SUPPORTING INFORMATION

Heterologous microcompartment assembly in *Bacillaceae*: establishing the components necessary for scaffold formation

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This file includes: Figures S1-S3, Tables S1-S4, Bioinformatic analysis, References

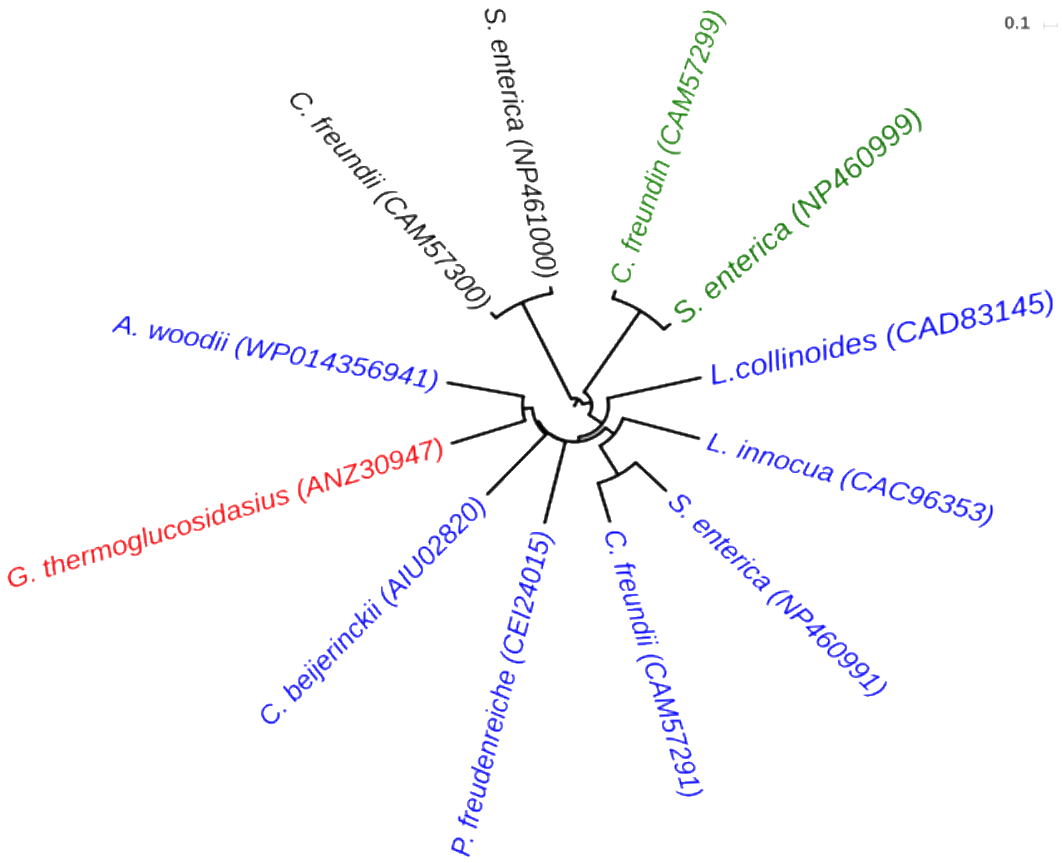

Figure S1. The translated BCV53_13080 gene from *P. thermoglucosidasius* NCIMB 11955 (shown in red) clustered within the PduK protein clade (blue) by phylogenetic analysis; PduT (green) and PduU (black) proteins were also included in the analysis. The tree was constructed using the neighbour-joining algorithm with the CheY chemotaxis protein (not shown) of *B.subtilis* 168 as the root. The GenBank NCBI accession numbers are quoted in brackets.

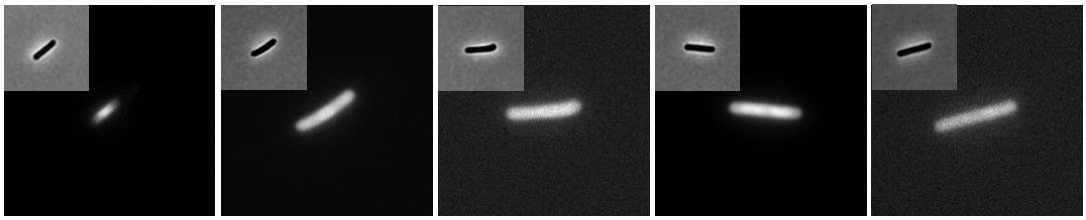

Figure **S2**. Heterologous expression of the shell proteins, PduA, -B, -K, -J and –N with C terminal msfGFP fusions in *B. subtilis* cells after 4 hours induction with 1 mM IPTG in MCGT medium ascertained by fluorescence microscopy. Strains (relevant features present in brackets) used (from left to right): YW02 ($P_{hyperspank}$ - *pduA-msfgfp pduBKJN*), YW03 ($P_{hyperspank}$ - *pduAB-msfgfp pduKJN*), YW04 ($P_{hyperspank}$ - *pduABK-msfgfp*), YW05 ($P_{hyperspank}$ - *pduABKJ-msfgfp*), YW06 ($P_{hyperspank}$ - *pduABKJN-msfgfp*)

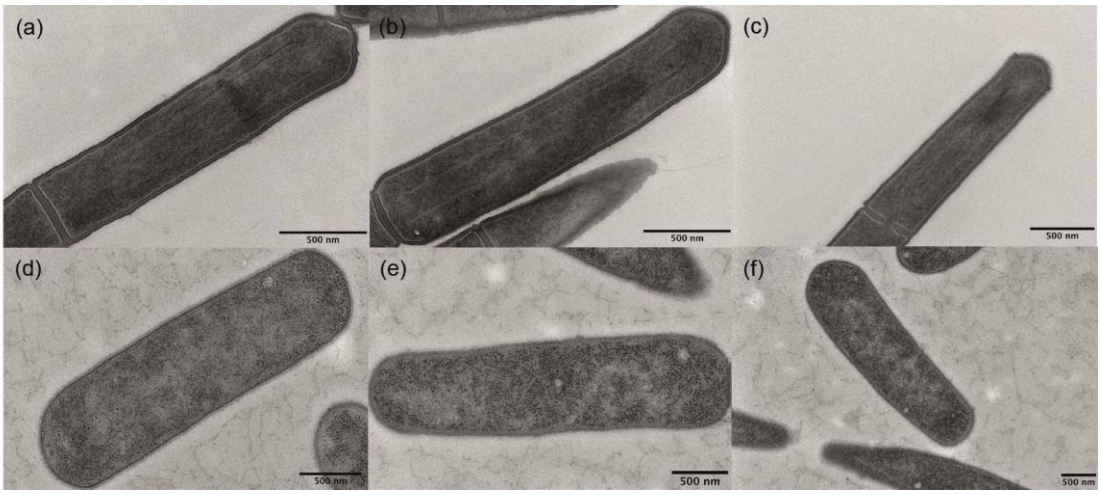

Figure S3. TEM longitudinal ultrathin sectioning of *B. subtilis* YW01 (a-c) and *P. thermoglucosidasius* YW11 (d-f) cells synthesising BMCs.

Table S1. List of the *Geobacillus* species genomes analysed in this study

#	Name	Strain	NCBI assembly #	Pfam00936/03319 domain-containing proteins	Reference
1	*P. thermoglucosidasius*	DSM 2542	GCA_001295365.1	+/+	Chen[1]
2	*P. thermoglucosidasius*	TNO-09.020	GCA_000258725.1	+/+	Zhao[2]
3	*P. thermoglucosidasius*	C56-YS93	GCA_000178395.2	+/+	Brumm[3]
4	*P. thermoglucosidasius*	NCIMB 11955	GCA_001700985.1	+/+	Sheng[4]
5	*P. thermoglucosidasius*	TM242	GCA_001902495.1	+/+	Sheng[4]
6	*G. thermocatenulatus*	KCTC 3921	GCA_002243665.1	+/+	nda*
7	*P. thermoglucosidasius*	Y4.1MC1	GCF_000166075.1	+/+	Brumm[3]
8	*Geobacillus* sp.	JS12	GCA_001592395.1	+/+	Jeon[5]
9	*P. toebii*	WCH70	GCA_000023385.1	-/-	Brumm[6]
10	*Geobacillus* sp.	Y412MC61	GCA_000024705.1	-/-	Brumm[3]
11	*Geobacillus* sp.	C56-T3	GCA_000092445.1	-/-	nda
12	*Geobacillus* sp.	Y412MC52	GCA_000174795.2	-/-	Mead[7]
13	*Geobacillus* sp.	GHH01	GCA_000336445.1	-/-	Wiegand[8]
14	*Geobacillus* sp.	12AMOR1	GCA_001028085.1	-/-	Wissuwa[9]
15	*Geobacillus* sp.	LC300	GCA_001191625.1	-/-	Cordova[10]
16	*G. kaustophilus*	HTA426	GCA_000009785.1	-/-	Takami[11]
17	*G. stearothermophilus*	10	GCA_001274575.1	-/-	Lewis[12]
18	*G. stearothermophilus*	DSM 458	GCA_002300135.1	-/-	Egan[13]
19	*G. thermodenitrificans*	NG80-2	GCA_000015745.1	-/-	Feng[14]
20	*G. thermodenitrificans*	KCTC3902	GCA_002072065.1	-/-	Lee[15]
21	*G. thermodenitrificans*	T12	GCA_002119625.1	-/-	Daas[16]
22	*G. thermoleovorans*	KCTC 3570	GCA_001610955.1	-/-	nda
23	*G. thermoleovorans*	CCB_US3_UF5	GCA_000236605.1	-/-	Sakaff[17]
24	*G. thermoleovorans*	FJAT-2391	GCA_001719205.1	-/-	Liu[18]
25	*G. thermoleovorans*	ID-1	GCA_002706565.1	-/-	Boonmak[19]
26	*G. subterraneus*	KCTC 3922	GCA_001618685.1	-/-	Lee[15]
27	*G. lituanicus*	N-3	GCA_002243605.1	-/-	Park[20]
28	*Geobacillus* genomosp. 3	JF8	GCA_000445995.2	-/-	Shintani[21]

*no data available

Table S2. Bacterial strains used in this study

Strain	Genotype	Source
B. subtilis: SG81	*trpC2 lacA::neo*	Daniel[22]
YW01	*trpC2 amyE::(*$P_{hyperspank}$*- pduABKJN spc) lacA::neo*	SG81 with pYW01 integrated at the *amyE* locus (this study)
YW01c	*trpC2 amyE::(*$P_{hyperspank}$*- spc) lacA::neo*	SG81 with pDR111 integrated at the *amyE* locus (this study)
YW02	*trpC2 amyE::(*$P_{hyperspank}$*– pduA-msfgfp pduBKJN spc) lacA::neo*	SG81 with pYW02 integrated at the *amyE* locus (this study)
YW03	*trpC2 amyE::(*$P_{hyperspank}$*– pduAB-msfgfp pduKJN sps) lacA::neo*	SG81 with pYW03 integrated at the *amyE* locus (this study)
YW04	*trpC2 amyE::(*$P_{hyperspank}$*– pduABK-msfgfp spc) lacA::neo*	SG81 with pYW04 integrated at the *amyE* locus (this study)
YW05	*trpC2 amyE::(*$P_{hyperspank}$*– pduABKJ-msfgfp spc) lacA::neo*	SG81 with pYW05 integrated at the *amyE* locus (this study)
YW06	*trpC2 amyE::(*$P_{hyperspank}$*– pduABJKN-msfgfp spc) lacA::neo*	SG81 with pYW06 integrated at the *amyE* locus (this study)
YW07	*trpC2 amyE::(*$P_{hyperspank}$*- pduABKJN spc) lacA::(*P_{xyl}*-pduP-msfgfp erm)*	YW01with pYW08 integrated at the *lacA* locus (this study)
YW08	*trpC2 amyE::(*$P_{hyperspank}$*- pduABKJN spc) lacA::(*P_{xyl}*- pduP72-msfgfp erm)*	YW01 with pYW09 integrated at the *lacA* locus (this study)
YW9	*trpC2 amyE::(*$P_{hyperspank}$*- pduABKJN spc) lacA::(*P_{xyl}*- pduP54-msfgfp erm)*	YW01 with pYW10 integrated at the *lacA* locus (this study)
YW07c	*trpC2 amyE::(*$P_{hyperspank}$*- spc) lacA::(*P_{xyl}*- pduP-msfgfp erm)*	YW01c with pYW08 integrated at the *lacA* locus (this study)
YW08c	*trpC2 amyE::(*$P_{hyperspank}$*- spc) lacA::(*P_{xyl}*-pduP72-msfgfp erm)*	YW01c with pYW09 integrated at the *lacA* locus (this study)
YW09c	*trpC2 amyE::(*$P_{hyperspank}$*- spc) lacA::(*P_{xyl}*-pduP54-msfgfp erm)*	YW01c with pYW10 integrated at the *lacA* locus (this study)
YW10	*trpC2 amyE::(*$P_{hyperspank}$*– pduA-msfgfp pduBKJN spc) lacA::neo aprE::(*P*xyl-m9r-walp-mch cat)*	YW02 with pCW integrated at the *aprE* locus (this study)
P. thermoglucosidasius: NCIMB 11955	Wild type	National Collection of Industrial Food and Marine Bacteria
YW11	ΩP*pdu*::(P*glv-pduABCDEGHKJLYZNOPUnQ*)	NCIMB 11955 with P*glv* replacing P*pdu*
E. coli: DH5α	*E. coli* F⁻ φ*80lacZΔM15, Δ(lacZYArgF)U196, recA1, endA1, hsdR17, (rk-, mk+), phoA, supE44, λ-, thi-1, gyrA96, relA1*	Invitrogen
S17-1 λpir	*Tp*^{R} *Sm*^{R} *recA, thi, pro, hsdR*$^-$ *M*$^+$*RP4: 2-Tc:Mu: Km Tn7 λpir*	Simon[23]

Table S3. Plasmids used in this study

Name	Features	Reference/Source
pAX01	*bla lacA' xylR* P_{xyl} *erm 'lacA, B. subtilis* integration vector	Hartl[24]
pDR111	*bla amyE' lacI* $P_{hyperspank}$ *spc 'amyE, B. subtilis* integration vector	Wagner[25]
pHJS105	Source of *msfgfp*	Henrik Strahl Lab collection
pCW	*bla aprE'* $P_{xyl\text{-}M9R}$ – *WALP-mch cat 'aprE, B. subtilis* integration vector	Jeff Errington Lab collection
pP2	Source of *G. thermoglucosidasius glv* promoter	David Leak Lab collection
pG2K	kan, *repB, Geobacillus* spp. shuttle vector	Reeve[26]
pG2KoriT	$oriT_{RP4}$ kan, *repB, Geobacillus* spp. shuttle vector	Ortenzi & Leak (unpublished)
pYW01	*bla amyE' lacI* $P_{hyperspank}$ – *pduABKJN spc 'amyE*	This study
pYW02	*bla amyE' lacI* $P_{hyperspank}$ – *pduA-msfGFP pduBKJN spc 'amyE*	This study
pYW03	*bla amyE' lacI* $P_{hyperspank}$ – *pduAB-msfGFP pduKJN spc 'amyE*	This study
pYW04	*bla amyE' lacI* $P_{hyperspank}$ – *pduABKm-sfGFP spc 'amyE*	This study
pYW05	*bla amyE' lacI* $P_{hyperspank}$ – *pduABKJ-msfGFP spc 'amyE*	This study
pYW06	*bla amyE' lacI* $P_{hyperspank}$ – *pduABKJN-msfGFP spc 'amyE*	This study
pYW07	*bla lacA' xylR* P_{xyl} – *msfGFP erm 'lacA*	This study
pYW08	*bla lacA' xylR* P_{xyl} – *pduP-msfGFP erm 'lacA*	This study
pYW09	*bla lacA' xylR* P_{xyl} – *pduP72 - msfGFP erm 'lacA*	This study
pYW10	*bla lacA' xylR* P_{xyl} – *pduP54 - msfGFP erm 'lacA*	This study
pYW11	$oriT_{RP4}$ *kan, repB,* P_{pdu} *'*P_{glv} *'*P_{pdu}	This study

Table S4. Oligonucleotides used in this study

Name	Sequence (5' → 3')	Purpose
01	ACAATAGCTAGCGTAGAGGGAGGTCCACGTATGGTTCG	*pduAB* gene amplification
02	CGTTAATCCCCAAAAATCTTCACCTCCTTGTTAAATATATG GAATGGTCTCTG	
03	CCTGTCTCAGAGACCATTCCATATATTTAACAAGGAGGTG AAGATTTTTGGGG	*pduKJ* gene amplification
04	ATTCCGATATACACGGATGATCACCTTCACTTAAATGCTTT TTGGCAACATTTTTTCCACATCTG	
05	GTGGAAAAAATGTTGCCAAAAAGCATTTAAGTGAAGGTGA TCATCCGTG	*pduN* gene amplification
06	TGGTACGCATGCCTATCCATTGATTTCCACAGAG	
07	GGAAGGAATTCTTCCTAAACTAGTTCAAGAAAGCGGAGCT GTTGGTATGAGCAAAGGAGAAGAACTTTTCACTGG	C-terminal fusion of *pduA* to *msfgfp*
08	CTCCTTCATGCCTAACACCTCCATTGCTTTATTTGTAGAGCT CATCCATGCC	
09	GTGCCTGTCTCAGAGACCATTCCATATATTAGCGGAGCTGT TGGTATGAGCAAAGGAGAAGAACTTTTCACTGG	C-terminal fusion of *pduB* to *msfgfp*
10	CGTTAATCCCCAAAAATCTTCACCTCCTTGTTATTTGTAGA GCTCATCCATGCC	
11	ATTATCAAGAGATTAGGAGGAAAGATAGCAAAAGCGGAG CTGTTGGTATGAGCAAAGGAGAAGAACTTTTCACTGG	C-terminal fusion of *pduK* to *msfgfp*
12	CTCGTTTCCACCGAATTAGCTTGCATGCTTATTTGTAGAGC TCATCCATGCC	
13	AGATGTGGAAAAAATGTTGCCAAAAAGCATTAGCGGAGCT GTTGGTATGAGCAAAGGAGAAGAACTTTTCACTGG	C-terminal fusion of *pduJ* to *msfgfp*
12	CTCGTTTCCACCGAATTAGCTTGCATGCTTATTTGTAGAGC TCATCCATGCC	
14	GCCATTGTCGACTCTGTGGAAATCAATGGAAGCGGAGCTG TTGGTATGAGCAAAGGAGAAGAACTTTTCACTGG	C-terminal fusion of *pduN* to *msfgfp*
12	CTCGTTTCCACCGAATTAGCTTGCATGCTTATTTGTAGAGC TCATCCATGCC	
15	GGATAAACTTGTTCACTTAAATCAAAGGTAAGGAGGAGGG CCCGACTCTGGTCTTGCTATGAGCAAAGGAGAAGAACTTT TCACTGG	*msfgfp* insertion into pAX01 vector
16	GAGTGCGGCCGCCCGCGGGAGCTCTTATTTGTAGAGCTCA TCCATGCC	
17	GGAGGGCCCATGAGCGTGGATGCACAAAAAATTG	*pduP* gene amplification
18	AGCTCAGACCAGAGTCTCTTATCGACAAAGCATCCACTAA G	
17	GGAGGGCCCATGAGCGTGGATGCACAAAAAATTG	*pduP 72 5' end* nucleotides amplification
19	AGCTCAGACCAGAGTCCTTCTTTTTCTCTTCCATTTCCTCC	
17	GGAGGGCCCATGAGCGTGGATGCACAAAAAATTG	*pduP 54* 5' end nucleotides amplification
20	AGCTCAGACCAGAGTCTTCCTCCAGTATTTTTCTTACAAG	
21	AGGTCTCAATAACGATCATGATGAATAAGAAGTTGG	*glv* promoter region amplification
22	AGGTCTCACATACGTGAGCGCTTTCACTAC	
23	AGGTCTCAACAGCCATCAAGAAGATGTTATTGCG	BCV53_13120 fragment amplification for homologous recombination in *Geobacillus*
24	AGGTCTCATTATATAAGCCAATTTCCATTGAG	
25	AGGTCTCATATGGTAGAGGGAGGTCCACGTATGG	pduA fragment amplification for homologous recombination in *Geobacillus*
26	AGGTCTCACTAGCATGGACACTAGAATCTACATTGGC	
27	GCTTCTCCTTCTCAATGGCAGCG	Confirmation of the *lacA* chromosome integration
28	GGCCAGCTATACGACATTTGCGG	

Bioinformatic analysis of the *pdu* operon from *P. thermoglucosidasius* NCIMB 11955

In addition to the shell proteins described in the manuscript, we have also analysed the other proteins encoded in the *pdu* operon of *P. thermoglucosidasius*. Based on translated sequence similarity, the operon encodes diol dehydratase (PduCDE), phosphotransacylase (PduL), propionaldehyde dehydrogenase (PduP) and 1-propanol dehydrogenase (PduQ) enzymes converting 1,2-PD into propanol and propionyl-phosphate. The last step in 1,2-PD metabolism is typically facilitated by propionate kinase (PduW) converting propionyl-phosphate to propionate, a reaction that acts as a source of ATP for the entire degradation pathway[27], but the relevant gene was not found in the studied *pdu* operon. However, the BCV53_18310 gene, which encodes a protein, with 56% identity to PduW from *Salmonella*, from the acetate/propionate kinase family was found further downstream from the *pdu* operon in the *P. thermoglucosidasius* genome and may function as a propionate kinase in 1,2-PD degradation. No gene encoding a 1,2-PD transporter, normally PduF, was identified in the operon or any other genomic region. In *S. enterica*, 1,2-PD degradation is linked with synthesis of cobalamin, which is an essential component for activity of propanediol dehydratase (PduCDE). Genes encoding diol dehydratase reactivase (PduGH) and adenosyltransferase (PduO), enzymes participating in the initial step of B12 production were identified in the operon. However, the genes encoding cobalamin reductase (PduS) and L-threonine kinase (PduX) enzymes were not found in the operon or elsewhere in the genome. Three translated genes, BCV53_13065, BCV53_13060 and BCV53_13040, did not have significant similarity to any of the Pdu proteins from *Salmonella* and, thus, a wider search including all homologs present in the NCBI database was performed. This showed that the product of BCV53_13065 aligned with proteins possessing the conserved domain of the HSP70 family of molecular chaperones and had close homologues in other thermophilic and thermo-tolerant species such as *Anoxybacillus flavithermus*, *Clostridium thermopalmarium*, *Geobacillus* spp. and *Quasibacillus thermotolerans*. The translated BCV53_13060 CDS had 100% similarity to a protein from the flavoprotein family (pfam02441), found in the BMC operon of *A. flavithermus* surrounded by the BMC and EutN/CcmL domain-containing proteins. A BLAST search (BLASTP 2.8.0+) of the product of the BCV53_13040 gene against *Bacillaceae* (NCBI taxonomic identifier: 186817) showed sequence identity from 37-72% to a hypothetical protein from members within the family. However, despite its high degree of conservation, this 279 amino acid hypothetical protein had no identifiable motifs based on accelerated protein-protein BLAST, standard protein-protein BLAST, or Position-Specific Iterated (PSI-)BLAST searches and, thus, we conclude it is representative of a novel protein. Since these genes are clustered within the *pdu* operon in *P. thermoglucosidasius*, the corresponding proteins are expected to be involved in either the formation of BMCs or the Pdu pathway, but their specific functions are unclear. Nevertheless, in order to allow for future referencing of the BCV53_13065,

BCV53_13060 and BCV53_13040 genes, we propose here to designate them with the novel identifiers *pduY, -Z* and *-Un*, respectively. Two genes, BCV53_13125 and BCV53_13120, were immediately upstream of the *pdu* operon and transcribed in the same direction. BCV53_13125 encodes a protein with a central domain with 78% similarity to a histidine kinase from *Bacillus azotoformans*, with additional N-terminal PocR and C-terminal ATPase domains. Identical proteins are encoded upstream of the *pdu* operon in the other *P. thermoglucosidasius* strains and *Anoxybacillus flavithermus*. The translated BCV53_13120 CDS suggests it is a DNA-binding response regulator of the AraC protein family containing an N-terminal phosphorylation site of the REC superfamily and HTH-ARAC domain at the C terminus. In *S. enterica* transcription of the *pdu* locus is regulated by a PocR regulatory protein, which binds to a specific recognition site upstream of the operon in response to the presence of 1,2-PD. PocR also regulates expression of the adjacent *cob* operon for the synthesis of cobalamin, the essential cofactor for the activity of the propanediol dehydratase enzyme in the initial step of 1,2-PD breakdown[28,29]. However, transcription initiation of the *pdu* operon may not be regulated in the same way for *Parageobacillus* and *Geobacillus* spp. due to differences in the control of carbon catabolism in Gram-positive and Gram-negative bacteria[30,31]. A catabolite-responsive element (cre) sequence in the intergenic area upstream of the first *pduA* gene of the *pdu* operon was proposed to regulate 1,2-PD metabolism in *Lactobacillus reuteri*[32]. Further empirical investigation to examine the nature of the *pdu* operon regulation in *P. thermoglucosidasius* NCIMB 11955 is needed. The *pdu* operon and regulatory elements are enclosed by genes that code an ABC transporter protein family and a divergently transcribed gene encoding a transposase protein upstream and downstream of the locus, respectively.

References

[1] Chen, J., Zhang, Z., Zhang, C., and Yu, B. (2015) Genome sequence of *Geobacillus thermoglucosidasius* DSM2542, a platform hosts for biotechnological applications with industrial potential, *J Biotechnol 216*, 98-99.

[2] Zhao, Y., Caspers, M. P., Abee, T., Siezen, R. J., and Kort, R. (2012) Complete genome sequence of *Geobacillus thermoglucosidans* TNO-09.020, a thermophilic sporeformer associated with a dairy-processing environment, *J Bacteriol 194*, 4118.

[3] Brumm, P., Land, M. L., Hauser, L. J., Jeffries, C. D., Chang, Y. J., and Mead, D. A. (2015) Complete genome sequences of *Geobacillus sp*. Y412MC52, a xylan-degrading strain isolated from obsidian hot spring in Yellowstone National Park, *Stand Genomic Sci 10*, 81.

[4] Sheng, L. L., Zhang, Y., and Minton, N. P. (2016) Complete Genome Sequence of *Geobacillus thermoglucosidasius* NCIMB 11955, the Progenitor of a Bioethanol Production Strain, *Microbiol Resour Ann 4*.

[5] Jeon, S. J., Park, A. K., Kim, B. K., Park, H., Lee, J. H., Kim, H. W., and Shin, S. C. (2016) Complete genome sequence of the crude oil-degrading thermophilic bacterium *Geobacillus sp*. JS12, *J Biotechnol 230*, 28-29.

[6] Brumm, P. J., Land, M. L., and Mead, D. A. (2016) Complete genome sequences of *Geobacillus sp*. WCH70, a thermophilic strain isolated from wood compost, *Stand Genomic Sci 11*, 33.

[7] Mead, D. A., Lucas, S., Copeland, A., Lapidus, A., Cheng, J. F., Bruce, D. C., Goodwin, L. A., Pitluck, S., Chertkov, O., Zhang, X., Detter, J. C., Han, C. S., Tapia, R., Land, M., Hauser, L. J., Chang, Y. J., Kyrpides, N. C., Ivanova, N. N., Ovchinnikova, G., Woyke, T., Brumm, C., Hochstein, R., Schoenfeld, T., and Brumm, P. (2012) Complete Genome Sequence of Paenibacillus strain Y4.12MC10, a Novel *Paenibacillus lautus* strain Isolated from Obsidian Hot Spring in Yellowstone National Park, *Stand Genomic Sci 6*, 381-400.

[8] Wiegand, S., Rabausch, U., Chow, J., Daniel, R., Streit, W. R., and Liesegang, H. (2013) Complete Genome Sequence of *Geobacillus sp*. Strain GHH01, a Thermophilic Lipase-Secreting Bacterium, *Genome Announc 1*, e0009213.

[9] Wissuwa, J., Stokke, R., Fedoy, A. E., Lian, K., Smalas, A. O., and Steen, I. H. (2016) Isolation and complete genome sequence of the thermophilic *Geobacillus sp*. 12AMOR1 from an Arctic deep-sea hydrothermal vent site, *Stand Genomic Sci 11*, 16.

[10] Cordova, L. T., Long, C. P., Venkataramanan, K. P., and Antoniewicz, M. R. (2015) Complete genome sequence, metabolic model construction and phenotypic characterization of Geobacillus LC300, an extremely thermophilic, fast growing, xylose-utilizing bacterium, *Metab Eng 32*, 74-81.

[11] Takami, H., Nishi, S., Lu, J., Shinamura, S., and Takaki, Y. (2004) Genomic characterization of thermophilic Geobacillus species isolated from the deepest sea mud of the Mariana Trench, *Extremophiles 8*, 351-356.

[12] Lewis, S. A., Clifton, S. W., Najar, F. Z., and Roe, B. A. (2016) Complete genome Sequence of *Geobacillus stearothermophilus* strain 10, a Yellowstone hot spring isolate, (Submitted JUL-2014) EMBL/GenBank/DDBJ databases.

[13] Egan, K., Kelleher, P., Field, D., Rea, M. C., Ross, R. P., Cotter, P. D., and Hill, C. (2017) Genome Sequence of *Geobacillus stearothermophilus* DSM 458, an

Antimicrobial-Producing Thermophilic Bacterium, Isolated from a Sugar Beet Factory, *Genome Announc 5*.

[14] Feng, L., Wang, W., Cheng, J., Ren, Y., Zhao, G., Gao, C., Tang, Y., Liu, X., Han, W., Peng, X., Liu, R., and Wang, L. (2007) Genome and proteome of long-chain alkane degrading *Geobacillus thermodenitrificans* NG80-2 isolated from a deep-subsurface oil reservoir, *Proc Natl Acad Sci U S A 104*, 5602-5607.

[15] Lee, Y. J., Park, M. K., Park, G. S., Lee, S. J., Lee, S. J., Kim, B. S., Shin, J. H., and Lee, D. W. (2017) Complete genome sequence of the thermophilic bacterium *Geobacillus subterraneus* KCTC 3922(T) as a potential denitrifier, *J Biotechnol 251*, 141-144.

[16] Daas, M. J. A., Vriesendorp, B., van de Weijer, A. H. P., van der Oost, J., and van Kranenburg, R. (2018) Complete Genome Sequence of *Geobacillus thermodenitrificans* T12, A Potential Host for Biotechnological Applications, *Curr Microbiol 75*, 49-56.

[17] Sakaff, M. K. L. M., Rahman, A. Y. A., Saito, J. A., Hou, S. B., and Alam, M. (2012) Complete Genome Sequence of the Thermophilic Bacterium *Geobacillus thermoleovorans* CCB_US3_UF5, *Journal of Bacteriology 194*, 1239-1239.

[18] Liu, G. H., Liu, B., Wang, J. P., Che, J. M., Zhu, Y. J., Chen, Q. Q., and Ruan, C. Q. (2015) Genome Sequence of *Paenibacillus sp*. Strain FJAT-28004 for the Genome Sequencing Project for Genomic Taxonomy and Phylogenomics of Bacillus-Like Bacteria, *Microbiol Resour Ann 3*.

[19] Boonmak, C., Takahasi, Y., and Morikawa, M. (2013) Draft Genome Sequence of *Geobacillus thermoleovorans* Strain B23, *Genome Announc 1*.

[20] Park, M.-K., Lee, Y.-J., Yi, H., Bahn, Y.-S., Kim, J. F., and Lee, D.-W. (2016) The whole genome sequencing and assembly of *G. thermoleovorans* ID-1 strain, (Submitted OCT-2016) EMBL/GenBank/DDBJ databases.

[21] Shintani, M., Ohtsubo, Y., Fukuda, K., Hosoyama, A., Ohji, S., Yamazoe, A., Fujita, N., Nagata, Y., Tsuda, M., Hatta, T., and Kimbara, K. (2014) Complete Genome Sequence of the Thermophilic Polychlorinated Biphenyl Degrader *Geobacillus sp*. Strain JF8 (NBRC 109937), *Genome Announc 2*.

[22] Daniel, R. A., Haiech, J., Denizot, F., and Errington, J. (1997) Isolation and characterization of the lacA gene encoding beta-galactosidase in *Bacillus subtilis* and a regulator gene, lacR, *J Bacteriol 179*, 5636-5638.

[23] Simon, R., Priefer, U., and Puhler, A. (1983) A Broad Host Range Mobilization System for Invivo Genetic-Engineering - Transposon Mutagenesis in Gram-Negative Bacteria, *Bio-Technol 1*, 784-791.

[24] Hartl, B., Wehrl, W., Wiegert, T., Homuth, G., and Schumann, W. (2001) Development of a new integration site within the *Bacillus subtilis* chromosome and construction of compatible expression cassettes, *Journal of Bacteriology 183*, 2696-2699.

[25] Wagner, J. K., Marquis, K. A., and Rudner, D. Z. (2009) SirA enforces diploidy by inhibiting the replication initiator DnaA during spore formation in *Bacillus subtilis*, *Mol Microbiol 73*, 963-974.

[26] Reeve, B., Martinez-Klimova, E., de Jonghe, J., Leak, D. J., and Ellis, T. (2016) The Geobacillus Plasmid Set: A Modular Toolkit for Thermophile Engineering, *Acs Synth Biol 5*, 1342-1347.

[27] S. Palacios, V. J. Starai and J. C. Escalante-Semerena, "Propionyl Coenzyme A Is a Common Intermediate in the 1,2-Propanediol and Propionate Catabolic

Pathways Needed for Expression of the prpBCDE Operon during Growth of *Salmonella enterica* on 1,2-Propanediol," *J. Bacteriol.,* vol. 185, no. 9, pp. 2802-2810, 2003.

[28] P. Chen, M. Ailion, T. A. Bobik, G. D. Stormo and J. R. Roth, "Five promoters integrate control of the cob/pdu regulon in *Salmonella typhimurium*," *J. Bacteriol.,* vol. 177, no. 19, pp. 5401-5410, 1995.

[29] M. R. Rondon and J. C. Escalante-Semerena, "In vitro analysis of the interactions between the PocR regulatory protein and the promoter region of the cobalamin biosynthetic (*cob*) operon of *Salmonella typhimurium* LT2," *J. Bacteriol.,* vol. 178, no. 8, pp. 2196-2203, 1996.

[30] M. H. Saier, S. Chauvaux Jr., J. Deutscher, J. Reizer and J. J. Ye, "Protein phosphorylation and regulation of carbon metabolism in gram-negative versus gram-positive bacteria," *Trends Biochem. Sci.,* vol. 20, pp. 267-271, 1995.

[31] J. Stülke and W. Hillen, "Regulation of Carbon Catabolism in Bacillus Species," *Annu. Rev. Microbiol.,* vol. 54, no. 1, pp. 849-880, 2000.

[32] D. D. Sriramulu, M. Liang, D. Hernandez-Romero, E. Raux-Deery, H. Lünsdorf, J. B. Parsons, M. J. Warren and M. B. Prentice, "*Lactobacillus reuteri* DSM 20016 Produces Cobalamin-Dependent Diol Dehydratase in Metabolosomes and Metabolizes 1,2-Propanediol by Disproportionation," *J. Bacteriol.,* vol. 190, no. 13, pp. 4559-4567, 2008.