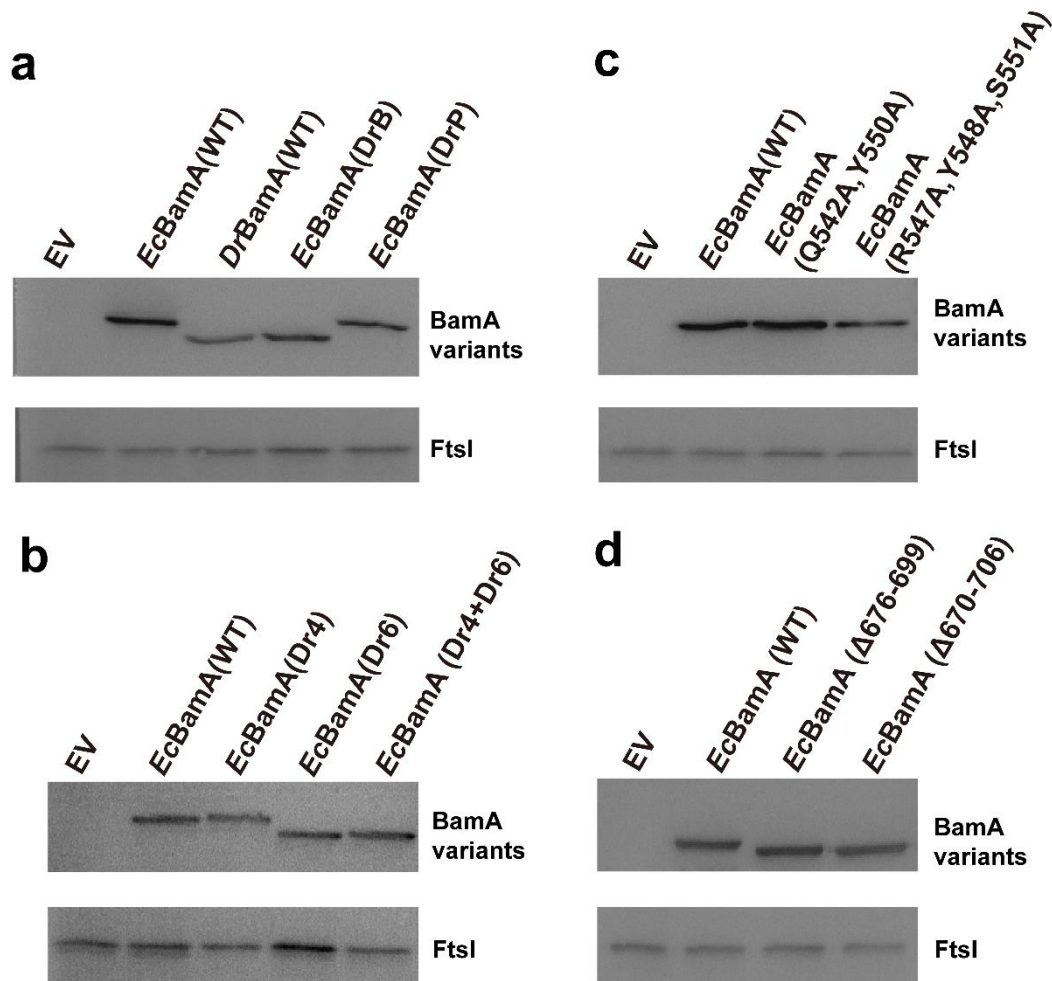
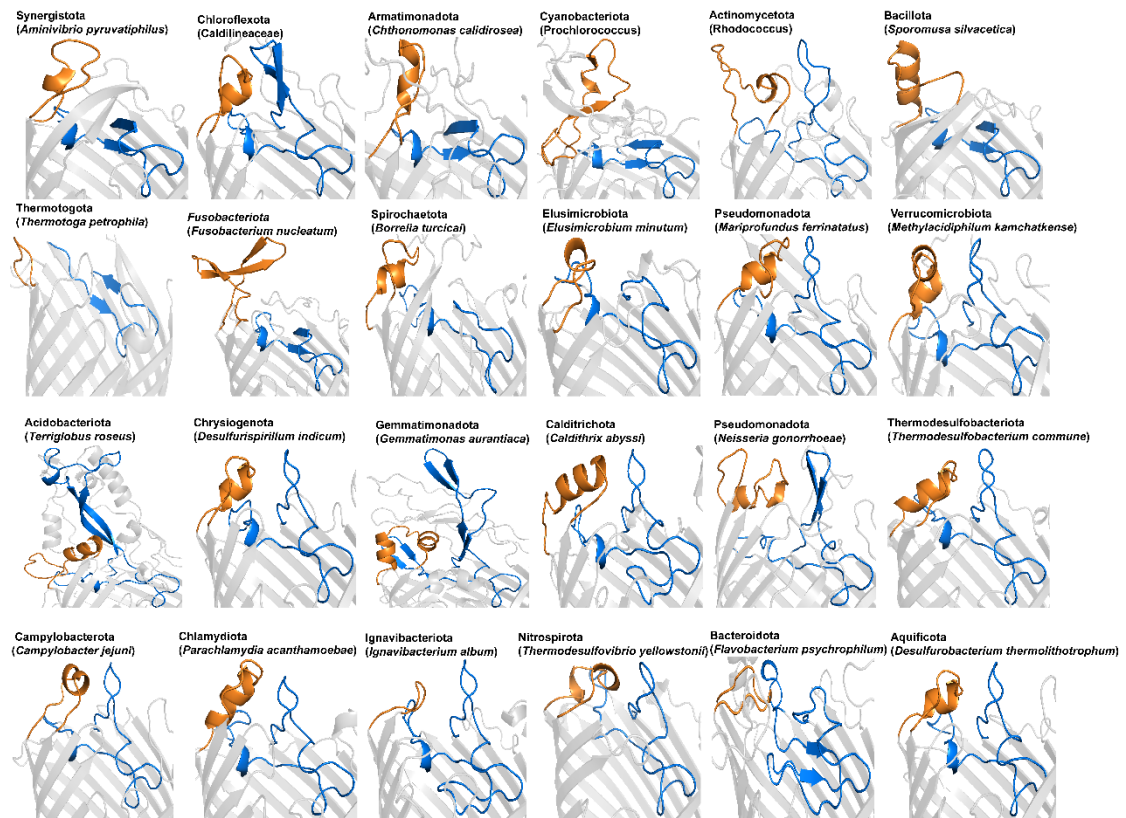


**Figure S2.** Cryo-EM maps of representative structural elements of *DrBamA*. The densities were all prepared in PyMol<sup>[1]</sup> with the density of selected  $\beta$ -strands (a) and ECL6 (b) contoured  $5 \sigma$  and the density of ECL4 (c) contoured  $3 \sigma$ .



**Figure S3. Expression test of BamA variants.** (a) Western blot of chimeric BamA carrying different domains of *EcBamA* and *DrBamA*. (b) Western blot of *EcBamA* replaced the ECL4 or ECL6 with the corresponding region of *DrBamA*. (c,d) Western blot of *EcBamA* mutants carrying mutations in ECL4 (c) or ECL6 (d). EV: empty vector.



**Figure S4. Comparative Structure of ECL4 (orange) and ECL6 (navy blue) Across Various Bacterial Species.** This figure highlights the structures of ECL4 and ECL6 in the barrel domains of BamA obtained from existing studies such as *Neisseria gonorrhoeae* BamA<sup>[2]</sup> or predicted by AlphaFold2<sup>[3]</sup>. A comprehensive summary of these structures is presented in Figure 4.

**Table S1. Strains, plasmids and primers used in this study**

<b>Strain</b>	<b>Genotype</b>	<b>Reference/source</b>
DH5 $\alpha$	F <sup>-</sup> , $\Delta$ ( <i>argF-lac</i> )169, $\phi$ 80dlacZ58(M15), $\Delta$ <i>phoA8</i> , <i>recA1</i> , <i>endA1</i> , <i>thiE1</i> , <i>hsdR17</i> , $\lambda$ <sup>-</sup> , <i>deoR481</i> ,	CGSC #14231 <sup>[4]</sup>
BL21(DE 3)	F <sup>-</sup> , <i>lon-11</i> , $\Delta$ ( <i>ompT-nfrA</i> )885, $\Delta$ ( <i>galM-ybhJ</i> )884, $\lambda$ DE3, $\Delta$ 46, [ <i>mal</i> <sup>+</sup> ] <sub>K-12</sub> ( $\lambda$ S), <i>hsdS10</i>	CGSC #12504 <sup>[5]</sup>
ZW01	BL21(DE3), P <sub>T7</sub> :: <i>pelB-Flag-10</i> × <i>His-DrbamA</i>	This study
BW25113	F <sup>-</sup> , $\Delta$ ( <i>araD-araB</i> )567, $\Delta$ <i>lacZ4787</i> (:: <i>rrnB-3</i> ), <i>rph-1</i> , $\Delta$ ( <i>rhaD-rhaB</i> )568, <i>hsdR514</i>	CGSC #8590 <sup>[6]</sup>
JC20	BW25113, $\Delta$ <i>bamA</i> (:: <i>aph</i> ) , P <sub>BAD</sub> :: <i>bamA-sacB</i>	This study
ZW02	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i>	This study
ZW03	JC20 P <sub>T5lac</sub> :: <i>Flag-DrbamA</i>	This study
ZW04	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> ( <i>DrB</i> ) <sup>1</sup>	This study
ZW05	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> ( <i>DrP</i> ) <sup>2</sup>	This study
ZW06	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> ( <i>Dr4</i> ) <sup>3</sup>	This study
ZW07	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> ( <i>Dr6</i> ) <sup>4</sup>	This study
ZW08	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> <sup><math>\Delta</math>670-706</sup>	This study
ZW09	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> <sup><math>\Delta</math>676-699</sup>	This study
ZW11	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> <sup>Q542A, Y550A</sup>	This study
ZW12	JC20 P <sub>T5lac</sub> :: <i>Flag-bamA</i> <sup>R547A, Y548A, S551A</sup>	This study
<b>Plasmid</b>	<b>Genotype</b>	<b>Reference/source</b>
pSB-13	ColE1, <i>cat araC</i> P <sub>BAD</sub> :: <i>sb13-His6</i>	Lab stock
pKD46	pSC101, <i>bla repA</i> <sup>ts</sup> <i>araC</i> P <sub>BAD</sub> :: <i>gam bet exo</i>	[6]
pKD13	R6K $\gamma$ , <i>bla</i>	[6]
pBAD33	pACYC, <i>cat araC</i> P <sub>BAD</sub> ::	[7]
pYD48	ColE1, <i>bla lacI</i> <sup>Q1</sup> P <sub>T5lac</sub> :: <i>Halo-gpg-murJ</i>	Lab stock
pJC06	pACYC, <i>cat araC</i> P <sub>BAD</sub> :: <i>bamA</i>	This study
pCDFD <sub>ue</sub>	CloDF13, <i>aadA</i> P <sub>T7</sub> ::	[8]
pZW01	CloDF13, <i>aadA</i> P <sub>T7</sub> :: <i>Flag-His<sub>10</sub>-DrbamA</i>	This study

pZW02	CloDF13, <i>aadA</i> P <sub>T7</sub> :: <i>pelB-Flag-His<sub>10</sub>-DrbamA</i>	This study
pZW23	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> ::	This study
pZW24	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA</i>	This study
pZW25	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-DrbamA</i>	This study
pZW26	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA(DrB)<sup>a</sup></i>	This study
pZW27	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA(DrP)<sup>b</sup></i>	This study
pZW28	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA(Dr4)<sup>c</sup></i>	This study
pZW29	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA(Dr6)<sup>d</sup></i>	This study
pZW30	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA<sup>A670-706</sup></i>	This study
pZW31	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA<sup>A676-699</sup></i>	This study
pZW33	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA<sup>Q542A, Y550A</sup></i>	This study
pZW34	ColE1, <i>bla lacI<sup>Q1</sup></i> P <sub>T5lac</sub> :: <i>Flag-bamA<sup>R547A, Y548A, S551A</sup></i>	This study
<b>Primer</b>	<b>Sequence (5' - 3')</b>	<b>note</b>
oZW1	<u>ATAAGGAGATATAACCATGCGACACCCCCTG</u> ACGTT	Amplification of the gene encoding <i>DrBamA</i> (Met1-Ala21) for pZW01
oZW2	<u>GTCCTTGTAATCAGAGCCGGCCAGCGCGGGT</u> GCGGACGCGATCAGAA	
oZW3	<u>CACGGTTCTGACGAAGTTGATGCCGGATCCC</u> AGCAGGCCGGCACCGTGCAGGACAT	Amplification of the gene encoding <i>DrBamA</i> (Gln22-Trp846) for pZW01
oZW4	<u>TAGGTTAATTAAGCTGCGTTACCAGAAGTTG</u> CCGATGC	
oZW5	<u>GGCTCTGATTACAAGGACGACGATGACAAG</u> TCTGGCCAT	Amplification of the dsDNA fragment encoding Flag- <i>His<sub>10</sub></i> tag
oZW6	<u>ACTTCGTCAGAACCGTGATGATGATGGTGAT</u> GATGGTGGTGATGGCCAGACTTGTCATC	
oZW7	CGCAGCTTAATTAACCTAGG	Amplification of vector backbone for pZW01
oZW8	CATGGTATATCTCCTTATTAAG	
oZW9	<u>ATAAGGAGATATAACCATGAAATATCTGCTGC</u> CGACCG	Amplification of the <i>pelB</i> sequence for pZW02
oZW10	<u>GTCCTTGTAATCAGAGCCGGCCATTGCAGGC</u> TGGGCTGC	
oZW29	<u>GAGAAATTA ACTACTAGTGCGGCCGCTAAG</u> GGTCGA	Amplification of pZW23 with oZW35
oZW31	<u>GAGAAATTA ACTACTAGTATGGCGATGAAA</u> AAGTTGCTCATAG	Amplification of the gene encoding <i>EcBamA</i> (Met1-Gly20) for pZW24 and pZW25
oZW32	<u>GTCCTTGTAATCAGAGCCACCGTATACGGTG</u> GCGCTGC	

oZW33	<u>GGCTCTGATTACAAGGACGACGATGACAAG</u> GGTTCTGCTGAAGGGTTCGTAGTG	Amplification of the gene encoding Flag- <i>Ec</i> BamA (Ala21-Trp810) for pZW24
oZW34	<u>TCGACCCTTAGCGGCCGCTTACCAGGTTTTA</u> CCGATGTTA	
oZW35	ACTAGTAGTTAATTTCTCCTCTT	Amplification of vector backbone of pYD48 for pZW24 and pZW25
oZW36	GCGGCCGCTAAGGGTCGA	
oZW37	<u>GGCTCTGATTACAAGGACGACGATGACAAG</u> GGTTCTCAGCAGGCCGGCACCGT	Amplification of the gene encoding Flag- <i>Dr</i> BamA (Gln22-Trp846) for pZW25
oZW38	<u>TCGACCCTTAGCGGCCGCTTACCAGAAGTTG</u> CCGATGC	
oZW39	GGTGTGCGCTCTTTTACCT	Amplification of vector backbone with oZW36 for pZW26
oZW40	<u>GTAAAAGAGCGCAACACCGGCATCCCGGTC</u> AACCTC	Amplification of the gene encoding the barrel domain (Ile481-Trp846) of <i>Dr</i> BamA for pZW26
oZW41	<u>TCGACCCTTAGCGGCCGCTTACCAGAAGTTG</u> CCGATGC	
oZW42	GGTAGCTTCAACTTTGGTATTG	Amplification of vector backbone with oZW32 for pZW27
oZW43	<u>GGCTCTGATTACAAGGACGACGATGACAAG</u> GGTTCTGCCGGCACCGTGCAGGA	Amplification of the gene encoding the POTRA domain (Gln22-Gly480) of <i>Dr</i> BamA for pZW27
oZW44	<u>ACCAAAGTTGAAGCTACCGCCCGTGCGACCT</u> TCGC	
oZW45	<u>GGACAGGGAGTTATGTACATAA</u>	Amplification of vector backbone for pZW28
oZW46	<u>AGCTTCAAACGGACGACTTC</u>	
oZW47	<u>GTACATAACTCCCTGTCCCTCGAACCCAAGC</u> AGGAAG	Amplification of the gene encoding ECL4 of <i>Dr</i> BamA for pZW28
oZW48	<u>GTCGTCCGTTTTGAAGCTGGTCGCGGGCAGC</u> AGAG	
oZW49	ACCATCACCATAACCCAGC	Amplification of vector backbone for pZW29
oZW50	GCCATGGCGGTTGCCAGC	
oZW51	<u>TGGGGTTATGGTGATGGTGACGGCACCGGCT</u> ACTTCAT	Amplification of the gene encoding ECL6 of <i>Dr</i> BamA for pZW29
oZW52	<u>GCTGGCAACCGCCATGGCGGTGCCGAACAG</u> TTGCCCAT	
oZW53	<u>GGTGGTTCTGGATCAGGCGGTAACGCCATG</u> GCGG	Mutagenesis of <i>bamA</i> gene for pZW30
oZW54	<u>GCCTGATCCAGAACCACCAATGGTATTGGAC</u> TGGAAGCC	

oZW55	<u>TACTTCGGTTCTGGATGTAAATCGGATGATG</u> CTGTAG	Mutagenesis of <i>bamA</i> gene for pZW31
oZW56	<u>ACATCCAGAACCGAAGTAAACTGCTTTCGG</u> ACC	
oZW59	<u>ACGCCACATCGCAACTGCAGGCTGCATGTTG</u> GACAGG	Mutagenesis of <i>bamA</i> gene for pZW33
oZW60	<u>GCAGTTGCGATGTGGCGTTATCTGGCATCTA</u> TGGGTGAACATCCGAG	
oZW61	<u>TGGGCAGCACTGTACGCAATGGGTGAACAT</u> CCGAGCAC	Mutagenesis of <i>bamA</i> gene for pZW34
oZW62	<u>TGCGTACAGTGCTGCCACATCGCAACCTGA</u> GGCTGC	
oSY9	CGTTTCACTCCGAGCTCGAATTC	Amplification of vector backbone for pJC06
oSY10	GCATGCAAGCTTGGCTGTTTTG	
oJC38	<u>GCTCGGAGTGAAACGATGGCGATGAAAAAG</u> TTGCTCATAG	Amplification of the gene encoding <i>EcBamA</i> for pJC06
oJC39	<u>GCCAAGCTTGCATGCTTACCAGGTTTTACCG</u> ATGTTAAA	
oJC40	<u>TGCACTTTTCAATGATTTCTCTCGGTTATGAG</u> <u>AGTTAGTTAGGAAGAACGCATAATAACGTG</u> TAGGCTGGAGCTGCTTCG	Amplification of the kanamycin resistance cassette
oJC41	<u>CTTATATTGATCGCCTAAAGTCATCGCTACA</u> <u>CTACCACTACATTCCTTTGTGGAGAACACAT</u> TCCGGGGATCCGTCGACC	

<sup>a</sup> *bamA(DrB)*: gene of the chimera BamA carrying the POTRA domain (Met1-Thr423) of *EcBamA* and the barrel domain(Ile481-Trp846) of *DrBamA*.

<sup>b</sup> *bamA(DrP)*: gene of the chimera BamA carrying the POTRA domain (Gln22-Gly480) of *DrBamA* and the signal peptide (Met1-Gly20) and barrel domain (Gly424-Trp810) of *EcBamA*.

<sup>c</sup> *bamA(Dr4)*: gene of the *EcBamA* replaced the ECL4 (Asn538-Asn563) with the correponding region (Leu614-Thr637) of *DrBamA*.

<sup>d</sup> *bamA(Dr6)*: gene of the *EcBamA* replaced the ECL6 (Leu641-Asn709) with the correponding region (Asp727-Thr755) of *DrBamA*.



**Table S2. Summary of data collection and model statistics**

	<i>DrBamA</i> (EMD-38500) (PDB 8XNB)
<b>Data collection and processing</b>	
Magnification	81,000
Voltage (kV)	300
Electron exposure (e- /Å <sup>2</sup> )	50
Defocus range (μm)	-1.6 ~ -1.8
Pixel size (Å)	1.07
Symmetry imposed	C2
Initial particle images(no.)	5,755,354
Final particle images (no.)	607,784
Resolution (Å)	3.8
FSC threshold	0.143
Map resolution range (Å)	3.1 ~ 4.7
<b>Refinement</b>	
Initial model	Alphafold2
Map sharpening B factor (Å <sup>2</sup> )	300
Model composition	
Non-hydrogen atoms	5580
Protein residues	738
Ligands	0
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.885
Validation	
MolProbity score	1.95
Clashscore	10.81
Poor rotamers (%)	0.54
Ramachandran plot	
Favored (%)	94.01
Allowed (%)	5.99
Disallowed (%)	0

**Table S3. Characteristic of BamA from different species**

Group	phylum	Species	Uniprot entry	Helix in ECL4	Helix interacts with other ECLs	Stem region in ECL6
Terrabacteria	Deinococcota	<i>Deinococcus radiodurans</i>	Q9RXD5	yes	no	no
	Thermotogota	<i>Thermotoga petrophila</i>	A0A3B8N0X3	no		no
	Synergistota	<i>Aminivibrio pyruvatiphilus</i>	A0A4R8MJ79	yes	yes	no
	Bacillota	<i>Sporomusa silvacetica</i>	A0A259UR18	yes	yes	no
	Actinomycetota	unclassified Rhodococcus	A0A520ELS9	yes	no	yes
	Cyanobacteriota	unclassified Prochlorococcus	A0A163CN93	yes	yes	no
	Chloroflexota	unclassified Caldilineaceae	A0A957UFE8	yes	yes	yes
	Armatimonadota	<i>Chthonomonas calidirosea</i>	S0EX68	yes	yes	no
Gracilliales	Fusobacteriota	<i>Fusobacterium canifelinum</i>	A0A7T4FP41	no		no
	Spirochaetota	<i>Borrelia turcica</i>	A0A386PMY4	yes	yes	no
	Elusimicrobiota	<i>Elusimicrobium minutum</i>	B2KAU4	yes	yes	yes
	Pseudomonadota	<i>Mariprofundus ferrinatatus</i>	A0A2K8L4U4	yes	yes	yes
	Nitrospirota	<i>Thermodesulfobacterium yellowstonii</i>	B5YKV4	yes	yes	yes
	Chrysiogenota	<i>Desulfurispirillum indicum</i>	E6W0D0	yes	no	yes
	Acidobacteriota	<i>Terriglobus roseus</i>	I3ZBF8	yes	yes	yes
	Thermodesulfobacteriota	<i>Thermodesulfobacterium commune</i>	A0A124FKV8	yes	yes	yes
	Aquificota	<i>Desulfurobacterium thermolithotrophum</i>	F0S063	yes	yes	yes
	Verrucomicrobia	<i>Methylacidiphilum kamchatkense</i>	A0A516TNK1	yes	yes	yes

Chlamydiota	<i>Parachlamydia acanthamoebae</i>	F8KYI9	yes	yes	yes
Gemmatimonadota	<i>Gemmatimonas aurantiaca</i>	A0A3D4VC68	yes	no	yes
Calditrichota	<i>Caldithrix abyssi</i>	A0A1J1C9H6	no	no	yes
Bacteroidota	<i>Flavobacterium psychrophilum</i>	A6H1D4	no		yes
Ignavibacteriota	<i>Ignavibacterium album</i>	I0AGV2	no		yes
Campylobacterota	<i>Campylobacter jejuni</i>	A0A431EK96	yes	yes	yes
Pseudomonadota	<i>Escherichia coli</i>	P0A940	yes	yes	yes
Pseudomonadota	<i>Haemophilus influenzae</i>	O32629	yes	yes	yes
Pseudomonadota	<i>Pasteurella multocida</i>	Q9CJL1	yes	yes	yes
Pseudomonadota	<i>Salmonella enterica</i>	Q8ZRP0	yes	yes	yes
Pseudomonadota	<i>Pseudomonas aeruginosa</i>	Q9HXY4	yes	yes	yes
Pseudomonadota	<i>Neisseria gonorrhoeae</i>	Q5F5W8	yes	yes	yes
Pseudomonadota	<i>Neisseria meningitidis</i>	Q9K1H0	yes	yes	yes

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