A

Streptococcus pneumoniae
Bacillus subtilis
Bacillus cereus
Mycobacteroides abcessus
Staphylococcus epidermidis
Mycobacterium tuberculosis
Listeria monocytogenes
Vibrio vulnificus
Pseudomonas amygdali
Rhodococcus qingshengii
Escherichia coli
Clostridium botulinum
Clostridium perfringens
Methanosaeta
Bacillus anthracis
Chlamydia trachomatis
Paeniclostridium sordellii
Clostridioides difficile
Intestinibacter bartlettii

0.2

B

B. subtilis   *C. difficile*   *L. monocytogenes*

C. trachomatis   *P. amygdali*   *V. vulnificus*

C

α1   α2

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Bs --SSEQERLLVSIDEKRKLMIDAARKQGFTGHDTIRHSQELDCLINEYHQLM  53
Cd MANNELEALKNEIEAVREEINTYIEYPEIFQEELVESSKKIDILINKYMYLS  52
Lm LRTAGSIYPQYSIDYLRTHLIEIGMLQGLSHPETIKSSQELDKLLYKFQRNI  61
Ct MNSRCATEICSEIRMKRWEMMELAKMYGIGHEYTLRQSEQLDKLINEYLILQ  52
Pa YKTVVKQILVTEIEIMRERMIVTGLEEGFHSNNTIKMSEIMDQLLNELQKVD  53
Vv -----MEELHEKIEMLRKELITTGLIYGFTSPETLHKSQELDKLLNLLGH--  45
        .*  *  :           :     :. *: :* *:
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S5_Figure. Spo0E-like proteins are conserved and prevalent in Gram-positive and Gram-negative bacteria. A) Unrooted neighbor-joining tree based on the full amino acid sequence of Spo0E and Spo0E-like proteins. Tree generated using MEGA 11. **B)** The predicted Spo0E 3D structures generated with Phyre2 from representative Gram-positive and Gram-negative species. The residues comprising the signature Spo0E motif (SQELD/SxxxD) are colored red in each structure. Structures were edited in PyMOL (The PyMOL Molecular Graphics System, Version 2.4.0 Schrödinger, LLC). **C)** Multiple sequence alignment of Spo0E and Spo0E-like proteins overlayed against *B. subtilis* Spo0E secondary structure determined by AlphaFold and consisting of two α-helices. Spo0E motif residues are shaded red. Multiple sequence alignment performed using ClustalW.