR    | BSU09570| No        | 2.6.1.1 | 3ELE    | (Eubacterium rectale, 34%) | Putative aspartate aminotransferase | Unknown | Unknown | Nicolas et al., 2012 |
| YhxA    | BSU09260| No        | –        | 2N6M    | (B. anthracis, 64%) | Putative adenosylmethionine-8-amino-7-oxononanoate aminotransferase | Unknown | SigA, YodT (35%), BioA (33%), GabT (32%)/class III PLP AAT family | Holmberg et al., 1990; Richards et al., 2011 |
| YmE     | BSU15380| No        | –        | 1W8G    | (E. coli, 33%) | Unknown | PLP homeostasis, Ile and Val metabolism | Spo0A (–) | YggS/PROSC family | Mole et al., 2003b; Ito et al., 2013; Prunet et al., 2016 |
| YnbB    | BSU17440| No        | –        | 3L2L    | (L. monocytogenes str. 4b 2365, 67%) | Putative C-S lyase | Modification of Efp | Unknown | Unknown | Nicolas et al., 2012; Witzky et al., 2018 |
| YodT    | BSU19740| No        | –        | 3M4I    | (Deinococcus radiodurans, 39%) | Putative adenosylmethionine-8-amino-7-oxononanoate aminotransferase | Unknown | SigE, YhxA (35%), RocD (31%), ArgD (31%)/class III PLP AAT family | Feucht et al., 2003 |
| YfP     | BSU29970| No        | 2.5.1.47 | 2EGU    | (Geobacillus kaustophilus, 57%) | Putative cysteine synthase | Unknown | CysK (58%), McoA (42%)/cysteine synthase, cystathionine β-synthase family | Nicolas et al., 2012 |
| Yhar    | BSU29860| No        | –        | 3KL9    | (Streptococcus pneumoniae, 44% identity) | Putative glutamyl aminopeptidase | Unknown | YadC (48%)/peptidase M42 family | Kim D. et al., 2010 |
| YwG     | BSU36910| No        | –        | 1VBD    | (T. thermophilus, 48%) | Unknown | Affects Efp modification level | TnrA (–) | Unknown | Mrouze et al., 2015; Witzky et al., 2018 |
| AitA    | BSU81400| No        | –        | 1DJU    | (Pyrococcus horikoshii, 47%) | Methionine aminotransferase | Unknown | AspB (41%), PatA (41%); class I PLP AAT family | Matsu et al., 2000; Nicolas et al., 2012 |

a The proteins are essential for growth of B. subtilis in LB medium supplemented with glucose (Reuss et al., 2016).
b The overall amino acid sequence identity to other proteins from B. subtilis is shown in brackets.
c The protein family according to UniProt (www.uniprot.org).
d A large-scale mass spectrometry-based screen revealed that the proteins probably bind PLP in S. aureus (Hoegl et al., 2018). It has to be experimentally validated that HemH, HemQ, YtoP and YwlG are functional PLP-dependent proteins.
and Streptomyces species and inhibits cell wall synthesis in microorganisms (Dolak et al., 1980; Milner et al., 1996; Inaoka et al., 2004; Inaoka and Ochi, 2011; Vetter et al., 2013). The PLP-dependent enzymes SufS, GlgP, and PucG are involved in iron-sulfur cluster formation, glycogen biosynthesis, and purine utilization, respectively (Table 1). Homologs of SufS and GlgP are also present in E. coli (48 and 44% overall sequence identity, respectively). However, in B. subtilis the glycogen phosphorylase seems to be involved in a sporulation-specific process because the glgP gene is expressed early during sporulation in the mother cell (Kiel et al., 1994). Glycogen biosynthesis exclusively occurs in the presence of carbon sources allowing efficient sporulation (Kiel et al., 1994). Eight PLP-dependent enzymes are involved in the biosynthesis of the co-factors biotin, folate, heme and NAD (Table 1). While the biochemical and structural characterization of BioA, BioF, PabC, GsaB, HemL, and NiF revealed that the proteins require PLP for enzyme activity, it has to be investigated whether HemH and HemQ are bona fide PLP-dependent proteins. HemH and HemQ were recently identified in a mass spectrometry approach in the Gram-positive pathogen Staphylococcus aureus (Hoegl et al., 2018). B. subtilis possesses in total five PLP-dependent enzymes (Alr, Dat, PatA, NiF, and YrvO) that are involved in cell wall metabolism and in information processing (Table 1). The alanine racemase Alr, the D-alanine aminotransferase Dat and the N-acetyl-L,L-diaminopimelate aminotransferase PatA generate precursors for the peptidoglycan of the cell wall. The tRNA-modifying enzymes NiZ and YrvO are both cysteine desulfurases that are active in biosynthesis of 4-thiouridine and 2-thiouridine, respectively, for the formation of modified tRNA molecules. YrvO transfers sulfur to the TrmU tRNA methyltransferase, which is essential for 2-thiouridine biosynthesis (Black and Dos Santos, 2015). Finally, four PLP-dependent enzymes play a role in sporulation and biofilm formation in B. subtilis (Table 1). While it has been shown that Spd proteins such as SpdC are required for spore germination (Cangiano et al., 2014), the role of the alanine racemase YncD in sporulation is currently unknown. The two biofilm-related enzymes, the arginine decarboxylases SpeA and the UDP-2,6-dideoxy-2-acetamido-4-keto-glucose aminotransferase EpsN are important for biosynthesis of polyamines such as spermidine and extracellular polysaccharides (Burrell et al., 2010; Marvasi et al., 2010). Indeed, B. subtilis strains lacking either SpeA or EpsN are defective in biofilm formation (Burrell et al., 2010; Pozsgai et al., 2012). SpeA also possesses a paralog (YaaO, 34% overall sequence identity), However, this proteins does not seem to be involved in biofilm formation (see below). To conclude, B. subtilis possesses several PLP-dependent enzymes that are involved in different cellular processes. Moreover, many PLP-dependent enzymes do have paralogs that have similar activities or fulfill specific functions in the cell, probably due to specialization during evolution (Table 1).

PLP-DEPENDENT TRANSCRIPTION FACTORS IN BACILLUS SUBTILIS

B. subtilis possesses seven PLP-dependent DNA-binding transcription factors of which only one has been intensively characterized (see below; Table 1). The PLP-dependent transcription factors belong to the MocR-subfamily and contain a GntR-family DNA-binding domain at the N-terminus and an aminotransferase-like sensory domain at the C-terminus (Bramucci et al., 2011; Milano et al., 2015; Tramonti et al., 2015, 2017; Suvorova and Rodionov, 2016). The MocR-family-type PLP-dependent transcription factors that have been characterized are involved in controlling the expression of genes involved in PLP, γ-aminobutyrate, ecotoine, and taurine metabolism (Suvorova and Rodionov, 2016; Schulz et al., 2017; Tramonti et al., 2018). B. subtilis can utilize γ-aminobutyrate (GABA) as a source of nitrogen (Belitsky and Sonenshein, 2002). The catabolism of GABA requires the activities of the GABA aminotransferase GabT and the succinic semi-aldehyde dehydrogenase GabD that are encoded in the bicistronic gabT-gabD operon. The MocR-family-type regulator GabR
POORLY CHARACTERIZED PLP-DEPENDENT ENZYMES

In addition to the 6 transcription factors whose DNA-binding activities depend on PLP and additional unknown effectors (see above), *B. subtilis* possesses several poorly characterized PLP-dependent enzymes (Table 1; Figure 2). The DsdA protein from *B. subtilis* shows about 58% overall sequence identity with the *E. coli* D-serine deaminase (D-serine ammonia lyase) DsaA, which catalyzes the deamination of D-serine to form pyruvate and ammonia (Gale and Stephenson, 1938; McFall, 1964). The PLP-dependency and the structure of the enzyme from *E. coli* have been determined (Schnackerz et al., 1999; Urusova et al., 2012). Phylogenetic analyses suggest that the *E. coli* and *B. subtilis* D-serine deaminases and threonine synthases with similarities in the catalytic mechanisms may have evolved from a common ancestor (Parsot, 1986). The primary function of DsaA seems to be the detoxification of D-serine, which inhibits bacterial growth because it is a competitive antagonist of β-alanine in the pantothenate (vitamin B5) biosynthetic pathway, generating the precursor for coenzyme A biosynthesis (Cosloy and McFall, 1973). Previously, it has also been shown that *E. coli* mutants constitutively expressing DsaA are able to use D-serine as the sole source of carbon and nitrogen (Bloom and McFall, 1975). In *B. subtilis* the *dsdA* gene is located in the *yqjP-yqjQ-dsdA-coaA-yjgT* operon, containing three genes of unknown function as well as the *dsdA* and *coaA* genes, of which the latter encodes the major pantothenate kinase CoaA (Ogata et al., 2014). The genetic context of the *dsdA* gene strongly suggests that the D-serine deaminase may be involved in the detoxification of D-serine that probably also interferes with pantothenate synthesis in *B. subtilis*. The presence of a D-serine deaminase may be explained by the fact that L-serine is more rapidly racemized than most other amino acids (Reitzer, 2005). It will be interesting to elucidate whether the physiological function of the D-serine deaminase may be involved in the detoxification of D-serine.

The *B. subtilis* KamA enzyme shows about 60% overall sequence identity with the PLP-, S-adenosyl-L-methionine and [4Fe-4S]-dependent lysine-2,3-aminomutase from the obligate anaerobe bacterium *Clostridium subterminale* (Table 1) (Lepore et al., 2005). The lysine-2,3-aminomutase catalyzes the conversion of L-lysine to L-β-lysine, which is the first step in the anaerobic degradation of lysine in clostridia (Chirpich et al., 1970). In vitro characterization of KamA from *B. subtilis* revealed that enzyme also catalyzes the conversion of L-lysine to L-β-lysine under anaerobic conditions (Chen et al., 2000). The KamA enzyme is only produced during sporulation of *B. subtilis* (Feucht et al., 2003). Therefore, the enzyme does not seem to play a role during vegetative growth. The lysine-2,3-aminomutase EpmB from *E. coli*, which shows about 31% overall sequence identity with KamA from *B. subtilis*, has low lysine-2,3-aminomutase activity, indicating that L-lysine does not seem to be the natural substrate (Chen et al., 2000; Yanagisawa et al., 2010). Recently, it has been shown that the *E. coli* lysine-2,3-aminomutase EpmB enhances the lysislation of the elongation factor EF-P by the aminoacyl-tRNA synthetase GenX (Yanagisawa et al., 2010). The lysislation of EF-P is a post-translational modification that is essential for cell survival (Yanagisawa et al., 2010; Park et al., 2012). However, the physiological function of the lysine-2,3-aminomutase KamA from *B. subtilis* remains to be determined.

The *B. subtilis* YaaO enzyme, which belongs to class I Orn/Lys/Arg decarboxylases, encodes a putative arginine decarboxylase (Table 1). Arginine decarboxylases are important for biosynthesis of polyamines such as spermidine, substances that are crucial for biofilm formation (Burrell et al., 2010). The *B. subtilis* arginine decarboxylase SpeA, which can be considered as a paralog of YaaO (34% overall sequence identity), is indeed essential for the synthesis of polyamines and thus biofilm formation (see above) (Burrell et al., 2010). However, no biofilm-related phenotype has been reported so far for a *B. subtilis* mutant lacking YaaO. Recently, it has been reported that YaaO and two other proteins of unknown function (YfkA and YwlG, see below) influence the level of the post-translational aminopentanol modification of the elongation factor EF-P (Witzky et al., 2018). However, the precise role of YaaO in the modification of EF-P in *B. subtilis* remains to be elucidated.

The uncharacterized PLP-dependent proteins YcbU, AlaT, YhhA, and its paralog YodT are probably PLP-dependent amino acid transferases (Table 1). YcbU might be a cysteine desulfurase that is involved in co-factor biosynthesis (Mihara and Esaki, 2002). However, it remains to be elucidated whether YcbU is functional in *B. subtilis* because a mutant lacking YcbU shows no obvious phenotype (Koo et al., 2017). AlaT is similar
to PLP-dependent methionine amino acid transferases and the protein shares about 47% overall sequence identity with an amino acid transferase from *Pyrococcus horikoshii* that acts on aromatic amino acids (Matsui et al., 2000). However, not experimental evidence supporting the annotation of AlaT is available. Both, YhxA and YodT are annotated as putative adenosylmethionine-8-amino-7-oxononanoyl aminotransferases, enzymes that were shown to be involved in biotin biosynthesis in bacteria (Izumi et al., 1975). YhxA shares about 35 and 33% sequence identity with YodT and BioA, respectively. The PLP-dependent lysine-8-amino-7-oxononanoyl aminotransferase BioA is required for biotin biosynthesis in *B. subtilis* (Table 1). Therefore, it is tempting to speculate that YhxA and YodT are also involved in biotin metabolism in this organism. The expression of the yhxA and yodT genes depends on SigA and on the sporulation-specific sigma factor SigE, respectively. Therefore, these enzymes seem to be active in different cellular differentiation processes of *B. subtilis*.

The *B. subtilis* YlmE protein of unknown function shows about 33% overall sequence identity with the YggS protein from *E. coli*. Recently, it has been shown that YggS is a PLP-binding protein, which belongs to a highly conserved COG0325 protein family and exists in almost all kingdoms of life, including bacteria, fungi and animals (Ito et al., 2013). The high conservation of YggS indicates that the protein fulfills an important function in bacteria. Indeed, the lack of YggS in *E. coli* affects balance of PLP homeostasis, sensitivity toward the B6 vitamer PN and perturbation of biosynthesis of branched-chain amino acids (Prunetti et al., 2016). Similar phenotypes have been associated to a mutant strain of *Synechococcus elongatus* PCC 7942, lacking the pipY gene, which encodes a COG0325 homolog (Labella et al., 2017; Tremiño et al., 2017). It will be very interesting to elucidate the precise function of COG0325 homologs in controlling vitamin B6 homeostasis.

The proteins YhdR, YnbB, and YwlG cannot be assigned to a specific protein family (Table 1). YhdR shares 34% overall sequence identity with an amino acid transferase from *Eubacterium rectale* but its role in amino acid metabolism is unknown (Table 1). Interestingly, like YaaO, YnbB, and YwlG are involved in the post-translational aminopentanol modification of the elongation factor EF-P (Witzky et al., 2018). While YwlG influences the level of the post-translational modification, YnbB seems to be required for the modification. The precise function of the proteins in controlling the activity of the elongation factor EF-P in *B. subtilis* needs further investigation. Moreover, it remains to be experimentally determined whether the function of YwlG depends on PLP.

The YtkP protein is a putative cysteine synthase that shares sequence similarity with the bifunctional cysteine synthase CysK and the O-acetylserylseryl-thiol lyase McCA (Table 1). Since a *cysK* *mcca* double mutant is auxotrophic for cysteine, YtkP does not seem to be involved in cysteine biosynthesis. Thus, the function of YtkP remains elusive. The YtoP protein has been annotated as a putative glutamyl aminopeptidase because it shares about 44% overall sequence identity with PepA, a protease from *Streptococcus pneumoniae* that has been structurally and biochemically analyzed (Kim D. et al., 2010). Interestingly, YsdC, the paralog of YtoP (44% overall sequence identity), is annotated as an endo-1,4-β-glucanase (Table 1). Therefore, it is tempting to speculate whether YtoP is indeed involved in protein turnover. The binding of PLP to YtoP has to be experimentally validated. To conclude, *B. subtilis* contains several poorly characterized PLP-dependent proteins, which need to be studied in the future.

### CONCLUSIONS AND FUTURE PERSPECTIVES

For a complete understanding of the vitamin B6 metabolism of *B. subtilis* it is crucial to identify and characterize all the proteins that require the essential co-factor to fulfill their function. However, even for well-studied model bacteria like *B. subtilis* the complete set of the enzymes involved in vitamin B6 metabolism and the PLP-dependent proteins remains to be identified. Several bioinformatics-driven approaches have been performed to identify and classify PLP-dependent enzymes (Percudani and Peracchi, 2003, 2009). Even though the PLP-dependent proteins often show low sequence similarities, using sensitive Hidden Markov Model-base sequence similarity searches PLP-dependent proteins can be identified (Yoon, 2009). However, in case the protein has a fold that is different from the known PLP-dependent fold it is difficult to identify novel PLP-dependent proteins by sequence comparison. Even though structural similarity searches allowed assigning the PLP-dependent proteins to five distinct fold types (Mehta et al., 1993; Mehta and Christen, 2000; Catazaro et al., 2014), other approaches have to be pursued to uncover the full repertoire of PLP-dependent enzymes in a given organism. Indeed, mass spectrometry and biochemical approaches have been performed to identify proteins that were modified by PLP (Simon and Allison, 2009; Whittaker et al., 2015; Wu et al., 2018). As described above, a recent mass spectrometry approach identified proteins in the Gram-positive pathogen *S. aureus* that might depend on the B6 vitamer PLP (Hoegl et al., 2018). It will be interesting to evaluate whether the same approach will lead to the identification of novel PLP-dependent proteins in *B. subtilis* and related bacteria. Moreover, the transport systems for the uptake and export of the B6 vitamers PN and PL have to be identified by *B. subtilis*. The phosphatase involved in the dephosphorylation of PNP is so far unknown (Commichau et al., 2014, 2015). Furthermore, the function of the conserved YlmE protein (YggS in *E. coli*) in vitamin B6 homeostasis has to be studied. Finally, it has to be elucidated how the PLP molecules are delivered to their target proteins.

### SPECIALITY SECTION

**PLP-Dependent Enzymes: Extraordinary Versatile Catalysts and Ideal Biotechnological Tools for the Production of Unnatural Amino Acids and Related Compounds, in Process and Industrial Biotechnology**

A section of the journal Frontiers in Bioengineering and Biotechnology.
AUTHOR CONTRIBUTIONS
BR, JR, and FC performed the database search. FC coordinated the work and wrote the manuscript with input from all authors.

FUNDING
This work was supported by the Fonds der Chemischen Industrie (to FC), the Max-Buchner-Forschungstiftung (MBFSSt 3381 to FC), and the Deutsche Forschungsgemeinschaft (DFG grants CO 1139/1-2 and GSC 226/2 to FC and JR, respectively). This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 720776.

ACKNOWLEDGMENTS
We are grateful to members of the Commichau laboratory for fruitful comments and suggestions. We also acknowledge the foundational work on the metabolism of vitamin B6 in bacteria and other organisms that was published beforehand and cannot be fully covered because of the limit of references.

REFERENCES
Acevedo-Rocha, C. G., Gronenberg, L., Mack, M., Commichau, F. M., and Genee, H. J. (2019). Microbial cell factories for the sustainable manufacturing of B vitamins. Curr. Opin. Biotechnol. 56, 18–29. doi: 10.1016/j.copbio.2018.07.006

Albrecht, A. G., Netz, D. J., Miethke, M., Pierik, A. J., Burghaus, F., Lil, R., et al. (2010). SuU is an essential iron-sulfur cluster scaffold protein in Bacillus subtilis. J. Bacteriol. 192, 1643–1651. doi: 10.1128/JB.01536-09

Al-Zyoud, W. A., Hynson, R. M., Ganuelas, L. A., Coster, A. C., Du ff, A. P., Baker, M. A., et al. (2016). Binding of transcription factor GabR to DNA requires recognition of DNA shape at a location distinct from its cognate binding site. Nucleic Acids Res. 44, 1411–1420. doi: 10.1093/nar/gkv1466

Amidani, D., Tramonti, A., Canosa, A. V., Campanini, B., Maggi, S., Milano, T., et al. (2017). Study of DNA binding and bending by Bacillus subtilis GabR, a PLP-dependent transcription factor. Biochim. Biophys. Acta Gen. Subj. 1861, 3474–3489. doi: 10.1016/j.bbaglob.2016.09.013

Arrieta-Ortiz, M. L., Hafemeister, C., Bate, A. R., Chu, T., Greenfield, A., Shuster, B., et al. (2015). An experimentally supported model of the Bacillus subtilis global transcriptional regulatory network. Mol. Syst. Biol. 11:839. doi: 10.15252/msb.20156236

Auger, S., Gomez, M. P., Danchin, A., and Martin-Verstraete, I. (2005). The PatB protein of Bacillus subtilis is a C-S-lyase. Biochimie 87, 231–238. doi: 10.1016/j.biochi.2004.09.007

Auger, S., Yuan, W. H., Danchin, A., and Martin-Verstraete, I. (2002). The metIC operon involved in methionine biosynthesis in Bacillus subtilis is controlled by transcription antitermination. Microbiology 148, 507–518. doi: 10.1099/00221287-148-2-507

Babitzke, P., Gollnick, P., and Yanofsky, C. (1992). The mtrAB operon of Bacillus subtilis is controlled by transcription antitermination. J. Bacteriol. 174, 2059–2064. doi: 10.1128/JB.174.7.2059-2064.1992

Bramucci, E., Milano, T., and Pascarella, S. (2011). Genomic distribution of MtrB protein of Bacillus subtilis is a C-S-lyase. Biochem. Biophys. Res. Commun. 411, 729–738. doi: 10.1016/j.bbrc.2011.06.012

Brettman, S. R., Alexander, E. L., Livny, J., Stettner, A. J., Segrè, D., Rhee, K. Y., et al. (2014). Hierarchical expression of genes controlled by the Bacillus subtilis global regulatory protein CodY. Proc. Natl. Acad. Sci. U.S.A. 111, 8227–8232. doi: 10.1073/pnas.1321308111

Burns, K. D., Pieper, P. A., Liu, H. W., and Stankovich, M. T. (1996). Studies on the formation. J. Biol. Chem. 271, 1952–1962. doi: 10.1016/j.jbc.2011.06.012

Burrell, M., Hanfrey, C. C., Murray, E. J., Stanley-Wall, N. R., and Mich ael, H. J. (2019). Microbial cell factories for the sustainable manufacturing of B vitaminas. Curr. Opin. Biotechnol. 56, 18–29. doi: 10.1016/j.copbio.2018.07.006

Belitsky, B. R., and Sonenshein, A. L. (2002). GabR, a member of a novel protein family, regulates the utilization of gamma-amino butyrate in Bacillus subtilis. Mol. Microbiol. 45, 569–583. doi: 10.1046/j.1365-2958.2002.03036.x

Belitsky, B. R., and Sonenshein, A. L. (2008). Genetic and biochemical analysis of CodY-binding sites in Bacillus subtilis. J. Bacteriol. 190, 1224–1236. doi: 10.1128/JB.01780-07

Belitsky, B. R., and Sonenshein, A. L. (2011). Roadblock repression of transcription by Bacillus subtilis CodY. J. Mol. Biol. 411, 729–743. doi: 10.1016/j.jmb.2011.06.012

Berger, B. J., English, S., Chan, G., and Knodel, M. H. (2003). Methionine regeneration and aminotransferases in Bacillus subtilis, Bacillus cereus, and Bacillus anthracis. J. Bacteriol. 185, 2418–2431. doi: 10.1128/JB.185.8.2418-2431.2003

Bilski, P., Li, M. Y., Ehrenshaft, M., Daub, M. E., and Chingell, C. F. (2000). Vitamin B6 (pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants. Photochem. Photobiol. 71, 129–134. doi: 10.1562/0031-8655(2000)071<0129:SIPVBP>2.0.CO;2

Black, K. A., and Dos Santos, P. C. (2015). Abbreviated pathway for biosynthesis of 2-thiosorbitide in Bacillus subtilis. J. Bacteriol. 197, 1952–1962. doi: 10.1128/JB.02625-14

Bloom, F. R., and McFall, E. (1975). Isolation and characterization of D-serine deaminase constitutive mutants by utilization of D-serine as sole carbon or nitrogen source. J. Bacteriol. 121, 1078–1084.

Bochi-Muller, S., Azza, S., Pollastro, D., Corbier, C., and Brancel, G. (1997). Comparative enzymatic properties of GapB-encoded erythrose-4-P dehydrogenase of Escherichia coli and phosphorylating glyceraldehyde-3-phosphate dehydrogenase. J. Biol. Chem. 272, 15106–15112. doi: 10.1074/jbc.272.24.15106

Burns, K. D., Perks, J. R., Yocum, R. R., Howitt, C. L., Rahaim, P., and Pero, J. (1996). Cloning, sequencing, and characterization of the Bacillus subtilis biotin biosynthetic operon. J. Bacteriol. 178, 4122–4130. doi: 10.1128/JB.178.14.4122-4130.1996

Bramucci, E., Milano, T., and Pascarella, S. (2011). Genomic distribution and heterogeneity of MocR-like transcriptional factors containing a domain belonging to the superfamily of the pyridoxal-5-phosphate dependent enzymes of fold type I. Biochem. Biophys. Res. Commun. 415, 88–93. doi: 10.1016/j.bbrc.2011.10.017

Brintsma, S. R., Alexander, E. L., Livny, J., Stettner, A. J., Segre, D., Rhee, K. Y., et al. (2014). Hierarchical expression of genes controlled by the Bacillus subtilis global regulatory protein CodY. Proc. Natl. Acad. Sci. U.S.A. 111, 8227–8232. doi: 10.1073/pnas.1321308111

Burns, K. D., Pieper, P. A., Liu, H. W., and Stankovich, M. T. (1996). Studies on the redox properties of CDP-D-deoxy-L-threo-D-glycero-4-hexulose-3-dehydrode (E1) and CDP-D-deoxy-L-threo-D-glycero-4-hexulose-3-dehydroreductase (E3): two important enzymes involved in the biosynthesis of ascorbyl. Biochemistry 35, 7879–7889. doi: 10.1021/bi960284t

Burrell, M., Hanfrey, C. C., Murray, E. J., Stanley-Wall, N. R., and Michael, A. J. (2010). Evolution and multiplicity of arginine decarboxylases in polyamine biosynthesis and essential role in Bacillus subtilis biofilm formation. J. Biol. Chem. 285, 39224–39238. doi: 10.1074/jbc.M110.163154
Dolak, L. A., Castle, T. M., Dietz, A., and Laborde, A. L. (1980). 3-Amino-3-deoxyglucose produced by a Streptomyces sp. J. Antibiot. 33, 900–901. doi: 10.7164/antibiotics.33.900

Domke, A., Großklaus, R., Niemann, B., Przyrembel, H., Richter, K., Schmidt, E., et al. (2005). Use of Vitamins in Foods - Toxicological and Nutritional-Physiological Aspects. Berlin: BfR-Wissenschaft.

Dong, Q., and Fromm, H. J. (1990). Chemical modification of adenylylsuccinate synthetase from Escherichia coli by pyridoxal 5′-phosphate. Identification of an active site lysyl residue. J. Biol. Chem. 265, 6235–6240.

Drewke, C., Klein, M.,Clade, D., Arentz, A., Müller, A., and Leistner, E. (1996). 4-O-phosphoryl-L-threonine, a substrate of the pxd six (criscer) gene product involved in vitamin B6 biosynthesis. FEBS Lett. 390, 179–182. doi: 10.1016/S0014-5793(96)00652-7

Drewke, C., Noethis, C., Hansen, U., Leistner, E., Hemscheidt, T., Hill, R. E., et al. (1993). Growth response to 4-hydroxy-L-threonine of Escherichia coli mutants blocked in vitamin B6 biosynthesis. FEBS Lett. 318, 125–128. doi: 10.1016/0167-4838(93)80005-F

Edathyhumalgalam, R., Wu, R., Garcia, R., Wang, Y., Wang, W., Kreinbring, C., et al. (2013). Crystal structure of Bacillus subtilis GabR, an autorepressor and transcriptional activator of gabB. Proc. Natl. Acad. Sci. U.S.A. 110, 17820–17825. doi: 10.1073/pnas.1315887110

Eggersdorfer, M., Lauert, D., Létois, U., Mcclaymont, T., Medlock, J., Netscher, T., et al. (2012). One hundred years of vitamins - a success story of natural sciences. Angew. Chem. Int. Ed. Engl. 51, 12960–12990. doi: 10.1002/anie.201205886

Ehrenshin, M., and Dauh, M. E. (2001). Isolation of PXD2, a second gene in the pyridoxine biosynthesis pathway of eukaryotes, archaea, and a subset of eubacteria. J. Bacteriol. 183, 3383–3390. doi: 10.1128/JB.183.11.3383-3390.2001

Eichenberger, P., Fujita, M., Jensen, S. T., Conlon, E. M., Rudner, D. Z., Wang, T. S., et al. (2004). The program of gene transcription for a single differentiating cell type during sporulation in Bacillus subtilis. PLoS Biol. 2:e328. doi: 10.1371/journal.pbio.0020328

El Qaidi, S., Yang, J., Zhang, J. R., Metzger, D. W., and Bai, G. (2013). The vitamin B6 pathway in Streptococcus pneumoniae is controlled by pyridoxal 5′-phosphate and the transcription factor PdxR and has an impact on ear infection. J. Bacteriol. 195, 2187–2196. doi: 10.1128/JB.00041-13

Eliot, A. C., and Kirsch, J. F. (2004). Pyridoxyl phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu. Rev. Biochem. 73, 383–415. doi: 10.1146/annurev.biochem.73.011303.074021

Ewen, S., Burguérié, P., Auger, S., Soutourina, O., Danchin, A., and Martin-Verstraete, I. (2006). Global control of cysteine metabolism by CymR in Bacillus subtilis. J. Bacteriol. 188, 2184–2197. doi: 10.1128/JB.188.8.2184-2197.2006

Farrington, G. K., Kumar, A., Shames, S. L., Ewaskiwiecz, J. I., Ash, D. E., and Wedler, F. C. (1993). Threonine synthase of Escherichia coli: inhibition by classical and slow-binding analogues of homoserine phosphate. Arch. Biochem. Biophys. 307, 165–174. doi: 10.1016/0003-9861(93)86950-Z

Feucht, A., Evans, J., and Errington, J. (2003). Identification of sporulation genes by genome-wide analysis of the sigmaE regulon of Bacillus subtilis. Microbiology 149, 3023–3034. doi: 10.1099/mic.0.26413-0

Fitzpatrick, T. B., Mocand, C., and Roux, C. (2010). Vitamin B6 biosynthesis: charting the mechanistic landscape. ChemBioChem 11, 1185–1193. doi: 10.1002/cbic.201000084

Gale, E. F., and Stephenson, M. (1938). Factors influencing bacterial deamination: factors influencing the activity of d-serine deaminase in Bacterium coli. Biochem. J. 32, 392–404. doi: 10.1042/bj3200392

Gardan, R., Rapoport, G., and Débarbouillé, M. (1995). Expression of the rocDEFG operon involved in arginine catabolism in Bacillus subtilis. J. Mol. Biol. 249, 843–856. doi: 10.1006/jmbi.1995.0342

Ge, H., Ly, X., Fan, J., Guo, Y., Teng, M., and Niu, L. (2010). Crystal structure of glutamate-1-semialdehyde aminotransferase from Bacillus subtilis with bound pyridoxamine 5′-phosphate. Biochem. Biophys. Res. Commun. 402, 356–360. doi: 10.1016/j.bbrc.2010.03.033

Ghatge, M. S., Contestabile, R., di Salvo, M. L., Desai, J. V., Gandhi, A. K., Camara, C. M., et al. (2012). Pyridoxal 5′-phosphate is a slow tight binding inhibitor
of E. coli pyridoxal kinase. PLoS ONE. 7:e41680. doi: 10.1371/journal.pone.0041680
Grundy, F., and Henkin, T. (1998). The S box regulon: a new global transcription termination control system for methionine and cysteine biosynthesis genes in Gram-positive bacteria. Mol. Microbiol. 30, 737–749. doi: 10.1046/j.1365-2958.1998.01185.x
Gutiérrez-Peciado, A., Henkin, T. M., Grundy, F. I., Yanofsky, C., and Merino, E. (2009). Biochemical features and functional implications of the RNA-based T-box regulatory mechanism. Microbiol. Mol. Biol. Rev. 73, 36–61. doi: 10.1128/MMBR.00026-08
György, P. (1956). The history of vitamin B6. Am. J. Clin. Nutr. 4, 313–317.
Hansson, M., Rutberg, L., Schröder, I., and Hederstedt, L. (1991). The gyrase of Streptococcus bovis and Bacillus subtilis. Mol. Gen. Microbiol. Virol. 1, 34–37.
Karatas, A. Y., Cetin, S., and Ozencgiz, G. (2003). The effects of insertional mutations in comQ, comP, strA, spo0H, spo0A and abrB genes on bclisylin biosynthesis in Bacillus subtilis. Biochim. Biophys. Acta. 1626, 51–56. doi: 10.1016/S0167-4878(03)00037-X
Kears, D. B., Chu, F., Branda, S. S., Kolter, R., and Losick, R. (2005). A master regulator for biofilm formation by Bacillus subtilis. Mol. Microbiol. 55, 739–749. doi: 10.1111/j.1365-2958.2004.04440.x
Kiel, J. A., Boels, J. M., Beldman, G., and Venema, G. (1994). Glycogen in Bacillus subtilis: molecular characterization of an operon encoding enzymes involved in glycogen biosynthesis and degradation. Mol. Microbiol. 11, 203–218. doi: 10.1111/j.1365-2958.1994.tb03010.x
Kim, D., San, B. H., Moh, S. H., Park, H., Kim, D. Y., Lee, S., et al. (2010). Structural basis for the substrate specificity of Pepα from Streptococcus pneumoniae, a dodecameric tetrahedral protease. Biochem. Biophys. Res. Commun. 391, 431–436. doi: 10.1016/j.bbrc.2009.11.075
Kim, J., and Copley, S. D. (2012). Inhibitory cross-talk upon introduction of a new metabolic pathway into an existing metabolic network. Proc. Natl. Acad. Sci. U.S.A. 109, E266–E2664. doi: 10.1073/pnas.1208799109
Kosugi, T. E., Ogulur, I., Mutlu, A., Yazgan-Karatas, A., and Ozencgiz, G. (2011). Global regulatory systems operating in bacilysin biosynthesis in Bacillus subtilis. J. Mol. Microbiol. Biotechnol. 20, 144–155. doi: 10.1159/000328639
Kraemer, K., Sembra, R. D., Eggersdorfer, M., and Schaumburg, D. A. (2012). Introduction: the diverse and essential biological functions of vitamins. Ann. Nutr. Metab. 61, 185–191. doi: 10.1159/000343103
Kriel, A., Brinsmade, S. R., Tje, I. L., Tehranchi, A. K., Bittner, A. N., Sonenchein, A. L., et al. (2014). GTP dysregulation in Bacillus subtilis cells lacking (p)ppGpp results in phenotypic amino acid auxotrophy and failure to adapt to nutrient downshift and regulate biosynthetic genes. J. Bacteriol. 196, 189–201. doi: 10.1128/JB.00818-13
Labella, J. I., Cantos, R., Espinosa, J., Forcada-Nadal, A., Rubio, V., and Contreras, A. (2017). PipP, a member of the conserved COG0325 family of PLP-binding proteins, expands the cyanobacterial nitrogen regulatory network. Front. Microbiol. 8:1244. doi: 10.3389/fmicb.2017.01244
Lader, B., Maurer, W., Scharf, S., Stepuskin, K., and Schmidt, F. S. (1999). Vitamin B6 biosynthesis: formation of pyridoxine 5′-phosphate from 4′-(phosphohydroxy)-L-threonine and L-deoxy-D-xylulose-5-phosphate by PdxA and PdxJ proteins. FEBS Lett. 449, 45–48. doi: 10.1016/S0014-5793(99)0393-2
Lee, W. M., Elliot, J. E., and Brownsey, R. W. (2005). Inhibition of acetyl-CoA carboxylase isoforms by pyridoxal phosphate. J. Biol. Chem. 280, 41385–41843. doi: 10.1074/jbc.M510728200
Lepore, B. W., Rutzick, F. J., Frey, P. A., and Ringe, D. (2005). The X-ray crystal structure of lysine-2,3-aminomutase from Clostridium subterminalis. Proc. Natl. Acad. Sci. U.S.A. 102, 13829–13824. doi: 10.1073/pnas.0505726102
Lerma-Ortiz, C., Jeffryes, J. G., Cooper, A. J., Niehaus, T. D., Thamm, A. M., Frelin, O., et al. (2016). “Nothing of chemistry disappears in biology”: the top 30 damage-prone endogenous metabolites. Biochim. Biophys. Acta. 44, 961–971. doi: 10.1016/j.bbagen.2015.12.008
Liao, S., Bitoun, J. P., Nguyen, A. H., Bozner, D., Yao, X., and Wen, Z. T. (2015). Deficiency of PdxR in Streptococcus mutans affects vitamin B6 metabolism, acid tolerance response and biofilm formation. Mol. Oral Microbiol. 30, 255–268. doi: 10.1111/omi.12090
to isopenoids, thiamin, and pyridoxol. *Proc. Natl. Acad. Sci. U.S.A.* 94, 12857–12862. doi: 10.1073/pnas.94.24.12857

Stel, L., Serrano, M., Henriques, A. O., and Völker, U. (2005). Genome-wide analysis of temporally regulated and compartment-specific gene expression in sporulating cells of *Bacillus subtilis*. *Microbiology* 151, 399–420. doi: 10.1099/mic.0.26854-0

Stolz, J., and Vielreicher, M. (2003). Tn10p1, the plasma membrane vitamin B6 transporter of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 21, 18990–18996. doi: 10.1074/jbc.M300942000

Strohmeier, M., Raschle, T., Mazurkiewicz, J., Rippe, K., Sinning, I., Fitzpatrick, T. B., et al. (2006). Structure of a bacterial pyridoxal 5′-phosphate synthase complex. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19284–19289. doi: 10.1073/pnas.0604950103

Sugimoto, R., Saito, N., Shimada, T., and Tanaka, K. (2017). Identification of YsbA as the pyridoxal 5′-phosphate (PLP) phosphatase in *Escherichia coli*: importance of PLP homeostasis on the bacterial growth. *J. Gen. Appl. Microbiol.* 63, 362–368. doi: 10.2332/jgim.2017.02.008

Sun, D., and Setlow, P. (1993). Cloning, nucleotide sequence, and regulation of the *Bacillus subtilis* nadB gene and a nif-like gene, both of which are essential for NAD biosynthesis. *J. Bacteriol.* 175, 1432–1432. doi: 10.1128/jb.175.5.1432-1432.1993

Suvorova, I. A., and Rodionov, D. A. (2016). Comparative genomics of pyridoxal 5′-phosphate-dependent transcription factor regions in bacteria. *Microbiol. Genom.* 2:e000047. doi: 10.1099/mgen.0.000047

Szydłowski, N., Bürkle, L., Pourcel, L., Moulin, M., Stolz, J., and Fitzpatrick, T. B. (2013). Recycling of pyridoxine (vitamin B6) by PUP1 in Arabidopsis. *Plant J.* 70, 45–50. doi: 10.1111/tjp.12195

Takiff, H. E., Baker, T., Copeland, T., Chen, S. M., and Court, D. L. (1992). Identification of SUN, D., and Setlow, P. (1993). Cloning, nucleotide sequence, and regulation of the *Bacillus subtilis* nadB gene and a nif-like gene, both of which are essential for NAD biosynthesis. *J. Bacteriol.* 175, 1432–1432. doi: 10.1128/jb.175.5.1432-1432.1993

Suvorova, I. A., and Rodionov, D. A. (2016). Comparative genomics of pyridoxal 5′-phosphate-dependent transcription factor regions in bacteria. *Microbiol. Genom.* 2:e000047. doi: 10.1099/mgen.0.000047

Tomas, J., McDaniel, B. A., Grundy, F., and Henkin, T. M. (2008). EF-P posttranslational modification has variable impact on polypeptide translation in *Bacillus subtilis*. *MBio* 9, e00306–e00318. doi: 10.1128/mBio.00306-18

Wu, Y., Chen, J., Liu, Z., and Wang, F. (2018). Identification of pyridoxal-modified proteins using mass spectrometry. *Rapid Commun. Mass Spectrom.* 32, 195–200. doi: 10.1002/rcm.8030

Yang, Y., Tsui, H. C., Man, T. K., and Winkler, M. E. (1998). Identification and function of the pdxY gene, which encodes a novel pyridoxal kinase involved in the salvage pathway of pyridoxal 5′-phosphate biosynthesis in *Escherichia coli K-12*. *J. Bacteriol.* 180, 1814–1821.

Yang, Y., Zhao, G., and Winkler, M. E. (1996). Identification of the pdxK gene that encodes pyridoxine (vitamin B6) kinase in *Escherichia coli* K-12. *FEBS Microbiol. Lett.* 141, 89–95. doi: 10.1111/1577-6698.1996.tb08368.x

Yoon, B. J. (2009). Hidden markov models and their applications in biological sequence analysis. *Curr. Genomics* 10, 402–415. doi: 10.2187/138920209789177575

Yoshikane, Y., Yokochi, N., Ohnishi, K., Hayashi, H., and Yagi, T. (2006). A paralog of lysyl-tRNA synthetase aminoacylates a conserved lysine residue in translation elongation factor P. *Nat. Struct. Mol. Biol.* 13, 598–605. doi: 10.1038/nsmb.1037
Zhao, G., Pease, A. J., Bharani, N., and Winkler, M. E. (1995). Biochemical characterization of gapB-encoded erythrose 4-phosphate dehydrogenase of *Escherichia coli* K-12 and its possible role in pyridoxal 5′-phosphate biosynthesis. *J. Bacteriol.* 177, 2804–2812. doi: 10.1128/JB.177.10.2804-2812.1995

Zhao, G., and Winkler, M. E. (1995). Kinetic limitation and cellular amount of pyridoxine (pyridoxamine) 5′-phosphate oxidase of *Escherichia coli* K-12. *J. Bacteriol.* 177, 883–891. doi: 10.1128/JB.177.4.883-891.1995

Zhao, H., Roistacher, D. M., and Helmann, J. D. (2018). Aspartate deficiency limits peptidoglycan synthesis and sensitizes cells to antibiotics targeting cell wall synthesis in *Bacillus subtilis*. *Mol. Microbiol.* 109, 826–844. doi: 10.1111/mmi.14078

Zhu, B., and Stülke, J. (2017). SubtiWiki in 2018: from genes and proteins to functional network annotation of the model organism *Bacillus subtilis*. *Nucleic Acids Res.* 46, D743–D748. doi: 10.1093/nar/gkx908

Zhu, J., Burgner, J. W., Harms, E., Belitsky, B. R., and Smith, J. L. (2005). A new arrangement of (beta/alpha)8 barrels in the synthase subunit of PLP synthase. *J. Biol. Chem.* 280, 27914–27923. doi: 10.1074/jbc.M503642200

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Richts, Rosenberg and Commichau. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.