

## Supplementary Material

# Three Novel Marine Species of *Paracoccus*, *P. aerodenitrificans* sp. nov., *P. sediminicola* sp. nov. and *P. albus* sp. nov., and the Characterization of Their Capability to Perform Heterotrophic Nitrification and Aerobic Denitrification

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**Table S1.** Energy sources utilization of SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup> and SCSIO 80058<sup>T</sup>.

SCSIO 75817 <sup>T</sup>	SCSIO 76264 <sup>T</sup>	SCSIO 80058 <sup>T</sup>
It can utilize dextrin, D-maltose, D-trehalose, D-cellobiose, D-turanose, D-raffinose, $\beta$ -methyl-D-glucoside, D-salicin, <i>N</i> -acetyl-D-glucosamine, $\alpha$ -D-glucose, D-mannose D-fructose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, D-fructose-6-PO <sub>4</sub> , D-aspartic acid, gelatin, glycyl-L-prolin, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, pectin, D-gluconic acid, <i>p</i> -hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, Tween 40, $\gamma$ -amino-butyric acid $\alpha$ -hydroxy-butyric acid, $\beta$ -hydroxy-D,L-butyric acid, $\alpha$ -keto-butyric acid.	It can utilize dextrin, D-maltose, D-trehalose, D-cellobiose, sucrose, D-turanose, $\alpha$ -D-lactose, D-salicin, <i>N</i> -acetyl-D-glucosamine, <i>N</i> -acetyl- $\beta$ -D-mannosamine, <i>N</i> -acetyl-D-galactosamine, $\alpha$ -D-glucose, D-fructose, D-galactose, D-fucose, L-fucose, inosine, D-mannitol, D-arabitol, myo-inositol, glycerol, D-aspartic acid, L-alanine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, pectin, D-gluconic acid, mucic acid, <i>p</i> -hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, $\alpha$ -keto-glutaric acid, D-malic acid, L-malic acid, Tween 40, $\gamma$ -amino-butyric acid $\alpha$ -hydroxy-butyric acid, $\beta$ -hydroxy-D,L-butyric acid, $\alpha$ -keto-butyric acid, acetoacetic acid, propionic acid, acetic acid.	It can utilize dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-melibiose, $\beta$ -methyl-D-glucoside, D-salicin, <i>N</i> -acetyl- $\beta$ -D-mannosamine, <i>N</i> -acetyl-D-galactosamine, $\alpha$ -D-glucose, D-mannose, D-fructose, D-Galactose, D-fucose, L-fucose, L-rhamnose, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-PO <sub>4</sub> , D-fructose-6-PO <sub>4</sub> , D-aspartic acid, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-histidine, L-pyroglutamic acid, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, $\alpha$ -keto-glutaric acid, D-malic acid, L-malic acid, Tween 40, $\gamma$ -amino-butyric acid $\alpha$ -hydroxy-butyric acid, $\beta$ -hydroxy-D,L-butyric acid, acetoacetic acid, propionic acid, acetic acid.

**Table S2.** Fatty acid comparison of strains SCSIO 75817<sup>T</sup>, SCSIO76264<sup>T</sup>, SCSIO 80058<sup>T</sup> and the closely related neighbors.

Strains: 1, SCSIO 75817<sup>T</sup>; 2, SCSIO 76264<sup>T</sup>; 3, SCSIO 80058<sup>T</sup>; 4, DSM 22220<sup>T</sup>; 5, MCCC 1A16381<sup>T</sup>; 6, CGMCC 1.13898<sup>T</sup>. All data were obtained from this study. Values are percentages of total fatty acids. The major fatty acids (greater than 10%) are shown in bold. TR, less than 1%. –, data not available.

Fatty acid	1	2	3	4	5	6
C <sub>16:0</sub>	TR	TR	TR	TR	TR	2.0
C <sub>18:0</sub>	5.2	5.5	8.1	6.3	6.0	<b>12.0</b>
C <sub>19:0</sub>	TR	TR	TR	TR	2.4	TR
C <sub>10:0</sub> 3OH	2.9	2.1	2.8	2.7	2.7	2.9
Anteiso-C <sub>15:0</sub>	–	1.8	TR	TR	TR	TR
Anteiso- C <sub>17:0</sub>	–	2.5	TR	TR	TR	TR
C <sub>18:1</sub> $\omega$ 7c	<b>86.8</b>	<b>84.0</b>	<b>78.2</b>	<b>85.4</b>	<b>84.2</b>	<b>77.8</b>
C <sub>18:1</sub> $\omega$ 7c 11-methyl	–	TR	1.2	TR	–	1.4
C <sub>19:0</sub> cyclo $\omega$ 8c	–	–	6.9	TR	1.1	1.5
C <sub>20:1</sub> $\omega$ 7c	2.2	1.1	1.1	1.7	TR	TR

**Table S3.** General genome features of the three new species and the reference strains. Strains: 1, SCSIO 75817<sup>T</sup>; 2, SCSIO 76264<sup>T</sup>; 3, SCSIO 80058<sup>T</sup>; 4, *P. isopora* DSM 22220<sup>T</sup>; 5, *P. aurantiacus* CGMCC 1.13898<sup>T</sup>; 6, *P. xiamenensis* MCCC 1A16381<sup>T</sup>.

Parameter	1	2	3	4	5	6
GenBank accession number	CP115780	CP15768	CP11575	FNAH000000000	VOPL000000000	JAAOHY0000000
Genome size (bp)	3,318,305	3,175,232	3,318,811	3,523,473	3,788,400	3,643,702
Number of Contigs	1	1	1	31	42	21
No. of genes	3,256	3,040	3,299	3,427	3,786	3,576
Plasmid	4	6	4	0	0	0
16S rRNA	2	2	2	1	1	1
23S rRNA	2	2	2	1	2	1
5S rRNA	2	2	2	1	2	1
tRNA	50	47	47	44	45	46
G+C content (mol%)	60.6	64.1	60.1	65.8	62.5	63.9

**Table S4.** Comparison of COG functions of three new species and the reference strains. Strains: 1, SCSIO 75817<sup>T</sup>; 2, SCSIO 76264<sup>T</sup>; 3, SCSIO 80058<sup>T</sup>; 4, *P. isopora* DSM 22220<sup>T</sup>; 5, *P. aurantiacus* CGMCC 1.13898<sup>T</sup>; 6, *P. xiamenensis* MCCC 1A16381<sup>T</sup>.

Code	1	2	3	4	5	6	Description
B	0	1	0	0	0	0	Chromatin structure and dynamics
C	212	210	209	188	194	199	Energy production and conversion
D	44	44	33	26	26	46	Cell cycle control, cell division, chromosome partitioning
E	315	347	377	289	283	322	Amino acid transport and metabolism
F	75	79	79	75	80	91	Nucleotide transport and metabolism
G	191	214	253	201	232	277	Carbohydrate transport and metabolism
H	104	97	110	78	80	124	Coenzyme transport and metabolism
I	130	161	166	101	115	190	Lipid transport and metabolism
J	178	184	187	164	171	212	Translation, ribosomal structure and biogenesis
K	228	209	263	169	212	228	Transcription
L	296	201	259	148	159	119	Replication, recombination and repair
M	206	195	182	188	175	199	Cell wall/membrane/envelope biogenesis
N	44	48	43	32	32	36	Cell motility
O	154	125	140	126	132	159	Posttranslational modification, protein turnover, chaperones
P	226	192	250	203	188	191	Inorganic ion transport and metabolism
Q	93	117	125	84	69	83	Secondary metabolites biosynthesis, transport and catabolism
R	0	0	0	0	0	180	General function prediction only
S	642	641	690	959	1195	248	Function unknown
T	89	82	89	80	83	104	Signal transduction mechanisms
U	92	96	67	52	71	38	Intracellular trafficking, secretion, and vesicular transport
V	44	31	40	49	51	86	Defense mechanisms
W	0	0	0	0	0	1	Extracellular structures
X	0	0	0	0	0	38	Mobilome: prophages, transposons
Z	2	1	0	0	0	1	Cytoskeleton

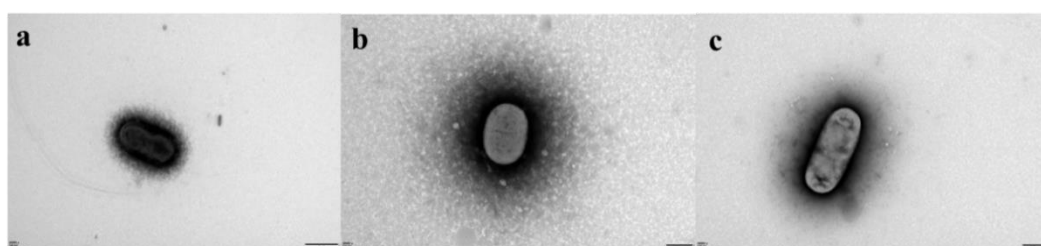
**Table S5.** The differences in the KEGG metabolic pathways of the genomes of strains SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup> and SCSIO 80058<sup>T</sup>.

Complete KEGG metabolic pathways	1	2	3
<b>Energy metabolism</b>			
<b>Nitrogen metabolism</b>			
M00531 Assimilatory nitrate reduction, nitrate => ammonia	+	-	-
<b>Lipid metabolism</b>			
<b>Fatty acid metabolism</b>			
M00086 beta-Oxidation, acyl-CoA synthesis	+	+	+
M00087 beta-Oxidation	-	+	+

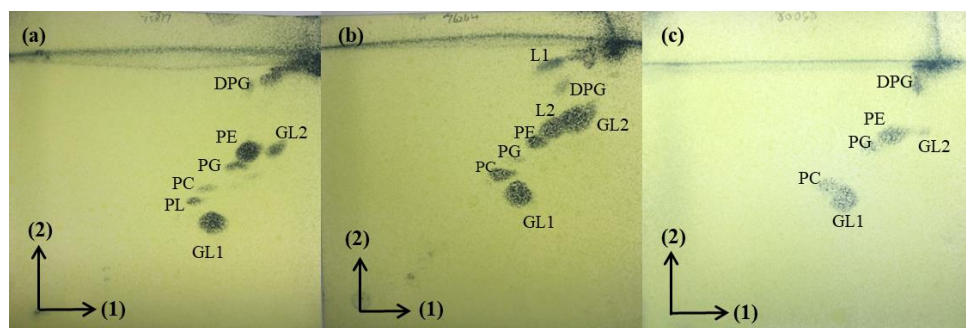
**Table S6.** Denitrification related genes of strains SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup> and SCSIO 80058<sup>T</sup> in nitrogen metabolism.

Genes	Gene function	SCSIO 75817 <sup>T</sup>	SCSIO 76264 <sup>T</sup>	SCSIO 80058 <sup>T</sup>
<i>nirS</i>	Cytochrome cd1 nitrite reductase (EC:1.7.2.1)	+	-	-
<i>nirK</i>	Copper-containing nitrite reductase (EC 1.7.2.1)	-	-	+
<i>nirV</i>	Nitrite reductase accessory protein NirV	-	-	+
<i>cnorB</i>	Nitric-oxide reductase subunit B (EC 1.7.99.7)	+	-	+
<i>cnorC</i>	Nitric-oxide reductase subunit C (EC 1.7.99.7)	+	-	+
<i>norE</i>	Nitric oxide reductase activation protein NorE	+	-	+
<i>norD</i>	Nitric oxide reductase activation protein NorD	+	-	+
<i>norQ</i>	Nitric oxide reductase activation protein NorQ	+	-	+
<i>nosZ</i>	Nitrous-oxide reductase (EC 1.7.99.6)	-	-	+
<i>nosD</i>	Nitrous oxide reductase maturation protein NosD	-	-	+
<i>nosF</i>	Nitrous oxide reductase maturation protein NosF (ATPase)	-	-	+
<i>nosY</i>	Nitrous oxide reductase maturation transmembrane protein NosY	-	-	+
<i>nosL</i>	Nitrous oxide reductase maturation protein, outer-membrane lipoprotein NosL	-	-	+

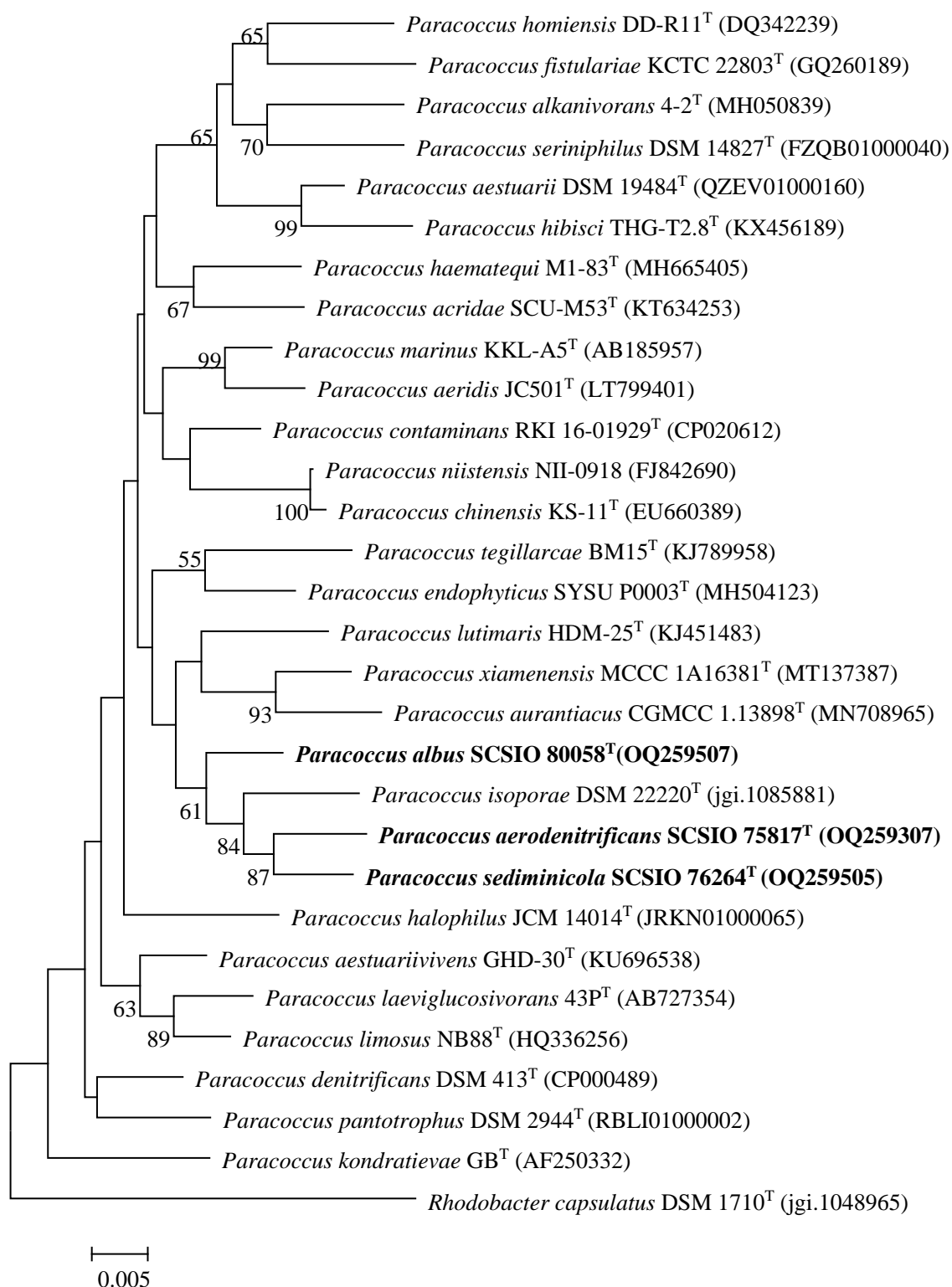
<i>nosX</i>	Nitrous oxide reductase maturation periplasmic protein NosX	+	-	-
<i>nosR</i>	Nitrous oxide reductase maturation protein NosR	+	-	+
<i>nnrS</i>	NnrS protein involved in response to NO	+	-	+
<i>nnrU</i>	NnrU family protein, required for expression of nitric oxide and nitrite reductases (Nir and Nor)	+	-	-



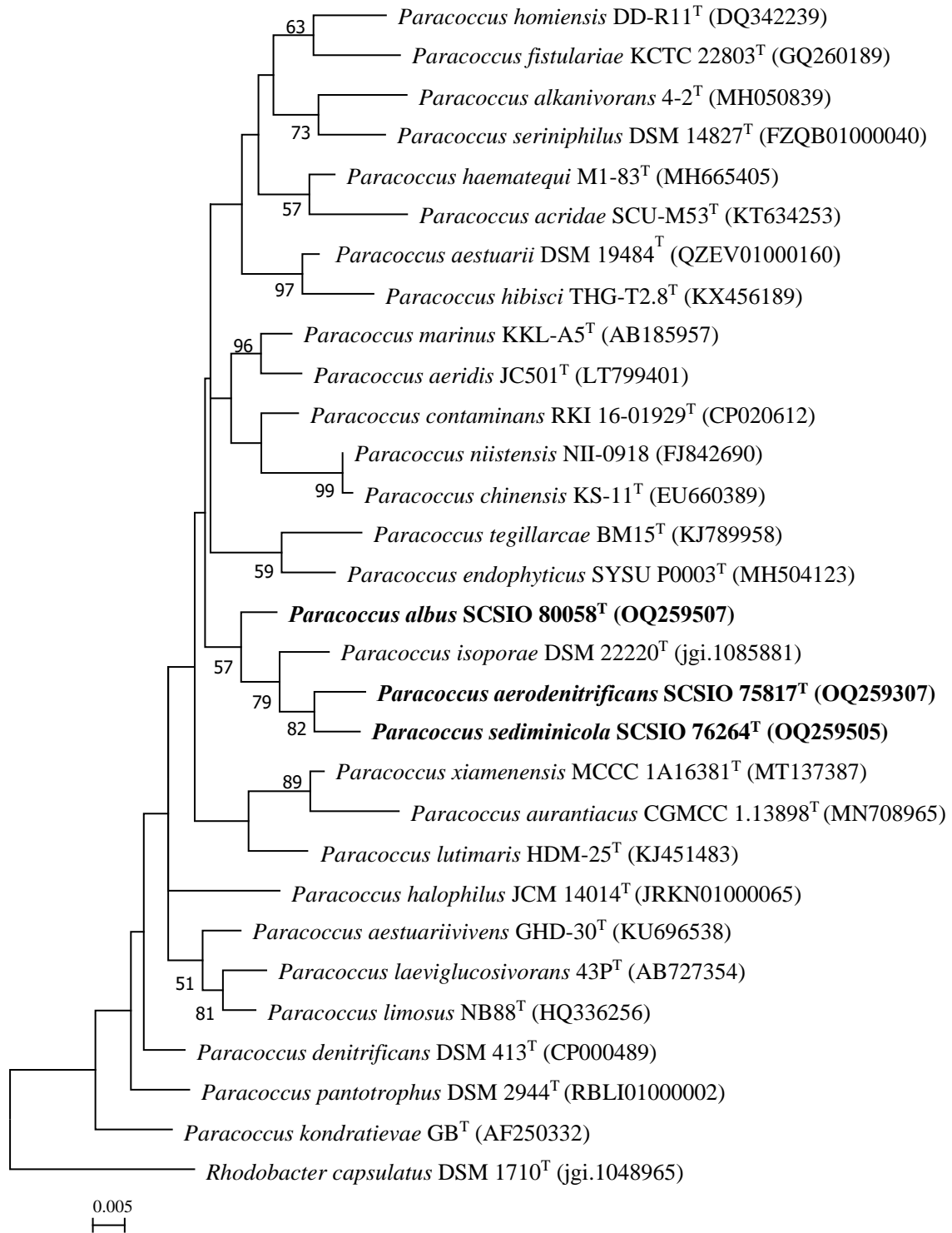
**Figure S1.** Transmission electron microscopy images show the cell morphology of strains SCSIO 75817<sup>T</sup> (a), SCSIO 76264<sup>T</sup> (b) and SCSIO 80058<sup>T</sup> (c) after grown on MA medium at 28 °C for 3 days. **a:** Bar, 1 µm; **b:** Bar, 0.5 µm; **c:** Bar, 0.5 µm.



**Figure S2.** Two-dimensional thin-layer chromatography of polar lipids of strains SCSIO 75817<sup>T</sup> (a), SCSIO 76264<sup>T</sup> (b) and SCSIO 80058<sup>T</sup> (c). The first direction developed in chloroform: methanol: water (65:25:4, v/v/v), and the second in chloroform: methanol: acetic acid: water (80:12:15:4, v/v/v/v). DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidyl choline; PL, unidentified phospholipid; GL, unidentified glycolipid; L, unidentified lipid.

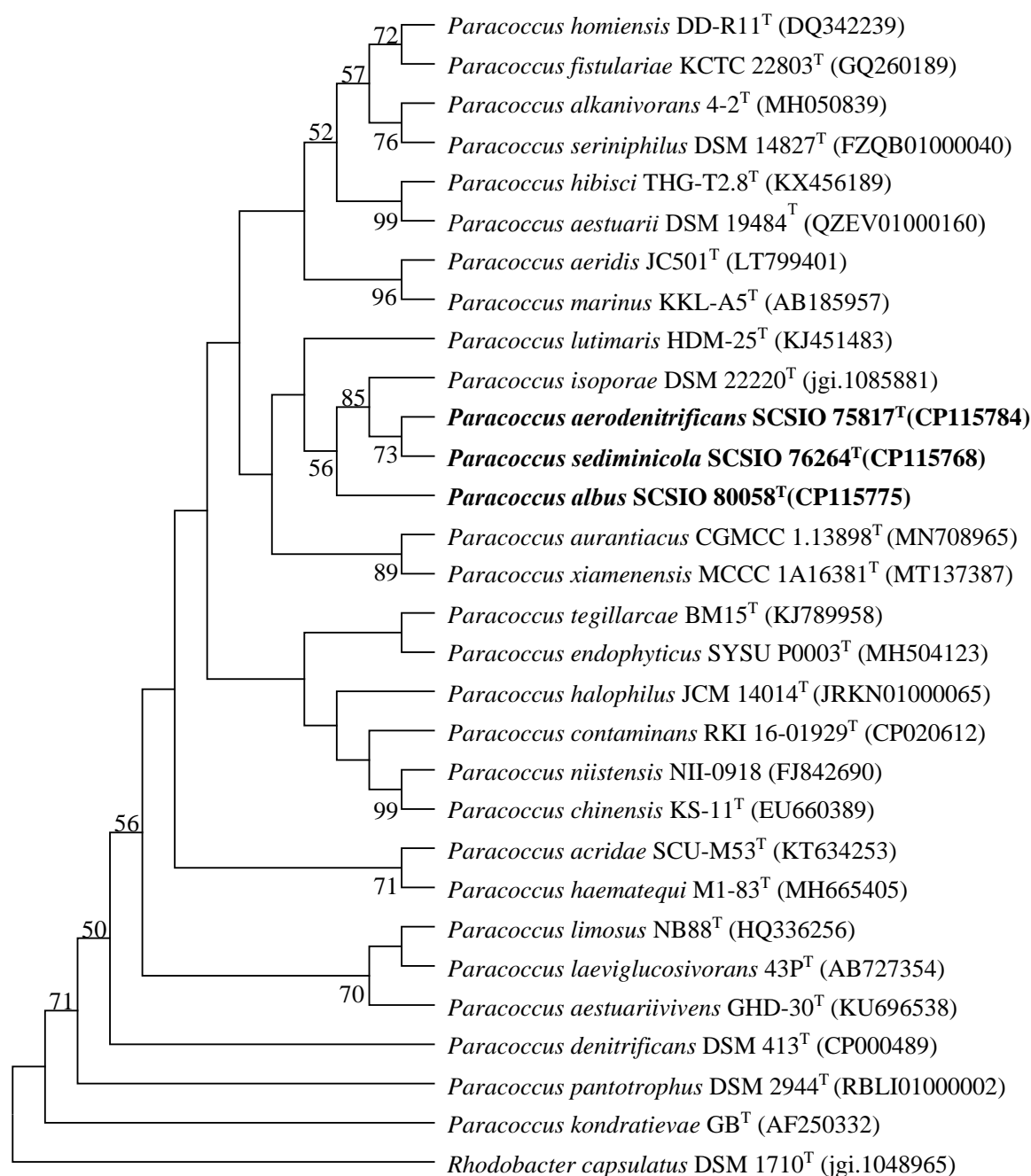


**Figure S3.** Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences of strains SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup> and SCSIO 80058<sup>T</sup> with their closest related taxa. *R. capsulatus* DSM 1710<sup>T</sup> was used as an outgroup. Numbers at nodes indicate the percentage of 1000 bootstrap replicates. Only bootstrap values above 50% are shown. Bar, 0.005 substitutions per nucleotide position.



**Figure S4.** The maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of strains SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup> and SCSIO 80058<sup>T</sup> with related taxa. Numbers at nodes indicate the bootstrap values (> 50%) based on 1000 replications. *Rhodobacter capsulatus* DSM 1710<sup>T</sup> (jgi.1048965) was an outgroup. Bar, 0.005 substitutions per nucleotide position.





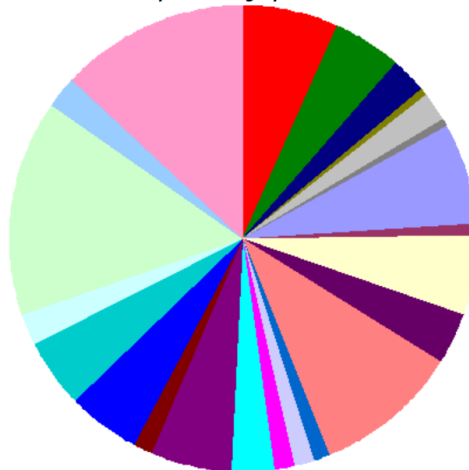
**Figure S5.** The maximum-parsimony phylogenetic tree shows relationships between strains SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup>, SCSIO 80058<sup>T</sup> and related taxa based on 16S rRNA gene sequences. Numbers at nodes refer to bootstrap values (1000 replicates; only values > 50% are shown). *Rhodobacter capsulatus* DSM 1710<sup>T</sup> was used as an outgroup.

### SCSIO 75817<sup>T</sup>

#### Subsystem Coverage



#### Subsystem Category Distribution



#### Subsystem Feature Counts

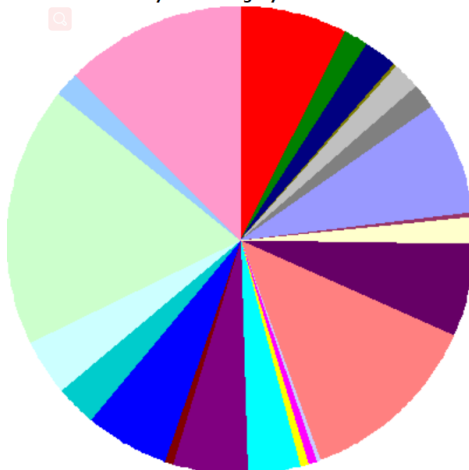
Cofactors, Vitamins, Prosthetic Groups, Pigments	(168)
Cell Wall and Capsule	(112)
Virulence, Disease and Defense	(63)
Potassium metabolism	(17)
Photosynthesis	(0)
Miscellaneous	(42)
Phages, Prophages, Transposable elements, Plasmids	(17)
Membrane Transport	(172)
Iron acquisition and metabolism	(20)
RNA Metabolism	(135)
Nucleosides and Nucleotides	(88)
Protein Metabolism	(242)
Cell Division and Cell Cycle	(30)
Motility and Chemotaxis	(30)
Regulation and Cell signaling	(35)
Secondary Metabolism	(4)
DNA Metabolism	(75)
Fatty Acids, Lipids, and Isoprenoids	(137)
Nitrogen Metabolism	(37)
Dormancy and Sporulation	(1)
Respiration	(121)
Stress Response	(111)
Metabolism of Aromatic Compounds	(58)
Amino Acids and Derivatives	(364)
Sulfur Metabolism	(4)
Phosphorus Metabolism	(54)
Carbohydrates	(310)

### SCSIO 76264<sup>T</sup>

#### Subsystem Coverage



#### Subsystem Category Distribution



#### Subsystem Feature Counts

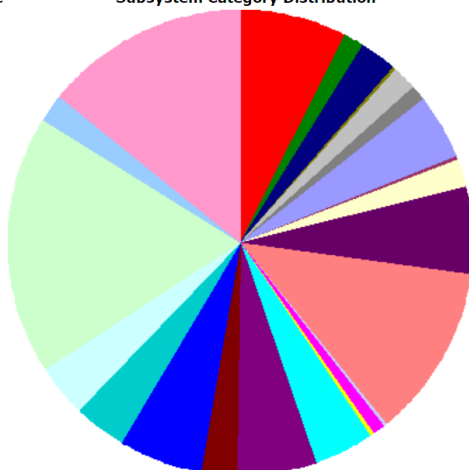
Cofactors, Vitamins, Prosthetic Groups, Pigments	(106)
Cell Wall and Capsule	(24)
Virulence, Disease and Defense	(30)
Potassium metabolism	(5)
Photosynthesis	(0)
Miscellaneous	(26)
Phages, Prophages, Transposable elements, Plasmids	(23)
Membrane Transport	(109)
Iron acquisition and metabolism	(5)
RNA Metabolism	(27)
Nucleosides and Nucleotides	(90)
Protein Metabolism	(176)
Cell Division and Cell Cycle	(0)
Motility and Chemotaxis	(5)
Regulation and Cell signaling	(9)
Secondary Metabolism	(5)
DNA Metabolism	(51)
Fatty Acids, Lipids, and Isoprenoids	(75)
Nitrogen Metabolism	(7)
Dormancy and Sporulation	(1)
Respiration	(80)
Stress Response	(41)
Metabolism of Aromatic Compounds	(53)
Amino Acids and Derivatives	(252)
Sulfur Metabolism	(2)
Phosphorus Metabolism	(25)
Carbohydrates	(168)

### SCSIO 80058<sup>T</sup>

#### Subsystem Coverage



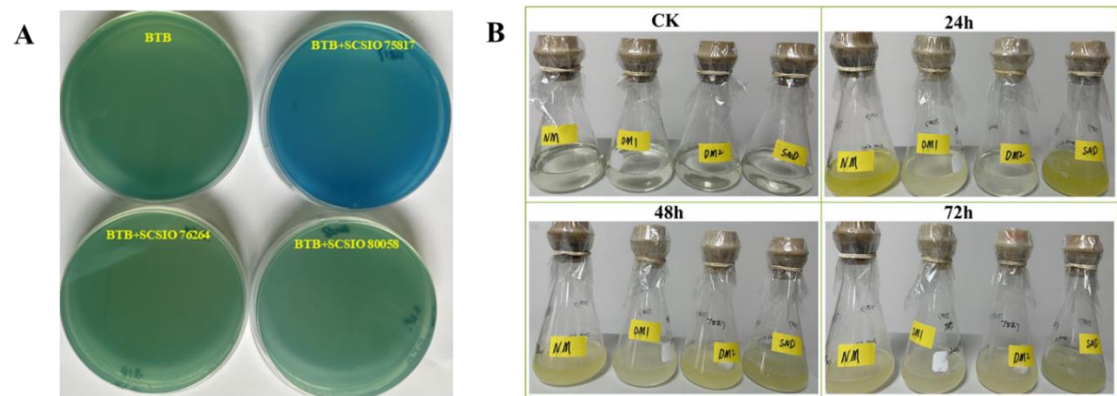
#### Subsystem Category Distribution



#### Subsystem Feature Counts

Cofactors, Vitamins, Prosthetic Groups, Pigments	(113)
Cell Wall and Capsule	(22)
Virulence, Disease and Defense	(37)
Potassium metabolism	(5)
Photosynthesis	(0)
Miscellaneous	(27)
Phages, Prophages, Transposable elements, Plasmids	(16)
Membrane Transport	(65)
Iron acquisition and metabolism	(7)
RNA Metabolism	(27)
Nucleosides and Nucleotides	(91)
Protein Metabolism	(180)
Cell Division and Cell Cycle	(0)
Motility and Chemotaxis	(5)
Regulation and Cell signaling	(13)
Secondary Metabolism	(5)
DNA Metabolism	(62)
Fatty Acids, Lipids, and Isoprenoids	(86)
Nitrogen Metabolism	(35)
Dormancy and Sporulation	(1)
Respiration	(89)
Stress Response	(53)
Metabolism of Aromatic Compounds	(55)
Amino Acids and Derivatives	(272)
Sulfur Metabolism	(3)
Phosphorus Metabolism	(28)
Carbohydrates	(209)

**Figure S6.** The subsystem category number of genes in strains SCSIO 75817<sup>T</sup>, SCSIO 76264<sup>T</sup> and SCSIO 80058<sup>T</sup> detected by RAST annotation server.



**Figure S7.** Screening of denitrifying bacteria using BTB plate (A) and culture of different sole nitrogen sources (B) including  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$ .