

Archives of Microbiology

Supplementary materials

***Aoguangibacterium sediminis* gen. nov., sp. nov., a noval denitrifying and carbon-fixing bacterium isolated from marine sediment**

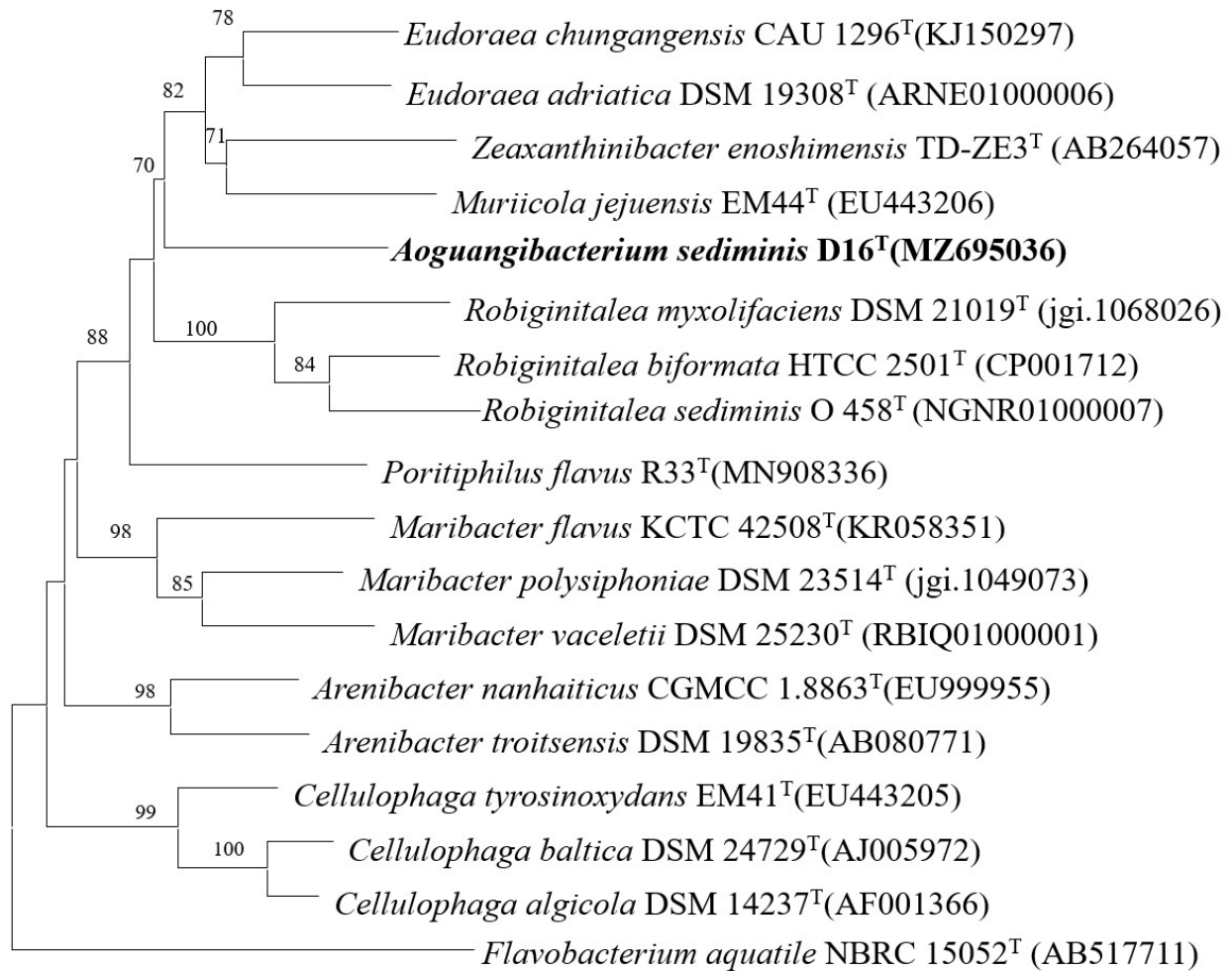
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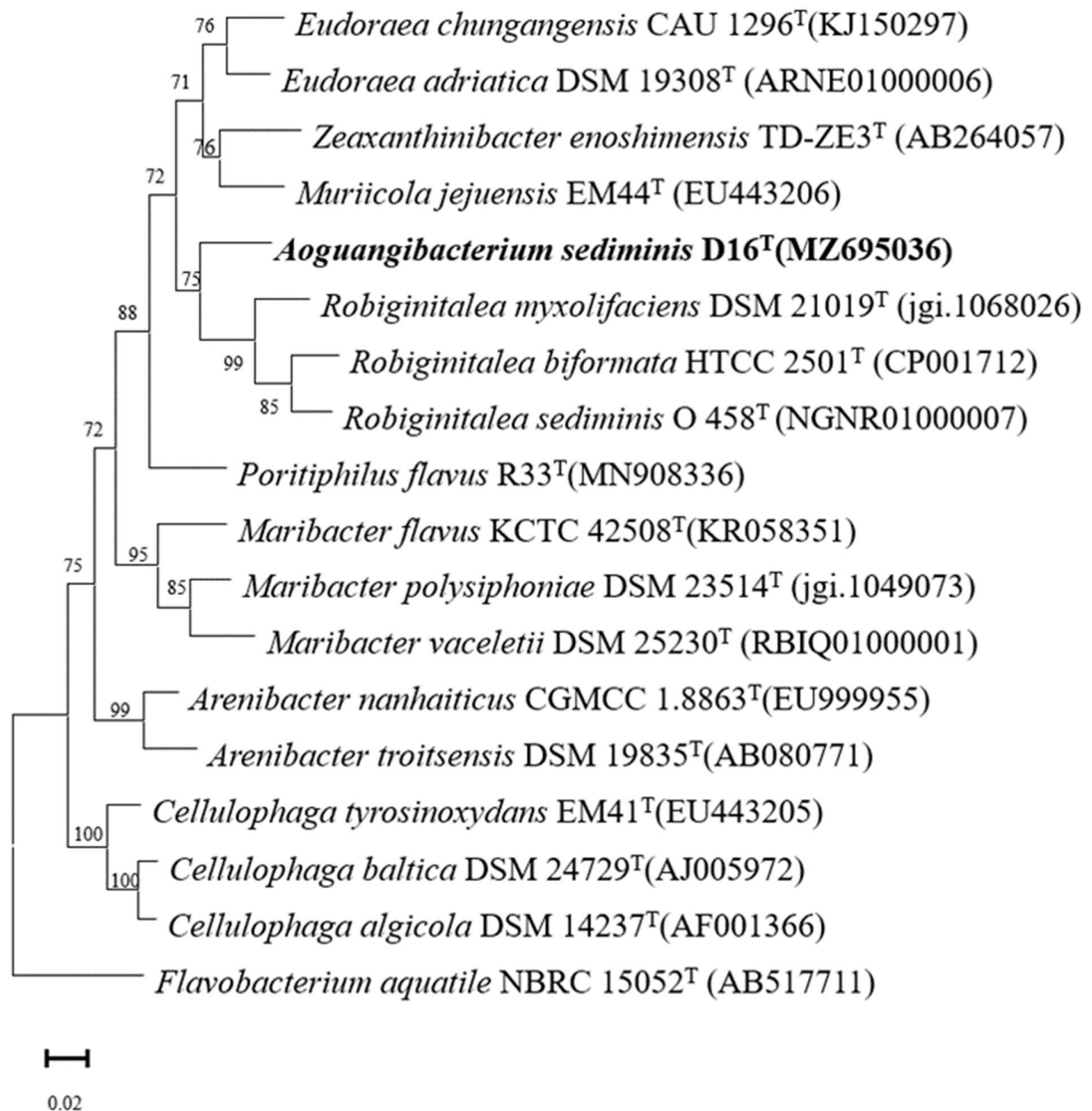
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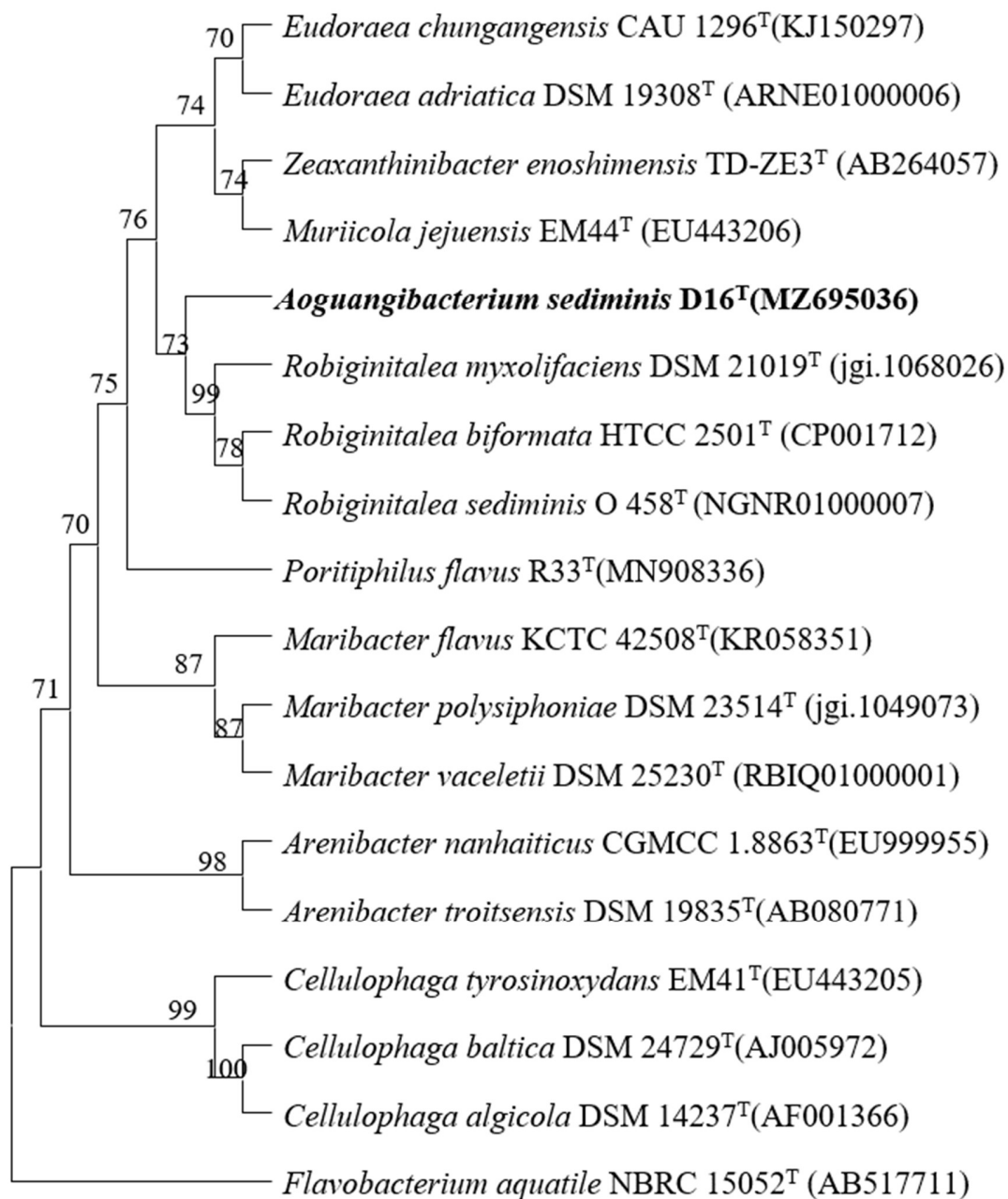


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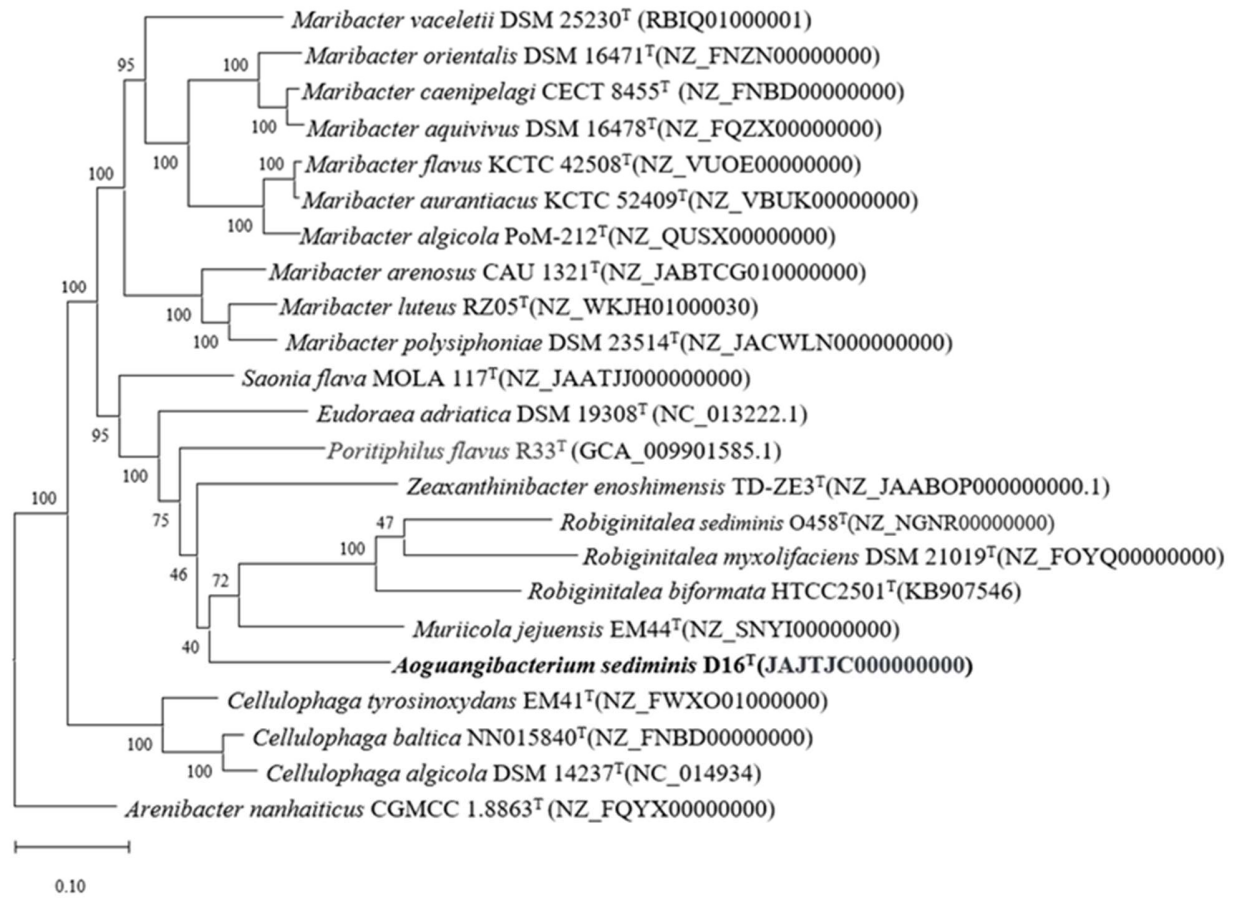
Supplementary Figure 1. Phylogenetic tree constructed based on 16S rRNA gene sequences of strain D16^T and its closely related species of the family *Flavobacteriaceae*. The tree was constructed using the neighbor-joining method (NJ). Bootstrapping was carried out with 1,000 replicates. The numbers at the branch node values are estimated from NJ. The node values below 70% are not shown. *Flavobacterium aquatile* NBRC 15052^T was selected as the outgroup. Scale bars: 0.01 represented the nucleotide substitution per position.



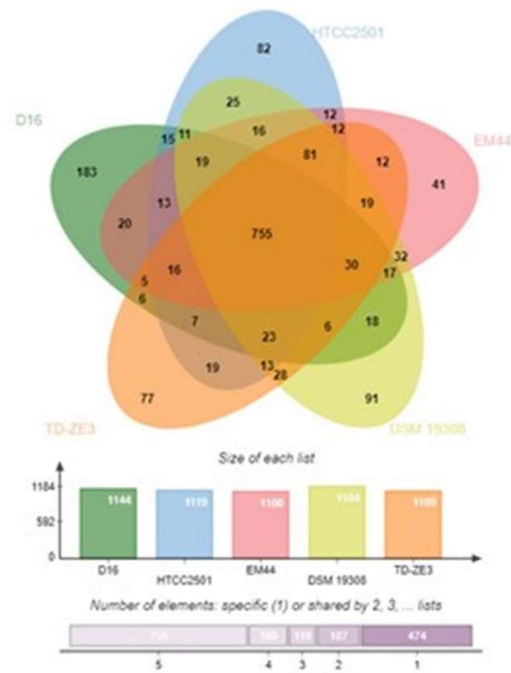
Supplementary Figure 2. Phylogenetic tree constructed based on 16S rRNA gene sequences of strain D16^T and its closely related species of the family *Flavobacteriaceae*. The tree was constructed using the maximum-likelihood method (ML). Bootstrapping was carried out with 1,000 replicates. The numbers at the branch node values are estimated from ML. The node values below 70% are not shown. *Flavobacterium aquatile* NBRC 15052^T was selected as the outgroup. Scale bars: 0.02 represented the nucleotide substitution per position



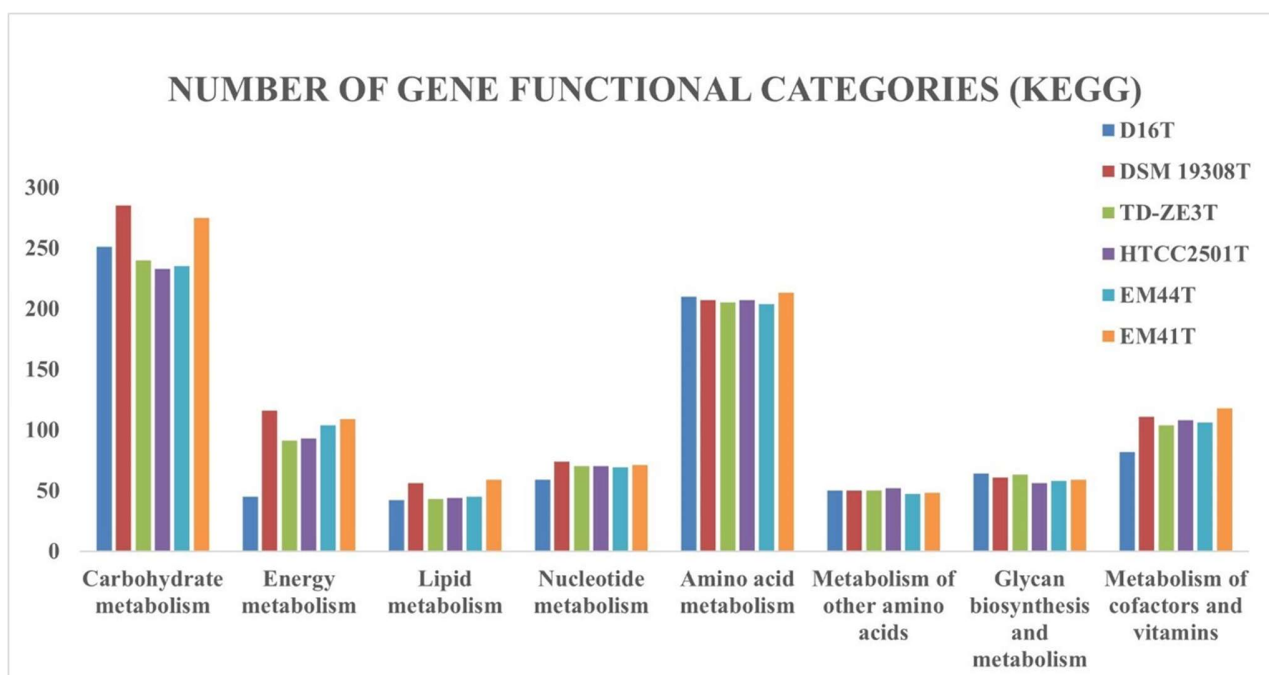
Supplementary Figure 3. Phylogenetic tree constructed based on 16S rRNA gene sequences of strain D16^T and its closely related species of the family Flavobacteriaceae. The tree was constructed using the maximum-parsimony method (MP). Bootstrapping was carried out with 1,000 replicates. The numbers at the branch node values are estimated from MP. The node values below 70% are not shown. *Flavobacterium aquatile* NBRC 15052^T was selected as the outgroup.



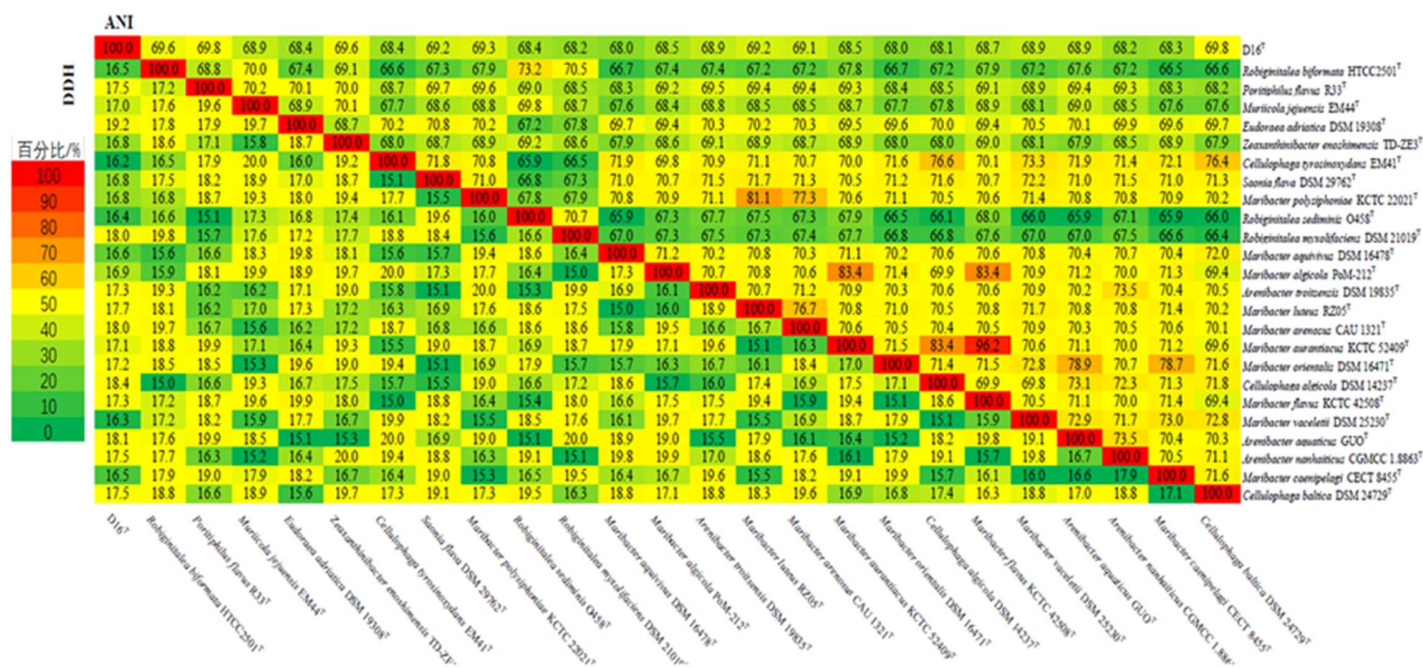
Supplementary Figure 4. Phylogenetic trees based on nucleotide sequences from 25 genomes of related strains show the taxonomic position of strain D16^T.



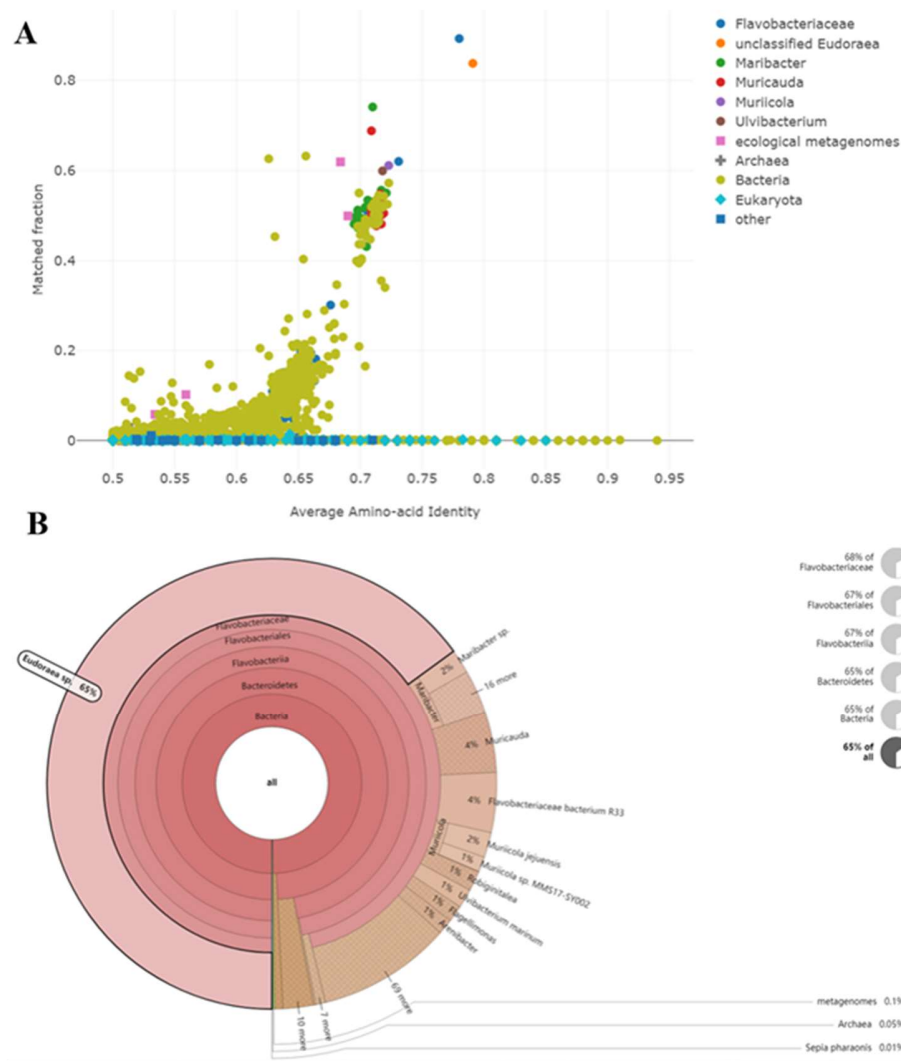
Supplementary Figure 5. Venn diagram of the KEGG annotated genes the six *Flavobacteriaceae* species. The size of the core genome is shown in the centre, while the numbers of strain-specific genes in each strain are shown in the petals. (Strain D16^T and *Eudoraea adriatica* DSM 19308^T, *Muriicola jejuensis* EM44^T, *Zeaxanthinibacter enoshimensis* TD-ZE3^T and *Robiginitalea biformata* HTCC 2501^T were tested by the KEGG.)



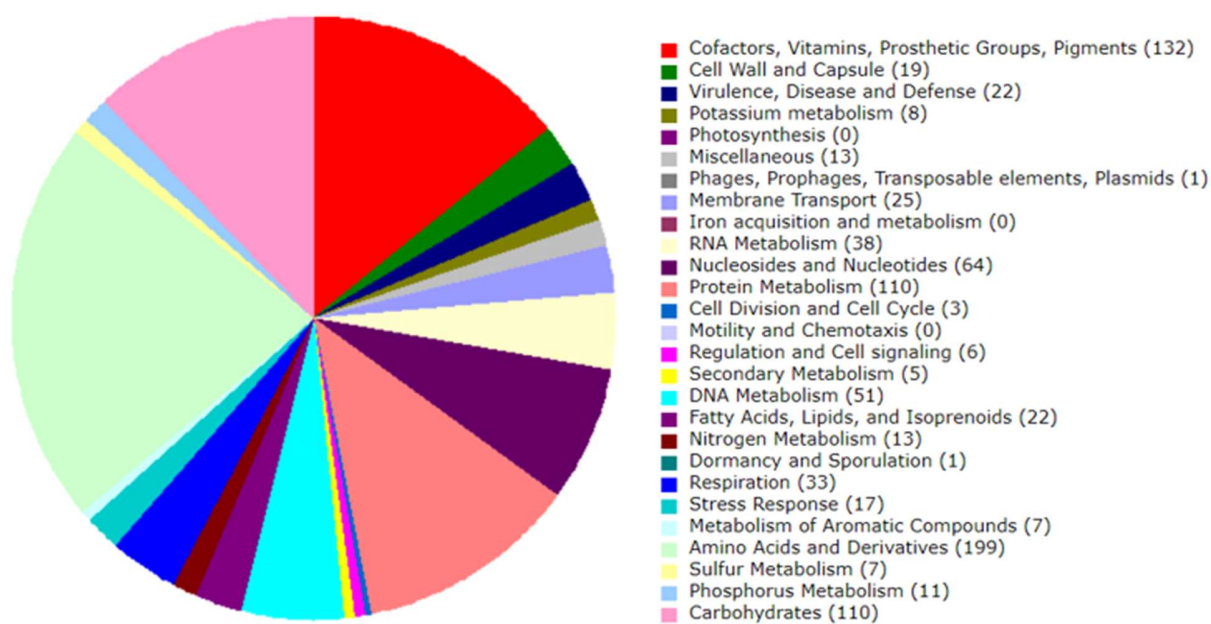
Supplementary Figure 6. Numbers of gene functional categories of six *Flavobacteriaceae* strains, D16^T, *Eudoraea adriatica* DSM 19308^T, *Robiginitalea biformata* HTCC2501^T, *Zeaxanthinibacter enoshimensis* TD-ZE3^T, *Cellulophaga tyrosinoydans* EM41^T and *Muriicola jejuensis* EM44^T, performed by KEGG.



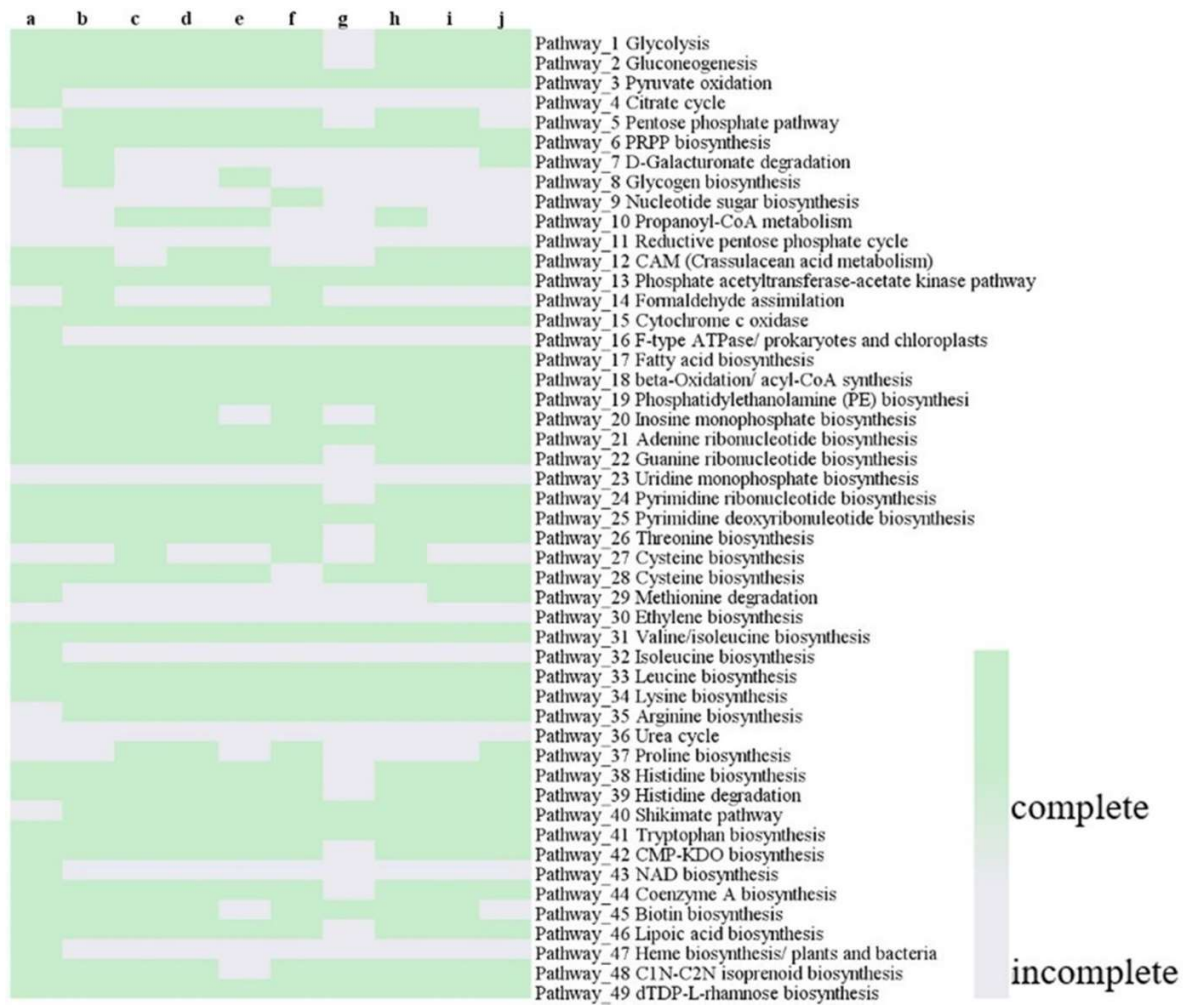
Supplementary Figure 7. Heat map of DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values of genomes of the isolate and related strains. The lower triangle displays the dDDH values, and upper triangle displays the ANI values. Isolates and related strains are given as row and column labels of the map.



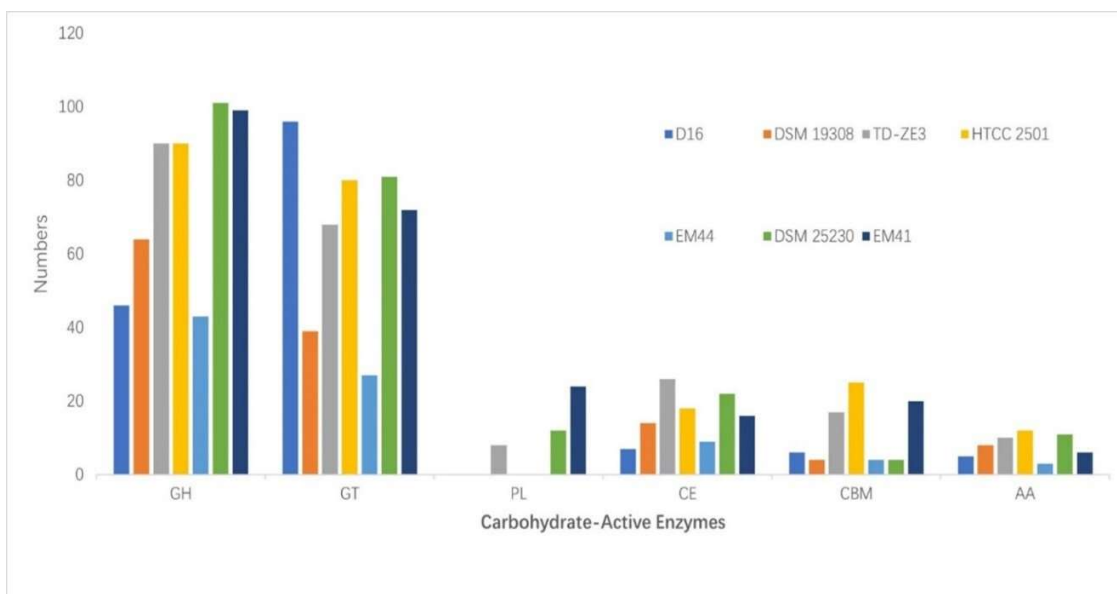
Supplementary Figure 8. Scatterplot and taxonomic profile of average amino acid identities (AAI) for isolate D16^T. (A) Each point represents a matched species in a fast homology search tool, AAI-profiler. Horizontal and vertical axes indicate AAI values and matched fractions of query proteins, respectively. (B) Diagram shows a taxonomic profile matched with the amino acid sequences of the isolate. The center to outside of the circle represents lineage from the class to genus level that is matched with the amino acid sequences of the isolate.



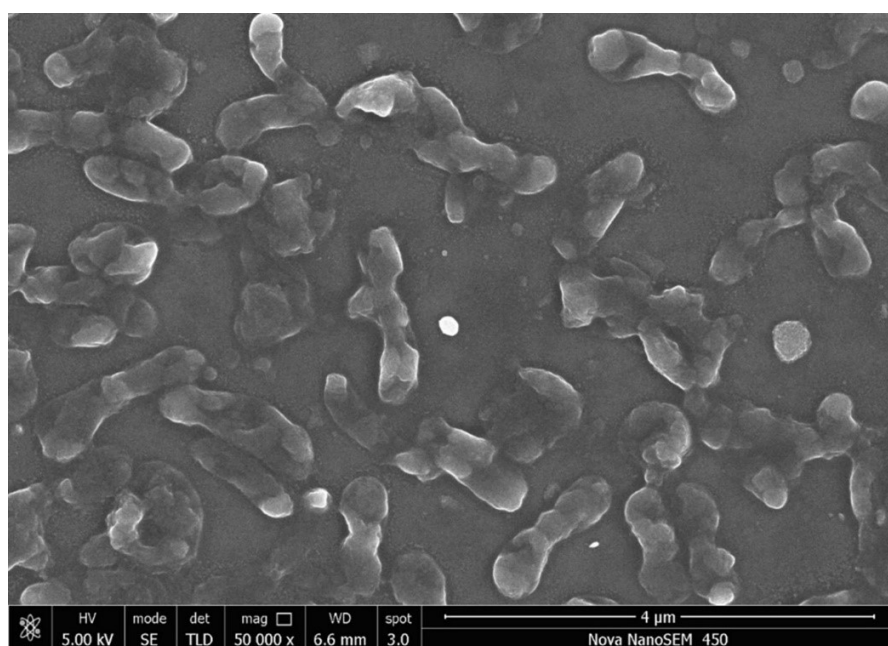
Supplementary Figure 9. Statistics of metabolic function of strain D16^T, the Figure comes from Genome annotation of RAST.



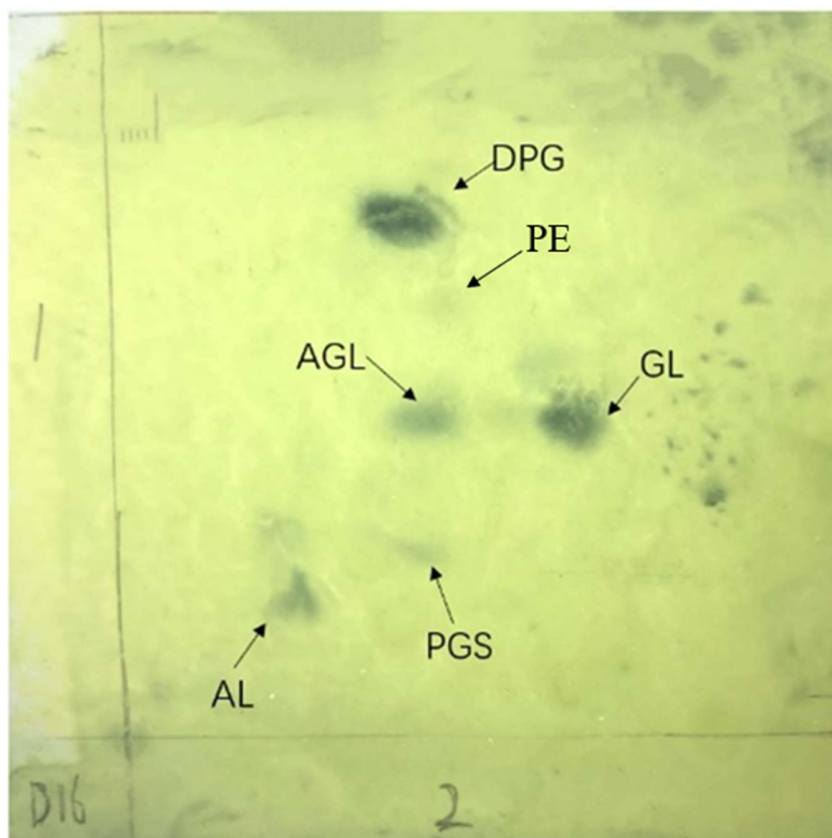
Supplementary Figure 10. Heatmaps of comparative genomic analysis between strain D16^T and related strains. heatmap clustered with 49 pathways according to the annotation of the KEGG. a, Strain D16^T; b, *Eudoraea adriatica* DSM 19308^T; c, *Zeaxanthinibacter enoshimensis* TD-ZE3^T; d, *Robiginitalea biformata* HTCC2501^T; e, *Muriicola jejuensis* EM44^T; f, *Cellulophaga tyrosinoydans* EM41^T; g, *Maribacter polysiphoniae* KCTC 22021^T; h, *Poritiphilus flavus* R33^T; i, *Maribacter aquivivus* DSM 16478^T; j, *Arenibacter troitsensis* DSM 19835^T.



Supplementary Figure 11. Histogram of predicted carbohydrate-active enzymes (CAZymes) in strain D16^T, *Eudoraea a driatica* DSM 19308^T, *Zeaxanthinibacter enoshimensis* TD-ZE3^T, *Robiginitalea biformata* HTCC 2501^T, *Muriicola jejue nsis* EM44^T, *Maribacter vaceletii* DSM 25230^T and *Cellulophaga tyrosinoxydans* EM41^T. Glycoside Hydrolase (GH)、G lycosyltransferase (GT)、Polysaccharide Lyase (PL)、Carbohydrate Esterase (CE)、Carbohydrate Binding Domain (CB M)、Accessory Enzyme (AA) .



Supplementary Figure 12. Scanning Electron Microscope image of strain D16^T cultivated at 30 °C for three days on 2216E agar medium. Bar, 4 μm.



Supplementary Figure 14. Polar lipid profile of strain D16^T grown on 2216E medium at 30°C for 3 days, separated by two-dimensional TLC. Spray reagent for the detection of spots and their color reaction; (a) 5% ethanolic molybdophosphoric acid for total lipids, (b) molybdenum blue reagent for phospholipids, (c) ninhydrin solution for amino lipids and (d) α -naphthol reagent for glycolipids. Diphosphatidylglycerol (DPG), Phosphatidylcholine (PE), Aminoglycolipid (AGL), glycolipid (GL), Aminolipid (AL) and unidentified lipids (PGS) was detected.

Supplementary Table 1. Cellular fatty acid compositions (percentages) of D16^T and related taxa of the family *Flavobacteriaceae*

Strains: 1, D16^T (data from this study); 2, *Eudoraea adriatica* DSM 19308^T (data from this study); 3, *Muriicola jejuensis* EM44^T (data from this study); 4, *Zeaxanthinibacter enoshimensis* TD-ZE3^T (data from this study); 5, *Robiginitalea biformata* HTCC2501^T (JC and SJ 2004); 6, *Maribacter arcticus* KOPRI 20941^T (Cho et al. 2008); 7, *Cellulophaga tyrosinoxydans* EM41^T (Kahng et al. 2009); 8, *Arenibacter troitsensis* DSM 198352^T (Nedashkovskaya et al. 2003b); 9, *Poritiphilus flavus* R33^T (Wang et al. 2020). Data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 1.0 % in all strains are not shown. Major components (>10.0 %) are highlighted in bold type; TR, trace amount (<1.0 %); -, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated acids:									
C _{14:0}	3.0	-	0.8	-	-	-	-	TR	8.0
C _{15:0}	-	-	-	7.7	5.5	3.9	16.6	-	4.2
C _{16:0}	10.5	TR	3.0	TR	1.3	TR	-	2.3	
Branched saturated acids:									
iso-C _{14:0}	3.2	TR	1.2	-	-	-	-	-	40.0
iso-C _{15:0}	32.0	29.4	25.6	18.9	26.0	12.5	9.1	12.7	-
anteiso-C _{15:0}	8.1	TR	4.4	2.3	3.7	9.0	-	6.4	-
iso-C _{16:0}	7.1	TR	3.9	1.1		1.0	-	-	-
Monounsaturated acids:									
C _{15:1} ω6c	TR	TR	-	TR	-	-	-	-	-
C _{16:1} ω7c	TR	-	-	-	-	14.8	13.3	-	-
C _{17:1} ω8c	TR	TR	-	TR	-	-	-	-	-
C _{17:1} ω6c	-	1.0	-	1.0	-	-	-	-	-
Branched monounsaturated acids:									
iso-C _{15:1}	-	-	-	-	-	-	20.9	-	-
anteiso-C _{15:1}	TR	-	1.1	-	-	-	-	-	20.8
iso-C _{15:1} G	1.8	16.0	21.2	9.9	17.2	8.0	-	10.0	-
iso-C _{16:1}	-	-	1.9	-			-	-	-
iso-C _{17:1} ω9c	-	-	1.5	13.3		7.9	-	8.6	-
iso-C _{17:1} ω7c	TR	9.0	-	-	-	-	-	-	-
Hydroxy acids:									
C _{15:0} 3-OH	TR	7.3	4.9	-	4.3		-	-	-
C _{16:0} 3-OH	TR	1.4		-		1.5	-	2.7	-
C _{17:0} 2-OH	-		1.1	TR	1.2	3.8	-	2.0	-
C _{17:0} 3-OH	TR	14.1		-	-	19.1	-	-	-
iso-C _{15:0} 3-OH	1.5	-	-	2.7	-	2.8	-	4.3	-
anteiso-C _{15:0} 3-OH	TR	-	-	-	-	-	-	-	-
iso-C _{16:0} 3-OH	TR	-	10.3	2.4	-	4.0	-	1.7	-
iso-C _{17:0} 3-OH	4.0		13.3	16.5	25.8	-	3.7	23.5	-
Summed feature 3	1.2	11.6	5.8	5.8	3.5	-		18.7	-
Others				2.1				-	2.6

Supplementary Table 2. Carbon, nitrogen and sulfur metabolism of the strain D16^T and relative genera

strain	CO2 fixation	Denitrification	Inorganic Sulfur Assimilation	[NiFe]
D16 ^T	+	+	-	+
<i>Robiginitalea biformata</i> HTCC2501 ^T	-	+	-	+
<i>Muriicola jejuensis</i> EM44 ^T	+	+	-	+
<i>Eudoraea adriatica</i> DSM 19308 ^T	-	+	-	-
<i>Zeaxanthinibacter enoshimensis</i> TD-ZE3 ^T	+	+	+	-
<i>Cellulophaga tyrosinoydans</i> EM41 ^T	-	-	+	-
<i>Saonia flava</i> DSM 29762 ^T	-	+	-	-
<i>Maribacter polysiphoniae</i> KCTC 22021 ^T	+	+	-	+
<i>Robiginitalea sediminis</i> O458 ^T	+	+	-	-
<i>Robiginitalea myxolifaciens</i> DSM 21019 ^T	-	+	-	-
<i>Maribacter aquivivus</i> DSM 16478 ^T	+	-	+	-
<i>Maribacter algicola</i> PoM-212 ^T	-	+	+	-
<i>Arenibacter troitsensis</i> DSM 19835 ^T	-	-	+	-
<i>Maribacter luteus</i> RZ05 ^T	-	+	-	-
<i>Maribacter arenosus</i> CAU 1321 ^T	-	+	-	+
<i>Maribacter aurantiacus</i> KCTC 52409 ^T	+	+	-	-
<i>Maribacter orientalis</i> DSM 16471 ^T	+	-	-	-
<i>Cellulophaga algicola</i> DSM 14237 ^T	-	+	-	-
<i>Maribacter flavus</i> KCTC 42508 ^T	+	+	-	-
<i>Maribacter vaceletii</i> DSM 25230 ^T	-	-	-	-
<i>Arenibacter aquaticus</i> GUO ^T	-	+	-	-
<i>Arenibacter nanhaiticus</i> CGMCC 1.8863 ^T	-	-	+	-
<i>Maribacter caenipelagi</i> CECT 8455 ^T	-	+	-	-
<i>Cellulophaga baltica</i> DSM 24729 ^T	-	-	-	-