Supplemental Materials

FlrA represses transcription of biofilm-associated *bpfA* operon in *Shewanella putrefaciens*

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FIG S1

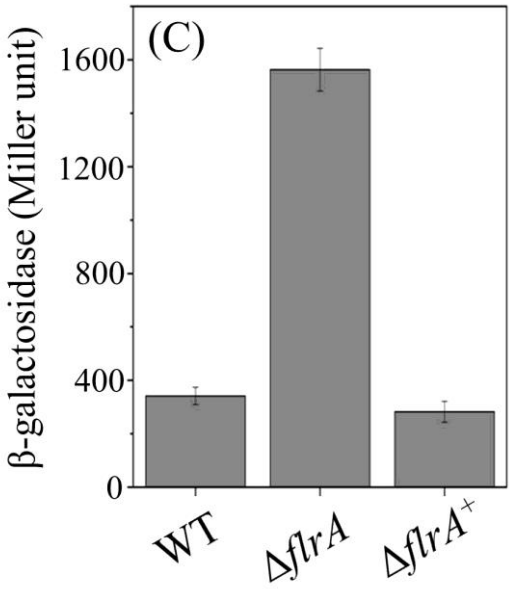
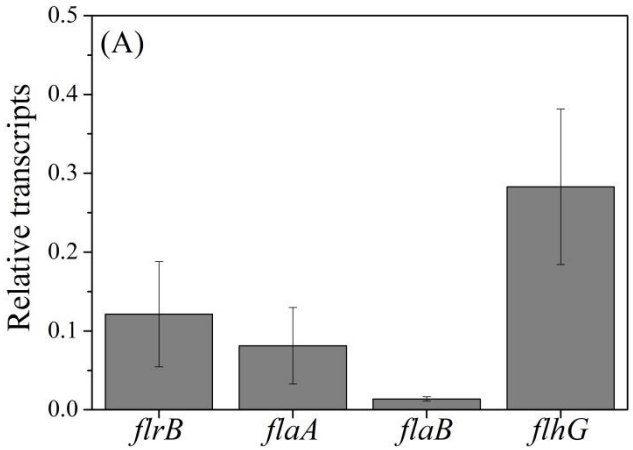


FIG S1 Complementation of *flrA* by a single copy recovered the *bpfA* expression in Δ*flrA* to the level of WT (3591Z). The gene of *flrA* was integrated into the neutral site of Δ*flrA* (3591ZΔ*flrA*) genome. The expression of *bpfA* was reported by *lacZ* transcriptionally fused into *bpfA* operon. The plus in superscript represented complementation strain. The error bars represented the standard deviation from three independent replicates.

FIG S2



(B)

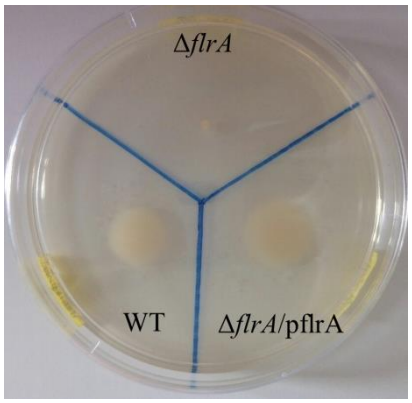


FIG S2 FlrA is essential for the transcription of flagella conding genes and consequent motility. **(A)** The transcription levels of *flrB, flaA, flaB and flhG,* genes in the class II, III and IV of flagella synthesis, in Δ*flrA* compared with that in WT. RNA was isolated from cultures grown to an OD of 1.0. Each value is the mean of three samples and the error bar indicates standard deviation. (B) Δ*flrA* showed a dramatic deficiency in motility in a 0.3% agar plate compared with WT. The motility of Δ*flrA* was recovered to comparable level of WT by introducing of plasmid-born *flrA* same as used in the assay of biofilm complementation.

FIG S3

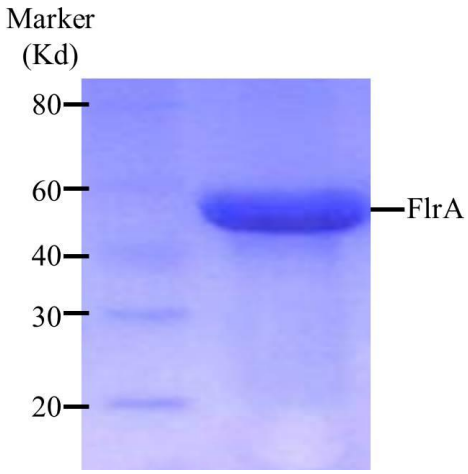
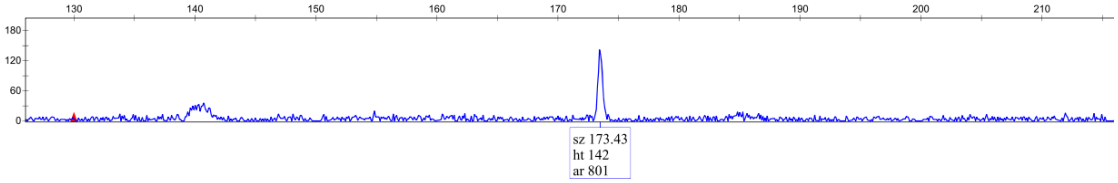


FIG S3 Purified recomminant FlrA examined by SDS-PAGE.

FIG S4

(A)



(B)

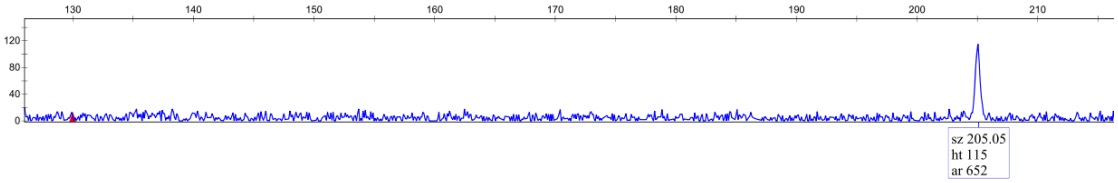


FIG S4 Determination of the transcriptional start site of *bpfA* operon by fluorescent-based primer extension. Total RNA was isolated from WT grown to an OD_{600} of 1.0. cDNA was synthesized using 5'-FAM labelled primers of P_{bpfA}-R1-FAM (A) and P_{bpfA}-R2-FAM (B), respectively. The size of cDNA was determined by the capillary electrophoresis**.**

FIG S5

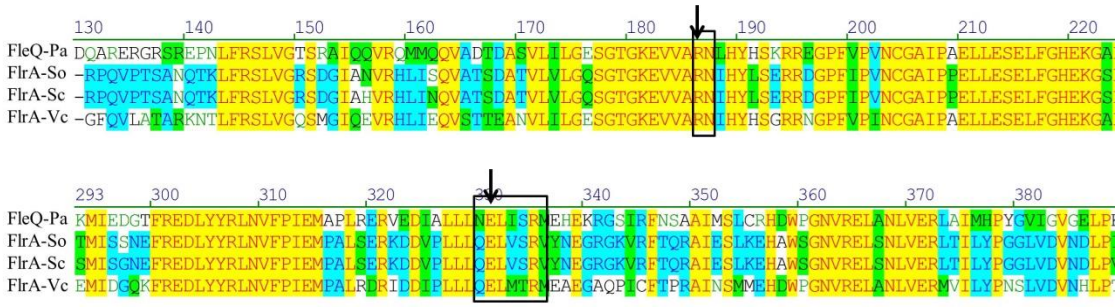
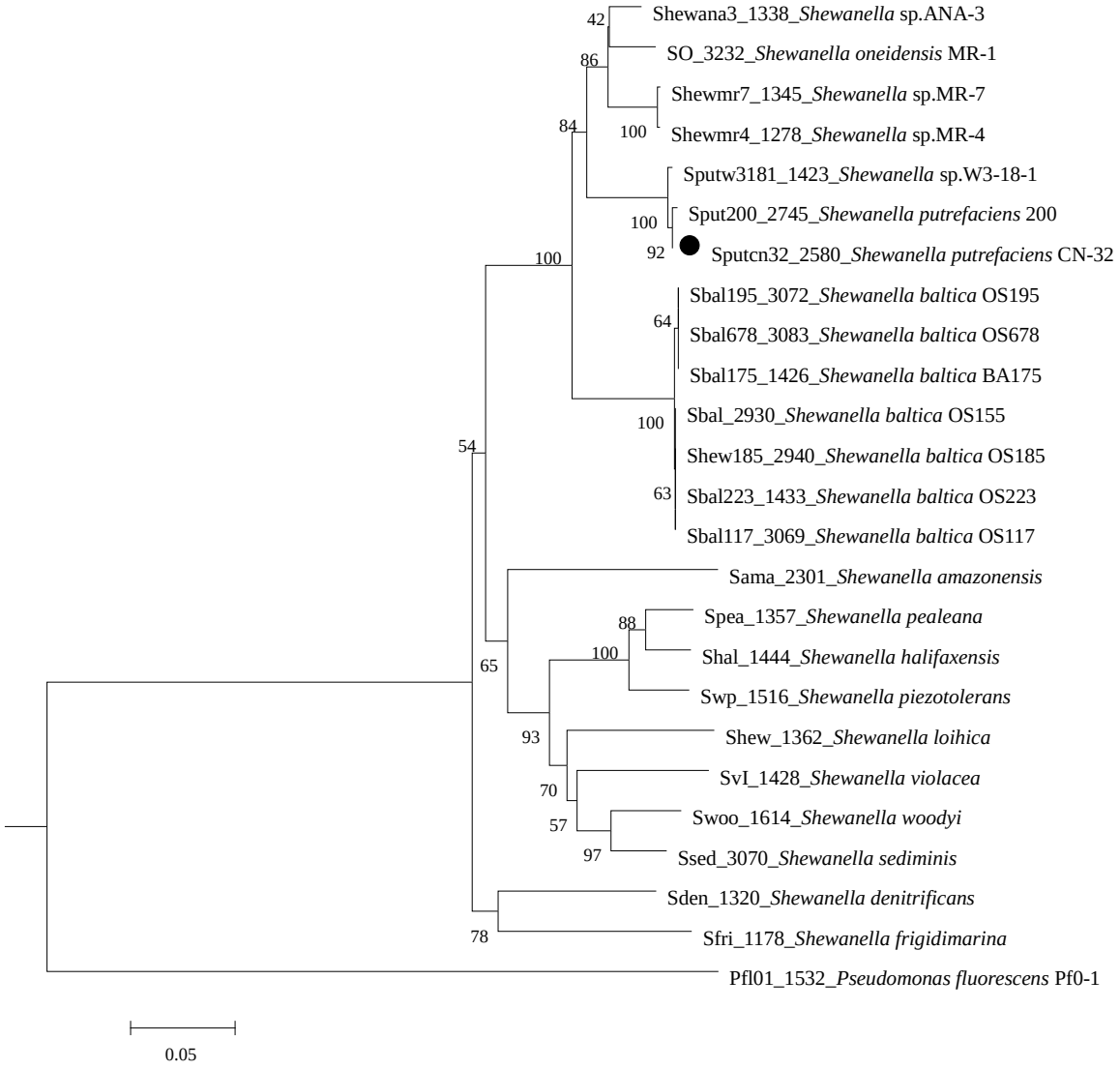


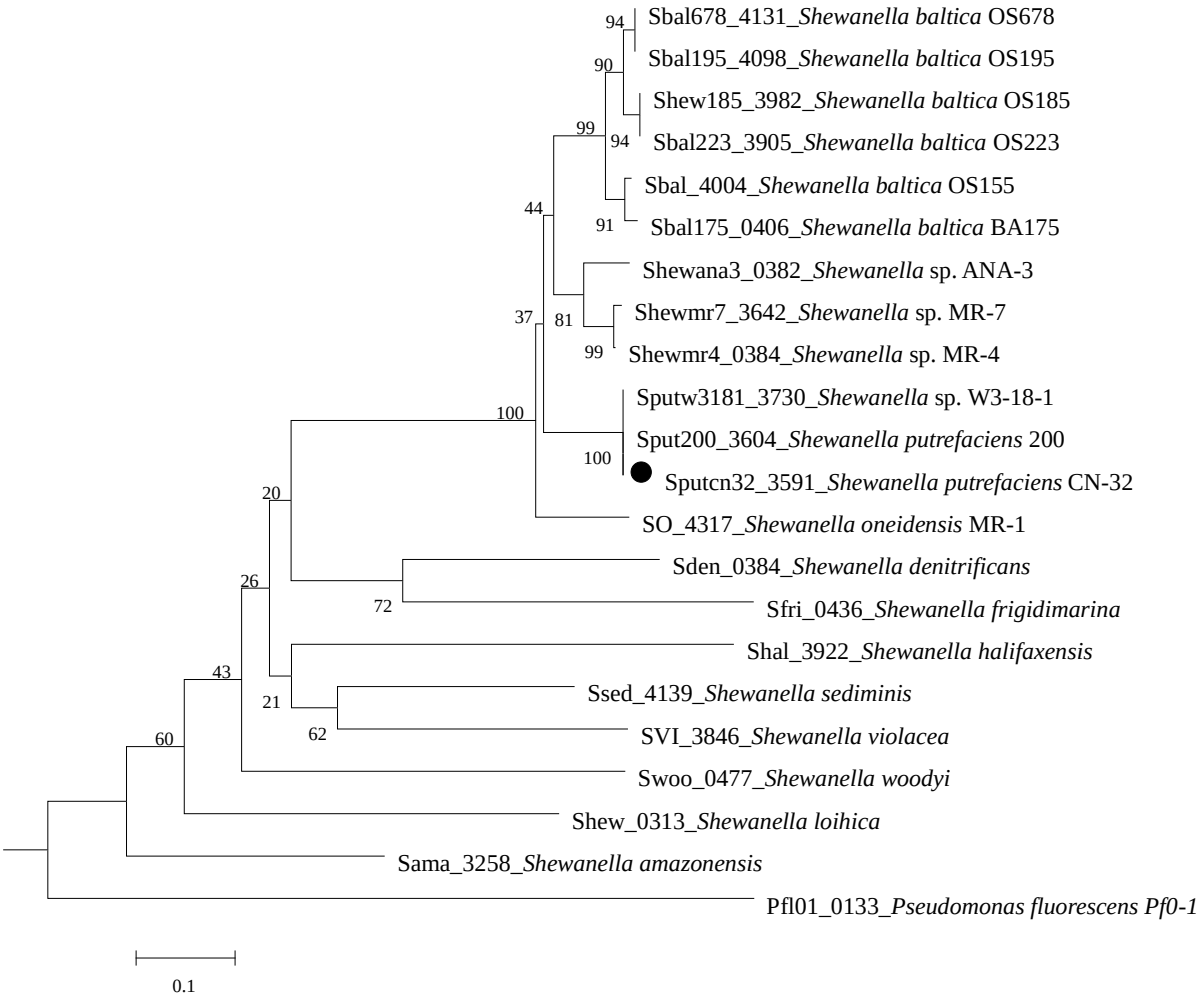
FIG S5 Alignment of sequences of FlrA from *S. putrefaciens* CN32(Sc), *S. oneidensis* MR-1 (So), and *V. cholerae* (Vc) with FleQ from *P. aeruginosa* PAO1 (Pa). Black arrows point to the conserved residues in FleQ from *P. aeruginosa* PAO1important for c-di-GMP binding.

FIG S6

(A)


Shewana3_1338_*Shewanella* sp.ANA-3
SO_3232_*Shewanella oneidensis* MR-1
Shewmr7_1345_*Shewanella* sp.MR-7
Shewmr4_1278_*Shewanella* sp.MR-4
Sputw3181_1423_*Shewanella* sp.W3-18-1
Sput200_2745_*Shewanella putrefaciens* 200
Sputcn32_2580_*Shewanella putrefaciens* CN-32
Sbal195_3072_*Shewanella baltica* OS195
Sbal678_3083_*Shewanella baltica* OS678
Sbal175_1426_*Shewanella baltica* BA175
Sbal_2930_*Shewanella baltica* OS155
Shew185_2940_*Shewanella baltica* OS185
Sbal223_1433_*Shewanella baltica* OS223
Sbal117_3069_*Shewanella baltica* OS117
Sama_2301_*Shewanella amazonensis*
Spea_1357_*Shewanella pealeana*
Shal_1444_*Shewanella halifaxensis*
Swp_1516_*Shewanella piezotolerans*
Shew_1362_*Shewanella loihica*
SvI_1428_*Shewanella violacea*
Swoo_1614_*Shewanella woodyi*
Ssed_3070_*Shewanella sediminis*
Sden_1320_*Shewanella denitrificans*
Sfri_1178_*Shewanella frigidimarina*
Pfl01_1532_*Pseudomonas fluorescens* Pf0-1
42
86
84
100
100
92
100
64
100
54
63
88
100
65
93
70
57
97
78
0.05

(B)


Sbal678_4131_*Shewanella baltica* OS678
Sbal195_4098_*Shewanella baltica* OS195
Shew185_3982_*Shewanella baltica* OS185
Sbal223_3905_*Shewanella baltica* OS223
Sbal_4004_*Shewanella baltica* OS155
Sbal175_0406_*Shewanella baltica* BA175
Shewana3_0382_*Shewanella* sp. ANA-3
Shewmr7_3642_*Shewanella* sp. MR-7
Shewmr4_0384_*Shewanella* sp. MR-4
Sputw3181_3730_*Shewanella* sp. W3-18-1
Sput200_3604_*Shewanella putrefaciens* 200
Sputcn32_3591_*Shewanella putrefaciens* CN-32
SO_4317_*Shewanella oneidensis* MR-1
Sden_0384_*Shewanella denitrificans*
Sfri_0436_*Shewanella frigidimarina*
Shal_3922_*Shewanella halifaxensis*
Ssed_4139_*Shewanella sediminis*
SVI_3846_*Shewanella violacea*
Swoo_0477_*Shewanella woodyi*
Shew_0313_*Shewanella loihica*
Sama_3258_*Shewanella amazonensis*
Pfl01_0133_*Pseudomonas fluorescens Pf0-1*
94
90
99
94
44
91
37
81
99
100
100
20
26
72
43
21
62
60
0.1

(C)

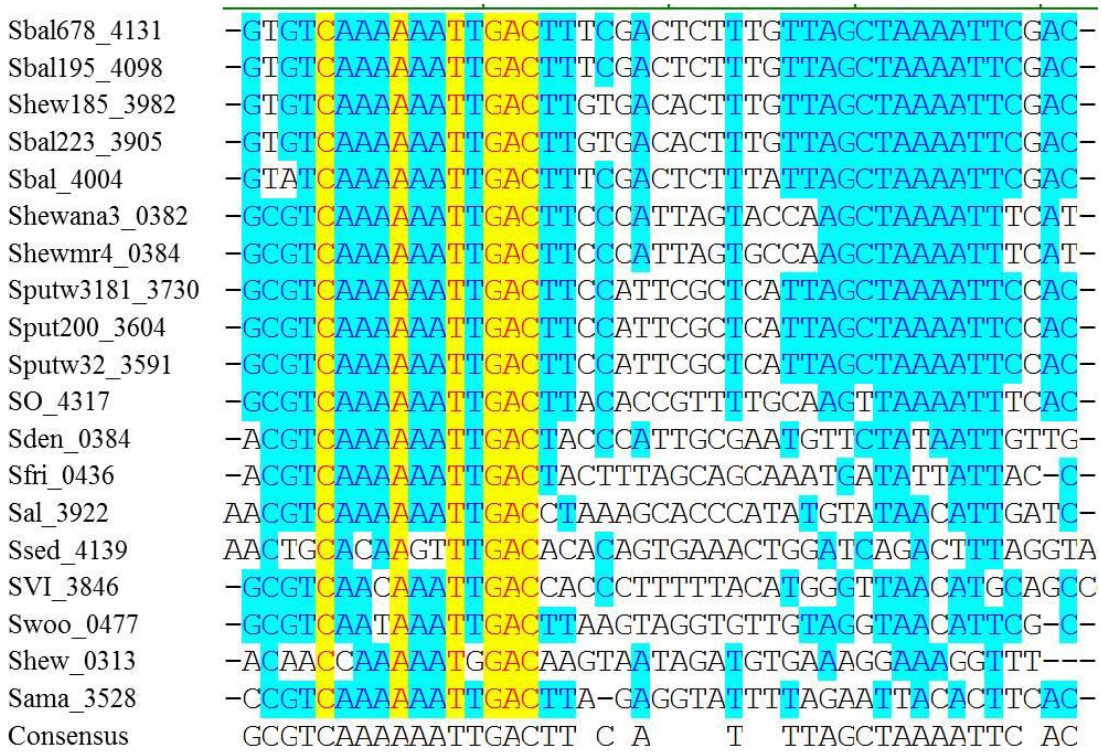


FIG S6 Co-occurrence of FlrA and BpfA orthologs across the *Shewanellaceae*. Phyogenetic analysis of FlrA (A) and BpfA(B). Locus tag of genes from NCBI database are presented and followed by species names. The tree is drawn to scale with the scale bar represnting substitutions per site. The analyses were conducted by MEGA6 (1). (C) The alignment of the promoter of these *bpfA*-coding genes. The DNA sequence of intergenic region bewteen the translation start site of *bpfA*-coding gene and its upstream gene was seleted. The alignment was conducted using Vector NTI (Invetrogen, USA). The DNA sequence of *bpfA* promoter in *S. putrefaciens* CN32 was selected as the profile during alignment. The neclotides labelled by yellow background indicate indentical ones, and those labelled by blue indicate conservative ones.

Reference

1. **Edgar R**. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. http://dx.doi.org/10.1093/nar/gkh340.