Supplementary Information

Characterization of the relationship between polar and lateral flagellar structural genes in the deep-sea bacterium *Shewanella piezotolerans* WP3

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Figures
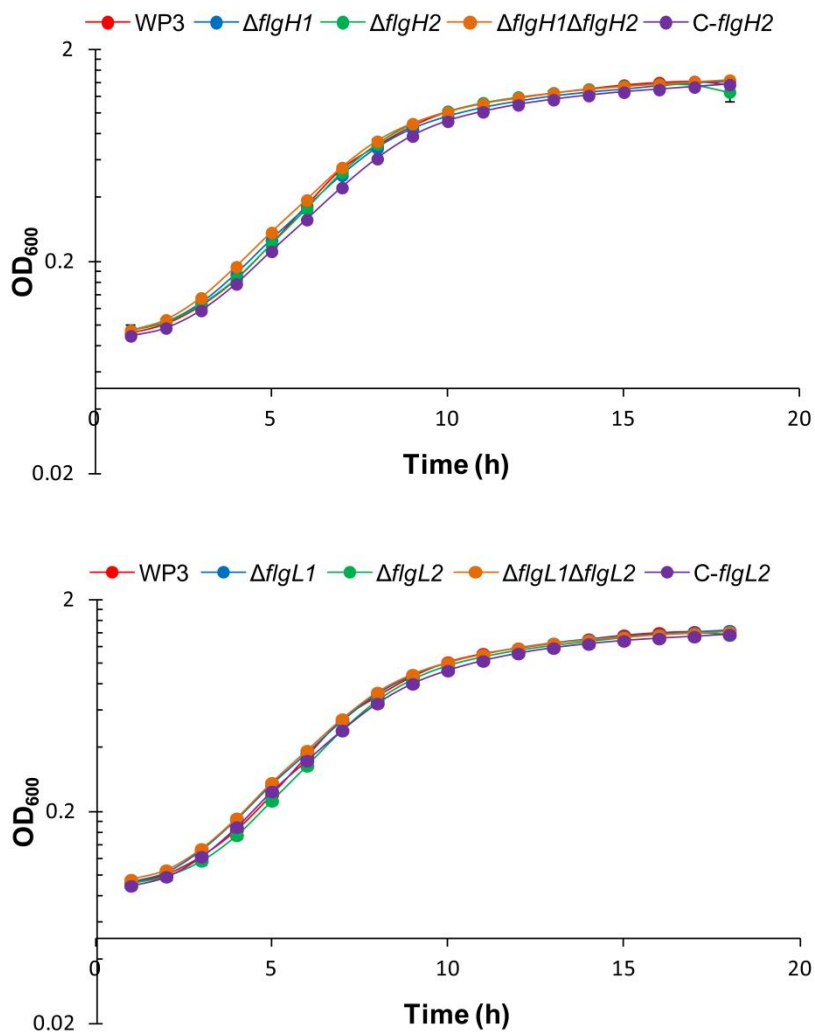
Figure S1. Growth assay of WP3 flagellar gene deletion mutants. The growth of WP3 and the mutants was monitored by measuring the optical density of the cultures at OD_{600}. The mean values with standard deviations (indicated by vertical bars) from triplicate experiments are given.
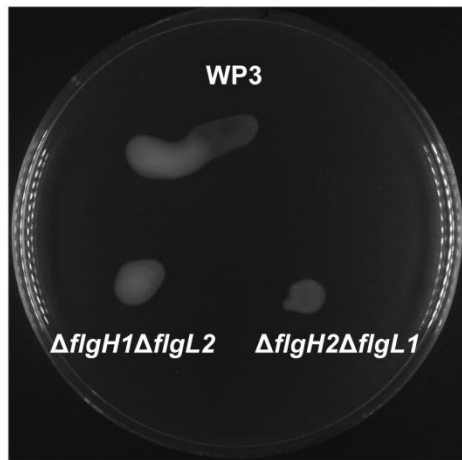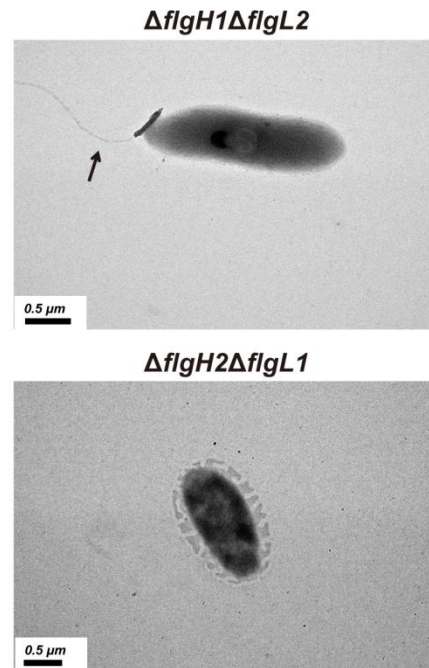
**Figure S2.** Effect of non-allelic flagellar genes mutation on the swarming motility and flagellar production on agar plates. (A) Swarming motility assays of the flagellar gene deletion mutants. The results represent two independent experiments. (B) TEM analysis of WP3 flagellar gene mutants that were cultured on swarming agar plate. Black arrow indicates the polar flagellum. The scale bars are indicated in the lower-left corner.
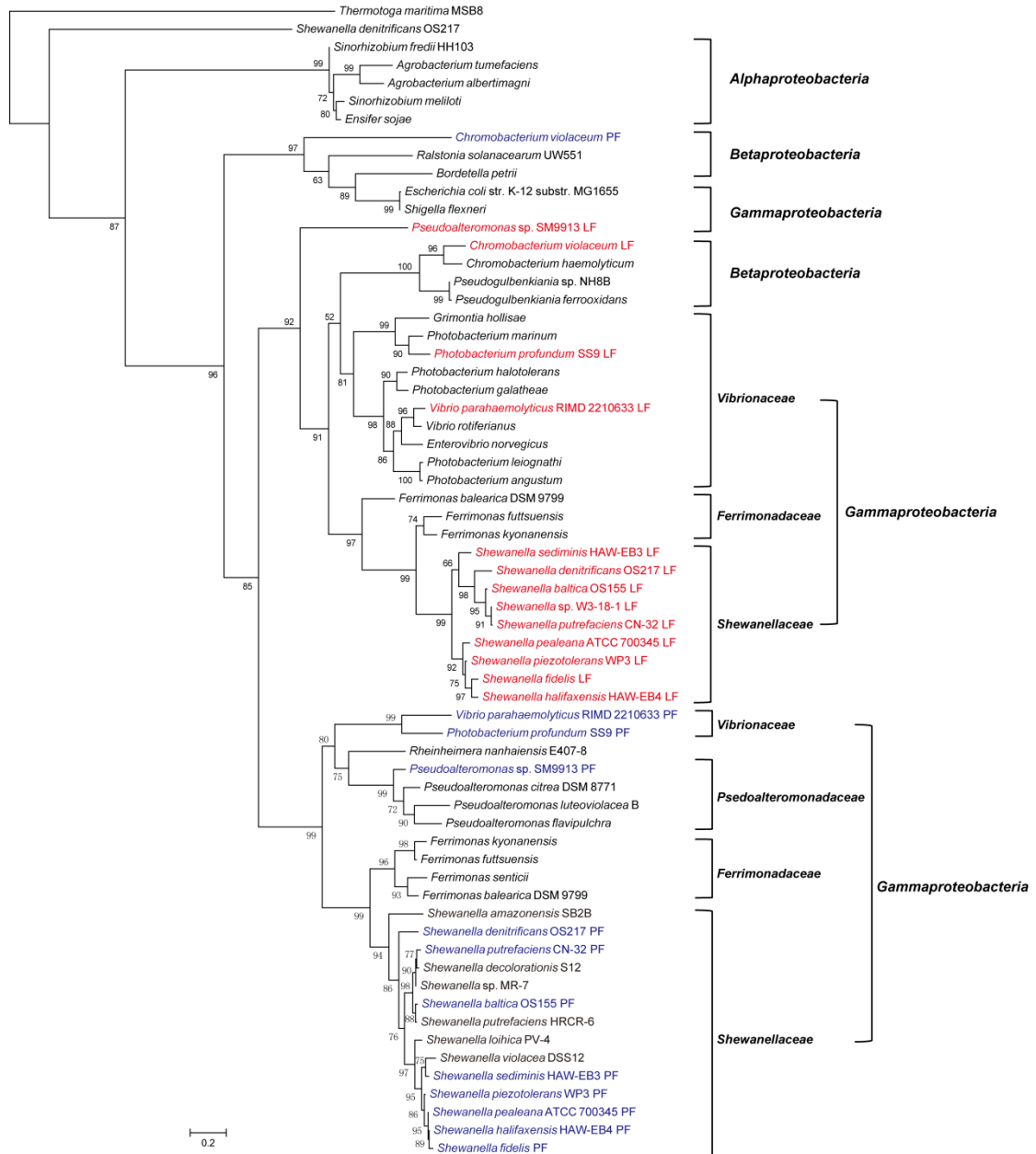
Figure S3. Phylogenetic tree of FlgH protein sequences of proteobacteria. "PF" and
"LF" after species names indicate polar and lateral flagellar systems, which are shown
in blue and red, respectively. FlgH of *Thermotoga maritima* was used as the out-group.
The scale bar represents amino acid substitutions per site, and numbers represent
the branch support values in percentage. For each data set, bootstrap values were
obtained with 1,000 replicates.
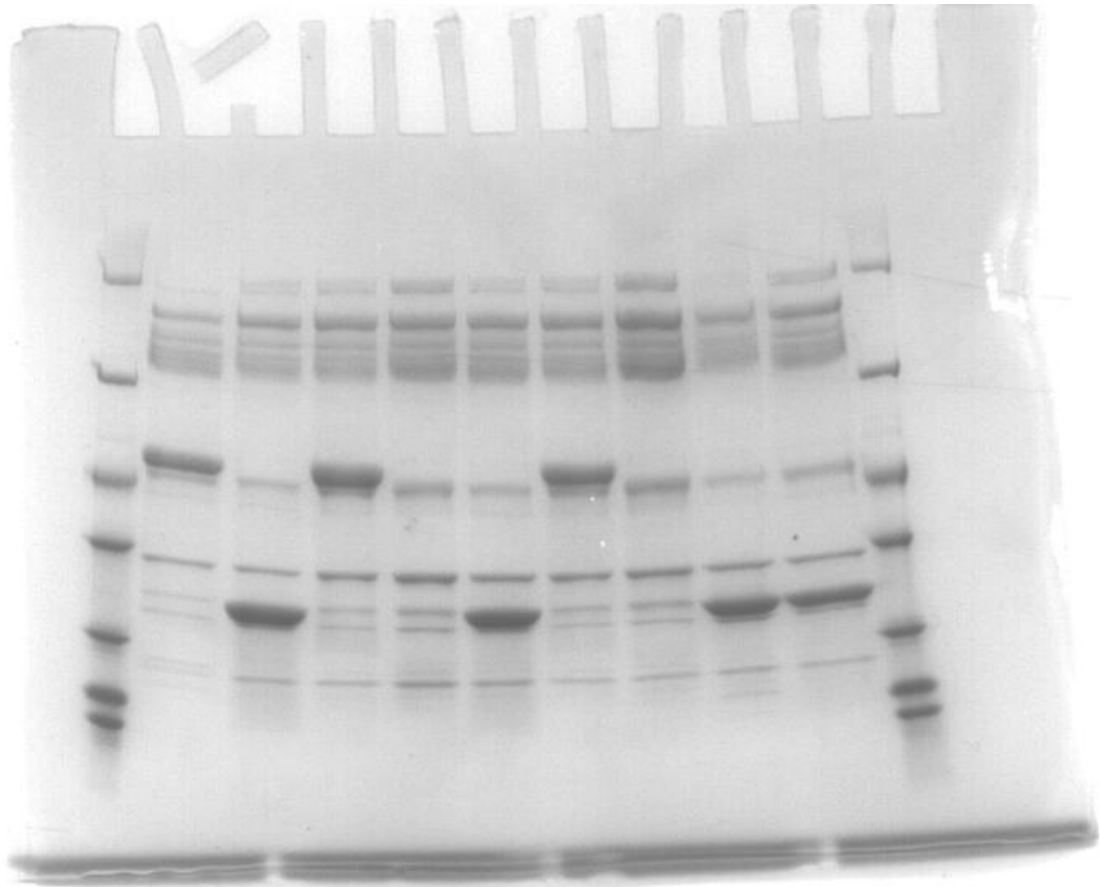
Figure S4. SDS-PAGE analysis of isolated flagellins from WP3 flagellar gene mutants

and complemented strains.
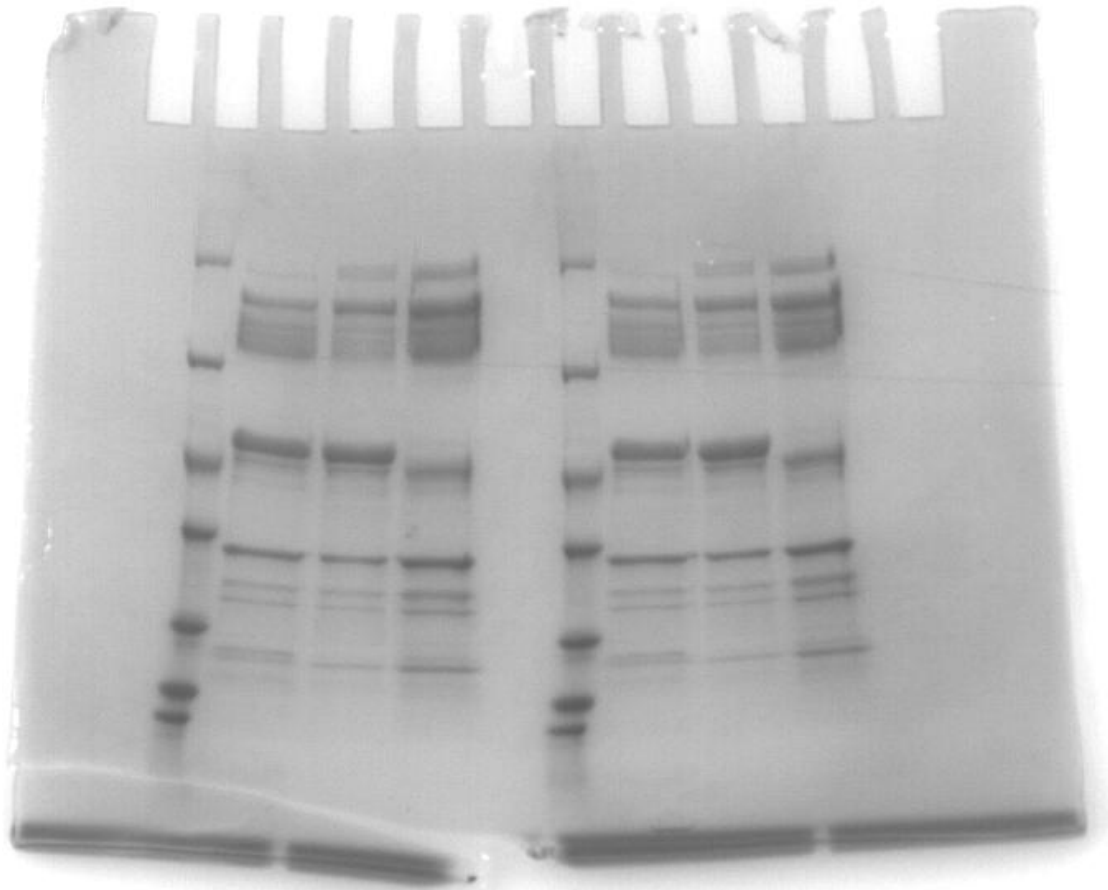
Figure S5. SDS-PAGE analysis of isolated flagellins from WP3 flagellar gene mutants.

Tables

Table S1. Primers and probe used in this study

Primer name	Sequence (5'-3')	Description
Swp1504UL	TATCTAGACAAGCGGGTGGGGTAAGTG	*flgH1* deletion
Swp1504UR	CGCTATATAGGATAAACAGGCGCATAATAAGG	*flgH1* deletion
Swp1504DL	TTTATCCTATATAGCGGCACTGGAACATTTG	*flgH1* deletion
Swp1504DR	TAGCATGCGGCGGCATATTGGCACTCACT	*flgH1* deletion
Swp5111UL	ATGAGCTCAGGTCAAGCCGATGCCCAAGAG	*flgH2* deletion
Swp5111UR	CGGATAAGCAAGCATCGCGATTCCAAGTCCTA	*flgH2* deletion
Swp5111DL	GATGCTTGCTTATCCGCGGCGAAAAATGG	*flgH2* deletion
Swp5111DR	TTTCTAGATTAATGCGCTGGGAGGAGATGTCA	*flgH2* deletion
Swp1508UL	AAGAGCTCGCGGCAAACTCGGGCAACA	*flgL1* deletion
Swp1508UR	ATTGATTGACCATCGCAGACTGGCTTTTCATCACAT	*flgL1* deletion
Swp1508DL	GCGATGGTCAATCAATCGGCACTGTCTT	*flgL1* deletion
Swp1508DR	AAGGTACCTCTGTATCACCATTGCCGCTGTTC	*flgL1* deletion
Swp5115UL	ATGAGCTCGCGGGAACAACAGCATCAAAAAT	*flgL2* deletion
Swp5115UR	TTGAGTGCATCATCAGAGGGGCGCAGTATCG	*flgL2* deletion
Swp5115DL	CTGATGATGCACTCAAGGTCACCCAGCAAACA	*flgL2* deletion
Swp5115DR	ATTCTAGAAGCGGGCAATAAGACTCGGGAACT	*flgL2* deletion
C-Swp5111For	GACTGGTACCTAATTGACTTGAAAAGGATC	*flgH2* complementation
C-Swp5111Rev	ACGGACGCGTAGTGATTAAACTTCATTGGA	*flgH2* complementation
C-Swp5115For	TGTAGGTACCATCCTACCAGGCTAACGC	*flgL2* complementation
C-Swp5115Rev	TCTACTGCAGATCAGTCAGTGGCACCTTA	*flgL2* complementation
Swp1504RTFor	CCATTGATTCACCGCGAGTA	qPCR
Swp1504RTRev	CAAATGTTCCAGTGCCGCTAT	qPCR
Swp1508RTFor	GATGCTGCGCTCAGTAAACG	qPCR
Swp1508RTRev	TCTCATCGGCAATTGTTTGC	qPCR
Swp5111RTFor	TTTGATCCGCACCGATGAT	qPCR
Swp5111RTRev	GCGATGCGCTGAGAAGAGA	qPCR
Swp5115RTFor	AGCCGCGCCGAAACTTAT	qPCR
Swp5115RTRev	TCTCGCATACGCGTTTGC	qPCR
Swp2079RTFor	TTAAGGCAATGGAAGCTGCAT	qPCR
Swp2079RTRev	CGTCTTTACCCGTTAATGATACGA	qPCR
Swp1538RTFor	GTCGCGGTCGATGTGGTT	qPCR
Swp1538RTRev	GCGACTCATAAGCAACATCATCA	qPCR
Swp5124RTFor	TTTTGGCCATCGAAGACATG	qPCR
Swp5124RTRev	GCCTTGCGGACTCGAGTAAC	qPCR
Swp1516RTFor	TCCGGTTAACTGTGGTGCAA	qPCR
Swp1516RTRev	TGGCCGAATAGCTCACTCTCA	qPCR
Swp1517RTFor	CAATAGCGGCACAAAAAAGCT	qPCR
Swp1517RTRev	CGAATACACTGTGTCGCATGATT	qPCR
Swp1518RTFor	ACGACAGCTTTGACATGGTGAT	qPCR
Swp1518RTRev	CCAAGGCCACCAATTCCTT	qPCR

| **Swp5090RTFor** | TCGCTGTTGCATCGATTACG | qPCR |
| **Swp5090RTRev** | AGCGCTGACGAGATCGTTTT | qPCR |

Table S2. The sequenced *Shewanella* strains and their flagellar system

No	Species or strain	Geographical origin	Isolation-site characteristics	Genome (Mb)	Flagellar system
1	S*hewanella amazonensis* SB2B	Amapa River, Brazil	Sediment; suboxic redox conditions; 1 m	4.31	PF
2	*Shewanella loihica* PV-4	Hawaiian Sea mount, United States	Iron-rich mat; hydrothermal vent; 1,325 m	4.6	PF
3	*Shewanella piezotolerans* WP3	West Pacific site WP (142°E, 8°N)	Sediment; under 1,914 m of water	5.4	PF+LF
4	*Shewanella pealeana* ATCC 700345	Woods Hole Harbor, Massachusetts, United States	Squid nidamental gland	5.17	PF+LF
5	S*hewanella halifaxensis* HAW-EB4	Halifax Harbor, Nova Scotia, Canada	Sediment; munitions dumping area; 215 m, Atlantic Ocean	5.23	PF+LF
6	*Shewanella psychrophila* WP2	West Pacific site WP (142°E, 8°N)	Sediment; under 1,914 m of water	6.35	PF+LF
7	S*hewanella violacea* DSS12	Ryuku Trench, Philippine Sea	Sediment; 5,110 m	4.96	PF
8	S*hewanella benthica* KT99	Tonga-Kermadec Trench	Deep sea; high-pressure zone; 9,000 m	4.35	PF
9	S*hewanella sediminis* HAW-EB3	Halifax Harbor, Nova Scotia, Canada	Sediment; 50 nautical miles from shore; 215 m	5.52	PF+LF
10	S*hewanella woodyi* ATCC 51908 (MS32)	Strait of Gibraltar, Mediterranean Sea	Detritus; 370 m	5.94	PF
11	S*hewanella denitrificans* OS217	Baltic Sea	Sea-water; oxic–anoxic interface; 120 m	4.55	PF+LF
12	S*hewanella frigidimarina* NCIMB 400	Coast of Aberdeen, United Kingdom	Sea-water; North Sea	4.85	PF
13	S*hewanella putrefaciens* 200	Alberta, Canada	Crude-oil pipeline	4.84	PF
14	S*hewanella putrefaciens* CN‑32	Albuquerque, New Mexico, United States	Subsurface; shale sandstone; 250 m	4.66	PF+LF
15	*Shewanella* sp. W3‑18‑1	Washington coast, Pacific Ocean	Marine sediment; under 997 m of oxic water	4.71	PF+LF
16	S*hewanella baltica* OS223	Baltic Sea	Sea-water; anoxic zone; 140 m	5.15	PF
17	S*hewanella baltica* BA175	Baltic Sea	ND	5.20	PF
18	S*hewanella baltica* OS117	Baltic Sea	ND	5.53	PF
19	S*hewanella baltica* OS155	Baltic Sea	Sea-water; oxic zone; 2 ml per litre of oxygen; 90 m	5.34	PF+LF
20	S*hewanella baltica*	Baltic Sea	Sea-water; oxic–anoxic interface;	5.31	PF

			OS185		120 m		
21	*Shewanella baltica* OS678	Baltic Sea	low-oxygen zone just above the chemocline (110 m)	5.37	PF
22	*Shewanella baltica* OS195	Baltic Sea	Sea-water; anoxic zone; 140 m	5.55	PF
23	*Shewanella oneidensis* MR-1	Lake Oneida, New York, United States	Sediment; anaerobic; Mn(IV) reduction	5.13	PF
24	*Shewanella* sp. ANA-3	Woods Hole, Massachusetts, United States	Brackish water; arsenic-treated wooden pier	5.25	PF
25	*Shewanella* sp. MR-7	Black Sea	Sea-water; anoxic zone; high NO_3; 60 m	4.79	PF
26	*Shewanella* sp. MR-4	Black Sea	Sea-water; oxic zone; 16℃; 5 m	4.71	PF
27	*Shewanella fidelis* ATCC BAA-318	The South China Sea	Sediment; depth of 73 m	4.8	PF+LF

Table S3. The protein sequence of FlgH used in the pylogenetic analysis

No	Class	Species	gi Number
1	*Thermotogae*	*Thermotoga maritima* MSB8	15644288
2	*Gammaproteobacteria*	*Shewanella denitrificans* OS217	91713419
3	*Alphaproteobacteria*	*Sinorhizobium fredii* HH103	365177968
4	*Alphaproteobacteria*	*Agrobacterium tumefaciens*	1086565
5	*Alphaproteobacteria*	*Agrobacterium albertimagni*	493777918
6	*Alphaproteobacteria*	*Sinorhizobium meliloti*	489625448
7	*Alphaproteobacteria*	*Ensifer sojae*	736851944
8	*Betaproteobacteria*	*Chromobacterium violaceum*	768336605
9	*Betaproteobacteria*	*Ralstonia solanacearum* UW551	83725703
10	*Betaproteobacteria*	*Bordetella petrii*	754361705
11	*Gammaproteobacteria*	*Escherichia coli* str. K-12 substr. MG1655	16129042
12	*Gammaproteobacteria*	*Shigella flexneri*	745761840
13	*Gammaproteobacteria*	*Vibrio parahaemolyticus* RIMD 2210633	28805768
14	*Gammaproteobacteria*	*Photobacterium profundum*	499536872
15	*Gammaproteobacteria*	*Rheinheimera nanhaiensis* E407-8	383704066
16	*Gammaproteobacteria*	*Pseudoalteromonas* sp. SM9913	315015864
17	*Gammaproteobacteria*	*Pseudoalteromonas citrea* DSM 8771	540253670
18	*Gammaproteobacteria*	*Pseudoalteromonas luteoviolacea* B ATCC 29581	441749577
19	*Gammaproteobacteria*	*Pseudoalteromonas flavipulchra*	498292150
20	*Gammaproteobacteria*	*Ferrimonas kyonanensis*	654652128
21	*Gammaproteobacteria*	*Ferrimonas futtsuensis*	654649162
22	*Gammaproteobacteria*	*Ferrimonas senticii*	737406071
23	*Gammaproteobacteria*	*Ferrimonas balearica* DSM 9799	307632559
24	*Gammaproteobacteria*	*Shewanella amazonensis* SB2B	119767947
25	*Gammaproteobacteria*	*Shewanella denitrificans* OS217	91714669
26	*Gammaproteobacteria*	*Shewanella putrefaciens* CN-32	145565377
27	*Gammaproteobacteria*	*Shewanella decolorationis* S12	553947544
28	*Gammaproteobacteria*	*Shewanella* sp. MR-7	113888283
29	*Gammaproteobacteria*	*Shewanella baltica* OS155	125998348
30	*Gammaproteobacteria*	*Shewanella putrefaciens*	655354729
31	*Gammaproteobacteria*	*Shewanella loihica* PV-4	126637577
32	*Gammaproteobacteria*	*Shewanella violacea* DSS12	293326657
33	*Gammaproteobacteria*	*Shewanella sediminis* HAW-EB3	157318587
34	*Gammaproteobacteria*	*Shewanella piezotolerans* WP3	212555835
35	*Gammaproteobacteria*	*Shewanella pealeana* ATCC 700345	157846174
36	*Gammaproteobacteria*	*Shewanella fidelis*	655362488
37	*Gammaproteobacteria*	*Shewanella halifaxensis* HAW-EB4	167353386
38	*Gammaproteobacteria*	*Pseudoalteromonas* sp. SM9913	315014501
39	*Gammaproteobacteria*	*Ferrimonas balearica* DSM 9799	307632211
40	*Gammaproteobacteria*	*Ferrimonas futtsuensis*	654647905
41	*Gammaproteobacteria*	*Ferrimonas kyonanensis*	654653971
42	*Gammaproteobacteria*	*Shewanella sediminis* HAW-EB3	157315578

43	*Gammaproteobacteria*	*Shewanella denitrificans* OS217	91716994
44	*Gammaproteobacteria*	*Shewanella baltica* OS155	125999358
45	*Gammaproteobacteria*	*Shewanella* sp. W3-18-1	120557401
46	*Gammaproteobacteria*	*Shewanella putrefaciens* CN-32	145566237
47	*Gammaproteobacteria*	*Shewanella pealeana* ATCC 700345	157844899
48	*Gammaproteobacteria*	*Shewanella piezotolerans* WP3	212559272
49	*Gammaproteobacteria*	*Shewanella fidelis*	655361441
50	*Gammaproteobacteria*	*Shewanella halifaxensis* HAW-EB4	167356180
51	*Gammaproteobacteria*	*Chromobacterium violaceum*	768336826
52	*Gammaproteobacteria*	*Chromobacterium haemolyticum*	759908292
53	*Gammaproteobacteria*	*Pseudogulbenkiania* sp. NH8B	503853881
54	*Gammaproteobacteria*	*Pseudogulbenkiania ferrooxidans*	496241021
55	*Gammaproteobacteria*	*Grimontia hollisae*	750224026
56	*Gammaproteobacteria*	*Photobacterium marinum*	494727063
57	*Gammaproteobacteria*	*Photobacterium profundum*	752746676
58	*Gammaproteobacteria*	*Photobacterium halotolerans*	653000184
59	*Gammaproteobacteria*	*Photobacterium galatheae*	636574688
60	*Gammaproteobacteria*	*Photobacterium angustum*	767119450
61	*Gammaproteobacteria*	*Photobacterium leiognath*	767076517
62	*Gammaproteobacteria*	*Enterovibrio norvegicus*	515572155
63	*Gammaproteobacteria*	*Vibrio rotiferianus*	657850858
64	*Gammaproteobacteria*	*Vibrio parahaemolyticus* RIMD 2210633	28900125