

Supplementary Materials

Draft Genome Sequence Analyses of Two Novel *Marinobacter suadae* sp. nov. and *Wenyngzhuangia gilva* sp. nov., Isolated from the Root of *Suaeda japonica* Makino

Department of Life Science, Dongguk University-Seoul, Goyang 10326, Republic of Korea;

eksvnd97@dgu.ac.kr (S.P.); duckling91@dgu.ac.kr (I.K.); lucky_salman@dongguk.edu (G.C.);

joh2395@dongguk.edu (Y.J.); woohj999@dongguk.edu (H.W.)

***Corresponding author:**

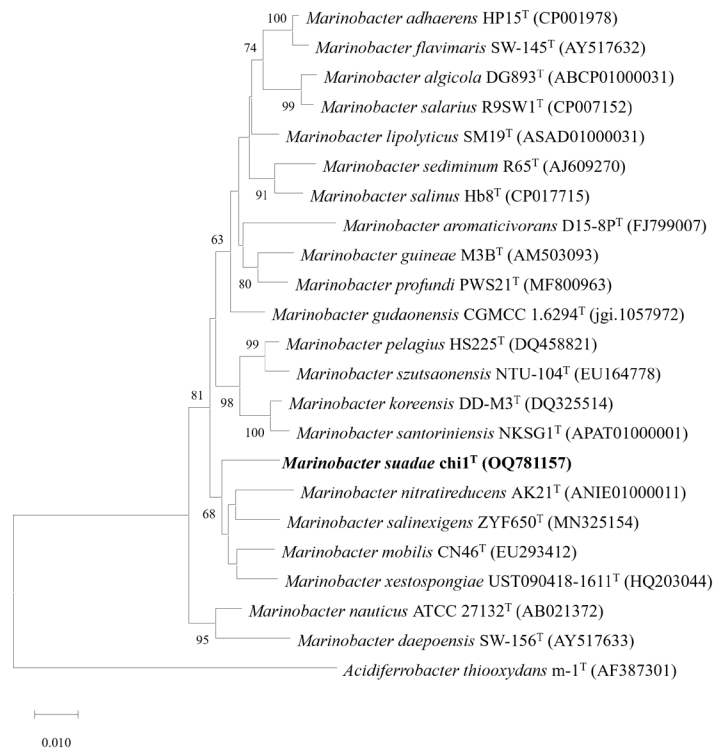
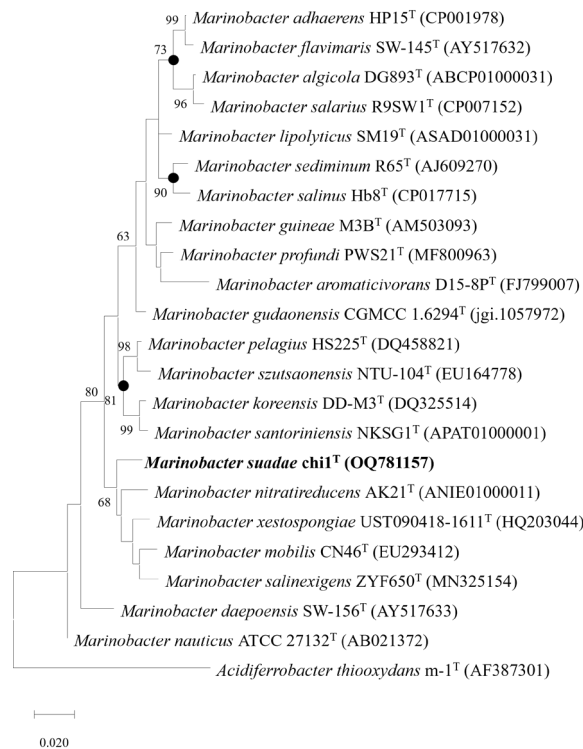
Taegun Seo

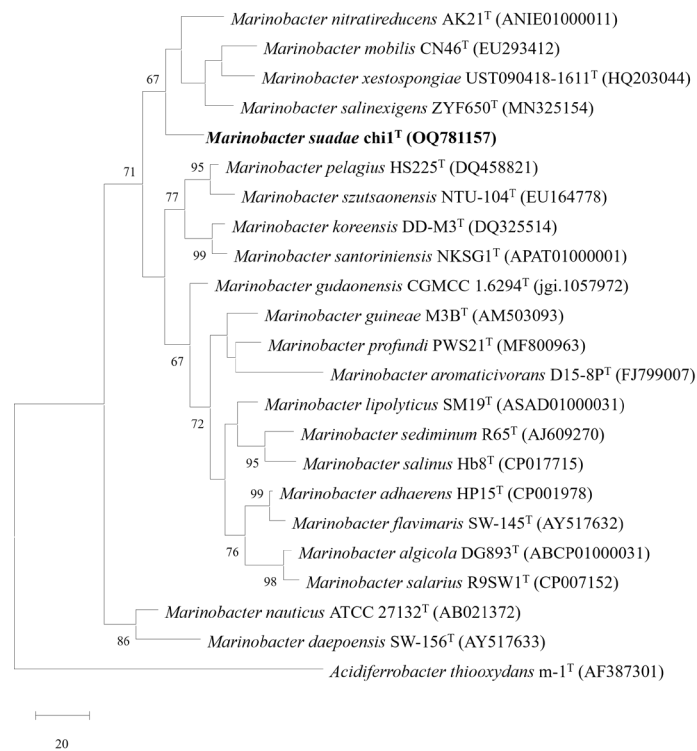
Tel: +82-31-961-5135

Fax: +82-31-961-5348

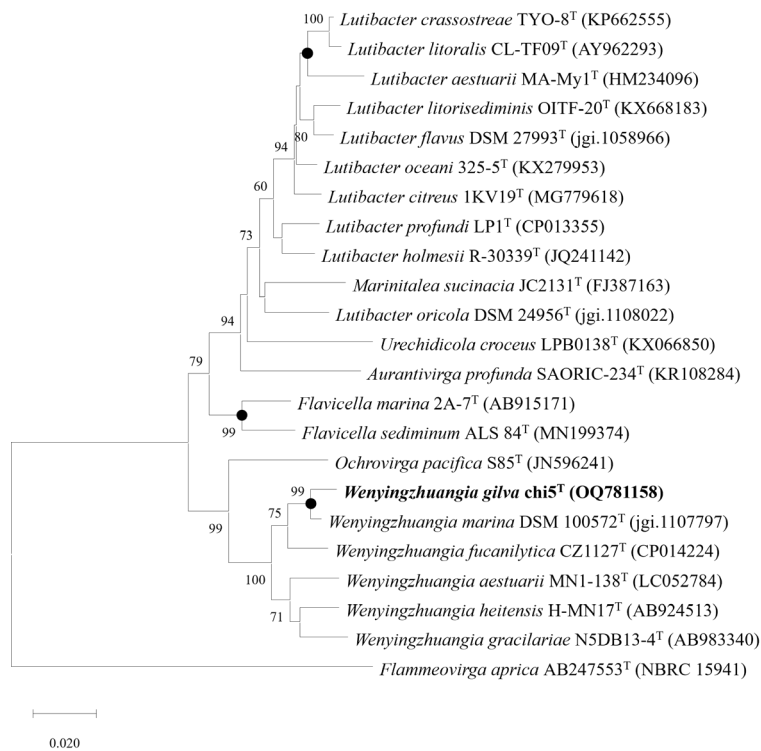
E-mail: tseo@dongguk.edu

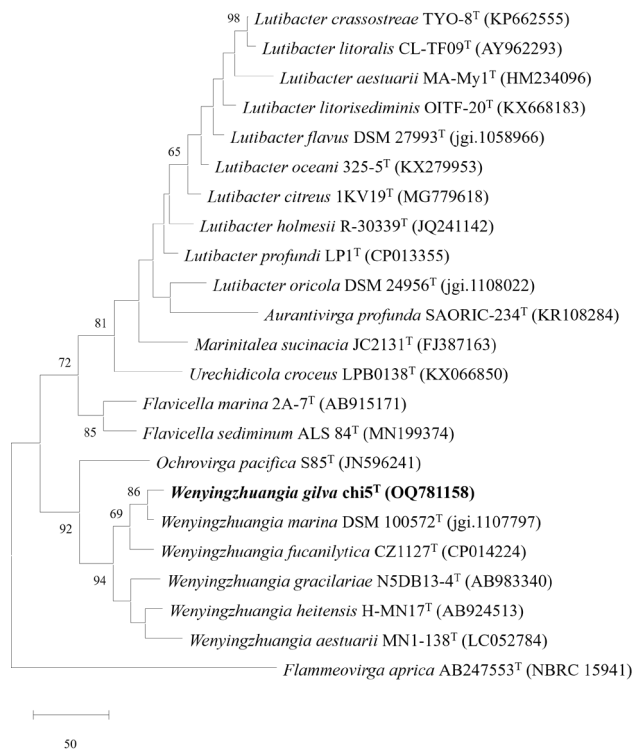
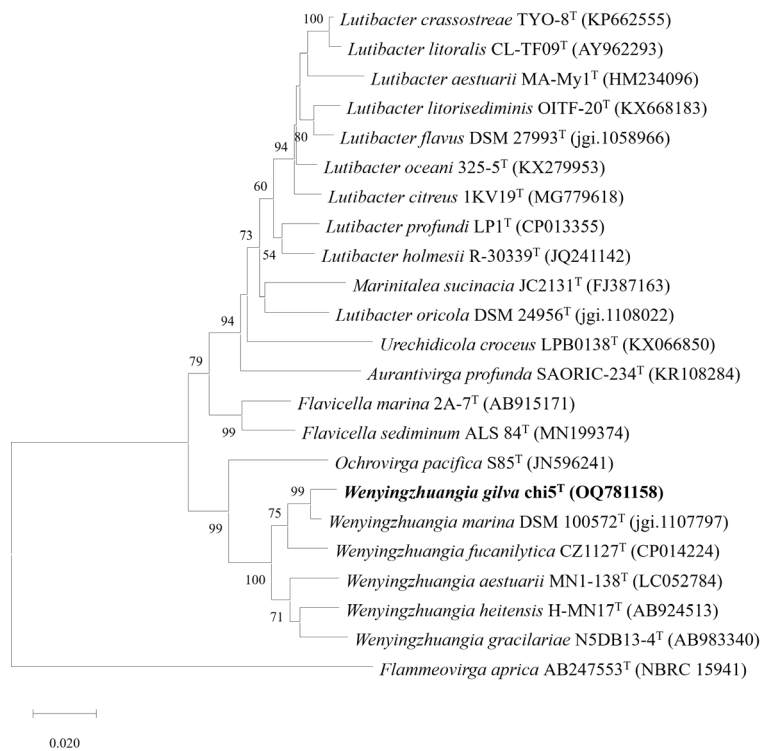
Fig. S1. 16S rRNA gene analysis based on phylogenetic trees constructed using the ML, NJ, and MP algorithms.





(A) Strain *chi1*^T within the genus *Marinobacter*.

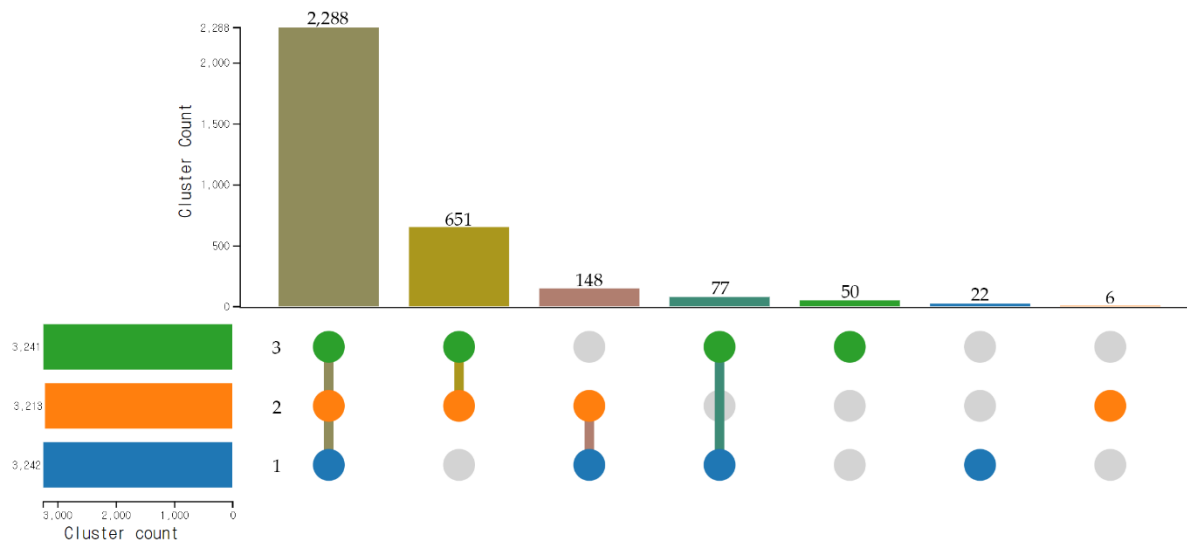




(B) Strain chi5^T within the genus *Wenyingzhuangia*.

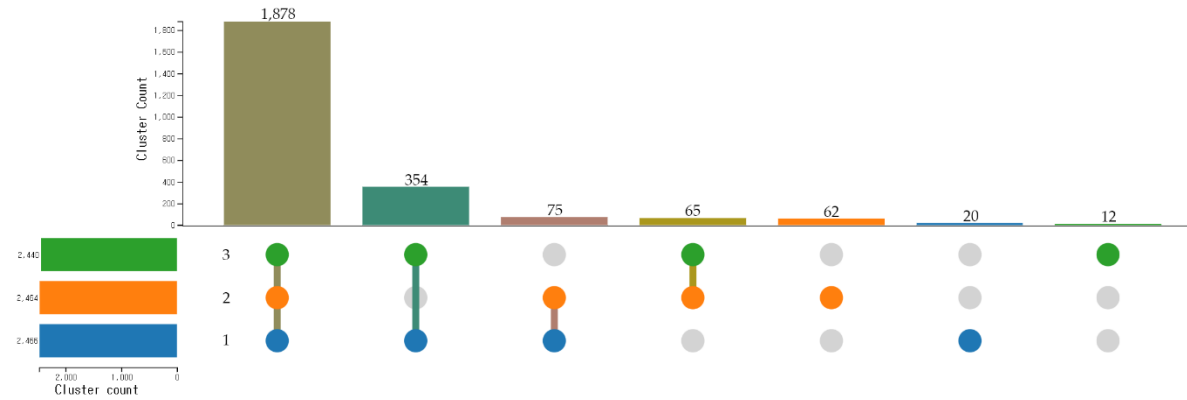
The bootstrap was set as a percentage of 1,000 replicates (above 60%). The outgroups used were *Acidiferrobacter thiooxydan* m-1^T (AF387301) and *Flammeovirga aprica* AB247553^T (NBRC 15941).

Fig. S2. UpSet table showing the orthologous clusters of strain chi1^T and their closely related reference strains (A), while strain chi5^T and their closely related reference strains (B).



(A) *Marinobacter suadae* sp. nov.

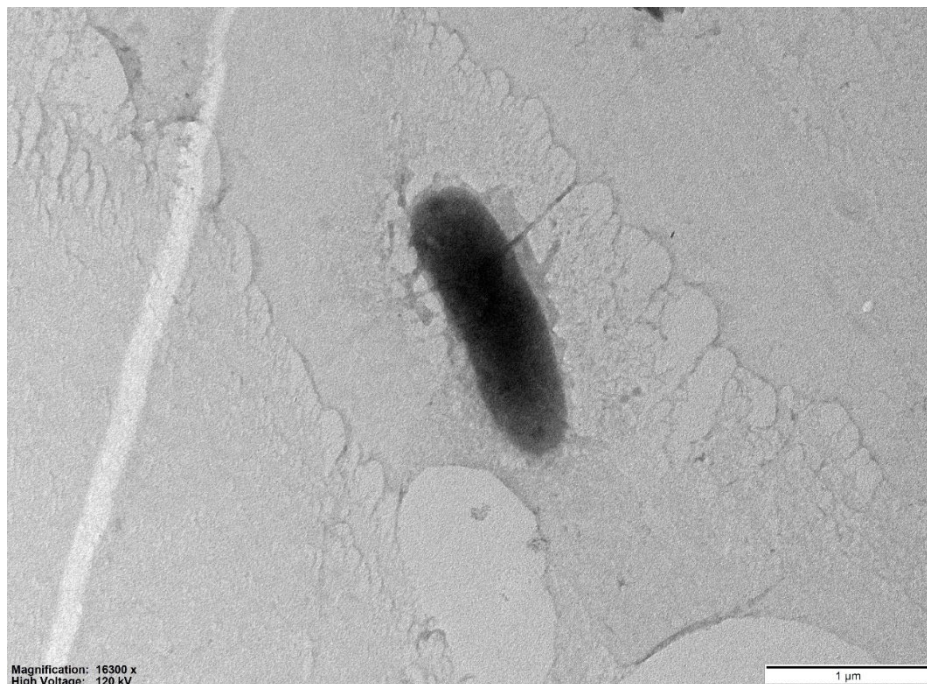
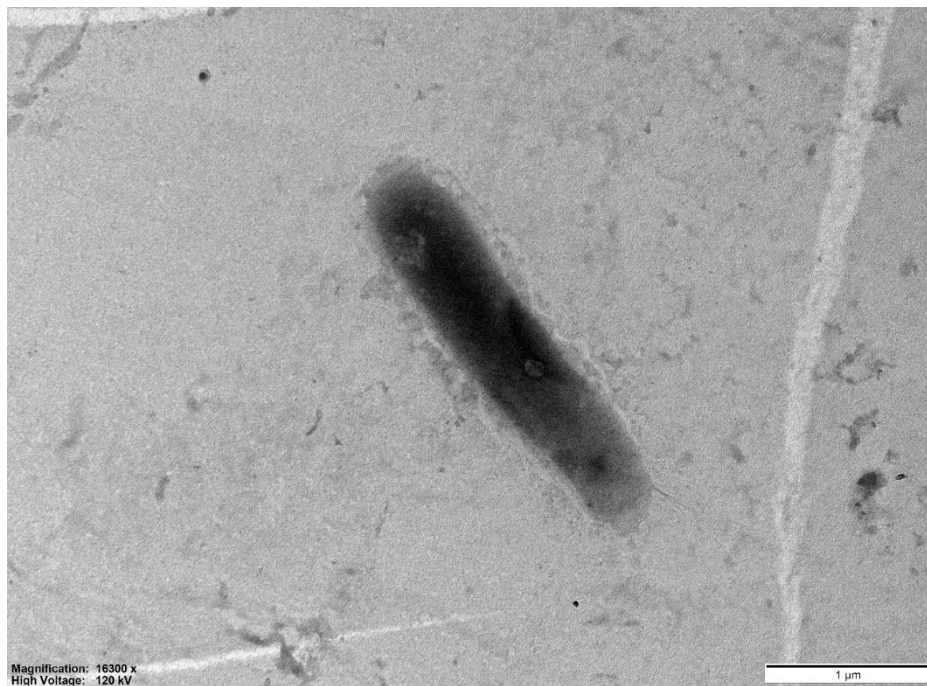
Strains: 1, *M. suadae* chi1; 2, *M. nitratireducens* AK21^T; 3, *M. salinexigens* ZYF650^T.



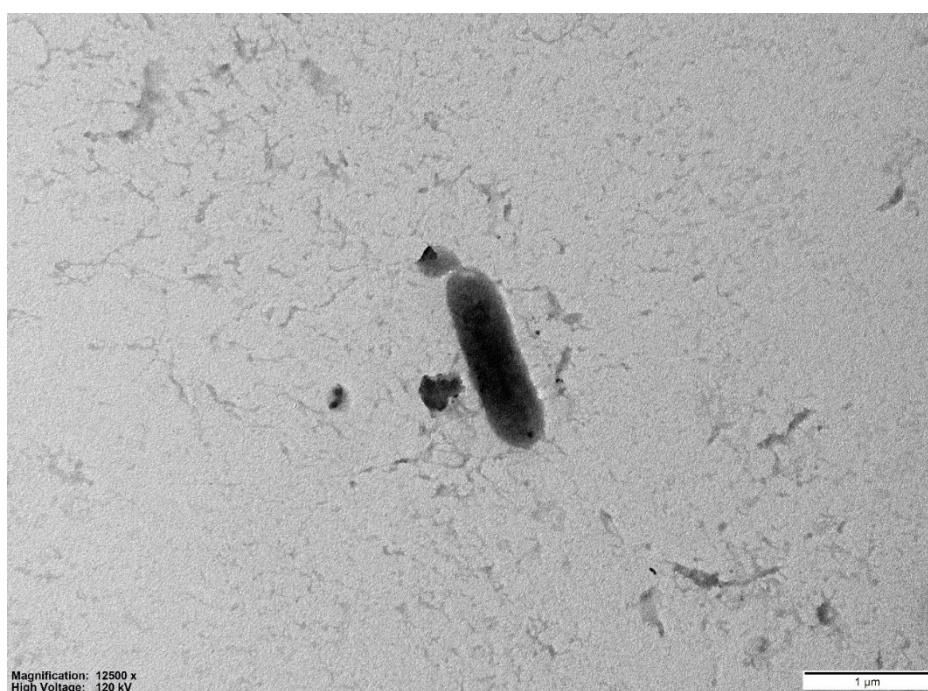
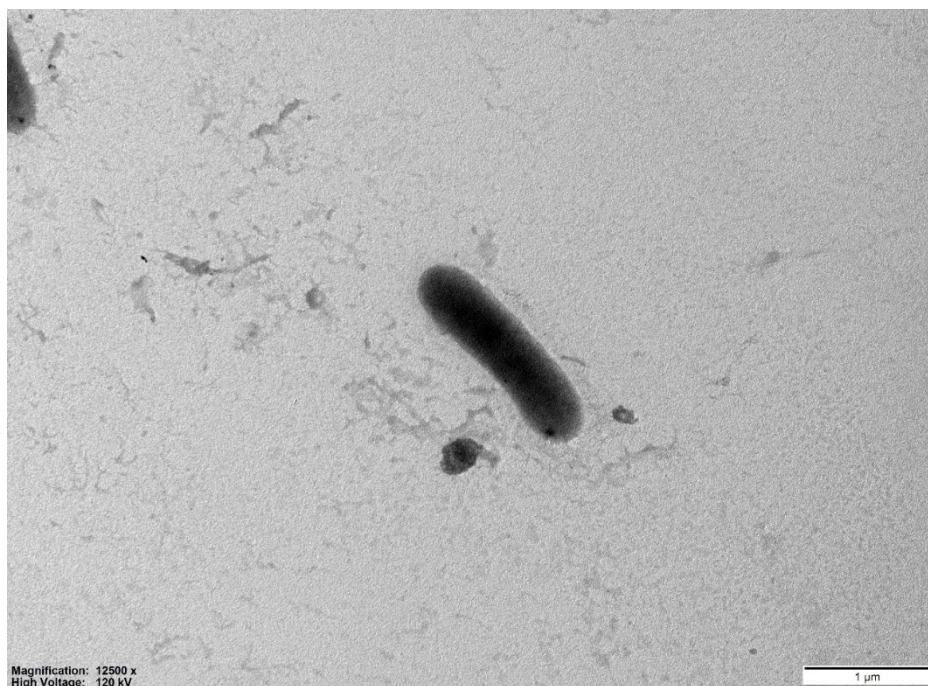
(B) *Wenyingzhuangia gilva* sp. nov.

Strains: 1, *W. gilva* chi5^T; 2, *W. fucanilytica* CZ1127^T; 3, *W. marina* DSM 100572^T.

Fig. S3. Strains *chi1^T* and *chi5^T* were incubated at 30°C for 3 days on marine agar and LB agar, respectively, and observed under a transmission electron microscope (JEM-1010; JEOL).

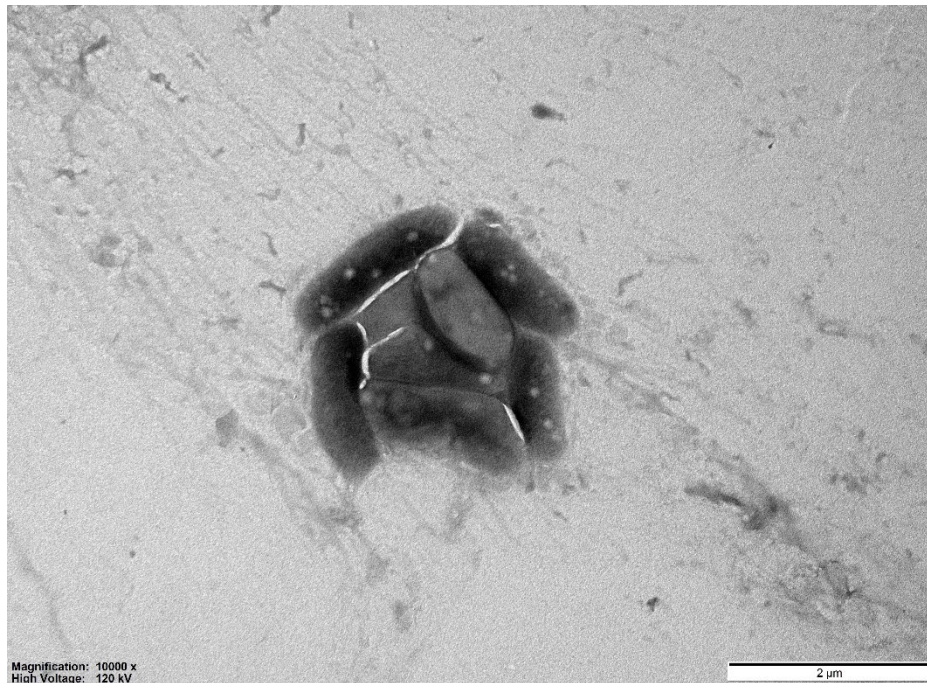


(A) *Marinobacter suadae* sp. nov.

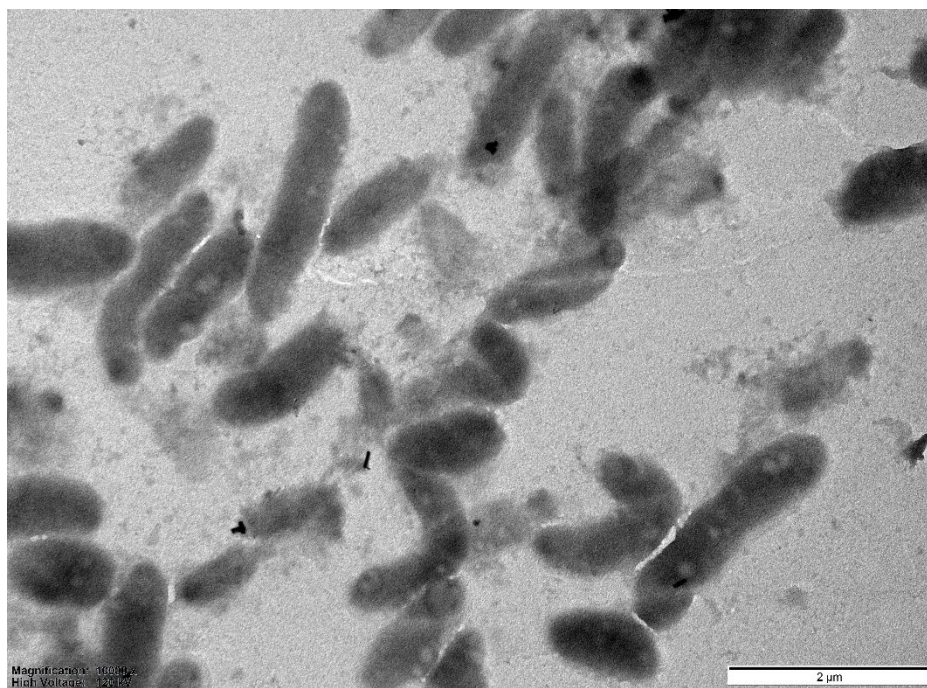


(B) *Wenyngzhuangia gilva* sp. nov.

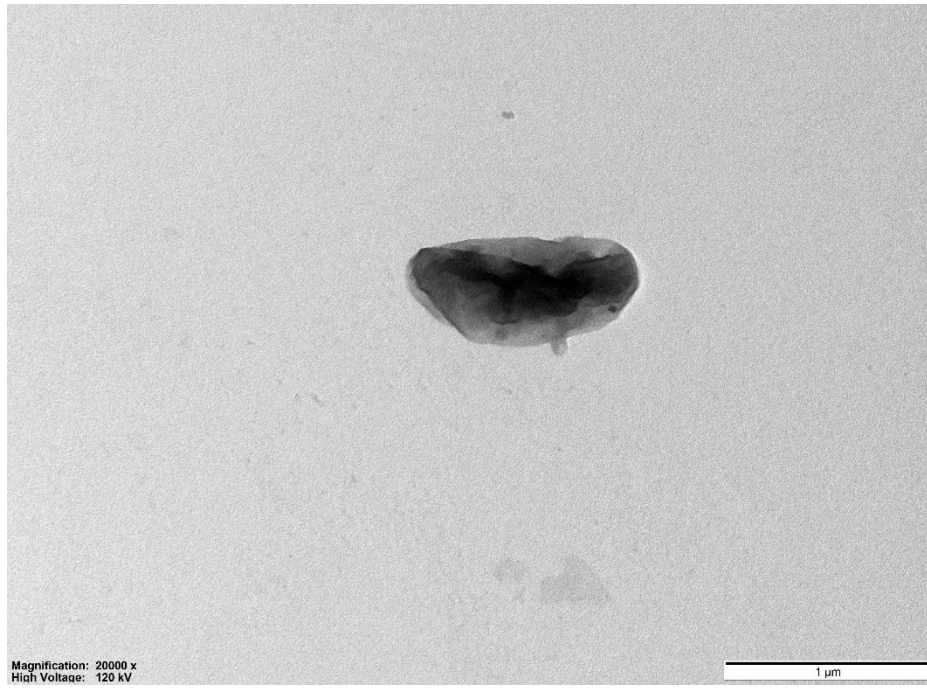
Fig. S4. Presence of flagella according to NaCl concentration (w/v) change in strain *chi1^T*.



(A) *Marinobacter suadae* sp. nov. at a NaCl concentration of 3.5%.

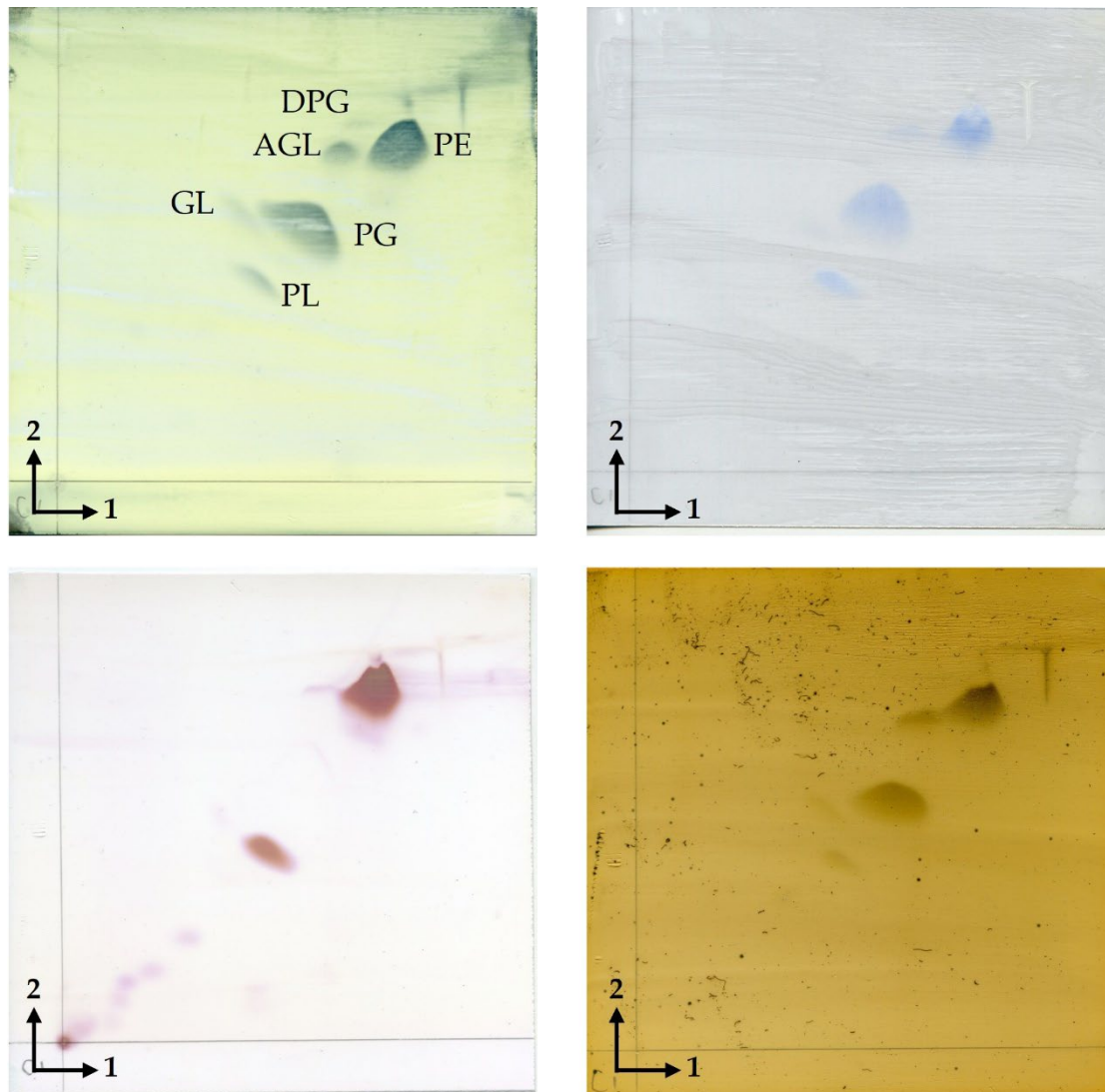


(B) *Marinobacter suadae* sp. nov. at a NaCl concentration of 9.0%.



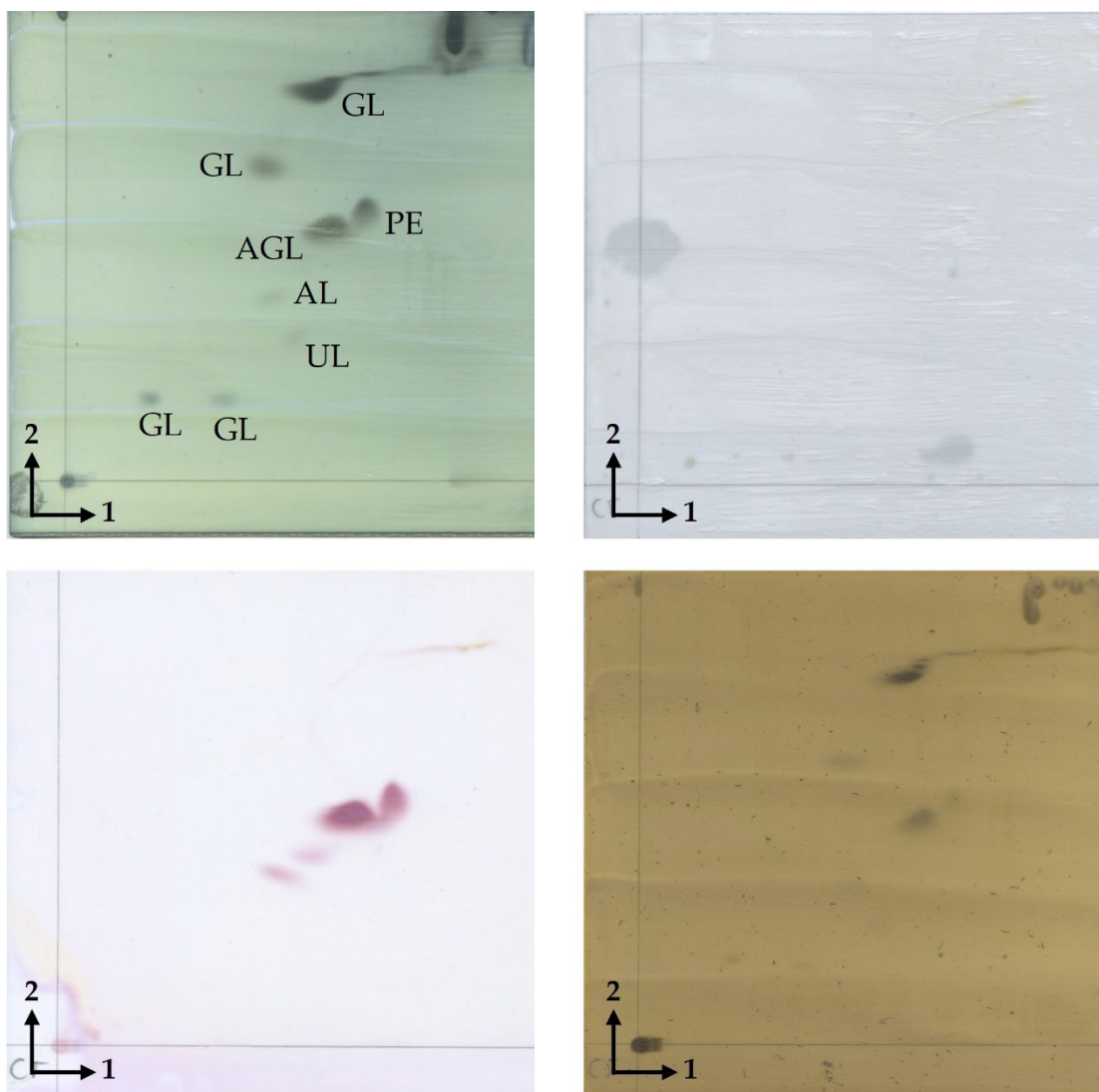
(C) *Marinobacter suadae* sp. nov. at a NaCl concentration of 15.0%.

Fig. S5. Results of two-dimensional thin-layer chromatography (TLC) showing the polar lipids profiles of the two novel strains *chi1^T* and *chi5^T*. A chloroform/methanol/water mixture (65:25:4, v/v/v) and a chloroform/acetic acid/methanol/water mixture (80:15:12:4, v/v/v/v) were used for the first and second directions, respectively.



(A) *Marinobacter suadae* sp. nov.

one phospholipid (PL), one phosphatidylglycerol (PG), one diphosphatidylglycerol (DPG), one aminoglycolipid (AGL), and one phosphatidylethanolamine (PE).



(B) *Wenyingzhuangia gilva* sp. nov.

one phosphatidylethanolamine (PE), four glycolipids (GLs), one aminolipid (AL), one aminoglycolipid (AGL), and one unidentified lipid (UL).

Table S1. Genomic features of strains chi1^T and chi5^T and reference strains belonging to the genera *Marinobacter* and *Wenyngzhuangia*, respectively.

(A) Genomic features of strain chi1^T and reference strains.

Characteristics	Species		
	1	2	3
Genome length (bp)	3,572,209	3,890,033	4,240,922
Contigs	6	22	149
Contig N50 bp	1,388,190	552,815	527,644
Number of rRNAs	6	3	7
Number of tRNAs	46	45	50
Total gene count	3,286	3,543	3,915
Total protein count	3,230	3,491	3,854
DNA G + C content (%)	57.2	56.7	55.6

Strains: 1, *M. suaedae* chi1^T; 2, *M. nitratireducens* AK21^T; 3, *M. salinexigens* ZYF650^T.

(B) Genomic features of strain chi5^T and reference strains.

Characteristics	Species				
	1	2	3	4	5
Genome length (bp)	3,353,634	3,526,759	3,427,057	3,668,833	3,242,070
Contigs	34	6	N/D	29	12
Contig N50 bp	383,849	201,914	N/D	1,935,568	707,963
Number of rRNAs	6	4	12	3	3
Number of tRNAs	45	45	52	45	45
Total gene count	2,909	3,045	2,871	3,123	2,810
Total protein count	2,854	2,961	2,792	3,033	2,737
DNA G+C content (%)	31.5	31.8	31.6	31.4	31.2

Strains: 1, *W. gilva* chi5^T; 2, *W. aestuarii* DSM 105044^T; 3, *W. fucanilytica* CZ1127^T; 4, *W. heitensis* DSM

101599^T; 5, *W. marina* DSM 100572^T. N/D, no data.

Table S2. Flagella and motility gene profiles of strain chi1^T.

Gene	Accession	Length (aa)
<i>FliL</i>	WP_302909520	51
<i>MotA/TolQ/ExbB</i>	WP_302909899	460

Table S3. Secondary metabolite gene cluster profiles of strains chi1^T and chi5^T.(A) Gene cluster profiles of strain chi1^T.

Region	Type	From	To	Most similar known cluster	Similarity (%)
1.1	Ectoine	1,503,503	1,513,898	-	-
1.2	Redox-cofactor	1,747,656	1,769,819	Lankacidin C	13
2.1	RiPP-like	287,731	299,923	-	-
2.2	NRPS-like, T1PKS, NRPS	605,619	658,653	-	-
2.3	Betalactone	702,342	726,527	-	-
2.4	Betalactone	1,091,039	1,122,812	Plipastatin	15

(B) Gene cluster profiles of strain chi5^T.

Region	Type	From	To	Most similar known cluster	Similarity (%)
1.1	Ripp-like	529,970	540,800	-	-
6.1	Terpene	154,597	175,433	Carotenoid	28

Table S4. Subsystem features of strains chi1^T and chi5^T and reference strains belonging to the genera *Marinobacter* and *Wenyinzhuangia*, respectively, as assessed using the RAST server.

(A) Subsystem features of strain chi1^T and reference strains.

Subsystem category distribution	Strains		
	1	2	3
Amino acids and derivatives	285	290	272
Carbohydrates	149	211	176
Cell division and cell cycle	0	0	0
Cell wall and capsule	22	28	26
Cofactors, vitamins, prosthetic groups, and pigments	124	169	155
DNA metabolism	59	73	69
Dormancy and sporulation	4	3	3
Fatty acids, lipids, and isoprenoids	89	103	93
Iron acquisition and metabolism	3	7	8
Membrane transport	100	101	93
Metabolism of aromatic compounds	4	58	42
Miscellaneous	15	13	10
Motility and chemotaxis	0	76	32
Nitrogen metabolism	14	53	50
Nucleosides and nucleotides	73	74	59
Phages, prophages, transposable elements, and plasmids	2	7	5
Phosphorus metabolism	24	21	23
Photosynthesis	0	0	0
Potassium metabolism	9	8	7
Protein metabolism	198	204	197
Regulation and cell signaling	14	31	20
Respiration	60	80	65
RNA metabolism	51	54	52
Secondary metabolism	4	4	4
Stress response	56	64	70
Sulfur metabolism	9	16	6
Virulence, disease, and defense	31	53	40

Strains: 1, *M. suaedae* chi1^T; 2, *M. nitratreducens* AK21^T; 3, *M. salinexigens* ZYF650^T.

(B) Subsystem features of strain chi5^T and reference strains

Subsystem category distribution	Strains		
	1	2	3
Amino acids and derivatives	151	230	237
Carbohydrates	114	201	271
Cell division and cell cycle	3	26	27
Cell wall and capsule	26	65	68
Cofactors, vitamins, prosthetic groups, and pigments	127	145	143
DNA metabolism	48	67	74
Dormancy and sporulation	2	3	4
Fatty acids, lipids, and isoprenoids	18	43	56
Iron acquisition and metabolism	0	3	3
Membrane transport	28	39	42
Metabolism of aromatic compounds	6	14	10
Miscellaneous	12	22	19
Motility and chemotaxis	0	0	0
Nitrogen metabolism	16	26	22
Nucleosides and nucleotides	49	63	64
Phages, prophages, transposable elements, and plasmids	1	1	1
Phosphorus metabolism	13	17	16
Photosynthesis	0	0	0
Potassium metabolism	11	17	15
Protein metabolism	99	127	166
Regulation and cell signaling	6	16	33
Respiration	24	47	42
RNA metabolism	29	120	121
Secondary metabolism	4	4	4
Stress response	16	48	46
Sulfur metabolism	13	35	27
Virulence, disease, and defense	24	49	44

Strains: 1, *W. gilva* chi5^T; 2, *W. fucanilytica* CZ1127^T; 3, *W. marina* DSM 100572^T.

Table S5. Number of ORFs in the novel strains chi1^T and chi5^T compared with those in related species belonging to the genera *Marinobacter* and *Wenyingzhuangia*, respectively.

(A) Number of ORFs in strain chi1^T and closely related species.

Characteristics	Species		
	1	2	3
Auxiliary activity	7	7	10
Carbohydrate-binding module	0	1	6
Carbohydrate esterase	7	6	1
Glycoside hydrolase	8	13	12
Glycosyl transferase	30	34	42
Polysaccharide lyase	0	1	0
Total ORF number	52	62	71

Strains: 1, *M. suaedae* chi1^T; 2, *M. nitratreducens* AK21^T; 3, *M. salinexigens* ZYF650^T.

(B) Number of ORFs in strain chi5^T and closely associated species.

Characteristics	Species				
	1	2	3	4	5
Carbohydrate-binding module	7	2	13	2	4
Carbohydrate esterase	22	13	14	9	19
Glycoside hydrolase	122	107	145	131	124
Glycosyl transferase	52	47	51	53	53
Polysaccharide lyase	15	5	17	8	10
Total ORF number	218	174	240	203	210

Strains: 1, *W. gilva* chi5^T; 2, *W. aestuarii* DSM 105044^T; 3, *W. fucanilytica* CZ1127^T; 4, *W. heitensis* DSM 101599^T; 5, *W. marina* DSM 100572^T.

Table S6. Results of pangenomic analysis of the novel strains chi1^T and chi5^T and reference strains belonging to the genera *Marinobacter* and *Wenyinzhuangia*, respectively, using Build Pangenome with OrthoMCL version 2.0.

(A) Pangenomic profiles of strain chi1^T and closely related species.

Genome	Genes	Homologous genes	Singleton genes	Homologous families
<i>M. suaedae</i> chi1 ^T	3,230	3,033	197	2,969
<i>M. guineae</i> M3B ^T	4,130	3,876	254	3,780
<i>M. adhaerens</i> HP15 ^T	4,410	3,895	515	3,773
<i>M. flavimaris</i> KCTC 12185 ^T	4,077	3,843	234	3,732
<i>M. sediminum</i> R65 ^T	3,356	3,209	147	3,166
<i>M. gudaonensis</i> CGMCC 1.6294 ^T	3,434	3,312	122	3,259
<i>M. algicola</i> DG893 ^T	4,127	3,817	310	3,655
<i>M. salinus</i> Hb8 ^T	3,732	3,540	192	3,476
<i>M. lipolyticus</i> SM19 ^T	3,620	3,458	162	3,370
<i>M. pelagius</i> HS225 ^T	3,473	3,326	147	3,257
<i>M. nitratreducens</i> AK21 ^T	3,491	3,402	89	3,331
<i>M. santoriniensis</i> NKSG1 ^T	3,690	3,509	181	3,416
<i>M. profrundi</i> PWS21 ^T	3,605	3,446	159	3,340
<i>M. nauticus</i> ATCC 49840 ^T	3,614	3,481	133	3,419
<i>M. koreensis</i> DD-M3 ^T	3,475	3,347	128	3,305
<i>M. salinexigens</i> ZYF650 ^T	3,855	3,592	263	3,474
<i>M. xestospongiae</i> UST090418-1611 ^T	4,253	3,573	680	3,442
<i>M. mobilis</i> CGMCC 1.7059 ^T	3,603	3,420	183	3,184
<i>M. daepoensis</i> DSM 16072 ^T	3,484	3,257	227	3,206
<i>M. aromaticivorans</i> D15-8P ^T	3,734	3,367	367	3,279
<i>M. salarius</i> R9SW1 ^T	3,168	2,786	382	2,691

B) Pangenomic profiles of strain chi5^T and closely related species.

Genome	Genes	Homologous genes	Singleton genes	Homologous families
<i>W. gilva</i> chi5 ^T	2,855	2,487	368	2,411
<i>W. aestuarii</i> DSM 105044 ^T	2,992	2,708	284	2,601
<i>W. fucanilytica</i> CZ1127 ^T	2,803	2,503	300	2,353
<i>W. heitensis</i> DSM 101599 ^T	3,071	2,683	388	2,558
<i>W. marina</i> DSM 100572 ^T	2,758	2,491	267	2,417

Table S7. Cellular fatty acid profiles of strains chi1^T and chi5^T and closely related reference strains.(A) Cellular fatty acid profiles of strain chi1^T and closely related species.

Fatty acid	Strains		
	1	2	3
C _{12:0}	12.1	9.5	12.2
C _{14:0}	0.8	2.4	2.9
C _{16:0}	23.7	24.7	21.7
C _{17:0}	2.1	1.0	2.6
C _{12:0} 3OH	7.5	10.6	14.0
Iso-C _{17:0}	1.0	TR	TR
C _{16:1} ω 5 <i>c</i>	0.8	TR	TR
C _{17:1} ω 8 <i>c</i>	3.5	1.1	1.7
C _{18:1} ω 9 <i>c</i>	1.1	19.9	22.0
Summed feature 3*	31.4	5.3	2.25
Summed feature 8*	6.7	N/D	N/D
Summed feature 9*	2.6	–	1.2

Strains: 1, *M. suaedae* chi1^T; 2, *M. nitratreducens* AK21^T; 3, *M. salinexigens* ZYF650^T.

*Summed features are fatty acids that cannot be resolved reliably from other fatty acids using the chosen chromatographic conditions. The MIDI system groups these fatty acids into one feature with a single percentage of the total. Summed feature 3 consists of C_{16:1} ω 6*c* and/or C_{16:1} ω 7*c*. TR, the amount is less than 1% of the total; –, not detected; N/D, no data.

(B) Cellular fatty acid profiles of strain chi5^T and closely related species.

Fatty acid	Strains		
	1	3	5
C _{10:0}	1.0	–	–
C _{12:0}	9.9	–	–
C _{14:0}	4.6	–	–
C _{15:0}	1.4	–	–
C _{16:0}	3.5	3.5	2.2
C _{18:0}	1.2	–	–
C _{16:0} 3OH	1.5	3.7	1.5
Iso-C _{15:0} 3OH	11.1	12.2	15.0
Iso-C _{17:0} 3OH	14.7	17.4	15.2

Iso-C _{13:0}	6.5	3.8	5.7
Iso-C _{15:0}	24.1	17.6	18.0
Iso-C _{15:1} G	6.2	6.3	12.0
Summed feature 3*	6.2	13.1	10.3

Strains: 1, *W. gilva* chi5^T; 2, *W. fucanilytica* CZ1127^T; 3, *W. marina* DSM 100572^T.

*Summed features are fatty acids that cannot be resolved reliably from other fatty acids using the chosen chromatographic conditions. The MIDI system groups these fatty acids into one feature with a single percentage of the total. Summed feature 3 consists of C_{16:1} ω 6c and/or C_{16:1} ω 7c. –, not detected.

Table S8. Polar lipids profiles of strains chi1^T and chi5^T and closely related reference strains.

Polar lipids	Strains		
	1	2	3
AGL	1	0	0
AL	0	0	2
APL	0	1	0
DPG	1	1	1
PE	1	1	1
PG	1	1	1
PL	1	2	2
UL	0	1	3

Strains: 1, *M. suaedae* chi1^T; 2, *M. nitratreducens* AK21^T; 3, *M. salinexigens* ZYF650^T.

Polar lipids	Strains		
	4	5	6
AGL	1	0	0
AL	1	3	0
GL	4	0	0
PE	1	1	1
PL	0	1	0
UL	1	10	4

Strains: 1, *W. gilva* chi5^T; 2, *W. fucanilytica* CZ1127^T; 3, *W. marina* DSM 100572^T.

Abbreviations: APL; unidentified aminophospholipid.