
Structure of a distinct β -barrel assembly machinery complex in the Bacteroidota

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Discovery of a distinct BAM complex in the Bacteroidota

Supplementary Information

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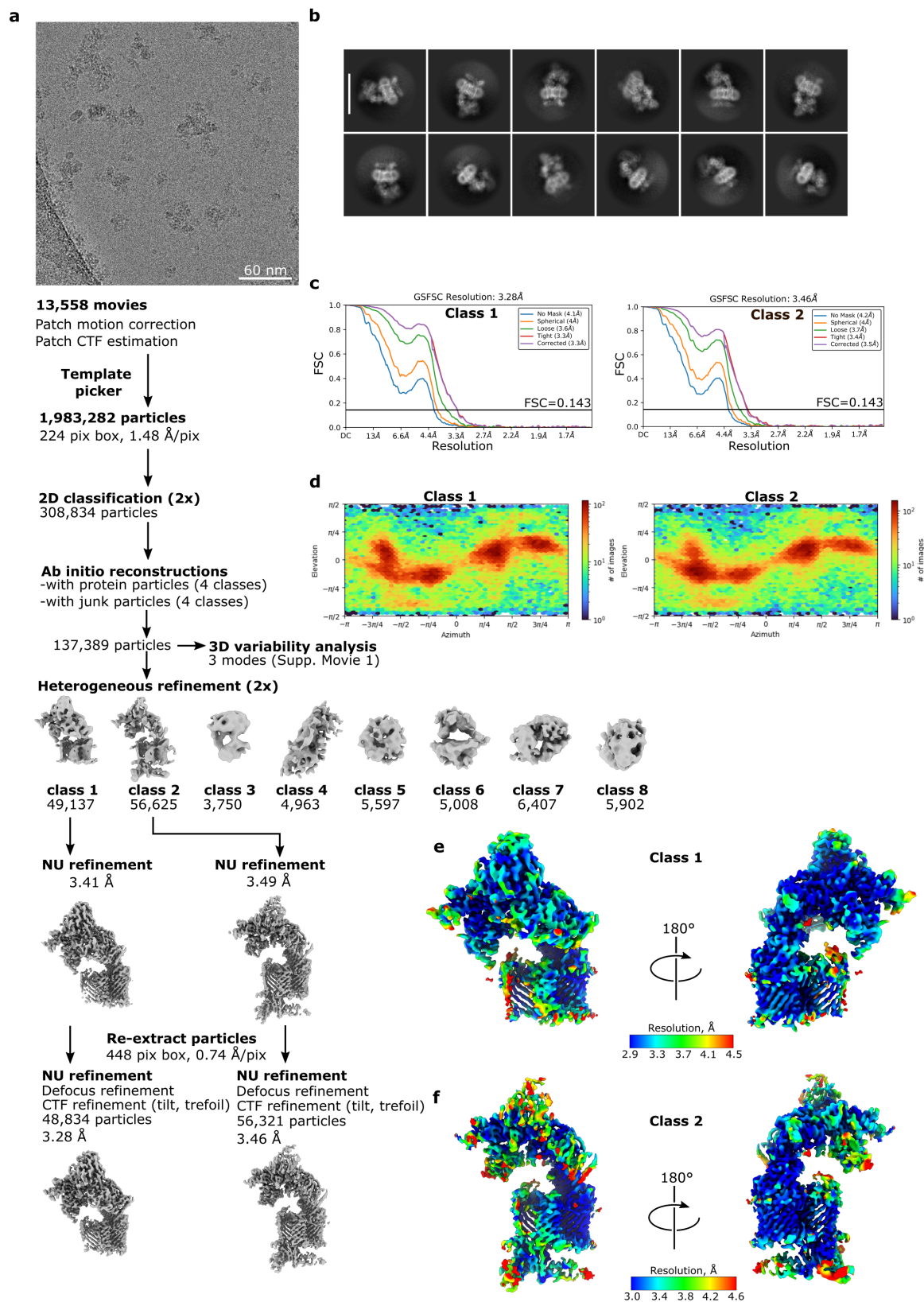
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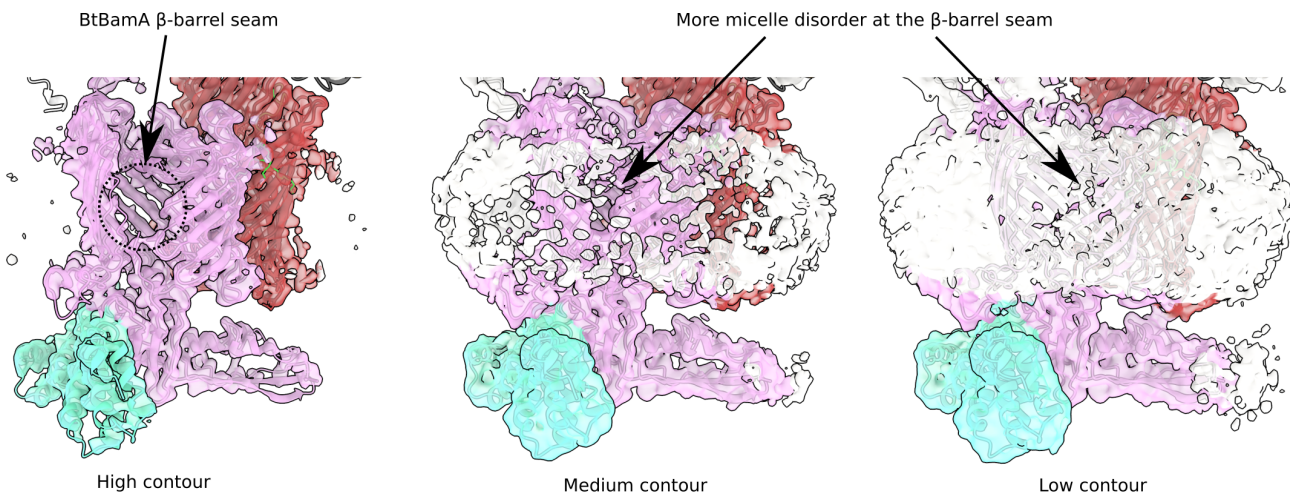
mariusz.madej@uj.edu.pl

Supplementary Figures



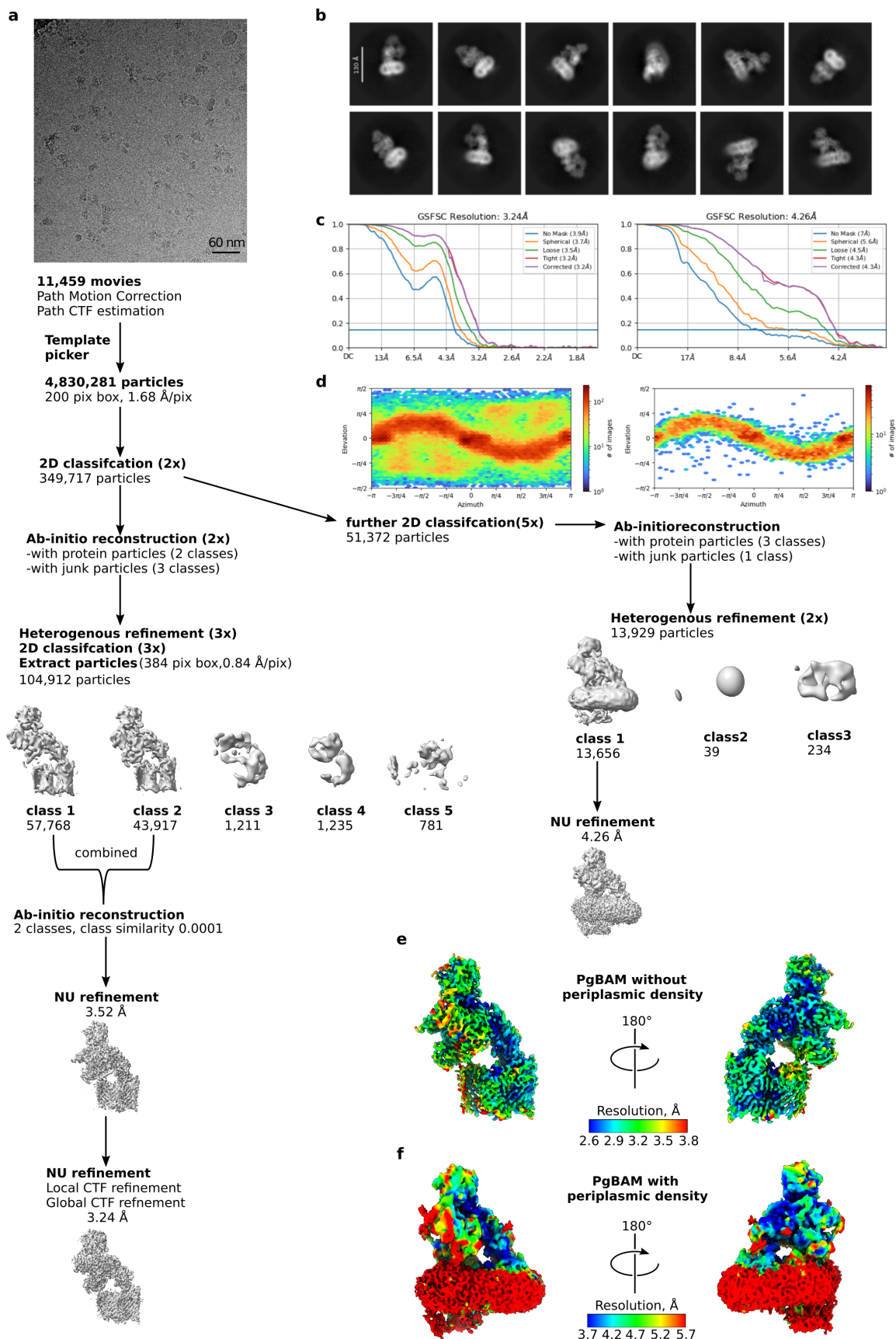
32 **Supplementary Figure 1. BtBAM cryo-EM data processing workflow.** **a**, Representative
33 motion-corrected movie (n=13,558) and cryoSPARC data processing workflow. 3D variability
34 analysis results are shown in Supplementary Movie 1. **b**, Representative 2D class averages. The
35 white bar represents a length of ~165 Å. **c**, Global gold-standard Fourier shell correlation (FSC)
36 curves. **d**, Viewing direction distribution plots. **e** and **f**, Local resolution estimation for the final
37 class 1 and class 2 maps, respectively.

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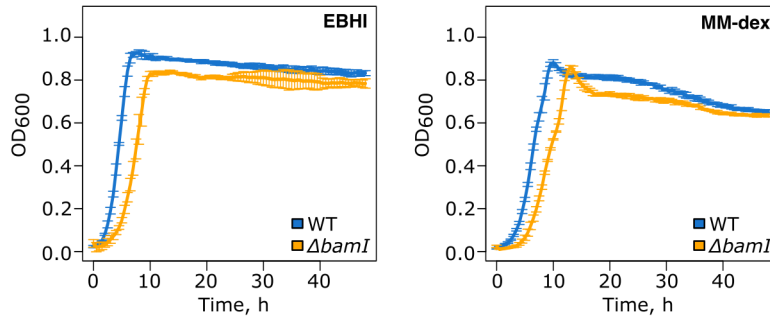


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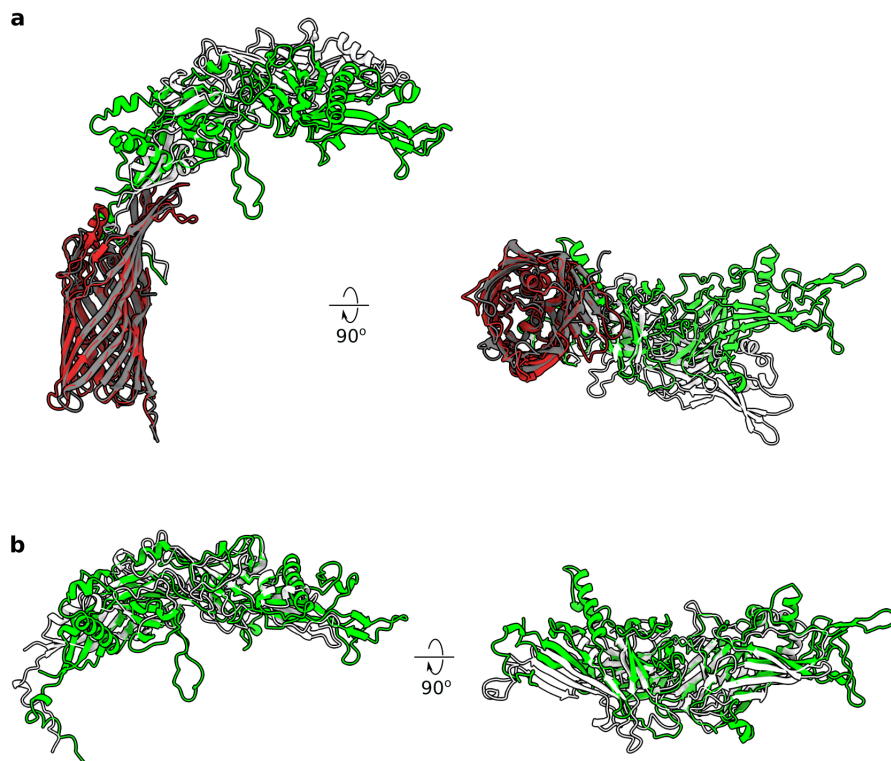
40 **Supplementary Figure 2. Increased disorder at the BtBamA β-barrel seam.** The BtBamAD^G
 41 model (cartoon) overlaid with the cryo-EM class 2 map (transparent surface) is displayed at three
 42 different contour levels: high, medium and low. As the threshold is lowered, the detergent micelle
 43 density does not appear uniformly around the BamA β-barrels. The density is weaker at the β-
 44 barrel seam, suggesting that there is more disorder in this region.



46 **Supplementary Figure 3. PgBAM cryo-EM data processing workflow.** **a**, Representative
47 motion-corrected movie (n=11,459) and cryoSPARC data processing workflow. **b**, Representative
48 2D class averages. The white bar represents a length of 130 Å. **c**, Global gold-standard Fourier
49 shell correlation (FSC) curves. **d**, Viewing direction distribution plots. **e** and **f**, Local resolution
50 estimation for the final reconstructions without and with periplasmic density, respectively.



Supplementary Figure 4. Growth curves of *B. theta* $\Delta bamI$ strain. Cells were cultured either in EBHI or in minimal medium supplemented with 0.4% dextran 40 and 10% BHI (MM-dex). Each trace is an average of n=3 technical repeats. The error bars show the standard deviation. Growth curves are representative for 2 biological replicates.



Supplementary Figure 5. Structural comparison of BtBamGH and BtBamG2H2 (Bt1785-86).

a, Superposition of the BtBamGH cryo-EM structure (red, green) and AF3¹ prediction of the Bt1785-86 complex (grey, white), renamed BamG2H2. The superposition was generated using Matchmaker in ChimeraX² on BtBamG and BtBamG2 (Bt1785); C α -C α RMSD was 1.01 Å between 249 pruned atom pairs. **b**, Superposition of the BtBamH cryo-EM structure and the Bt1786 AF3 prediction. The superposition was generated using Matchmaker in ChimeraX on BtBamH and BtBamH2 (Bt1786); C α -C α RMSD was 1.25 Å between 67 pruned atom pairs, and 15.41 Å between all 385 pairs.

Supplementary Movie Legends

Supplementary Movie 1. 3D variability analysis results showing cryo-EM density variability along three modes. 20 volumes per mode were reconstructed and filtered to 4 Å.

Supplementary Movie 1. The movie shows a trajectory starting from the PgBAM conformation, morphing to the BtBAM conformation, and back to the PgBAM conformation. The trajectory was generated using the 'morph' command in ChimeraX. The protein models are displayed as coloured solvent-accessible surfaces.

Supplementary Tables

Supplementary Table 1. *B. theta* *bamA-his* and *bamA-hisΔbamH* pulldown proteomics results compared to the wild-type strain. Significant hits are coloured red (enriched relative to wild type) or blue (depleted relative to wild type).

Supplementary Table 2. Cryo-EM data collection, processing and model refinement parameters.

	BtBAM			PgBAM	
Data collection					
Electron microscope	Titan Krios			Titan Krios	
Voltage (kV)	300			300	
Spherical aberration (μm)	2.7			2.7	
Camera	Falcon 4i (counting)			Gatan K3	
Energy filter	Selectris (10 eV slit)			BioQuantum (20 eV slit)	
Magnification	165,000x			105,000x	
Pixel size (Å)	0.74			0.84	
Total dose (e ⁻ /Å ²)	35			41	
Defocus range (μm)	-0.8 to -2.0			-0.6 to 1.5	
Number of movies collected	13,558			11,459	
Image Processing	Class 1	Class 2		Class 1	Class 2
Symmetry	C1	C1		C1	C1
Initial number of particles	2,266,553	2,266,553		4,830,281	4,830,281
Final number of particles	48,834	56,321		86,584	13,929
Global resolution (FSC = 0.143)	3.28 Å	3.46 Å		3.24	4.26
Map sharpening B-factor (Å ²)	-58.0	-64.9		-82.6	-49.3
Refinement	BtBamHIJK	BtBamADG	Complete BtBAM	PgBAM	Class 2
Model composition					
Non-hydrogen atoms	10,517	9,004	19,521	15,298	-
Protein residues	1,325	1,119	2,444	1,917	-
R.m.s. deviations					
Bonds lengths (Å)	0.004	0.005	0.006	0.005	-
Bond angles (°)	0.792	0.964	0.934	0.785	-
Validation					
MolProbity score	1.48	1.96	1.99	2.05	-
Clash score	2.70	11.45	11.21	10.48	-
Rotamer outliers (%)	0	0	0	0	-
Ramachandran plot					
Favoured (%)	93.52	94.31	93.51	91.74	-
Outliers (%)	0.08	0	0.04	0.21	-
PDB	9HIS	9HIV	9HJ3	9HJM	-
EMDB	EMD-52200	EMD-52202	EMD-52209	EMD-52218	EMD-52219

Supplementary Table 3. BlastP search results using BamG sequence as query against the NCBI RefSeq database.

Supplementary Table 4. *B. theta* *bamH* deletion vs wild type total membrane fraction proteomics results shown as protein abundance differences. OMP categories are coloured as follows: yellow, SLP; green, β -barrel; blue, SusC (*i.e.* a barrel that assembles with one or more SLPs).

Supplementary Table 5. *P. gingivalis* *bamH* deletion vs wild type whole cell proteomics results shown as protein abundance differences. OMP categories are coloured as follows: yellow, SLP; green, β -barrel; blue, SusC (*i.e.* a barrel that assembles with one or more SLPs; grey, T9SS component; purple, T9SS substrate).

Supplementary Table 6. Strains used in this study.

Strain	Relevant genotype	Source
<i>E. coli</i>		
TOP10	F- <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) Φ 80/ <i>lacZ</i> Δ M15 Δ <i>lacX74</i> <i>recA1</i> <i>araD139</i> Δ (<i>araleu</i>)7697 <i>galU</i> <i>galK</i> <i>rpsL</i> (Str ^R) <i>endA1</i> <i>nupG</i>	Invitrogen
S17-1λpir	<i>TpR</i> <i>SmR</i> <i>recA</i> <i>thi</i> <i>pro</i> <i>hsdR-M</i> + <i>RP4</i> :2- <i>Tc</i> : <i>Mu</i> : <i>Km</i> Tn7 <i>λpir</i> .	ATCC
<i>P. gingivalis</i>		
ATCC33277	Wild type	3
RagB-8His ATCC33277	<i>ragB</i> p.N ⁵⁰³ _extHHHHHHHHH (Tet ^R)	4
ΔbamH ATCC33277	Δ <i>bamH</i> (NCBI:PGN_1735)(Tet ^R)	This study
ΔbamI ATCC33277	Δ <i>bamI</i> (NCBI:PGN_0296)(Tet ^R)	This study
ΔbamK ATCC33277	Δ <i>bamK</i> (NCBI:PGN_1188)(Tet ^R)	This study
BamH-7His ATCC33277	<i>bamH</i> (NCBI:PGN_1735) p.N ⁴⁵⁵ _extHHHHHHHHH (Tet ^R)	This study
RagB-8His in ΔbamH ATCC33277	Δ <i>bamH</i> <i>ragB</i> p.N ⁵⁰³ _extHHHHHHHHH (Em ^R)(Tet ^R)	This study
<i>B. theta</i>		
VPI-5482 <i>tdk</i>⁻	<i>tdk</i> ⁻	5
Bt1761_{his}	<i>tdk</i> <i>bt1760</i> _{D42A} <i>bt1761</i> _{his}	6
Bt1761_{his} ΔbamH	<i>tdk</i> <i>bt1760</i> _{D42A} <i>bt1761</i> _{his} <i>bt4306</i> ⁻	This study
BtBamA_{his}	<i>tdk</i> <i>bt3725</i> _{his}	This study
BtBamA_{his} ΔbamH	<i>tdk</i> <i>bt3725</i> _{his} <i>bt4306</i> ⁻	This study
ΔbamI	<i>tdk</i> <i>bt3727</i> ⁻	This study
Bt1927-ON	<i>tdk</i> <i>bt1927</i> -ON	7
Bt1927-ON ΔbamH	<i>tdk</i> <i>bt1927</i> -ON <i>bt4306</i> ⁻	This study
SusA_{his}	<i>tdk</i> <i>bt3704</i> _{his}	J. Abellon-Ruiz (unpublished)

Supplementary Table 7. Plasmids used in this study.

Plasmids		
Plasmid	Relevant features	Source
pUC19	<i>E. coli</i> cloning vector, Amp ^R	Thermo Fisher Scientific
pExchange	Plasmid for <i>Bacteroides</i> spp. genome engineering by allelic exchange.	5
Δ bamH	Plasmid for deletion of <i>P. gingivalis</i> bamH gene, derivative of pUC19	This study
Δ bamI	Plasmid for deletion of <i>P. gingivalis</i> bamI gene, derivative of pUC19	This study
Δ bamK	Plasmid for deletion of <i>P. gingivalis</i> bamK gene, derivative of pUC19	This study
BamHall	Master plasmid for <i>P. gingivalis</i> bamH modifications, derivative of pUC19	This study
BamH-7His	Plasmid for insertion of His ₇ -tag at C-terminal of <i>P. gingivalis</i> BamH, used for purification of the BAM complex, derivative of BamHall	This study
pEx-bamA-his	Plasmid for inserting a His ₇ -tag at the N-terminus of <i>B. theta</i> BamA, derivative of pExchange	This study
pEx-bamH-KO	Plasmid for deletion of <i>B. theta</i> bamH gene, derivative of pExchange	This study
pEx-bamI-KO	Plasmid for deletion of <i>B. theta</i> bamI gene, derivative of pExchange	This study

Supplementary References

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