Supplementary Material

Cell-Penetrating Peptide–Peptide Nucleic Acid Conjugates as a Tool for Protein Functional Elucidation in the Native Bacterium

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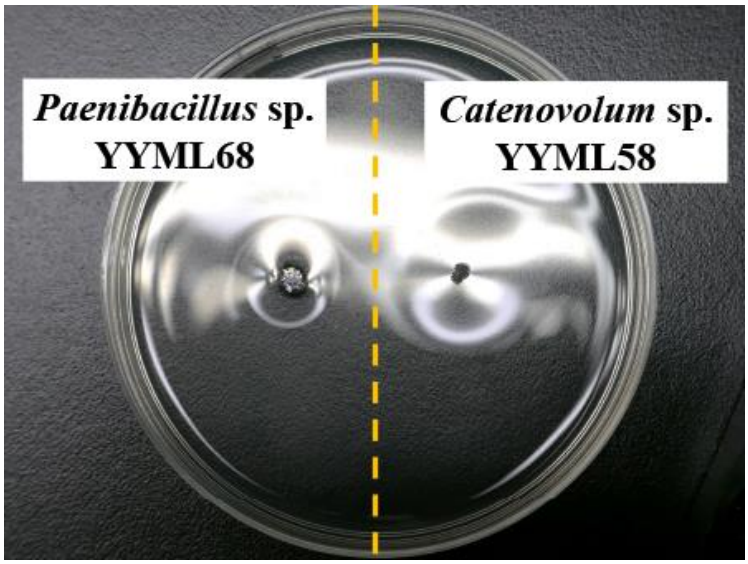

Figure S1. Degradation of crude carrageenan by *Paenibacillus* sp. YYML68. Strain YYML68 was cultivated on 4X diluted MB plates supplemented with 2% carrageenan for 2 days and carrageenan degradation was confirmed by "potholes" surrounding the bacterial colony. Another carrageenan-degrading isolate, *Catenovulum* sp. YYML58 was used as a control to indicate the efficiency of carrageenan degradation by strain YYML68.

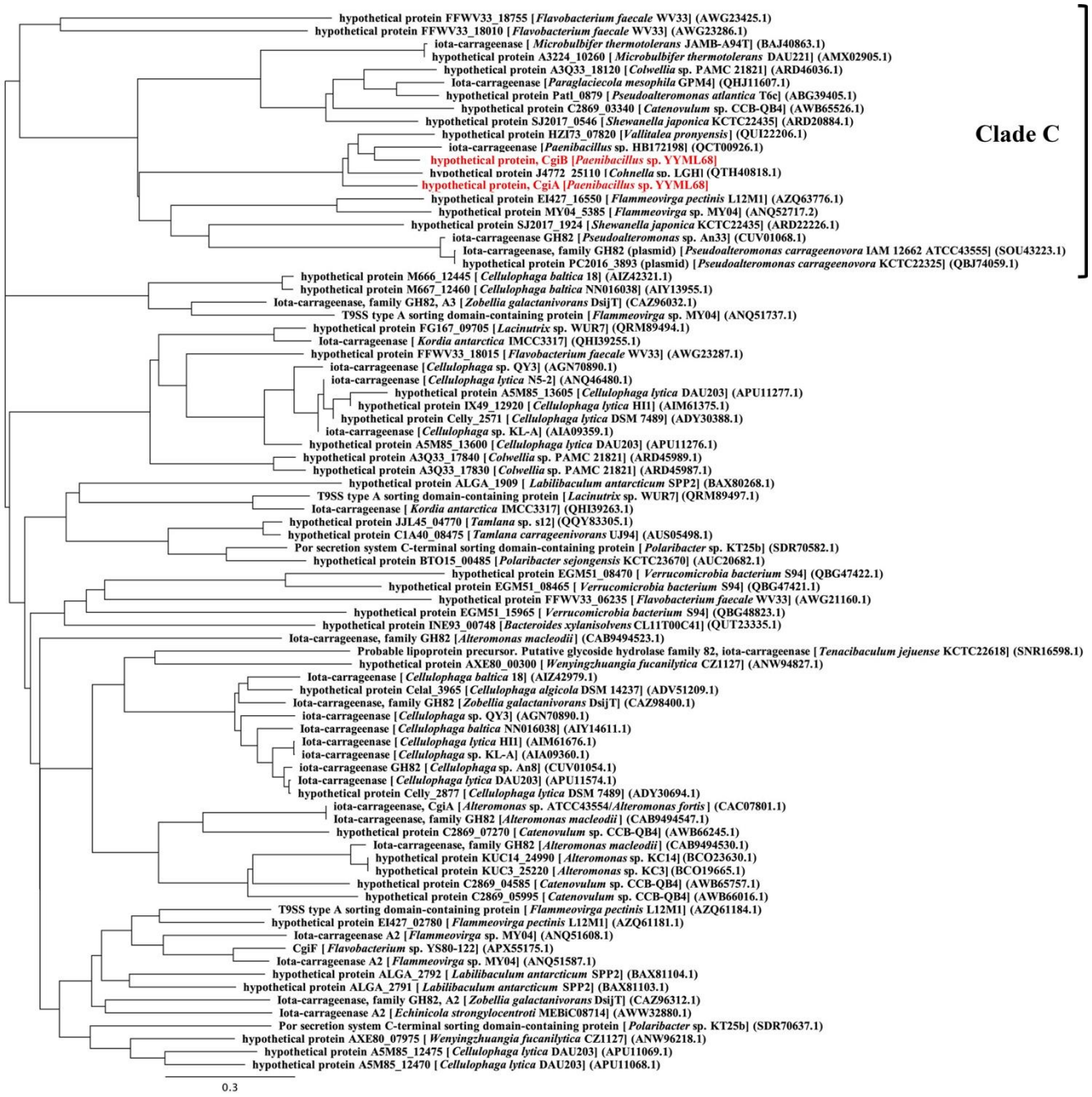

Figure S2. Phylogenetic representation of the CgiA and CgiB candidate hypothetical proteins with the proteins of the GH82 family from the CAZy database. The candidate genes are highlighted in red and were clustered within Clade C of the ι-carrageenase clades [1].

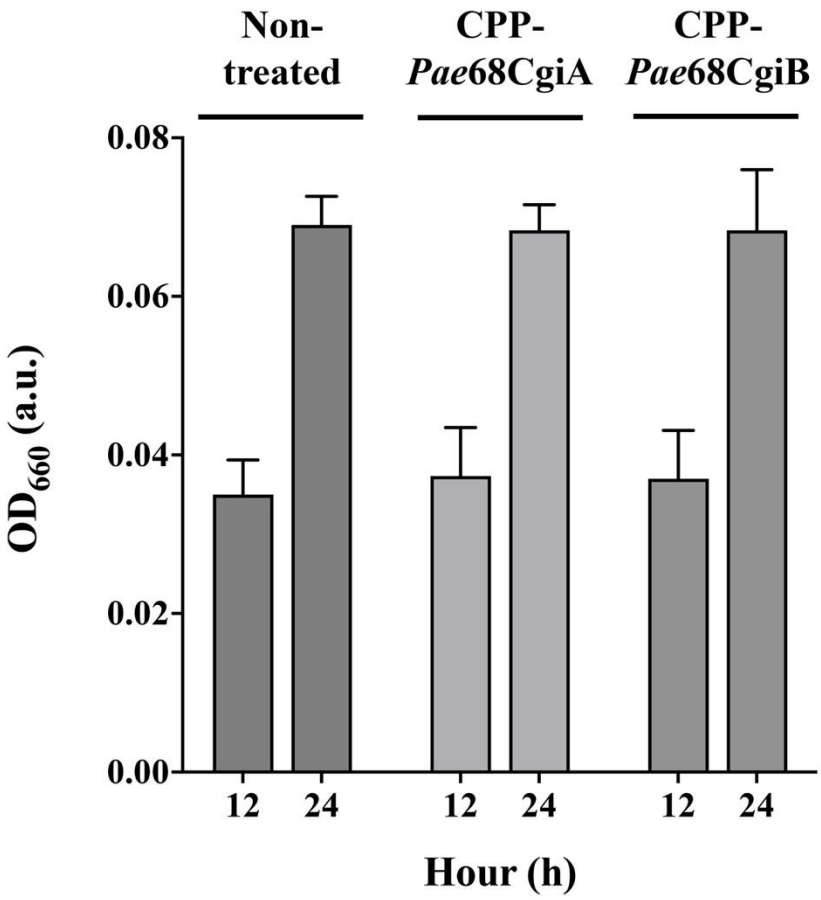

Figure S3. Growth inhibition effects of the CPP-*Pae*68CgiA and CPP-*Pae*68CgiB probes. 1.0 X 10^5 cells/mL were used as starter cultures and samples were cultivated in Marine Broth with the presence of the probes for 24 h. A non-probe treated samples was used as the negative control. The growth of the cells was measured at 12 and 24 h respectively.

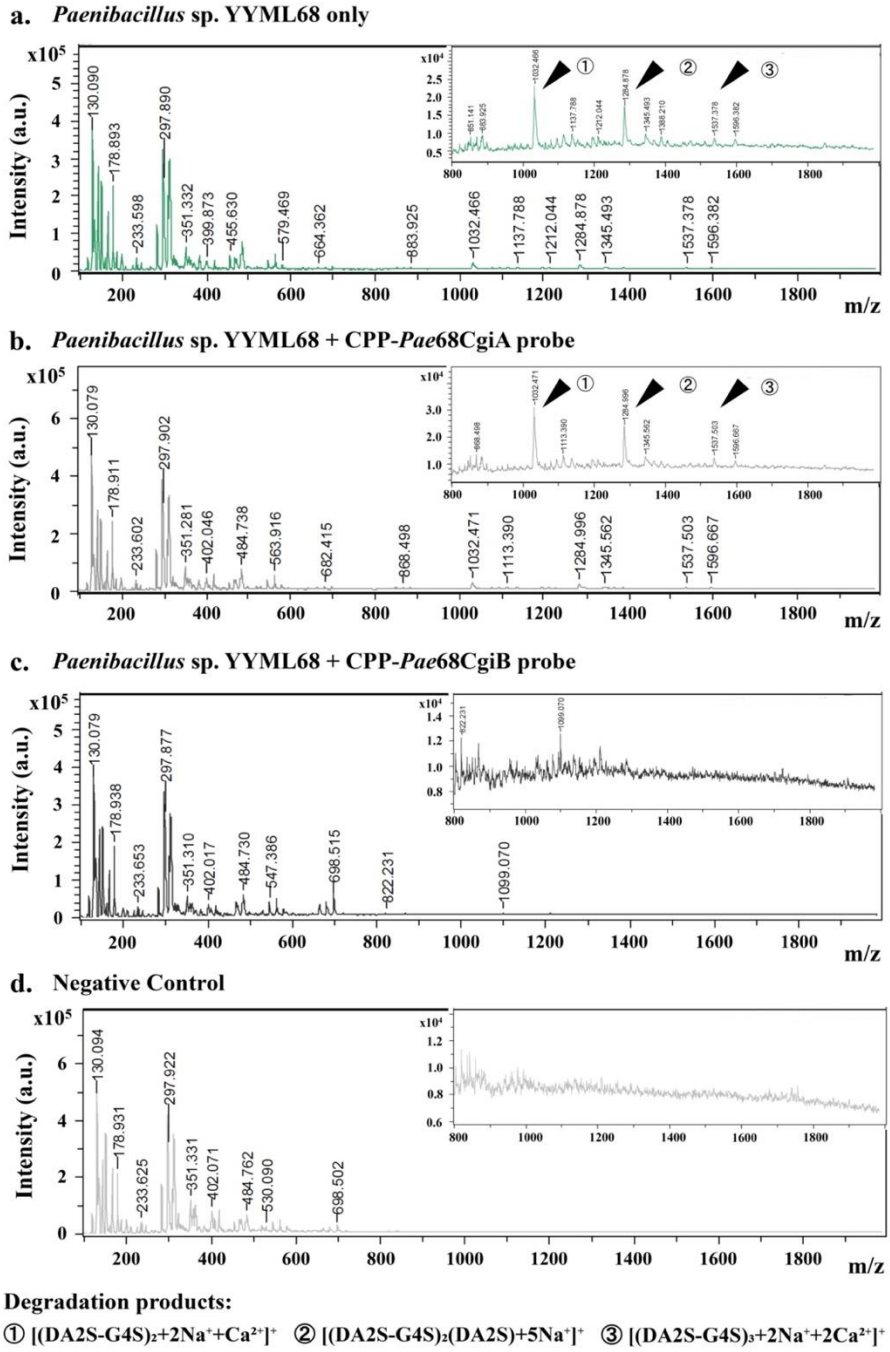

Figure S4. MALDI-TOF MS analysis of the degradation products by CgiA and CgiB. The measured m/z values of each peak averaged at ①1032.5 (1032.09 calculated), ②1284.9 (1285.15 calculated) and ③1537.4 (1536.2 calculated) respectively calculated based on the oligosaccharide values provided by Antonopoulos et al. [2]. Degradation experiments were performed in 0.01X Marine Broth (MB) supplemented with 0.5% ι-carrageenan with only the (a.) YYML68 strain, the (b.) YYML68 strain with the CPP-*Pae*CgiA probe, or the (c.) YYML68 strain with the CPP-*Pae*CgiB probe. A non-strain YYML68 inoculated sample was used as the (d.) negative control. MALDI-TOF MS analysis was performed using norharmane as the matrix [3] and measurements were performed at the positive ion mode.

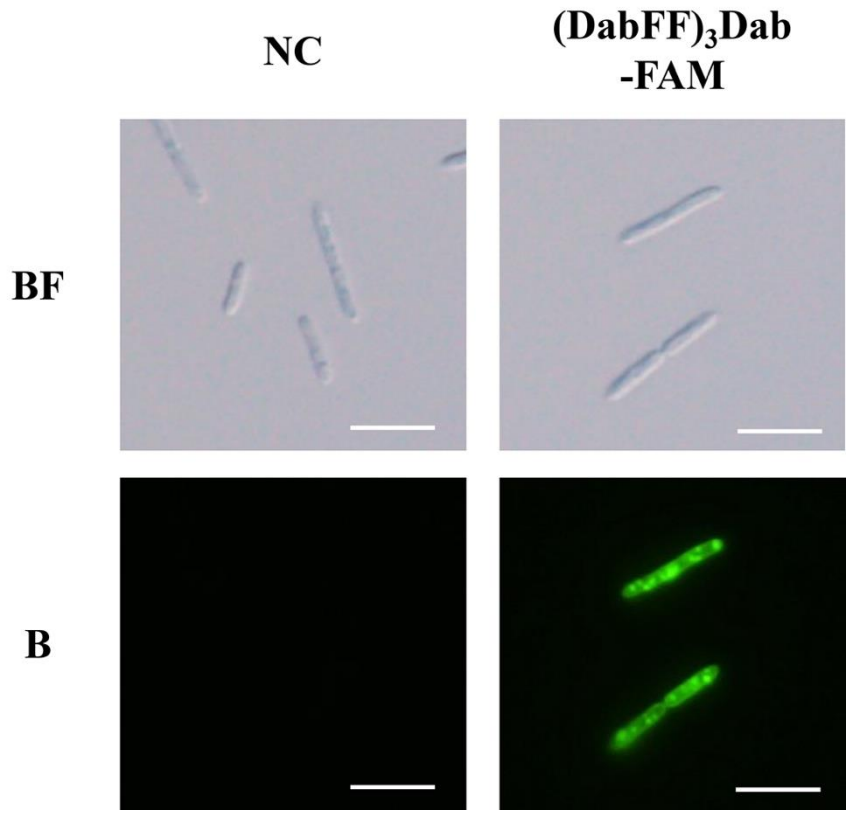

Figure S5. Evaluation of $(DabFF)_3K$-FAM permeation into *Paenibacillus* sp. YYML68. $(DabFF)_3$Dab-FAM *permeation* was performed at 2 µM concentration in PBS at room temperature (23°C) for 1 h. Untreated YYML8 strain samples were used as a negative control (NC). Green fluorescence images were captured using the blue wide-excitation mirror unit (U-FBWA; Olympus). Cell integrity was confirmed by bright field (BF) images. Images of $(DabFF)_3$Dab-FAM permeated cells and the negative controls were captured at similar exposure times to allow for comparison. Scale bar is 5 µm.

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