TABLE S1

Plasmids used in this study

Plasmid	Source, reference, or description of construction
pET-21b	Novagen
pET-42b	Novagen
pXJ58	(1)
pID2	*spoIIGA* was amplified by PCR using genomic DNA of *B. subtilis* PY79 (2) as template and primers IIGA-FN and IIGA-RB. *gfp* was amplified by PCR using pXJ58 as template and primers gfp-FB and gfp-RX. *Nde*I-*Bam*HI-digested *spoIIGA* fragment and *Bam*HI-*Xho*I-digested *gfp* fragment were cloned into *Nde*I-*Xho*I-digested pET-21b.
pID15	*spoIIR* and *sigE* were amplified by PCR using genomic DNA of *B. subtilis* PY79 (2) as template and primers IIR-FN and IIR-RF2B, and sigE-FrbsB and sigE-RX, respectively. *Nde*I-*Bam*HI-digested *spoIIR* fragment and *Bam*HI-*Xho*I-digested rbs-*sigE* fragment were cloned into *Nde*I-*Xho*I-digested pET-42b.
pID16	*spoIIGA* and *gfp* were amplified by PCR using pID2 as template and primers IIGA-FN and IIGA-RE, and gfp-FE and His-RB, respectively. *Nde*I-*Eco*RI-digested *spoIIGA* and *Eco*RI-*Bam*HI-digested *gfp* were cloned into *Nde*I-*Bam*HI-digested pID15.
pID17	*spoIIR* was amplified by PCR using pID15 as template and primers IIR-FN and IIR-RX. *Nde*I-*Xho*I-digested *spoIIR* fragment was cloned into *Nde*I-*Xho*I-digested pET-21b.
pID44	pID15 was subjected to site-directed mutagenesis using primers sigE(M17I)-F and sigE(M17I)-R.
pID48	*spoIIGA-gfp* was amplified by PCR using pID2 as template and primers IIGA-FN and gfp-RF2X. *Nde*I-*Bam*HI-digested and gel-extracted *spoIIGA* fragment and *Bam*HI-*Xho*I-digested and gel-extracted *gfp*-F2 fragment were cloned into *Nde*I-*Xho*I-digested pET-21b.
pID49	pID15 was subjected to site-directed mutagenesis using primers BamHItoSTOP-F and BamHItoSTOP-R.
pID50	pID49 was subjected to site-directed mutagenesis using primers sigE(M17I)-F and sigE(M17I)-R.
pID53 to pID86 and pID89 to pID107	pID48 was subjected to site-directed mutagenesis using primers indicated in Table S2.
pID87	rbs-*spoIIGA-gfp* was amplified by PCR using pID2 as template and primers IIGA-FrbsX and gfp-RX. *Xho*I-digested rbs-*spoIIGA-gfp* was cloned into *Xho*I-digested pID48.
pID88	*spoIIR*-F2 fragment from *Nde*I-*Bam*HI-digested pID15 was gel extracted and cloned into *Nde*I-*Bam*HI-digested pET-42b.

TABLE S2

Primers used in this study

Primer	Sequence[a]
IIGA-FN	5'-ggaattccatatgaaaatctatttagatgtcatttgg-3'
IIGA-RB	5'-cgcggatcctgaaacgtgtttgacagcctt-3'
gfp-FB	5'-cgcggatccagtaaaggagaagaacttttcac-3'
gfp-RX	5'-ccgctcgagtttgtatagttcatccatgcca-3'
IIR-FN	5'-ggaattccatatgaaaaaaacagtaatcatttgtatata-3'
IIR-RF2B	5'ggatccctacttgtcatcgtcatccttgtaatccttgtcatcgtcatccttgtaatcggaaaaaaggtcggaaatcca-3'
sigE-FrbsB	5'-ggatccaagaagggatatagatatgaaaaaactgaaattacggttgac-3'
sigE-RX	5'-ccgctcgagcaccattttgttgaactcttttc-3'
IIGA-RE	5'-ccggaattctgaaacgtgtttgacagcctt-3'
gfp-FE	5'-gaattcagtaaaggagaagaacttttcac-3'
His-RB	5'-cgcggatcctagcagccggatctcagtg-3'
IIR-RX	5'-ccgctcgagggaaaaaaggtcggaaatcca-3'
sigE(M17I)-F	5'-tataagctgctgat**t**aaacttgggctg-3'
sigE(M17I)-R	5'-cagcccaagttt**a**atcagcagctata-3'
gfp-RF2X	5'ctcgagctacttgtcatcgtcatccttgtaatccttgtcatcgtcatccttgtaatctttgtatagttcatccatgcca-3'
BamHItoSTOP-F	5'-gatgacaagtagg**t**ag**c**caagaagggat-3'
BamHItoSTOP-R	5'-atcccttcttgg**cta**cctacttgtcatc-3'
IIGA-FrbsX	5'-ctcgagagaagggatatagatatgaaaatctatttagatgtcatttgg-3'
IIGA(D183A)-F	5'-aggcctgattg**c**ttccggtaacc-3' (pID53)[b]
IIGA(D183A)-R	5'-ggttaccggaa**g**caatcaggcct-3'
IIGA(D183E)-F	5'-aggcctgattga**g**tccggtaacc-3' (pID54)
IIGA(D183E)-R	5'-ggttaccgga**c**tcaatcaggcct-3'
IIGA(S184A)-F	5'-ggcctgattgat**g**ccggtaaccagctg-3' (pID55)
IIGA(S184A)-R	5'-cagctggttaccgg**c**atcaatcaggcc-3'
IIGA(S184C)-F	5'-ggcctgattgatt**g**cggtaaccagctg-3' (pID56)
IIGA(S184C)-R	5'-cagctggttaccg**c**aatcaatcaggcc-3'
IIGA(S184T)-F	5'-ggcctgattgat**a**ccggtaaccagctg-3' (pID57)
IIGA(S184T)-R	5'-cagctggttaccgg**t**atcaatcaggcc-3'
IIGA(G185A)-F	5'-ctgattgattccg**c**taaccagctgtac-3' (pID58)

IIGA(G185A)-R	5'-gtacagctggtta**gc**ggaatcaatcag-3'
IIGA(D6A)-F	5'-aaaatctatttag**c**tgtcatttggctg-3' (pID59)
IIGA(D6A)-R	5'-cagccaaatgaca**g**ctaaatagatttt-3'
IIGA(D6E)-F	5'-aaaatctatttaga**g**gtcatttggctg-3' (pID60)
IIGA(D6E)-R	5'-cagccaaatgac**c**tctaaatagatttt-3'
IIGA(H177A)-F	5'-agccaaacactt**gc**tgtcagaggcc-3' (pID61)
IIGA(H177A)-R	5'-ggcctctgaca**gc**aagtgtttggct-3'
IIGA(H261A)-F	5'-gttaaaccggat**gc**cgtaacgattat-3' (pID62)
IIGA(H261A)-R	5'-ataatcgttacg**gc**atccggtttaac-3'
IIGA(H261S)-F	5'-gttaaaccggat**tc**cgtaacgattat-3' (pID63)
IIGA(H261S)-R	5'-ataatcgttacg**ga**atccggtttaac-3'
IIGA(H296A)-F	5'-gacgcgattatt**gc**tccgaaaatgc-3' (pID64)
IIGA(H296A)-R	5'-gcattttcgga**gc**aataatcgcgtc-3'
IIGA(H296S)-F	5'-gacgcgattatt**tc**tccgaaaatgc-3' (pID65)
IIGA(H296S)-R	5'-gcattttcgga**ga**aataatcgcgtc-3'
IIGA(H307A)-F	5'-caaggctgtcaaa**gc**cgtttcaggatcc-3' (pID66)
IIGA(H307A)-R	5'-ggatcctgaaacg**gc**tttgacagccttg-3'
IIGA(S286A)-F	5'-acaacaaagctg**g**cggcagatggagag-3' (pID67)
IIGA(S286A)-R	5'-ctctccatctgccg**cc**agctttgttgt-3'
IIGA(D190A)-F	5'-aaccagctgtacg**c**tcctcttacaaaa-3' (pID68)
IIGA(D190A)-R	5'-ttttgtaagagga**g**cgtacagctggtt-3'
IIGA(D190E)-F	5'-aaccagctgtacga**g**cctcttacaaaa-3' (pID69)
IIGA(D190E)-R	5'-ttttgtaagagg**c**tcgtacagctggtt-3'
IIGA(D230A)-F	5'-atcgaacagctcg**c**tgattcctttcgc-3' (pID70)
IIGA(D230A)-R	5'-gcgaaaggaatca**g**cgagctgttcgat-3'
IIGA(D230E)-F	5'-atcgaacagctcga**g**gattcctttcgc-3' (pID71)
IIGA(D230E)-R	5'-gcgaaaggaatc**c**tcgagctgttcgat-3'
IIGA(D230P)-F	5'-atcgaacagctc**cc**tgattcctttcgc-3' (pID72)
IIGA(D230P)-R	5'-gcgaaaggaatca**gg**gagctgttcgat-3'
IIGA(D260A)-F	5'-tgcgttaaaccgg**c**tcacgtaacgatt-3' (pID73)
IIGA(D260A)-R	5'-aatcgttacgtga**g**ccggtttaacgca-3'
IIGA(D260E)-F	5'-tgcgttaaaccgga**g**cacgtaacgatt-3' (pID74)
IIGA(D260E)-R	5'-aatcgttacgtg**c**tccggtttaacgca-3'
IIGA(R164A)-F	5'-atatgaggaa**gc**agtcagcgtgc-3' (pID75)
IIGA(R164A)-R	5'-gcacgctgact**gc**ttcctcatat-3'
IIGA(R164I)-F	5'-atatgaggaa**at**agtcagcgtgc-3' (pID76)
IIGA(R164I)-R	5'-gcacgctgact**at**ttcctcatat-3'
IIGA(R164K)-F	5'-atatgaggaa**aa**agtcagcgtgc-3' (pID77)
IIGA(R164K)-R	5'-gcacgctgact**tt**ttcctcatat-3'
IIGA(V165A)-F	5'-tgaggaacgag**c**cagcgtgcagg-3' (pID78)
IIGA(V165A)-R	5'-cctgcacgctg**g**ctcgttcctca-3'
IIGA(V165P)-F	5'-tgaggaacga**cc**cagcgtgcagg-3' (pID79)
IIGA(V165P)-R	5'-cctgcacgctg**gg**tcgttcctca-3'
IIGA(G180A)-F	5'-tcatgtcagag**c**cctgattgatt-3' (pID80)
IIGA(G180A)-R	5'-aatcaatcagg**g**ctctgacatga-3'

IIGA(L181A)-F	5'-tgtcagaggc**gc**gattgattccg-3' (pID81)
IIGA(L181A)-R	5'-cggaatcaatc**gc**gcctctgaca-3'
IIGA(I182A)-F	5'-cagaggcctg**gc**tgattccggta-3' (pID82)
IIGA(I182A)-R	5'-taccggaatca**gc**caggcctctg-3'
IIGA(D183N))-F	5'-aggcctgatt**a**attccggtaacc-3' (pID83)
IIGA(D183N))-R	5'-ggttaccggaat**t**aatcaggcct-3'
IIGA(G185V)-F	5'-gattgattccg**t**taaccagctgt-3' (pID84)
IIGA(G185V)-R	5'-acagctggtta**a**cggaatcaatc-3'
IIGA(H296Q)-F	5'-cgcgattattca**a**ccgaaaatgc-3' (pID85)
IIGA(H296Q)-R	5'-gcattttcgg**t**tgaataatcgcg-3'
IIGA(H296F)-F	5'-cgcgattatt**tt**tccgaaaatgc-3' (pID86)
IIGA(H296F)-R	5'-gcattttcgga**aa**aataatcgcg-3'
IIGA(G180D)-F	5'-cttcatgtcagag**a**cctgattgattcc-3' (pID90)
IIGA(G180D)-R	5'-ggaatcaatcagg**t**ctctgacatgaag-3'
IIGA(I182D)-F	5'-gtcagaggcctg**ga**tgattccggtaac-3' (pID91)
IIGA(I182D)-R	5'-gttaccggaatca**tc**caggcctctgac-3'
IIGA(Q252E)-F	5'-ggtcagcaaaat**g**aattttattatgc-3' (pID92)
IIGA(Q252E)-R	5'-gcataataaaaatt**c**attttgctgacc-3'
IIGA(T282E)-F	5'-atcggtatcagc**ga**aacaaagctgtcg-3' (pID93)
IIGA(T282E)-R	5'-cgacagctttgtt**tc**gctgataccgat-3'
IIGA(T283A)-F	5'-ggtatcagcaca**g**caaagctgtcggca-3' (pID94)
IIGA(T283A)-R	5'-tgccgacagctttg**c**tgtgctgatacc-3'
IIGA(I294A)-F	5'-gagtttgacgcgg**gc**tattcatccgaaa-3' (pID95)
IIGA(I294A)-R	5'-tttcggatgaata**gc**cgcgtcaaactc-3'
IIGA(I295A)-F	5'-tttgacgcgatt**gc**tcatccgaaaatg-3' (pID96)
IIGA(I295A)-R	5'-cattttcggatga**gc**aatcgcgtcaaa-3'
IIGA(H296E)-F	5'-gacgcgattatt**gaa**ccgaaaatgctt-3' (pID97)
IIGA(H296E)-R	5'-aagcattttcgg**ttc**aataatcgcgtc-3'
IIGA(H296G)-F	5'-gacgcgattatt**gg**tccgaaaatgctt-3' (pID98)
IIGA(H296G)-R	5'-aagcattttcgga**cc**aataatcgcgtc-3'
IIGA(H296I)-F	5'-gacgcgattatt**at**tccgaaaatgctt-3' (pID99)
IIGA(H296I)-R	5'-aagcattttcgga**at**aataatcgcgtc-3'
IIGA(K298A)-F	5'-attattcatccg**gc**aatgctttcgggc-3' (pID100)
IIGA(K298A)-R	5'-gcccgaaagcatt**gc**cggatgaataat-3'
IIGA(K298N)-F	5'-attattcatccgaa**t**atgctttcgggc-3' (pID101)
IIGA(K298N)-R	5'-gcccgaaagcat**a**ttcggatgaataat-3'
IIGA(ΔC-8)-F	5'-gaaaatgctttcgggatccagtaaag-3' (pID102)
IIGA(ΔC-8)-R	5'-ctttactggatcccgaaagcattttc-3'
IIGA(H296G K298N)-F	5'-gacgcgattatt**gg**tccgaa**t**atgctttcgggc-3' (pID107)
IIGA(H296G K298N)-R	5'-gcccgaaagcat**a**ttcgga**cc**aataatcgcgtc-3'

[a]Bold type indicates a mutated sequence. Single underlining indicates a restriction endonuclease recognition site. Double underlining indicates a ribosome binding site.

Italics indicate a FLAG coding sequence.

[b]For primer pairs used for site-directed mutagenesis of pID48, the substitution in SpoIIGA is indicated in parentheses in the name of the forward (F) and reverse (R) primers, and the name of the mutant plasmid is listed after the forward primer.

TABLE S3

Protease inhibitors tested for inhibition of pro-σ^E processing in E. coli

Inhibitor (Source)	Type(s) of protease inhibited	Concentration tested
Complete Mini EDTA-free (Roche)	Serine and cysteine proteases	One tablet per 10 ml
Phenylmethylsulfonyl fluoride (Boehringer Mannheim	Serine proteases	500 μM
Pefabloc (Roche)	Serine proteases	500 μM
Pepstatin A (Roche)	Aspartic proteases	10 μM
Acetyl pepstatin (Sigma)	Aspartic proteases	10 μM
β-secretase inhibitor IV (Calbiochem)	β-secretase (aspartic protease)	20 μM
Boc-FψFAF-OMe[a]	γ-secretase (aspartic protease)	5 μM
Boc-FψFFA-OMe	γ-secretase (aspartic protease)	5 μM
Boc-FψFFL-OMe	γ-secretase (aspartic protease)	5 μM
Boc-FψFLF-OMe	γ-secretase (aspartic protease)	5 μM
Boc-FψFVF-OMe	γ-secretase (aspartic protease)	5 μM
Saquinavir[b]	HIV protease (aspartic protease)	100 μM
Nelfinavir	HIV protease (aspartic protease)	100 μM
Indinavir	HIV protease (aspartic protease)	100 μM
Amprenavir	HIV protease (aspartic protease)	100 μM
Atazanavir	HIV protease (aspartic protease)	100 μM
PfPM2 cci	HIV protease (aspartic protease)	100 μM
PfPM4 cci	HIV protease (aspartic protease)	100 μM
PmPM4 cci	HIV protease (aspartic protease)	100 μM
PvPM4 cci	HIV protease (aspartic protease)	100 μM
PfPM4 ssi	HIV protease (aspartic protease)	100 μM

[a]Peptidomimetic transition state analogs differing at $P_{2'}$ and $P_{3'}$ were a gift from M. Wolfe.

[b]HIV protease inhibitors were a gift from B. Dunn.

References:

1. Jiang, X., Rubio, A., Chiba, S., and Pogliano, K. (2005) *Mol. Microbiol.* **58**, 102-115
2. Youngman, P., Perkins, J. B., and Losick, R. (1984) *Plasmid* **12**, 1-9

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Time-dependent processing of pro-σ^E. Western blot analysis of pro-σ^E(M17I)-H6/σ^E-H6 using antibodies against σ^E. *E. coli* bearing pID50 and pID48 were shaken at 120 rpm and collected at the indicated times after IPTG induction. Ten min after IPTG induction, chloramphenicol (34 mg/ml in ethanol) was added to a final concentration of 200 µg/ml (lanes 5-8) to block protein synthesis or, as a control, the same volume of ethanol was added (lanes 1-4).
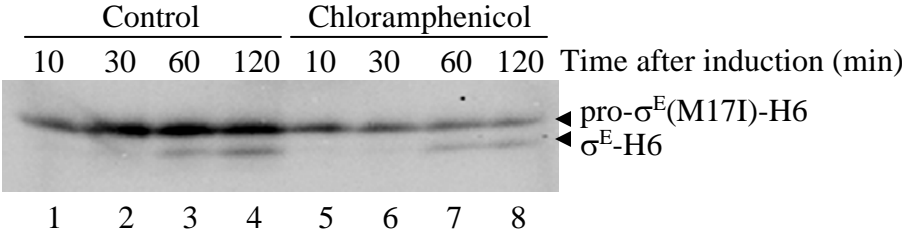
Supplemental Fig. 2. Similarity of *B. subtilis* SpoIIGA to aspartic proteases as detected by the HHpred algorithm. Residues S147 to P196 of SpoIIGA and short regions of known or predicted aspartic proteases (with sources indicated in parentheses) are shown with sequences matching the DS/TG sequence in bold.

Supplemental Fig. 3. Alignment of SpoIIGA orthologs. The predicted C-terminal intracellular domain of *B. subtilis* SpoIIGA was aligned with similar sequences (detected by BLAST analysis) (1) from the indicated bacteria using ClustalW (2). Numbers refer to residues in *B. subtilis* SpoIIGA. Stars, colons, and periods indicate identical, conserved, and semi-conserved residues.

REFERENCES

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* **215**, 403-410
2. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673-4680

Supplemental Fig. 1

	Control			Chloramphenicol			
10	30	60	120	10	30	60	120	Time after induction (min)

◄ pro-σ^E(M17I)-H6
◄ σ^E-H6

1　2　3　4　5　6　7　8

Supplemental Fig. 2

```
Cardosin A (C. cardunculus)        -------------DRDTSYFGEIGIGTPPQKFTVIFDTGSSV--------
Cathepsin D (H. sapiens)           ------------YMDAQYYGEIGIGTPPQCFTVVFDTG-----------
Candidapepsin (C. tropicalis)      ------DVPTTLINEGPSYAADIVVGSNQQKQTVVIDTGSSD--------
Candidapepsin-3 (C. albicans)      -----QTVPVKLINEQVSYASDITVGSNKQKLTVVIDTGSSD--------
Retropepsin (FIV)                  -------------TTLEKRPEILIFVNGYPIKFLLDTG-----------
DDI1 protease (S. cerevisiae)      --------------------INIEINNYPVKAFVDTG-----------
HIV protease (HIV-1)               -------------QITLWKRPLVTIKIGGQLKEALLDTGADDTIIEEMSL
HIV protease (Synthetic)           -------------QITLWKRPLVTIRIGGQLKEALLDTG-----------
HIV protease (HIV-2)               ------------QFSLWKRPVVTAYIEGQPVEVLLDTG-----------
EIAV protease (EIAV)               ------------TYNLEKRPTTIVLINDTPLNVLLDTG-----------
M-PMV protease (SRV-1)             --------------------LTLWLDDKMFTGLINTG-----------
SpoIIGA (B. subtilis)              SKRRIEDIETKNIQYEERVSVQADLGSQTLHVRGLIDSGNQLYDPLTKTP
Aspartic protease (B. germanica)   ---------YKLVHVFINTQYAGITKIGNQNFLTVFDSTSCN-------
RSV protease (RSV)                 -----------LVRVILTNTGSHPVKQRSVYITALLDSG-----------
```

Supplemental Fig. 3

```
Bacillus subtilis                 SKRRIEDIETKNIQYEERVSVQADLGSQTLHVRGLIDSGNQLYDPLTKTP 196
Bacillus amyloliquefaciens        SKKRIEDIETKSIQYDELVMVQAEFSGQTIRAKGLVDSGNQLYDPLTKTP
Bacillus licheniformis            SKKRFEDAEAKKIQYEERVRLEACIGEHTLHFTGLIDSGNQLYDPITKTP
Bacillus anthracis                SKKRIESVEVTKIHYDQIVKLKIQLAEEELELAGLIDSGNQLYDPLTKTP
Bacillus cereus                   SKKRIESVEVTKIHYDQIVKLKIQLAEEELELAGLIDSGNQLYDPLTKTP
Bacillus thuringiensis            SKKRIESVEVTKIHYDQIVKLKIQLAEEELELAGLIDSGNQLYDPLTKTP
Bacillus mycoides                 SKKRIESVEVTKIHYDQIVKVKIQLVGEELELAGLIDSGNQLYDPLTKTP
Bacillus megaterium               SKTRVDDLRIKNITFDQLVDVEIILNEQTLSMKGLIDSGNQLVDPLTKTP
Bacillus halodurans               SKQRLETIEVKKVQYERLVDVEVWLEQSKLTIRGIIDSGNQLQDPFTKAP
Geobacillus thermodenitrifican    SRRRVENIREKKLRFEHIIDVIVVWDGRPLALRGLIDSGNQLFDPVTKTP
Geobacillus kaustophilus          SRRRVEAIREKKLRFEYIIEVKIVWDGRPLMLRGLIDSGNQLVDPLTKTP
Bacillus clausii                  CKQQFGAVKSKQFHASQLAEVEFWIEGISFSAPGLIDTGNRLRDPLTKMP
Clostridium tetani                LERIVSFIRDRIVLDKLIYKIDIDLKNSKKSINAFLDTGNELVEPVTNLP
Clostridium perfringens           VSGVMNYFKNRAIINNFIYDLDVCIDSEVVNIKAFLDTGNGLVEPATALP
Moorella thermoacetica            GWLKKNIWQQML-----RLPVVITFGGRHLAVKALVDTGNSLREPLSQRP
Desulfotomaculum reducens         GNWGASIWHKRMGLIDNRVPLTVNLWGNRISVQALVDTGNHLVDPLSHYP
Alkaliphilus metalliredigens      -----GYIQKQISREKVFTKILIEINGISVEVQGLVDTGNSLTDPLTNYP
Clostridium thermocellum          -----DIVQQRISKEKLLMPLKIAFGNGTIELFALVDTGNSLCDPLTNSP
Pelotomaculum thermopropionicum   GRGFSALVKKGCFDGLFKIPLSVKSEGKQVKLEALLDTGNHLKDPLTRHP
Syntrophomonas wolfei             GIFGQKYLLRQVLPALLRFDVELRFNNYHCQGKGFLDTGNNLRDPITNRP
Oceanobacillus iheyensis          TKRRMDEHVGEQIKYDQMCEVFLTLNQKVQQSKGYIDSGNQLVDPLTKRP
Carboxydothermus hydrogenoform    GEYAYKQYQRIVRLKKYGFSLEIVYSGKKVTVQSFLDTGNRLYDPQLGWP
Thermoanaerobacter tengcongensis  LFLNWDYVVKKAKQDKMKYEIYIEIFGKKVKTYGFVDTGNKLFDPLSNLP
Symbiobacterium thermophilum      AQLLWQAYRERAEVDDGLYELEVRVDGRREVVEGLVDSGNLLRTPVGRMP
Desulfitobacterium hafniense      AWHRILGSTARHQASIYDLEIDMGGQGNPFRVKALLDTGNQLRDPLTGYP
Clostridium difficile             LKYIYKDLKMLKYIKELTKTININLLEKSFCCKALVDSGNLLKDPISQSD
                                         :                  . :*:**  *   *


Bacillus subtilis                 VMIIYIDKLEPIFGTAETMIIRNTDPLEAIEQLDDSF---RFLDKMRLIP 243
Bacillus amyloliquefaciens        VMILCIDKFAPILSEKETDLIKSASPLEVIEQLDDSF---RHADKLRLIP
Bacillus licheniformis            VMIVNIEKLKVVLGEEASVTIKEMSPLDAVGKLDEAL---PYIGRIRLIP
Bacillus anthracis                VMIMHISSLEHCLPAWLTEQIYSKTEIPQIPENDS-----GWATKLRLIP
Bacillus cereus                   VMIMHISSLEHCLPAWLTEQIYSKTEIPQIPENDS-----GWATKLRLIP
Bacillus thuringiensis            VMIMHISSLEHCLPAWLTEQIYSKTQIPQIPENDS-----GWATKLRLIP
Bacillus mycoides                 VMIMHISSLEHCLPVWLTEQIYSKTEIPQIPENDS-----GWATKLRLIP
Bacillus megaterium               VMIVTADSLKEILPEGLMELSKNVQSFSHSEDIDQ-----EWYSKVRFVP
Bacillus halodurans               VMIIQADVLRGQIPDEWIEQMLNVESIG-TTDGDE-----RFMRRMSVIP
Geobacillus thermodenitrifican    VMVVDREKAKEVLPEELT-MHMATMTFDDLPE--------QWAHRLRLIP
Geobacillus kaustophilus          VMVVEQEKAKAVLPEELLGCLSSPMMLASPPE--------RWVHRLRLIP
Bacillus clausii                  VMVAEAALLYSAFGKQQVDALLELEAENEPSP---------LMKRVRLIP
Clostridium tetani                VIVVEKDIFQKNE-------------------------IEDYDTVYIP
Clostridium perfringens           VIIAEREKFRGVN-------------------------IKEKDQFRIP
Moorella thermoacetica            VIIVEYSALKEIMPPEIIKAYDSQGGFDLDSLVNSLAA-TPWATRLRLIP
Desulfotomaculum reducens         VIIMEYEAIKPLLPNEISRLYSEGISQDGSDMLTLLSA-SPYAQRLRLIP
Alkaliphilus metalliredigens      VIIVEYEAITNLLPEEVKRIFNQSTTIDYKQVALLLDN-QSWMTRIRMIP
Clostridium thermocellum          VVVVEFNAIKNILPMEIQRIFDESKENDLDSVTKIVSD-SKWMSRFRLIP
Pelotomaculum thermopropionicum   VVVVEYAALKPLLPPEVQACFEKEGEPDVWQILNLLGE-KRFAGRFSAVP
Syntrophomonas wolfei             VIVAEYQFLKSCLPEDLREVLEIKQDENTMLDALGG---CSWANRLRLIP
Oceanobacillus iheyensis          VIICDRTFLKEWFTEDEWLQLEKAKNNLDMDEIPKR-----WQDKIQIIP
Carboxydothermus hydrogenoform    VVVVEYRAVKKILPVDFFQS----------------------KTIRRIP
Thermoanaerobacter tengcongensis  VVVVAYSSVKSLLPYDLEEFVAEGKIDFGKVLEIIKD--EKWHSRIGIIP
Symbiobacterium thermophilum      VAVVEGGRLRSLLPPAVLEAASSGPMGLDGLP-------AEWQSRCQLVP
Desulfitobacterium hafniense      VILIEEEAAAAGVPEELLPVLQGAWRDLDDPWPWLWQSDSLWIKQCAFIP
Clostridium difficile             VIIVKSSVLEGFIPEKLLNINYEDINMKKAQEIIGSLD-DKTSGRVRIIP
                                  *  :                                         :*
```

```
Bacillus subtilis              YRGVGQ-QNQFLLCVKPDHVTIM-TKEEMISADKCLIGISTTKLSADGEF 291
Bacillus amyloliquefaciens     YRGVGQ-DNQFLLCLKPDYVTIL-TKDEMIACEKCLIGISTKPLSADGEF
Bacillus licheniformis         YRGVGH-QHQFLLCLKPDHVLVC-TEREVIEAPKCLIGISTSPLSADGEF
Bacillus anthracis             FRAVGV-ENQFLWAIKPESVQIY-HEGSSIVVNKVLIGLNTQQLSTNGEY
Bacillus cereus                FRAVGV-ENQFLWAIKPESVQIY-HEGSSIVVNKVLIGLNTQQLSTNGEY
Bacillus thuringiensis         FRAVGV-ENQFLWAIKPESVQIY-HEGSSIVVNKVLIGLNTQQLSTNGEY
Bacillus mycoides              FRAVGV-ENQFLWAIKPDCVQVY-HEGSSIVVNKVLIGLNTQQLSSNGEY
Bacillus megaterium            YRSVGQ-ANQLLLALKPDMVRLV-HQSNTIEVTKVLVGISHTTLSVEKQY
Bacillus halodurans            YRVVGQ-PQRLLAVFKPDSVRIR-HEEEWMTVSKVLIGLNDKALSPEDTY
Geobacillus thermodenitrifican YRAVGS-GPQMMMAIKPDRIMLL-YEDQWLEVKQGLVALSANSLSTDGEY
Geobacillus kaustophilus       YRAVGS-GSQMMAAVKPDRIMLR-YENEWLEVAQGLVALSADPLSTDGEY
Bacillus clausii               YRAVGQ-GSSYMAALKPDRVKIR-YQNEAYETNHVLIGLSRETLSPEQHY
Clostridium tetani             FSVVNG-DRGMLKAIKPEMVSIY-KKR-EIEKKEVLIAFSDKKLSSSGDY
Clostridium perfringens        YKVVDG-NSGYMKGIKIDNIKLC-NVNGETMTRDAILCFCDNKLSKEGEY
Moorella thermoacetica         YHSLGQ-ERGMLLGLRPDEVVIV-TGQGMIKVKEVLIGLYRERLSPEGNY
Desulfotomaculum reducens      FHSLGN-NHGMLLGIRPDDIEVC-YKNKIQRIQNVVVGIYEKKLSPGTTY
Alkaliphilus metalliredigens   FQALGT-ENGMLVGFKPDRVSIE-NNSIMKETQDTIIAIYHKKLSKNEDY
Clostridium thermocellum       FTSLGK-ENGMLIGFRPDYIEVG-ENAEKKGITDVIIGIYNRALSKNNNY
Pelotomaculum thermopropionicum FQSLSR-IGGLMVGYRPDEVIIE-QKGRQLRAEKVVLAIYHSSLDPGNSY
Syntrophomonas wolfei          FSSIGK-KNGILLGVRADEIVVN-NGSKDVFHQNMVVGIYREKLSSQGEY
Oceanobacillus iheyensis       YQGVDG-GSSMMIGIRPERVIVN-YNDQQLLATNVIIGIQFGNLVRDDSY
Carboxydothermus hydrogenoform FRGVGR-GQGFFWGFIPDELKIW-QEGEVIRVKRAVVAITFQSLN--SEF
Thermoanaerobacter tengcongensis FSGVG--TKGLLLGIRPDRLIIN-GKE----IKDVMLGIS-----TDKME
Symbiobacterium thermophilum   FAAVGR-SDGWLLVIRPDGLSVRPRGRGDWVQVEGRVGLAAGPLDPEGRY
Desulfitobacterium hafniense   YQGVG--HKSWLLGIRPQKVVCT--SLSEPREMKATLAFVRQVLSPDRAY
Clostridium difficile          YKHAGSNKTSIIIGLKADYIEVD--------EQKIGNIILGISNFNDREY
                               :     .       :       : :                    :

Bacillus subtilis              DAIIHPKMLSGKAVKHVS---------------------- 309
Bacillus amyloliquefaciens     DGIVHPKMLTGKAVKHVS----------------------
Bacillus licheniformis         DAIVHPKMLAGNPVKHVS----------------------
Bacillus anthracis             QCIVHPKMLISQKMVIA-----------------------
Bacillus cereus                QCIVHPKMLISQKMVIA-----------------------
Bacillus thuringiensis         QCIVHPKMLISQKMVIA-----------------------
Bacillus mycoides              QCIVHPKMLISQKMVIA-----------------------
Bacillus megaterium            ECIVHPKLIVIGEVSSAS----------------------
Bacillus halodurans            QCIVHPKMIVHGITDKLA----------------------
Geobacillus thermodenitrifican SCIVHPKMVQSGKKLTAS----------------------
Geobacillus kaustophilus       DCIVHPKMVQAGRKLTAS----------------------
Bacillus clausii               RCIVHPQIMHEYAS--------------------------
Clostridium tetani             RALLSRGIIF-----------------------------
Clostridium perfringens        EALLSRGII------------------------------
Moorella thermoacetica         RALLHPDLLELSMSF------------------------
Desulfotomaculum reducens      RALLHPQLLAF----------------------------
Alkaliphilus metalliredigens   RALLHPDLV------------------------------
Clostridium thermocellum       SALLSPELI------------------------------
Pelotomaculum thermopropionicum HALVGPELIGLG---------------------------
Syntrophomonas wolfei          QFLIPAEIIENA---------------------------
Oceanobacillus iheyensis       HCLLHPQIMKQSIIHSA----------------------
Carboxydothermus hydrogenoform QALLSEEILKSA---------------------------
Thermoanaerobacter tengcongensis FALLNPEIMG----------------------------
Symbiobacterium thermophilum   AALLPTAMIAAARRAHGRARGRPVEAQSGERGEGRPDVHVR
Desulfitobacterium hafniense   QALLHPEHVQGAEES------------------------
Clostridium difficile          GAIMNPNILPGF---------------------------
                                 ::     :
```