Supplementary Information

Identification and Characterization of New Proteins Crucial for Bacterial Spore Resistance and Germination

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Supplementary Information List

Supplementary Table S1 Average parameters of the germination of multiple individual wt and *yetF* spores

Spores	PS832		PS4488	
	Valine	AGFK	Valine	AGFK
T_{lag}	20.3 ± 8.9	36.5 ± 16.6	44.6 ± 23.1	49.0 ± 20.5
$T_{release}$	24.0 ± 9.4	40.3 ± 16.9	48.2 ± 23.0	53.8 ± 20.9
T_{lys}	34.7 ± 9.2	52.0 ± 16.7	63.6 ± 17.6	74.5 ± 11.5
$\Delta T_{release}$	3.7 ± 1.4	3.8 ± 1.1	3.6 ± 1.2	4.8 ± 1.6
ΔT_{lys}	10.7 ± 2.8	11.7 ± 4.7	15.4 ± 8.0	20.7 ± 11.5
I_{lag}	0.88 ± 0.07	0.83 ± 0.06	0.77 ± 0.07	0.76 ± 0.09
$I_{release}$	0.21 ± 0.05	0.29 ± 0.07	0.29 ± 0.07	0.23 ± 0.07
ger/num†	97/139	80/104	44/133	14/40

Spores were germinated and individual germination parameters were measured as described in Methods. †Number of spores that germinated/number of spores examined.

Supplementary Table S2 Homologs of *B. subtilis yetF* or *2Duf* in completed genomes of *Bacilli*.

Bacilli spore forming species	Number of homologs to		Asporogenous *Bacilli* species	Number of homologs to	
	yetF	*2duf*		*yetF*	*2duf*
Aeribacillus pallidus	1	1	*Aerococcaceae bacterium*	1	0
Alicyclobacillus acidocaldaruis	0	1	*Auricoccus indicus*	1	0
Amphibacillus xylanus	2	0	*Brochothrix thermosphacta*	1	0
Aneurinibacillus soli	0	1	*Carnobacterium maltaromaticum*	1	0
Anoxyacillus flavithermus	2	1	*Enterococcus faecalis*	1	0
Bacillus amyloliquefaciens	1	0	*Gemella haemolysans*	1	0
Bacillus anthracis	3	1	*Jeotgalibaca dankookensis*	2	0
Bacillus atrophaeus	1	0	*Lactobacillus acidophilus*	1	0
Bacillus cereus	5	1	*Lactobacillus plantarum*	1	0
Bacillus firmus	1	0	*Lactobacillus reuteri_*	1	0
Bacillus halodurans	8	2	*Lactobacillus rhamnosus*	1	0
Bacillus licheniformis	6	0	*Lactococcus lactis*	1	0
Bacillus megaterium	1	0	*Leuconostoc kimchii*	1	0
Bacillus pumilus	1	0	*Marinilactibacillus 15R*	1	0
Bacillus subtilis	5	0	*Melissococcus plutonius*	1	0
Bacillus thuringiensis	5	1	*Planococcus antarcticus*	1	0
Bacillus weihenstephanensis	1	0	*Salimicrobium jeotgali*	1	0
Brevibacillus brevis	4	1	*Salinicoccus halodurans*	2	0
Cohnella candidum	5	1	*Streptococcus mutans GS-5*	1	0
Fictibacillus phosphorivorans	6	0	*Streptococcus pneumoniae*	1	0
Geobacillus kaustophilus	0	1	*Vagococcus penaei*	1	0
Geobacillus stearothermophilus	1	0			
Halobacillus halophilus	6	1			
Kyrpidia tusciae	0	2			
Laceyella sacchari	1	1			
Lentibacillus amyloliquefaciens	3	1			
Lysinibacillus sphaericus	4	0			
Novibacillus thermophilus	3	0			
Oceanobacillus iheyensis	2	1			
Paenibacillus polymyxa	2	1			
Paenisporosarcina cavernae	3	0			
Parageobacillus thermoglucosidasius	1	1			
Paraliobacillus zengyii	8	0			
Solibacillus silvestris	4	0			
Sporolactobacillus terrae	5	0			
Sporosarcina psychrophila	3	0			
Terribacillus goriensis	4	0			
Thermobacillus composti	2	0			
Virgibacillus halodenitrificans	3	2			
Average number in ones with ≥ 1	**3.2**	**1**	**Average number per species**	**1.1**	**0**

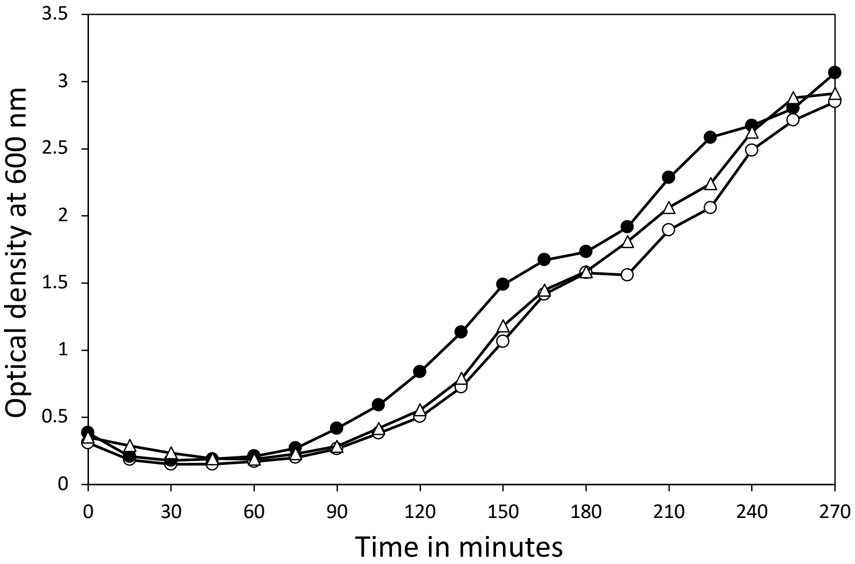
Supplementary Table S3 Homologs of *B. subtilis yetF/2Duf* in *Clostridia* genomes.

Clostridia spore formers	Number of homologs to		Likely *Clostridia* spore formers (have *gpr, spoVFA, spoVFB)*	Number of homologs to	
	yetF	*2duf*		*yetF*	*2duf*
Alkaliphilus metalliredigens	3	1	*Acetohalobium arabaticum*	5	0
Ammonifex degensii	2	0	*Anoxybacter fermentans*	2	0
Caldanaerobacter subterraneus	1	0	*Caldicellulosiruptor saccharolyticus*	1	0
Candidatus Arthromitus sp. SFB-mouse	1	0	*Carboxydocella thermautotrophica*	2	0
Candidatus Desulforudis audaxviator	1	0	*Ethanoligenens harbinense*	4	0
Caproiciproducens sp. NJN-50	2	0	*Faecalibacterium prausnitzii*	1	0
Cellulosilyticum lentocellum	1	0	*Flavonifractor plautii*	1	0
*Clostridioides difficile_*	1	0	*Halobacteroides halobius*	6	0
Clostridium acetobutylicum	5	0	*Halocella sp. SP3-1*	5	0
Clostridium botulinum	2	0	*Halothermothrix orenii*	6	0
Clostridium perfringens	1	0	*Herbinix luporum*	2	0
Clostridium tetani	2	0	*Natranaerobius thermophilus*	2	0
Dehalobacterium formicoaceticum	1	1	*Pelotomaculum thermopropionicum*	2	0
Desulfallas gibsoniae	2	1	*Ruminococcus albus*	1	0
Desulfitobacterium hafniense	3	0	*Syntrophomonas wolfei*	3	0
Desulfofarcimen acetoxidans	3	0	*Syntrophothermus lipocalidus*	2	0
Desulfofundulus kuznetsovii	2	0	*Thermincola potens*	3	0
Desulfosporosinus orientis	2	1	**Average number in ones with ≥1**	**2.8**	**0**
Desulfotomaculum ruminis	3	1			
Hungateiclostridium thermocellum	1	0	*Asporogenous Clostridia species*		
Intestinimonas butyriciproducens	1	0	*Aminipila sp. JN-39*	1	0
Lachnoanaerobaculum umeaense	2	0	*Carboxydothermus hydrogenoformans*	1	0
Lachnoclostridium phytofermentans	1	0	*Dehalobacter sp. CF*	1	0
Mahella australiensis	1	0	*Thermosediminibacter oceani*	1	0
Moorella thermoacetica	1	0	**Average number per species**	**1**	**0**
Paeniclostridium sordellii	2	0			
Pseudoclostridium thermosuccinogenes	1	1			
Ruminiclostridium cellulolyticum	3	0			
Symbiobacterium thermophilum	2	0			
Syntrophobotulus glycolicus	1	0			
Tepidanaerobacter acetatoxydans	1	0			
Thermacetogenium phaeum	2	0			
Thermoanaerobacter italicus	1	0			
Thermoclostridium stercorarium	1	0			
Average number in ones with ≥1	**1.6**	**1**			

Supplementary Table S4 Sequence identity between 2Duf homologs in *Bacilli* and *Clostridia* and 2Duf encoded in *spoVA*$^{\text{2mob}}$.

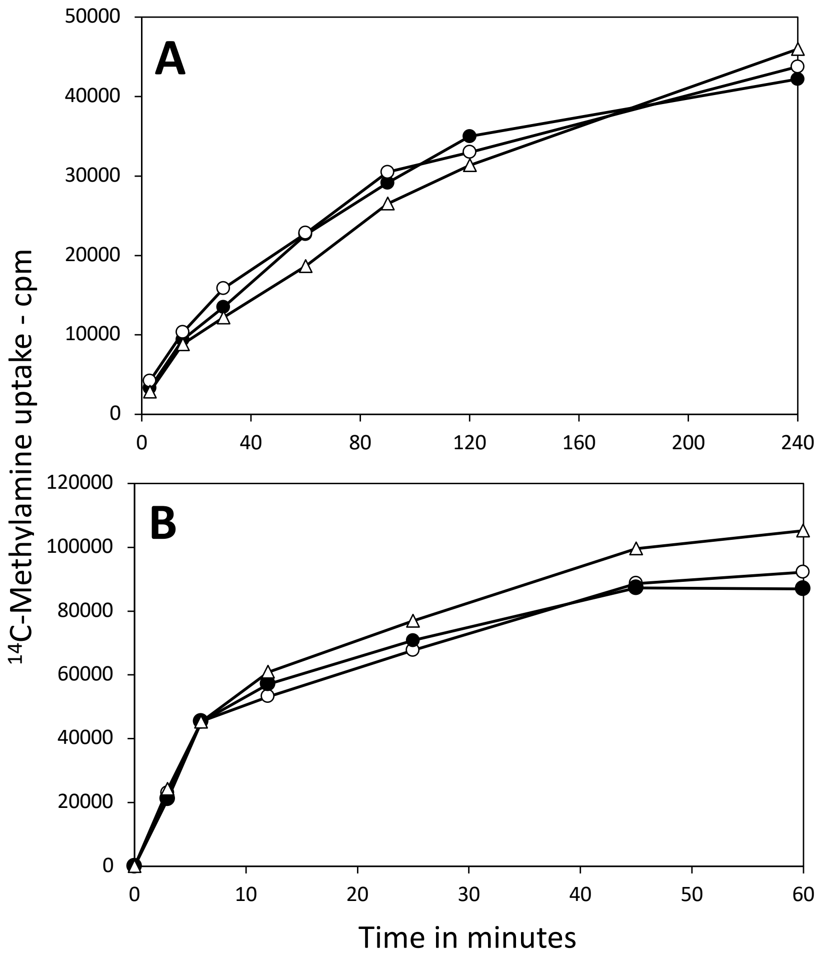
Bacilli	NCBI ID	Identity to 2Duf (%)
Group 1		
Aeribacillus pallidus	WP_066246610.1	55.2
Aneurinibacillus soli	WP_096463052.1	50.9
Anoxyacillus flavithermus	WP_012575624.	55.1
Bacillus anthracis	WP_000018934	59.1
Bacillus cereus	WP_000018922.1	60.5
Bacillus halodurans	WP_010897735.1	30.5
Bacillus thuringiensis	WP_000018924.1	60.8
Brevibacillus brevis	WP_015892354.1	52.4
Cohnella candidum	WP_123042938.1	53.1
Geobacillus kaustophilus	WP_011230358	54.5
Halobacillus halophilus	WP_014642772.1	57.3
Lentibacillus amyloliquefaciens	WP_068440573.1	55.1
Oceanobacillus iheyensis	WP_011065783.1	50.9
Parageobacillus thermoglucosidasius	WP_003252282.1	55.6
Virgibacillus halodenitrificans	WP_019376107.1	59.6
Group 2		
Alicyclobacillus acidocaldaruis	WP_041694969	48.6
Kyrpidia tusciae	WP_013075904.1	38.1
Laceyella sacchari	WP_102991708.1	47.9
Group 3		
Paenibacillus polymyxa	WP_013370894.1	39.9
Clostridia		
Alkaliphilus metalliredigens	WP_012065254.1	42.0
Dehalobacterium formicoaceticum	WP_089611718.1	54.0
Desulfallas gibsoniae	WP_006522674.1	46.5
Desulfosporosinus orientis	WP_014185970.1	38.6
Desulfotomaculum ruminis	WP_013842559.1	44.8
Pseudoclostridium thermosuccinogenes	WP_103081847.1	39.9

Groups 1, 2 and 3 have the genomic arrangements around *2duf* in *B. cereus, A. acidocaldarius* and *D. gibsoniae*, respectively, in Figure 11A.



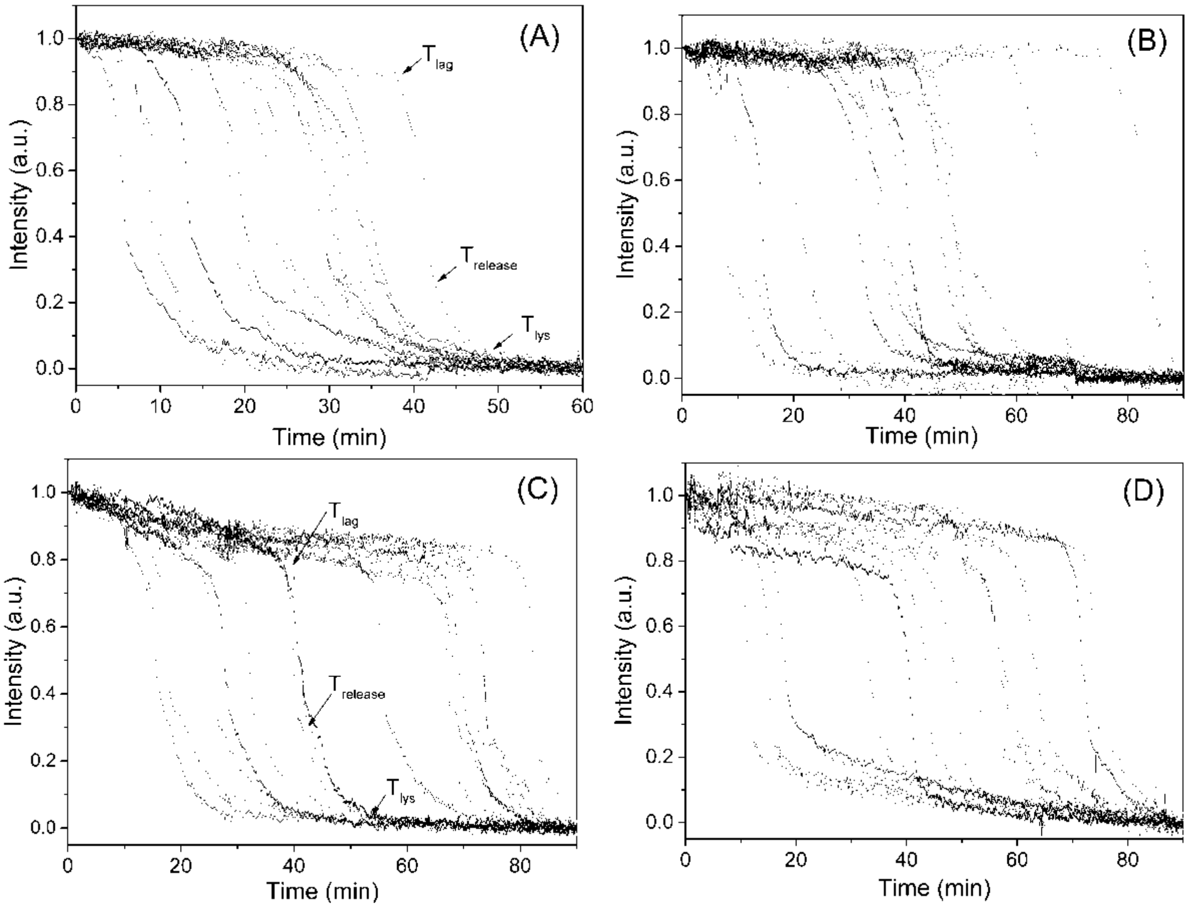
Supplementary Figure S1

Germination, outgrowth, and vegetative growth from dormant spores of various strains inoculated at ~10^8/ml in LB medium, incubated at 37°C and the culture's OD_{600} measured. The symbols used are: ○ – PS832; ● – PS4487; and △ – PS4488. A duplicate experiment found that all three strains again gave similar results.



Supplementary Figure S2

^{14}C-Methylamine uptake by (**A**) intact and (**B**) decoated spores of PS832 (wt), PS4487 (*ydfS*) and PS4488 (*yetF*) spores. ^{14}C-Methylamine uptake by spores of various strains, either intact or decoated, was as described in Methods. Symbols used are: ○ – PS832; ● – PS4487; and △ – PS4488. These results were from single measurements in one experiment.



Supplementary Figure S3

Germination of multiple individual (**A,B**) PS832 (wt) and (**C,D**) PS4488 (*yetF*) spores germinating with (**A,C**) L-valine or (**B,D**) AGFK. Spores were heat-activated and germinated with L-valine or AGFK and the germination of 16 individual spores of each strain and with each germinant were followed as described in Methods. Arrows on one trace in panels (**A**) and (**C**) denote the times of T_{lag}, $T_{release}$ and T_{lys} and I_{lag} and I_{lys} were measured at T_{lag} and T_{lys}, respectively.

sspA

-35 **σ^G-10**

CAATTTTCAGCTCCTGTATACAATTACCAAAGTTTTTCT**GAATG**AAGCCATGTGTTTTGACA**CATTCTA**TACTC

RBS **TSC**

AC**AAGGAGGTG**AGACAC**ATG**GCTAACA

ykjA

-35 **σ^F** **-10**

TGTTCAGGCGATATGCACGTTCGCCTAGACACTGAT**GCATA**GAGGGCCTGCCACG**GG**G**CACAATA**GGCTC

RBS **TSC**

AATATGACCAGCGG**ATGGAGGTA**AAGATCG**GTG**TTATGGA

yrbG

-35 **σ^G-10**

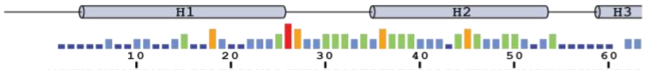
GACGTAAAAGCTTTGT**GAAAT**GCAATCGGTTTTTCTTA**CATAAAT**TGATCATTTTGTTGCAACCTATAAT

RBS **TSC**

GAG**TTGGAGGTG**CATA**ATG**GAAGAGC

Supplementary Figure S4

Likely promoter sequences for the *ykjA* and *yrbG* genes and comparison with the very strong σ^G-dependent promoter sequence of *sspA*. The *sspA*, *ykjA* and *yrbG* upstream sequences include: -35 and -10 promoter sequences; a GG sequence just upstream of the putative -10 promoter sequence for y*kjA* indicative of a σ^F promoter; the ribosome binding sites (RBS) that were identified based on the optimal *B. subtilis* RBS sequence (AAGGAGGTG) and the translation start codon (TSC). The *sspA* promoter sequences were experimentally determined, and the promoter sequences for *ykjA* and *yrbG* were chosen to give the best matches to consensus σ^F and σ^G promoter sequences, respectively (Wang et al., 2006).



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Bsubtilis_YetF                     -----MGNYLSVAVELVCGLGILFIILKLLGKTQFSQITPFDFISALILGELVGNAVYDHEIKIKEII 63
Aeribac_WP_063387519               ------MEFVAIATELVFGFFALFLLTKILGKTQITQITTFDFISALVLGELVGNAVYDVKMGVEKIL 62
Amphibac_WP_015010493              ----MGYNYSSVFFETVFGYFALFVIAKILGKTQIRQLTAFDFISALLLGELVGNALYDENVGIPHVA 64
Anoxyibac_WP_012575618             -----MNEFGQIAVELIVGYIALFIMAKILGRTQITQITPFDFISALVLGELVGNGLYDANVGLAQVL 63
Bamylo_WP_013351317                -----MGHLFTVAWELVCGLGILFVILKLLGKTQFSQITPFDFISALILGELVGNAVYDKGVRIVEII 63
Banthracis_NP_844257               -----MNHLGQITIELFVGFFVLLIATKILGKTQISQLTPFDFISAIVIGELVGNTVYDPEVRVWSIL 63
Batrophaeus_WP_003328379           -----MEHYLAVAVELICGLGILFLILKCLGKTQFSQITPFDFISALILGELVGNAVYDHEVRIKEII 63
Bbrevis_WP_012685590               -----MDDLTMVLLRTLFSYFFLLILVRLMGKRELGKLSVFDVVISIMLAEMAALAIEDVDKPALRFY 63
Bcereus_WP_001015543               -----MNHIGQITIELLVGFFVLLIATKILGKTQISQLTPFDFISAIVLGELVGNSIYDPKIKVWSIL 63
Bfirmus_WP_174752055               ------MKYLHILSVLVIGYIFLFIMAKLLGKTQITQITPFDFISAIVLGELVGNALYDQETGILEIF 62
Bhalodurans_WP_010897400           ------MEYPTIILRTILIYFIILLVLRIMGKREIGQLSVLDFVVSIMIAELAVISIENIQVSMMNTI 62
Blicheniformis_WP_003185310        -----MSEHIEVILRSILAFGILLVGSRLLGKQTISQMNIFDFIASITLGAITANLAFNTSLKIHHMV 63
Bmegaterium_WP_013057585           -------MYKDIAIELICGFLALFIMLKLLGKTQFAQITPFDFITTLVLGNIVGDAALEKGVELTEIL 61
Bpumilus_WP_041815618              ------MPFISILLELFIGFFALFFITKLLGKTQFAQITPFDFISALILGEMVGNAIFDPEVKIQHIL 62
Bthuringiensis_WP_001015535        -----MNHIGQITIELLIGFFVLLIATKILGKTQISQLTPFDFISAIVLGELVGNSIYDPKIKVWSIL 63
Bweihenstephanensis_WP_002126773   -----MNHLGQITIELFFGFFVLLIATKILGKTQISQLTPFDFISAIVLGELVGNTIYDPEIKVWSIL 63
Ccandidums_WP_123043699            --------MTTILLRTLLMYVLVFALLRLMGKREIGKLSVFDLVISIMIAEIAVIIIEDTDKPILYAI 60
Fphosphorivorans_WP_066393213      ----MDIHVGQLTTELIVGFIALFLLTKVLGKTQISQITPFDFISSIFLGELVGNAMYDDETSIFVIL 64
Gstearothermophilus_WP_053414763   ------MKWLHLTIELVTGFILLFAVVKIAGKKLISQMSPFTFISAIVLGELLGNALYDDHIHLWYII 62
Hhalophilus_WP_014641757           ------MDVIELLLRLALAFTVLLVLTRIMGRKEISQLTFFNFVSAISIGTIGGSLAIDASLSVRNGL 62
Lamyloliquefaciens_WP_068444514    ----MNE-YLLMLADAIFGFIALFALVKILGKTQISSLTPFDFISAVILGELVGNALYDEEAGIPKIA 63
Lsaccgaru_WP_102992088             --------MGSILLRTVFIYVFLLVIMRVMGKREIGKLSIFDLVVSFMIADISSMAIENKEEPLITWI 60
Lsphaericus_WP_024363643           ---MKDVSYLQIAIETVVTFFVLLALTRFLGKKQLSQLTFFNYVTGITIGSIAANMIVLSTKDYMKDL 65
Nthermophilus_WP_077719037         ------MELMTITLRTIFIYFFVLLIMRLMGKREIGKLSVFDLVVSFMIAELAAISIEDTNVTFLRGM 62
Oiheyensis_WP_011065627            ----MSG-YFSMFFEMVIGFVALFLVTKILGKTQISQITPFDFIAALLLGELVGNALFDQNAGIIDIL 63
Paraliobacillus_WP_112180199       ------MSISTLSIRLVIAFATLLILTRMTGRKEISQMTFFNFVSGIAIGTIGASLAIDQTLSIRNGV 62
Pcavernae_WP_119884294             ------MIFMEMILRTTATFFVLLLLARLMGKEQVSQLTFFNYITGITIGSIAAEMAGQSETPFWNGM 62
Ppolymyxa_WP_013371121             -----MEVFVTIGVKLIISFFGLWLITFITGRKTLSQLTPLDFLTSLVLSEIVGNTLYDDKVTIWHLL 63
Pthermoglucosidasius_WP_003250158  ------MKWLHLTVELVTGFILLFLVVKVAGKKLIHQISPFTFISALVLGELLGNALYDNHIHLWYIV 62
Rstabekisii_WP_066786518           -------MMLTITLKVTLGFIALMLVIRLMGKKELSEVTPFDIVFLLMLGGILEETLYDNKVQVWHFL 61
Spsychrophila_WP_067207038         MDDFFEVDFWEMIMRVTVTFFVLLILARFMGKKQISQLTFFHYVTGITIGSIAGEIAGVSKTPLLDGL 68
Ssilvestris_WP_014824326           -----MLEHFEIILRSFTSFGLLLVGTRILGKQTISQMTMFDFVATISLGAISANLAFNTSLKVSHTI 63
Sterrae_WP_028976672               ------MHFVQVAVELIVGFVSLFLMTKILGKATLAQVTAFDFISAIVLGELVGNALYDKEIGIGSIV 62
Tavium_WP_087458819                -------MHLDTFYRPLAAFVALLVLSRLLGKKQIRQITAFNYISSIAFGASAAMVAFNPLIPFLHAL 61
Tcomposti_WP_015255871             -----MSHYLHITVDLLFGFAGLFVFNQVLGKAHFSKLTPFEFVAILVLGDLVGNAVYQPDVTAAQIV 63
Tgoriensis_WP_038563309            -MPELIHDNVIVLARIITIIPLMLAVTLFMGKRTIGEMPVFDFLIIVILGALVGADIADPEIEHLPTA 67
Vhalodenitrificans_WP_121616112    ------MAIPELILRICLSFALLFTLTRIMGRKEISQMTFFNFVSAIAIGSITAILATNQNFSMLNGI 62
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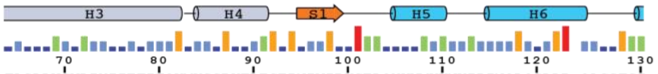

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Amphibac_WP_015010493              FAIATWGTLMYVTEFITQRFKGSRSLLEGRPAIIVRKGKIDREAMKKNKLDINQFQHLLR-LKDVFSL 131
Anoxyibac_WP_012575618             FAIALWGLLIYATEMITQKKKELRELLEGKPVIVISKGKILYDALKKTKLDLNQLQHLLR-ARNVFSI 130
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Banthracis_NP_844257               YAVFVWVILIYAIEVITQKFRRTRKFFEGYPSIIIRNGHIDREQLKSNHLDINQLQQMLRQQKDIFSI 131
Batrophaeus_WP_003328379           FASLLWGLLIYIIEFITQKLKGTRKFLEGEPNIVIQKGILKYEVLKKNKLDINQLQSLLR-QQGCFSI 130
Bbrevis_WP_012685590               LPMLLIALLEVAFAYLSLKSKKFRDTVDGSADLIIENGQIREQAMRRNRLNMDDLMVHLR-QKDVKNI 130
Bcereus_WP_001015543               YSVFVWVILIYTIEVITQKVRGTRRFFEGYPSIIIRNGKIDREQLSVNHLDINQLQQMLRQQKDIFSI 131
Bfirmus_WP_174752055               FAVTVWGTLIYATETLTQKFKRARKLLEGEPSIVIKKGKIIFEELKKNHLDLNQLQHLLR-SKDVFSI 129
Bhalodurans_WP_010897400           IPIVVLSLIQIVFAYVSLKSKTMRRLVDGKPTVLISQGKIDEREMRKQRYNFDDLLIQLR-QSNVRDI 129
Blicheniformis_WP_003185310        LAFFIFVFVILATAYISLWSQKGRKFFAGDPTVVIQNGKVLEHNMRKMRYTIDYLNQQLR-EKNVFKI 130
Bmegaterium_WP_013057585           YSVLIWGLLIYAVTKLSQTFTGFRGILEGKPSMIIYKGKILYKELKKNNLDLNQLQHLMR-QQGYFSL 128
Bpumilus_WP_041815618              FAVAVWGILIYTVEWFSQRFKGIRAFLEGRPTLVIDQGKIHYNRLKKNMLDLNQLQTLIR-AKGHFAL 129
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Bweihenstephanensis_WP_002126773   YAVLVWVVLIYTIEVITQKFRGTRSFFEGSPSIIIRNGYIDREQLSSNHLDINQLQQMLRQQKDIFSI 131
Ccandidums_WP_123043699            APIALLVVIQIVFAFLTIRSRKFRLWIDGTPSVLIRGGKLNRSEMRKQRYNLDDLMAQLR-EHEMINV 127
Fphosphorivorans_WP_066393213      YAIVVWGSMVYIVEIISQKFKGTRKFLEGAPTIVIRNGLIDRDMLKKSKLDINQLQNLVR-QKGFFGL 131
Gstearothermophilus_WP_053414763   YSITLWGAMLMTVEYASQKWLSFRLWSEGKPTVLIQNGVIDYEALKKSRLTLNQLQSLLR-KHETFSI 129
Hhalophilus_WP_014641757           IALVSWAVFTVFLGYLDIKSKDVRSFIDGQPLIVVKQGKVLEDELRKARLDLDSLNVLLR-KKNAFSL 129
Lamyloliquefaciens_WP_068444514    FLIALWGLLIYITEIITQKFKGSRYLLEGQPSMVIHKGHLVYDVLKQNRLDIDELQHMLR-SKDVFSI 130
Lsaccgaru_WP_102992088             SPIILLASLQILLSFILIKSERIRNWVDGSPVPLIENGKIVDKNMAKLRYNLDDLLTQLR-EKNIPDV 127
Lsphaericus_WP_024363643           VSLIIWCSLTTLIGYISLKSGKIRMLLDGQPTIVIKHGKIDRKALKRTGVNIDDLTMMIR-QYQVFSI 132
Nthermophilus_WP_077719037         VPVIVLMLLQVSMSFLSLKSSKIRKVIEGTPSYLIKNGEIQEQEMAKHRYNIADLLLQLR-EKNVEKV 129
Oiheyensis_WP_011065627            FVITIYAIIMYISEIITQKYKHTRHMIEGFPSIVIYNGKLIRDTMKKNKLDINQLQHLLR-TKDTFSI 130
Paraliobacillus_WP_112180199       YALAVWSAITIVIGIIDLKSTKFRYAVAGQPRVVIKEGKIMEEELRKVRLDIDALNVLLR-KKNIFAI 129
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Pthermoglucosidasius_WP_003250158  YSISLWGALLLLVEYMSQKYLSFRRFSEGEPTVLIRNGIIDDKALKKSRMTLNQLQSLLR-QNETFSL 129
Rstabekisii_WP_066786518           YAIILWTVLSLITNLVVRKYDKLRPFIKGEPSILINKGVLDIKELKKNKMETEQLLSLLR-QQGIFSI 128
Spsychrophila_WP_067207038         IAMVWWAFLTILMSYIAFKSKKARILLDDQPTIVVYEGKIVEASLKKLRLHLNDLGMMLR-EQSIFSV 135
Ssilvestris_WP_014824326           LAFVIYVFIIFMIALISLKSKKGRKFLAGDPTIVIENGKILEKNMNKMRYTLDYLNQQLR-EKDVFNI 130
Sterrae_WP_028976672               FAVALWGLMIYAVEWSTQKFRGTRQLFEGKPTIVIRNGHMDREAMKKEKLDINMLQNLLR-HKNVFSI 129
Tavium_WP_087458819                VSMVIWGGLSYVTEMLAQKSRKIRLLLEGEPTVVIKEGKILEKSLRKEKMNVEELIMLLR-QERIFSL 128
Tcomposti_WP_015255871             YGILIWGLIIYAIGRLTLKSKKMRTLFEGEPSIVIRRGHLQYDVMRRNMLDLDQLLSMLR-QQGYFSL 130
Tgoriensis_WP_038563309            IAIIAIGLFQKAIVKWKISNRKIGKLLTLGPTIVIQDGTLLYENMRKIQYSIDNILFMLR-QKDVFDI 134
Vhalodenitrificans_WP_121616112    IALTGWAGFTLIMGYIDIKSQKARKFTTGIPEIVIKDGKIMEEALRRTRLDNDSLQALLR-QKNVFSM 129
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▂▃▅▆▇ Conservation 30% -100%

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Bsubtilis_YetF                    QEVEYAILETNGMVSVLPKSD-------------FDKPTNKDLQIPSK-------SVSLP--ITLIID 176
Aeribac_WP_063387519              REVEYAVLETDGTISVLKKSE-------------FDKPTRNDLNILSS-------PATIP--YTIIVD 175
Amphibac_WP_015010493             RDVEYAILENDGTISVLNNTR-------------SQTPTRKDLKLQDE-------KVYLP--VTLIND 177
Anoxyibac_WP_012575618            REVEYAILETDGTVSVMKKAP-------------YEQPTRQDHNISAS-------EAVLP--VTVIID 176
Bamylo_WP_013351317               QEAEYAILETNGMVSVLPKFP-------------YDTTKNGDMQIKPK-------QVSLP--VTLIID 176
Banthracis_NP_844257              REVEYMILEPNGNISVLKKSK-------------YESPTINDLSLKHK-------PVYLP--ISLISD 177
Batrophaeus_WP_003328379          KEAEYAILETNGMISVLLKSK-------------YDTPKMQDVELAPK-------QVYLP--ITLILD 176
Bbrevis_WP_012685590              ADVEFALLEPTGQMSVFLKEQ---KE----------KVTREDLALMKKLQVGPVSYKGLP--IPLILD 183
Bcereus_WP_001015543              REVEYMILEPNGNISVLKKSK-------------YESPTINDLSLKHK-------PVYLP--ISLISD 177
Bfirmus_WP_174752055              RECEYAILETDGTVSAFKKPL-------------YATPTIQDLNLPIY-------NAELP--VTLILD 175
Bhalodurans_WP_010897400          SDVEFAILEPSGKLSVIEKR----------------PFSGKSSS-------------YLP--LPLILD 166
Blicheniformis_WP_003185310       EEVLYAIVETNGTLTVLKKPQ-------------FRNVTKKDLWVATTPEG------KLP--IELIMD 177
Bmegaterium_WP_013057585          YEAEYVILETNGEVSVAPKHE-------------FGPPTKNDLNIPYS-------QTNLP--IALIMD 174
Bpumilus_WP_041815618             NEIEYAILETDGSVSVLPKMK-------------YATATAEDVNVKGK-------EVKLP--RTFIID 175
Bthuringiensis_WP_001015535       REVEYMILEPNGNISVLKKSK-------------YESPTVNDLSLKHK-------PVYLP--ISLISD 177
Bweihenstephanensis_WP_002126773  REVEYMILEPNGNISVLKKSK-------------YESPTINDLSLKHK-------PVYLP--ISLISD 177
Ccandidums_WP_123043699           EDVELAVLEANGKLSVVPKTDRNSKETGITQRNVSAPSPGKSETPPKIRYE------LLP--LPLILD 187
Fphosphorivorans_WP_066393213     REVEYAILESNGSLSILPKYQ-------------YGPPNRQDLNLQSQ------EQPELP--ITLILD 178
Gstearothermophilus_WP_053414763  REVAFCFLEADGEISVLKKAH-------------YQKTTREDFQLPPH-------PVYVP--VTLIRD 175
Hhalophilus_WP_014641757          SEVSYAIFETDGTLSVMKKTG-------------EQPFTKKDGNVVSN------SPAVYPIPTTVVSD 178
Lamyloliquefaciens_WP_068444514   EEVEYAILEADGEVSVLKKPE-------------YQTPTIADLNLAPK-------PVKIG--RVLISD 176
Lsaccgaru_WP_102992088            SDVEFAVLETTGKLSVFPKEE---KR----------PAFKEDVRRDLK-QF------RMP--IPVIID 173
Lsphaericus_WP_024363643          DEIDFAILEPNGTLSILKKPP-------------FQGTQKIDINISPE------NPPFLP--IEIISD 179
Nthermophilus_WP_077719037        TDVEHAILETSGKLSVILKEG---KQ----------PVTKEEAGVELRGQH------HLP--VPLIID 176
Oiheyensis_WP_011065627           QEVEFAVLEANGTLSVLKKSD-------------YQIPTRKDLKMSTT-------EVDLS--TTLIND 176
Paraliobacillus_WP_112180199      NDVDYAIFETDGSLSVDKKEK-------------VKPLAKTD-LQIQN------KANVFPTPTMIVSD 177
Pcavernae_WP_119884294            QEVAYAVLETNGQLSIMKTEE-------------AKNPTKSDLGVAKK------TPKKIP--AAVFID 176
Ppolymyxa_WP_013371121            REVLYATLETNGSLSVMRKPE-------------YEPPAAQDMGIDAQ-------PDLFS--VTVIDK 176
Pthermoglucosidasius_WP_003250158 REVAFCYLETNGTISVLKKAK-------------YQKTTREDFQLPPH-------PVHVP--VTLIRD 175
Rstabekisii_WP_066786518          REVKYVILEPGGQISVMKR---------------------DSS-DQIT-------TDTLP--RLIIDE 165
Spsychrophila_WP_067207038        KDVHYAILETNGKLSVLKKAG-------------LEAATKKDVNAAIQ------VPKYIP--TEVIAE 182
Ssilvestris_WP_014824326          EEVLFAIIETNGTLTVLKKPQ-------------FRNVNKQDLMIPITPEF------NLP--IELIMD 177
Sterrae_WP_028976672              REVAFAILETDGSISVKQKSQ-------------YATPTMEDLQKQQK-------PVYLP--ITIIED 175
Tavium_WP_087458819               TEVDYAILEPDGQLSILKKTK-------------HLPASKHDVETGRA------NTAPFG--VQIVSD 175
Tcomposti_WP_015255871            REIEYAVLETNGMISVLPKRP-------------YGPPTRADLGLHGV-------GGRVP--ITLILD 176
Tgoriensis_WP_038563309           RDVETAIVEPNGALSVLKKQS-------------KMPATREDLQIQQQ-------PSAIA--FPVISD 180
Vhalodenitrificans_WP_121616112   KEVDYAIFETSGKLSVMKKEN-------------KQPATKSDIHQLTTY-----SPKIFP--TEIISD 177
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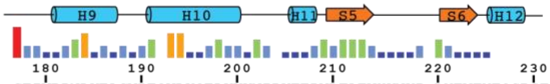

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Bsubtilis_YetF                    GEIVRDNLKEAGVDEQWLKQELKKKNIDKTEDVLFAEWHKNKP-LYTVTYEQSRST          231
Aeribac_WP_063387519              GEVVSENLKEAGFDEKWLQQQLLAYSVEDAKDVFYAEWDQNNG-LFVQKY                224
Amphibac_WP_015010493             GEIIEDNLAEINKDKDWLYDQIKKQKISSIEEIFYAEYLEDEP-LFIQKM                226
Anoxyibac_WP_012575618            GEVIWDNLRENGWDEQWLKKHIRHAGFENYSDILYAEWQDGKG-MHVQPY                225
Bamylo_WP_013351317               GEVLYENLKEAGVKEEQLIQDLKKQNIKRTEDVFYAEWKHDKP-LFVLPYEEKK            229
Banthracis_NP_844257              GKVVKDNLREAGFDEGWLYKQIKKKGITKFEDVLYAEWKTDDG-FFCQEMDR              228
Batrophaeus_WP_003328379          GEVVYDNLQEAGVDEEWLKQELASQGVNECKDVLFAEWHAGQP-LFFIKYDSGSDK          231
Bbrevis_WP_012685590              GKVRTEALSKIGQNELWLKREIRKYGIKDIREVSFCSIDE-RGIMYLDKKDKP             235
Bcereus_WP_001015543              GKVVKDNLREAGFDEGWLYKQIKQKGITKFEDVLYAEWKTDDG-FFCQEMQR              228
Bfirmus_WP_174752055              GEVVWDNLKSINWDETILKNEIKKYGASGVKDVLYAEWKKGEA-LHVQTY                224
Bhalodurans_WP_010897400          GKVQEKHLEKINKTSLWLRQQLRKLGYRDIKKISYCAIKE-DGTFFIDIKDEK             218
Blicheniformis_WP_003185310       GKMKNENLKDNQLTQAWLQSEMKKRGLSTDEVFYAVLSPD--GNIYIDTYEDHIHSSFDKE     236
Bmegaterium_WP_013057585          GKVVPGNLKEANVDEKWLKKQLAIKKIKKYSEVFYAEWQQERG-LEITKF                223
Bpumilus_WP_041815618             GEILKDDLQEAGFDETWLSEQLKRQHISSYRDVLFCEWIENEG-IYAMKYEKSKKHS         231
Bthuringiensis_WP_001015535       GKVVKDNLREAGFDEGWLYKQIKQKGITKFEDVLYAEWKTDDG-FFCQEMQR              228
Bweihenstephanensis_WP_002126773  GKVVKDNLREAGFDEGWLYKQIKQKGITKFEDVLYAEWKTDDG-FFCQEMPR              228
Ccandidums_WP_123043699           GQVQDDSLEKIGKNRFWLKTRLREQGIEQFRQVFFCSIDH-KGRFYVDRKK               237
Fphosphorivorans_WP_066393213     GELNHDNLSTAGLSRNKLLEQIHKQGYSDIKEVVYAEFSEGK--LLIMPCTKPKK           231
Gstearothermophilus_WP_053414763  GQLLADELAELGKTEQWLAAKLQKQGIASPKDVLIAEWLEGDG-LFVQTYQPAERQRPTRRQTAPE 240
Hhalophilus_WP_014641757          GKINDANMQSLNLDQKWLHHQLKSAGVPSISDVFYAEVQKDGS-LYIDKRNDTIH           232
Lamyloliquefaciens_WP_068444514   GEIVWDNLKEASLNKTWLEEELHKQNVQSIEDVFYAEWQENQQKLFVALYHTKKKRQN        234
Lsaccgaru_WP_102992088            GKVQEEGLKKLGQNRFWLKAEIQKRGLKDFKEVFYAAVNY-EGRLYIDRKDRPTR           227
Lsphaericus_WP_024363643          GKLLKRNLLEVGKNRQWLKDELKKMSIENIEEVFYAEIQSNGQ-LFIQKY                228
Nthermophilus_WP_077719037        GKVQDKRLEEIGQTRFWLKNEVQKQGYKDFKDIFFALYDVSNGELHIDRKQS              228
Oiheyensis_WP_011065627           GEVIQDNLKEKNLSIEWLMEQIKKEGYERIEDVFYAEYIKDKP-LFLLPYINRNHQKWDVD     236
Paraliobacillus_WP_112180199      GKIDNNNLEKLHLNKDWVMKQLESAGVGSIDEVFYAAVQKDGT-LYIDNTNDTVH           231
Pcavernae_WP_119884294            GNWMTDACHEIGVAQKTIEKQLKDSGYKNLDDVFLVQWQEDGT-LYVDKKTDVKQS          231
Ppolymyxa_WP_013371121            GRLLNESMRGKTIDVELIKAKTREQGYDSIDEIAYAELSEDGT-LHIVPMKEK             228
Pthermoglucosidasius_WP_003250158 GELLRDELQELGKDEQWLNEQLRAYGVTSHQDVFIAEWLEGDG-LFVQTYS               225
Rstabekisii_WP_066786518          GQIVNRTLNQIRKNEQWVINLLKKEGYEDIEKVYYAEWSEGKG-LYVQCNE               215
Spsychrophila_WP_067207038        GKISKGNLAELNLTEEWLFEQLKKQGIGNVKTVFFAEVQTDGS-LHVDMKAEGEK           236
Ssilvestris_WP_014824326          GEVMKQNLDQNNLSEQWLHSELKQRNLVQSNVLYAVLSGN--GNMYIDTYENHIHSPIDKE     236
Sterrae_WP_028976672              GEVDWDNLKEAGLNEEWLLKNLRRHHIRSYKDVFYCEWKKDEG-VFIEKMNANERR          230
Tavium_WP_087458819               GQLLTHNLKTIKRDEKWLEQQLKQQGIPTYKEVFYAEAQEGGG-LYVDRKKDQPY           229
Tcomposti_WP_015255871            GRLQSDNLGKASLSEPWLKQQLADRGITSYEGVLYADWDPEEG-LFVMPHQ               226
Tgoriensis_WP_038563309           GYIHHDTLTHFKLDEEWLRKQLAEQNIIGMNQIFFASISHPQS-LHISLKERKLNIPPIKH     240
Vhalodenitrificans_WP_121616112   GELNEANLKKLNLDTNWLNGQLKQAGITSLSEVFYAEVQPDGT-LYFDNKRDGLYH          232
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Conservation 30% -100%

Supplementary Figure S5

Sequence alignment of *B. subtilis* YetF with the best YetF match in other *Bacilli*. Sequence conservation is shown as a bar graph, with red bars indicating identity among YetF homologs. Secondary-structure assignments of YetF from the crystal structure (PDB ID: 3C6F) are shown as blue cylinders (helices) and orange arrows (β strands). Predicted secondary-structure elements by AlphaFold for the N-terminal TM region are shown as gray cylinders (α helices).

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Bsubtilis_YetF                          ------MGNYLS---VAVELVCGLGILFIILKLLGKTQFSQITPFDFISALILGELVGNAVYDHE-I 57
Rcellulolyticum_WP_015924636            --MN---EGL----VVFVRSLLSFFSLLIFTRILGKEQISQLTFFDYILGITIGSIAASLSTDLS-S 57
Sglycolicus_WP_013625014                ------MIIA------TIRTIILYLVIVIIIRIMGKKQIGQLQPFELVIILMISELAAIPSQNIG-I 54
Sthermophilum_WP_011197382              --MG---EVS----VVLVRSAITFFNLLILCRLLGKTQVSQLTFFEYVSGITLGSLAAELSTNLS-L 57
Tacetatoxydans_WP_013778043             ------MSILKDLLLHAYKSLILFMVSLTLVRLMGKRTVAQLSPFDLVFMIIMGSAIAIPLEDHD-I 60
Titalicus_WP_012994581                  ------MLIL------FFRTLILYALIVIFMRISGKQQIGQLQPYELVVAIMIADLVAIPMQNKG-I 54
Tphaeum_WP_015049695                    --MTDALLYL----QMALRAVIAFLAILLIARILEKEHVGELSLYEYLTGIAIGVIAGRIATTMQ-N 60
Tstercorarium_WP__015358013             -----MFFDI------VWKTALMYLVVIVSMRLMGKRMIGELQPFEFAIAIMISELAVFPLTEGN-T 55
Tthermosaccharolyticum_WP_015312322     ------MLII------FFRTLILYFLVVVVMRIMGKQQIGQLQPYELVVAIMIADLVAVPMQNKG-I 54
Adegensii_WP__015739924                 --MN---PFL----EILLRAVGAFLGVLFITRLVGKSQVGQLTISDYVNGIVIGSIAACLATDIK-E 57
Adegensii_WP_015739458                  ------MSVLG---QVVWRTVLIYFVVLVMMRLMGKREIAQLSPFDFAVAIMIAEVAVLPMESLE-I 57
Ametalliredigens_WP_012065355           ------MIVI------FIRAFVLYMIVVIVMRMMGKGQIAEMQPFELVITIMIADLAATPMENTS-V 54
Athermocellus_WP_003516115              ------MLII------VVRTLILYILVVIIMRIMGKRQIGQLQPFELVIAIMISELAAVPMQDKR-I 54
Cacetobutylicum_WP_010890760            --MED--PLI----NTVFRSVIVYLIAMFLTRLMGRKLISQMTFFDFVMGVSMGSIITDSIVGQK-F 58
Cacetobutylicum_WP_010964829            --MN---EGL----VVIVRSIIGFFTMLIFAKILGKQQISQLTFFDYILGITIGSIAATLTTDLS-S 57
Caproiciproducens_WP_128742257          ------MTIS------FIRTVLLYAVVILAVRLMGKRQISELQTSELVVTLLISDIASIPMQDSG-Q 54
Carthromitus_WP_005805303               ------MLIV------LSRTIIIFVVMFFTMKMMGKRYIAQLEPYEFVVSIMIAELATLPLEDVS-I 54
Cbotulinum_WP_011948896                 --MN---EAL----VVLVRAIIGFFTLLIFARMLGRQQISQLTFFDYVLGITIGSTASTLSTDLE-S 57
Cdesulforudis_WP_012303154              --MN---PFL----EIVIRAVGAFIAVILITRLVGKSQIGQLTVTDFVNGIVIGSIAAALAIDIR-S 57
Cdifficile_WP_009889750                 ------MTVV------LIRSIILYITVLIALRVMGKGEIAEMNCFDLVITLLIAEVASVPMENNN-I 54
Clentocellum_WP_013658886               ------MEVI----QAIITALGSAAALFTLSKLMGNREMSQLSMFDYINSITIGSIAAEMATCEF-T 56
Cperfringens_WP_003472738               ------MLIT------FIRTAILYFIVIISIRLMGKRQIGELQPYELVITLMLSDLASLPMQDTR-L 54
Csubterraneus_WP_009611278              ------MLIL------FFRTLILYALVVVFMRISGKQQIGQLQPYELVVAIMIADLVAIPMQNKG-I 54
Ctetani_WP_035111051                    --MN---EGL----VVVIRSIIGFFSLLIFARVLGKQQVSQLTFFEYVLGITIGSTASTLSTDLS-T 57
Dacetoxidans_WP_015757363               ------MLLI------LIRTLILYLGVVIVMRVMGKQEIGQLQPYELVIALMIADLAAIPMSNTG-V 54
Dformicoaceticum_WP_089609402           ------MET-------VLRTVGLLAFGFICYRLMGYRSMGDMEPTDFVIMLVIAETLGTPLADES-L 53
Dgibsoniae_WP_006522671                 MLIN---PYI----EIPLRALFAYLFLLLFTRINGREQISQLTYFEYVVGITIGSIAGTLTTTPE-D 59
Dhafniense_WP_011459753                 --MS---EGL----VVIVRSLIGFFSLLIFARIIGKSQISQLTFFDYVLGITIGSMAASLATDLS-S 57
Dkuznetsovii _AEG16849                  --MN---PYL----QILIRGIGAFLGVLVITRVVGKTQVGQLTVADFVNAIVIGSIAAAMVTDLK-E 57
Dorientis_WP_014187430                  ------MLIV------VVRTLILYALVIIALRLMGKREIGQLQPFELVVILMISELAAVPSENVG-V 54
Druminis_WP_041275606                   ------MALT-----YVWRTVIIYFSVLVIVRLMGKREIGQLSSFDFVVAIILAELAAIPMESEK-I 55
Ibutyriciproducens_WP_058117823         ------MIIA------FLRTIILYLLIIAGIRLMGKRQVGELEPSELVLDLIIADLAAVPMQDFG-I 54
Lphytofermentans_WP_012198749           ------MTII----RVILSSFASIVALFFLCKFIGYRQMSQMSLFDYINGITIGSIAAEMAT-DL-E 55
Lumeaense_WP_111524166                  ------MNAFLL---VAIKLLIGFFALVIIINISGKGNLAPSSASDQIVNYVLGGIIGGVIYNSS-V 57
Maustraliensis_WP_013780881             --MK---MIA----EIIIQTIMAFFAILIFTRLLGKQQITELTYYDYINGITFGSIAGVMATDIS-Q 57
Mtermoacetica_WP_011391764              --MK---FV-----EVFLQTLLAFFAILIYTRILGKQQIGQLTFFEYINGITFGSIAAVLATDTAPN 57
Psordellii_WP_055331643                 ----MINEIY----IVSIKSIISVSILFILTKIMGKKQISQLTLFDYVVGISIGSIAAELAINES-I 58
Pthermosuccinogenes_WP_103079909        ------MLVV------FVRTLILYAVVIVVMRLMGKRQIGELQPFELVVAILISDLAAVPMQNTG-I 54
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Bsubtilis_YetF                          KIKEIIFASLLWGVLIYIIEFITQKMKSSRKFLEGEPNIVIRKGELQYKVMKKNKIDINQLQSLLRQ 124
Rcellulolyticum_WP_015924636            RSWPHWIGLITWAALGLLLEYITLKWRYVAKYLENEPTIVIMNGKIMEKTLKKMRFRVSDITELLRN 124
Sglycolicus_WP_013625014                PLSAGLLPVIVLLLLGMTFSYITLKSEKARALICGTPTILIEKGKIIEKELVDLKYNLSDLLEQLRV 121
Sthermophilum_WP_011197382              RPWPVFVGLFAWGGFTLLTQLVALKSRWMAKMLDGEPVILVYRGQILEENLRRLRMRESELAELLRS 124
Tacetatoxydans_WP_013778043             RLTHGIFPVAITSLLNYLLAIIITKNRKVENILQGTSRILIKDGEVVVSNLKKERITITDLLVLLRE 127
Titalicus_WP_012994581                  PLLSGIIPILTLLVSQLFLSYLSMKSLRARAIICGTPTILIEKGKILTSQLQKERYNINDLLEELRV 121
Tphaeum_WP_015049695                    -PLPLLLALFVFAALTYLVRYFTLKSRAVRKLLAGEPAVVIQNGKIMERTMRRMHYSTDDLLMQLRT 126
Tstercorarium_WP__015358013             NILHGLLAIGVLVLCQFLLSLLSIKSLTVRTIICGRPRIVIKNGKILEKNLKKELYTINDLLEQLRM 122
Tthermosaccharolyticum_WP_015312322     PLLTGIIPIFTLLISQLFLSYISMKSLRGREMICGKPTVLIDKGKILTKELQKERYNINDLLEELRV 121
Adegensii_WP__015739924                 NPWYYVFGLAIFASLTILVQYVSLIYRPARKFLLDEPTVVVHNGRILERNMARMRYNVDDLMSQLRE 124
Adegensii_WP_015739458                  PLFRGLLPLLILTVLEIGFSYLSLHSRWLRRLVYGEPQLVIWEGKVLHREMRRARYNLDDLLAQLRE 124
Ametalliredigens_WP_012065355           PLINGVIPILTLLSGQVIISYLSLKSTKFRGLICGKPSILIHKGRIVQSELRRLRINVNDLLEQLRS 121
Athermocellus_WP_003516115              PLINGIIPILTLLFAQITFSFIGLKSTRARAIICGRPSILIQNGKINEAELRKEMYSVNDLLEQLRI 121
Cacetobutylicum_WP_010890760            TSISSVTALTVLSILVIITGYIHIKSLKLREIINSKPIILVDNGNIVEENMKESRITINELLMKLRE 125
Cacetobutylicum_WP_010964829            RAWPHWVGLLTWALLGYLMEYISTKWRYAGKYLEGEPTIVIMKGKIMEDAMKKMNFRATDIMELLRD 124
Caproiciproducens_WP_128742257          PLASGLIPIAALVMFEIVLSVLMLKSTKFRKLICGRPIIVINNGVVQQNEMKRLRMTTEDLSEQLRQ 121
Carthromitus_WP_005805303               PLLYGIICIFTILFIEFIISQIQLKNIKLRKLFGGKSVILVKDGKFIKENLKNEKLTINDVLEDLRS 121
Cbotulinum_WP_011948896                 TAWSHWIGLLTWCAIGFLLQWITLKWRYAAKYIEGEPTIVIMDGKIMEDTLRKMKYTVADVLEQLRG 124
Cdesulforudis_WP_012303154              PASFYFLALAVFSGLTLFLEWSTMKSRPVRKILEDEPTVVVHNGKILETKMRNMRYHLDDLMMQLRS 124
Cdifficile_WP_009889750                 PIINGVAAISGLVIMQTLISFLSLKSRKLSSFLSGKPSVLIDKGKIVYKELKKERISIDELLEQLRI 121
Clentocellum_WP_013658886               DLLKPFTAMIVFALFNILLSLLTNKSIKIRRLATGKPSILYDNGQLYYKTFAKAKMDLGEFLVQCRV 123
Cperfringens_WP_003472738               PLLLGIVPIITLLFVKILISEIQQHSRLFEKILDGTPSIIISDGEINLEMMKKQRLTMNDILEELRS 121
Csubterraneus_WP_009611278              PLVAGIIPVLTLLISQLVLSYLSMKSLRARVIICGTPTILIEKGKILTSQLQKERYNINDLLEELRV 121
Ctetani_WP_035111051                    RAWPHWVALISWAILGYIMQLITLKQRTMAKYIDGEPTIVIVNGKIMEEALKTMKYRAADILQLLRN 124
Dacetoxidans_WP_015757363               PLANGLVPIFGLLIAQVFISYAALKNIKFRTIVCGAPSILVKNGQLVESELRRIRYNIHDLLEQLRV 121
Dformicoaceticum_WP_089609402           SLTNTIIAVSTLTILQILFSWTGLKSTFLLQLMDGKPIPVIQNGKILEKNMRKARVNRSDLMEELRV 120
Dgibsoniae_WP_006522671                 PFGPGLLGMAVWTILPILTGLLVLKSVPARKIIEGEPIVVIQNGKIDEEALARQRTNLDDLMLMLRQ 126
Dhafniense_WP_011459753                 RAWPHWVALITWAALGYVMEKITIKWRYAAKFLEGEPVIVVMSGKIMDDALRKNNYRISELMGLLRN 124
Dkuznetsovii _AEG16849                  NGWYYAFGLLLFGLLTVISEYASLKYRPLRKLIEGEPTVVIHNGKILENNMKKLIYNVDDLMMQLRE 124
Dorientis_WP_014187430                  PMLSGVIPILVLLSASLTLAWISLKSEKARNIICGKPSILIDRGKISQDELRKNCYNITDLLEELRL 121
Druminis_WP_041275606                   PIWHGIVPIATLGLLEVTFSYLTLLNRPLRKIVYGSPQVIIENGKLLKHEMRSSRYNLDDLLSQLRE 122
Ibutyriciproducens_WP_058117823         PLLSGIIPILTLLSVTMVLSVLTMKSVKFRAIVCGRPSIIVENGRLNQQEMRRNRFTVDELNEELRV 121
Lphytofermentans_WP_012198749           NPLHALIAMAIYSVVAVALSIGTDKWLGFSRFVNGYPLVLLDNDKLLYENFKKAKIDIEEFQIQCRN 122
Lumeaense_WP_111524166                  RILDFIAILCIWCILVLGLKWIKTHNVKAKQLIDGKALTIIDGGKIIVENCKKAGLSAHDVSFKLRT 124
Maustraliensis_WP_013780881             RTRQHLMGLALFALLTFLMSYISMKSRPARKLLEGEPTIVVLNGQILEDNLRKMHYNIDDLISELRQ 124
Mtermoacetica_WP_011391764              QTWMHFLGLTLFAFFTWLAGYAVLVSRPARKLISGEPTVVVHNGKILEENMKKMRYNFDELAMQLRQ 124
Psordellii_WP_055331643                 SYIQGITGMTIYALFPILLSFISLKSHLARKILDGVPTILIQNGEINEKGLKKTKMNINDLIEECRL 125
Pthermosuccinogenes_WP_103079909        PLVNGIIPIFTLLIAQLAMSLISLKSVKARGIICGKPSILIEKGKINESVLRREMYTINDLLEQLRS 121
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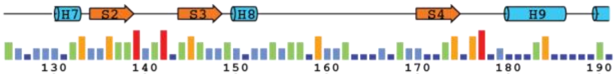

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Sthermophilum_WP_011197382              QSVFHFDEVELAVLEPRGTLSVLRTADTQPVTPADLGIPASSRGLGIELVVDGEVMDQNLRRLGVNR 191
Tacetatoxydans_WP_013778043             KDVTNINEVEEAAIEPNGKLSVIKKKYMQTVTPRDLGLWSNQGIFPTLVIDGGEVIQDNLDRVGVSI 194
Titalicus_WP_012994581                  KGYPNIADVEYAILETNGSLSVIPKSDKRPVTPQDLNLTPQYEGLPLPIIIDGRIMHQNMQKAGIDM 188
Tphaeum_WP_015049695                    KGVFNISDVEFAVVEPNGRLSVLLKSQKMPVTREDMQIPSQYRGISSELIVDGEVIYQNLRQNNLDE 193
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Tthermosaccharolyticum_WP_015312322     MGYPNIADVEYAILETNGCLSVIPKVDKRPVTPNDLNLTPQYEGLPLPIIIDGKIIHKNMKMANVDM 188
Adegensii_WP__015739924                 KGYFNLADVEFAIVEPNGSLSVLPKSQKRPVTPEDLGLPTKYEGVPSELIVDGQIIYQNLVQNNLTE 191
Adegensii_WP_015739458                  KGYHDPSEVACAVLESSGRLSVIPRSEYRPLTRGDLGLPPVPVGPVRVLVADGEILEENLKVVGVDR 191
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Athermocellus_WP_003516115              KNIYNIADVEFALLETNGQLSVIPKSQKRPVSPEDLNIPTKYEGLSLDIIVDGEISHENLKLANLDV 188
Cacetobutylicum_WP_010890760            KNIFNMADVEFAIIEIDGQLSVLPKASQKPLTPSNMNINVTSTGLTQDIIIDGNILDENLYKAGLDI 192
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Caproiciproducens_WP_128742257          KNVFSIQDVAYAIVETNGKMSVIKKPDKEQPTAGMLAVPLPDTGIETVVISDGVISDFSLQLCGKSA 188
Carthromitus_WP_005805303               GGNYDLSKIGFAILERSGKIAVIPK-NNEN----------EKVYLPTSIIIDGEIVKEGLKYINRNN 177
Cbotulinum_WP_011948896                 KDIFDLSKVDFAILESDGQLSVLKKPGEEPLTAKDLNIFKSKTGISRELIYDGEIVEDNLREINRDK 191
Cdesulforudis_WP_012303154              KGVFNIADVEFAVLEPNGEMSVQLKSQKRPVTPADLQLPTRYEGMPSELIVDGTVIEQNLIQNNLDE 191
Cdifficile_WP_009889750                 QGYFNLKDVQYAILETDGNLSVVPASSYNSTPPR------AFNHLPIPLILDGRIINKNLDIAQKDT 182
Clentocellum_WP_013658886               NDYFDLTDIQTALLEPNGKISFLPVSNKRPITGEDLNISPSQNYLVANVIIDGKIMLQNLKHIGNNE 190
Cperfringens_WP_003472738               AGYLDISDIQYAIIETNGTISIIPKSACDNVKRKDLKIKESESKIPIVLFEDGRLNKKALQGMNKDE 188
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Dformicoaceticum_WP_089609402           QGLSNYLDVEFAYLEPSGRFSIIRKKEVEPITPRYLG-----KKTSKTIMENGEIFADQLTQSGVTA 182
Dgibsoniae_WP_006522671                 KDIFNIGDVENAIFERNGELTVQRKSQLNPVTPADLNISTLYQGLPTTLVQDGVVIENRLKEISLSK 193
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Dkuznetsovii _AEG16849                  KNVFNIADVEFAVAEPNGGLSVLLKSHKQPLTPSDMQIPTKYQGIPSELIVDGVVIQQNLKQNNLTE 191
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Lumeaense_WP_111524166                  NRIYSTKDVKRAVLEQNGQLILIQSG--EENPK-------------FPVITDGQVQSDILEVIGKDE 176
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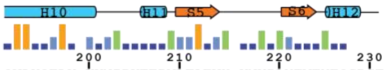

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Sthermophilum_WP_011197382              TWLMAKLK-EQGIRSPAEAFLAVVD-ADGKLYVDRYTDRVPK--SHDLSDY-PG----PN        242
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Titalicus_WP_012994581                  EWLNEQLK-MWKIQNVKEVLFASLD-TNKVLTVYRKED                              224
Tphaeum_WP_015049695                    AWLISELA-KQGIKSPREVMLAGLD-TQGNLYVDKRQDDQRH--VVQVRDD-PG--E           243
Tstercorarium_WP__015358013             DWLVGKLK-ENGWDNPRDIFYAYID-TAGNFQFQPKLKASKSRVMNTP                    235
Tthermosaccharolyticum_WP_015312322     KWLNDQLK-MWNVNNIENIILASLD-PNKVLTVYKKE                               223
Adegensii_WP__015739924                 EWLLKELE-KQGVKSLKEVLYASLD-SEGKLYIDKRQDKLEH--LTDVTDKLPG--QSGQ        245
Adegensii_WP_015739458                  EWLLRELE-KQGFREPEKVFLATLS-PQGKLFVSPKEEEEGELPN                       234
Ametalliredigens_WP_012065355           SWLLKQTE-NEGIKGLHNVFFAFIS-SEGNFHAQEKAK                              224
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Cacetobutylicum_WP_010890760            DWLLSQLN-SQNIKNESEVFYAGID-NTKKLYVSKKARKNTKY                         233
Cacetobutylicum_WP_010964829            DWLFKVLE-SRGIKNVSEVFLATLN-PGGSLYIDLYKDHIKK--VLDIGDY-KG----PY        242
Caproiciproducens_WP_128742257          DWLQGVLN-GQHLQAG-EIFLMTAN-TKGDFFIVKREAPK                            225
Carthromitus_WP_005805303               DWLMKQLK-ENKIKSPKDVLYAYTD-SNGTFKYQLETK                              213
Cbotulinum_WP_011948896                 EWLKAELK-KRNIKDSSDVFLATIN-ENNQIYIDTYKDHLKR--IIDIGDY-KG----PY        242
Cdesulforudis_WP_012303154              EWLYRELE-KQGVRSSEEVMYASLD-SEGKLYVDLKEDAIEH--YTDITDKVPD--KIPQ        245
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Ctetani_WP_035111051                    KWLKKELK-KRNINDPSEVFLATMD-NTGNFYADKYNDLMKKENVTKDGTY-KG----PL        244
Dacetoxidans_WP_015757363               NWLERELA-KFGIDSHQKVLFASLD-TNGKLFWQLKSD                              224
Dformicoaceticum_WP_089609402           AELAEILE-SFHIDDLNQLESIVIT-PDGHIALTKKQQ                              218
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Druminis_WP_041275606                   AWLKEKLA-ERGFH-PKKVLLATLN-TNGQLLIDCQNDDRQK                          228
Ibutyriciproducens_WP_058117823         GWLNKRLE-EHGVRAVRDVFLLSVD-EQNRVYFVPKEVGVK                           227
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Lumeaense_WP_111524166                  DWLLEELK-KQGIEKYSDVFLGEYV-NNSLILTIDK                                210
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Psordellii_WP_055331643                 KWLNSELD-KKGIKNTSDILLAYMD-SSKKINIYLKNKDIPITPTL                      236
Pthermosuccinogenes_WP_103079909        NWLKSELA-KHGISDARDVLFASLD-SSGNLFLQRKER                              224
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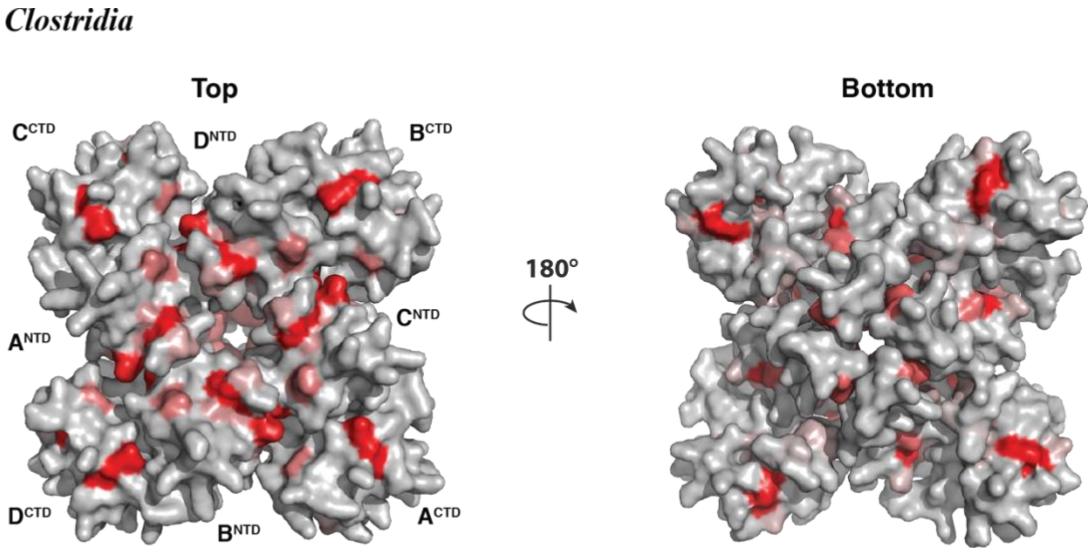
▂▃▄▅▆ Conservation 30% -100%

Supplementary Figure S6

Sequence alignment of *B. subtilis* YetF with the best YetF match in *Clostridia* species. Sequence conservation is shown as a bar graph, with red bars indicating identity among YetF homologs. Secondary-structure assignments of YetF from the crystal structure (PDB ID: 3C6F) are shown as blue cylinders (helices) and orange arrows (β strands). Predicted secondary-structure elements by AlphaFold for the N-terminal TM region are shown as gray cylinders (α helices).



Supplementary Figure S7

Surface representation of a tetrameric YetF, showing conservation among best matched YetF homologs identified in *Clostridia* species.