

Supplementary Material

Integrated ‘omic’ analyses provide evidence that a *Ca. Accumulibacter phosphatis* strain performs denitrification under micro-aerobic conditions

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Supplementary Figures

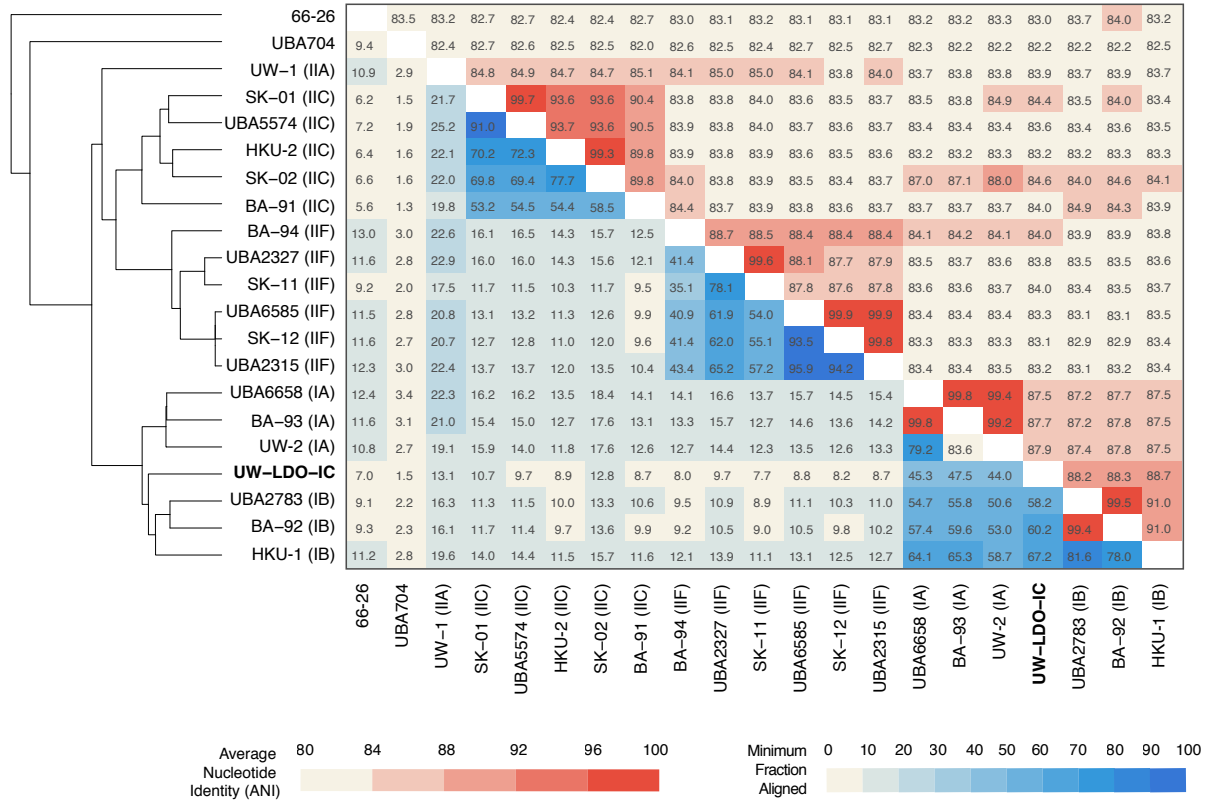


Figure S1. Comparison of the genome-wide average nucleotide identity and alignment percentage, of Accumulibacter-like genomes. The heatmap shows the average nucleotide identity (red low section of matrix) and the minimum percentage of the two genomes that aligned (blue upper section).

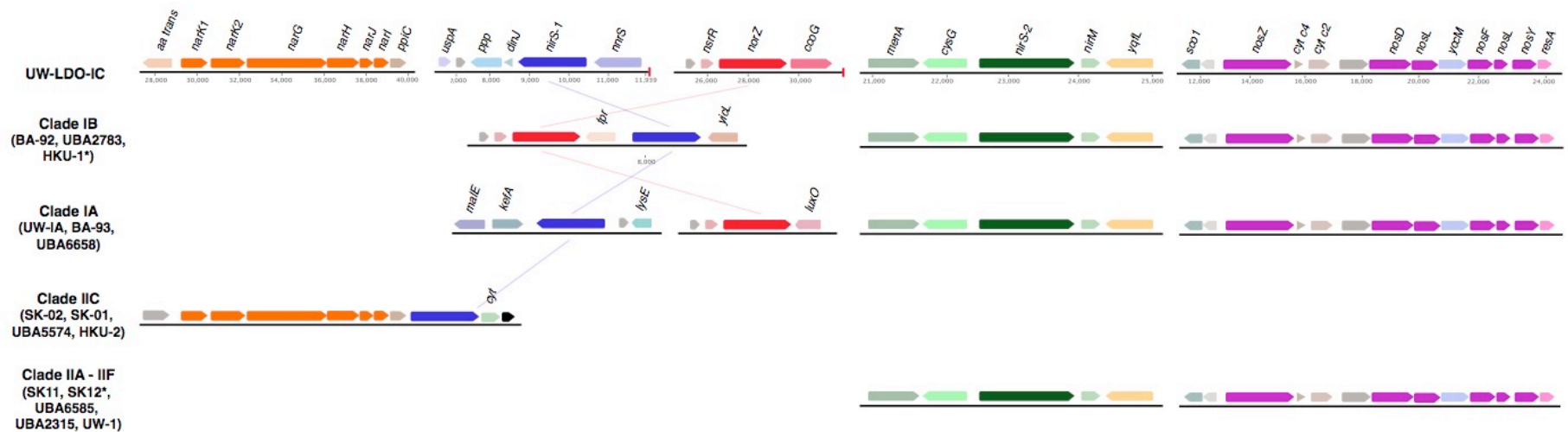


Figure S2. Schematic illustration of denitrifying genes loci in different genomes of *Accumulibacter*. Changes in the position of homologous genes are indicated by lines, the arrows show direction of transcription. Genes are drawn to scale. Proteins with unknown function are colored grey and transposases black. End of a scaffold is indicated with a red line. *aa trans*, aminoacid carrier family; *nar*, nitrate reductase; *ppiC*, peptidyl-prolyl cis-trans isomerase C; *uspA*, universal stress protein A, *ppp*, protein phosphatase; *dinJ*, DNA-damage-inducible protein J, *nir*, nitrite reductase; *nsrR*, Nitric oxie-sensitive repressor; *nor*, nitric oxide reductase; *ccoG*, type cbb3 cytochrome oxidase biogenesis protein; *menA*, 1,4-dihydroxy-2-naphthoate octaprenyltransferase; *cysG*, siroheme synthase; *yqjL*, ATP/GTP-binding protein; *sco1*, cytochrome c oxidase biogenesis protein; *nos*, nitrous oxide reductase; *cyt*, cytochrome; *resA*, cytochrome c-type biogenesis protein; *fpr*, Ferredoxin—NADP reductase; *yicL*, carboxylate/amino acid/amine transporter; *malE*, carboxylate/amino acid/amine transporter; *kefA*, potassium efflux system protein; *lysE*, L-lysine exporter family protein; *luxO*, two-component signal response regulator.
*Genome lacks one of the scaffolds.

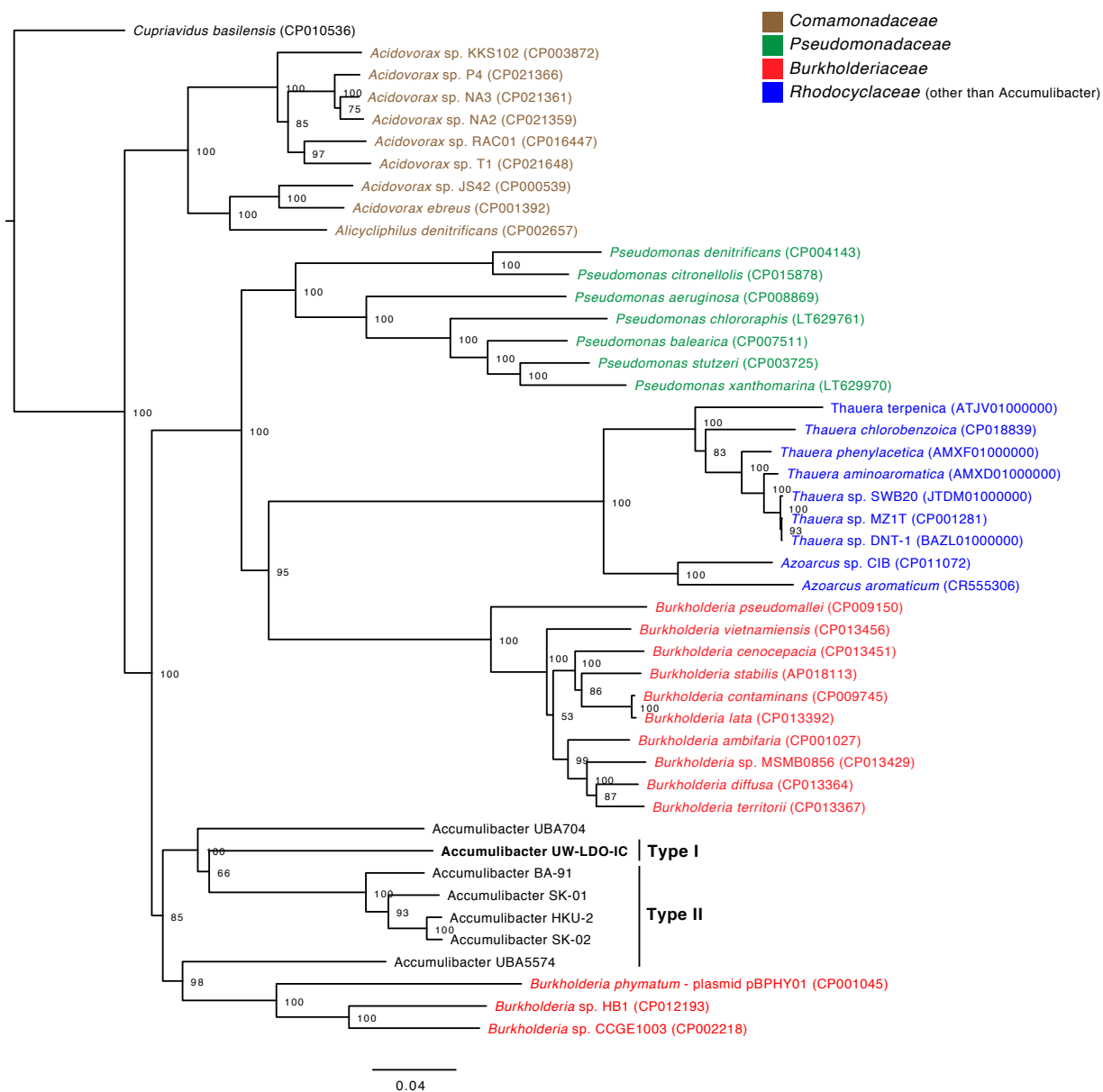


Figure S3. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to *narG* found in the genome of Accumulibacter and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

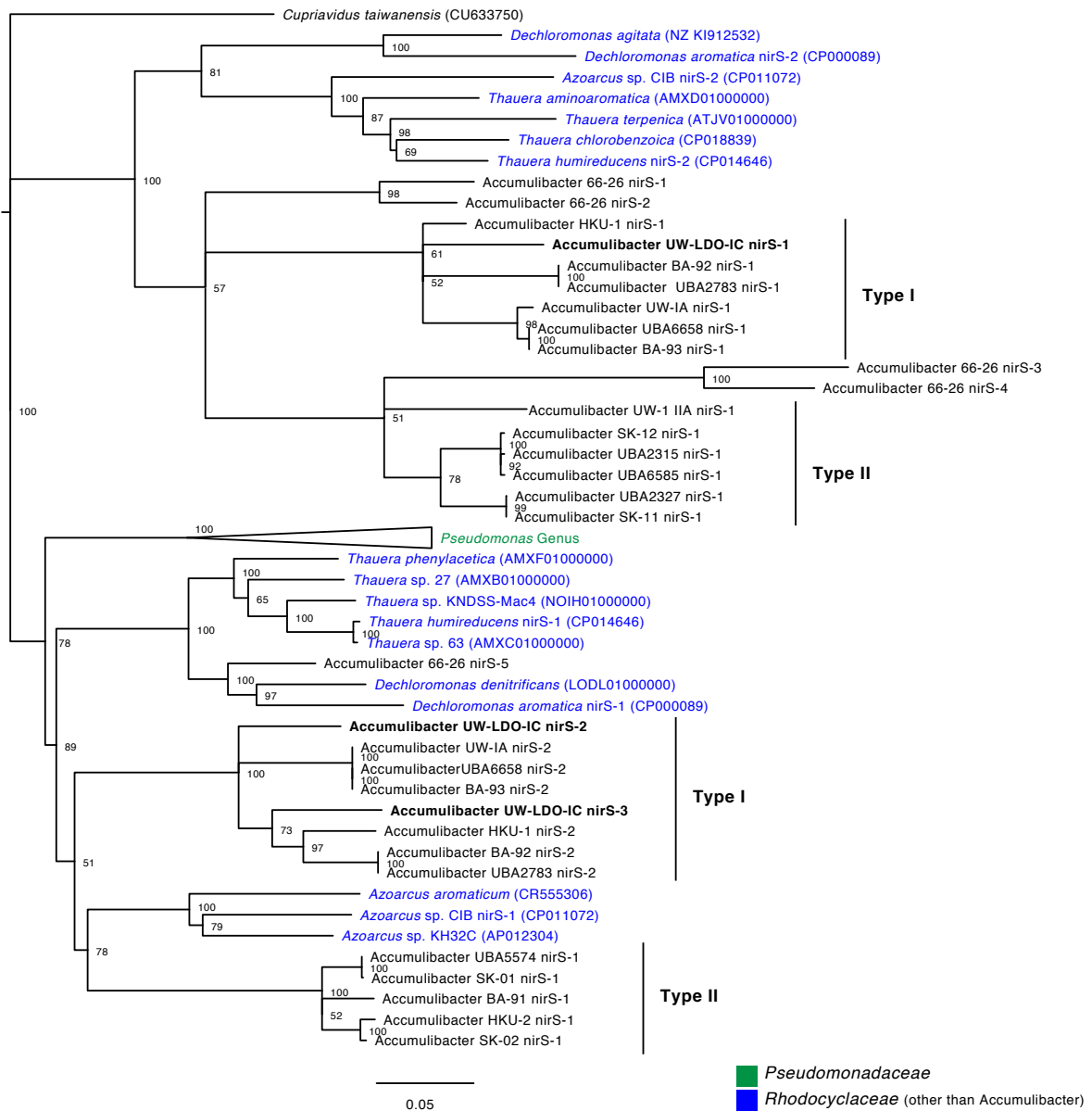


Figure S4. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to *nirS* found in the genome of *Accumulibacter* and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

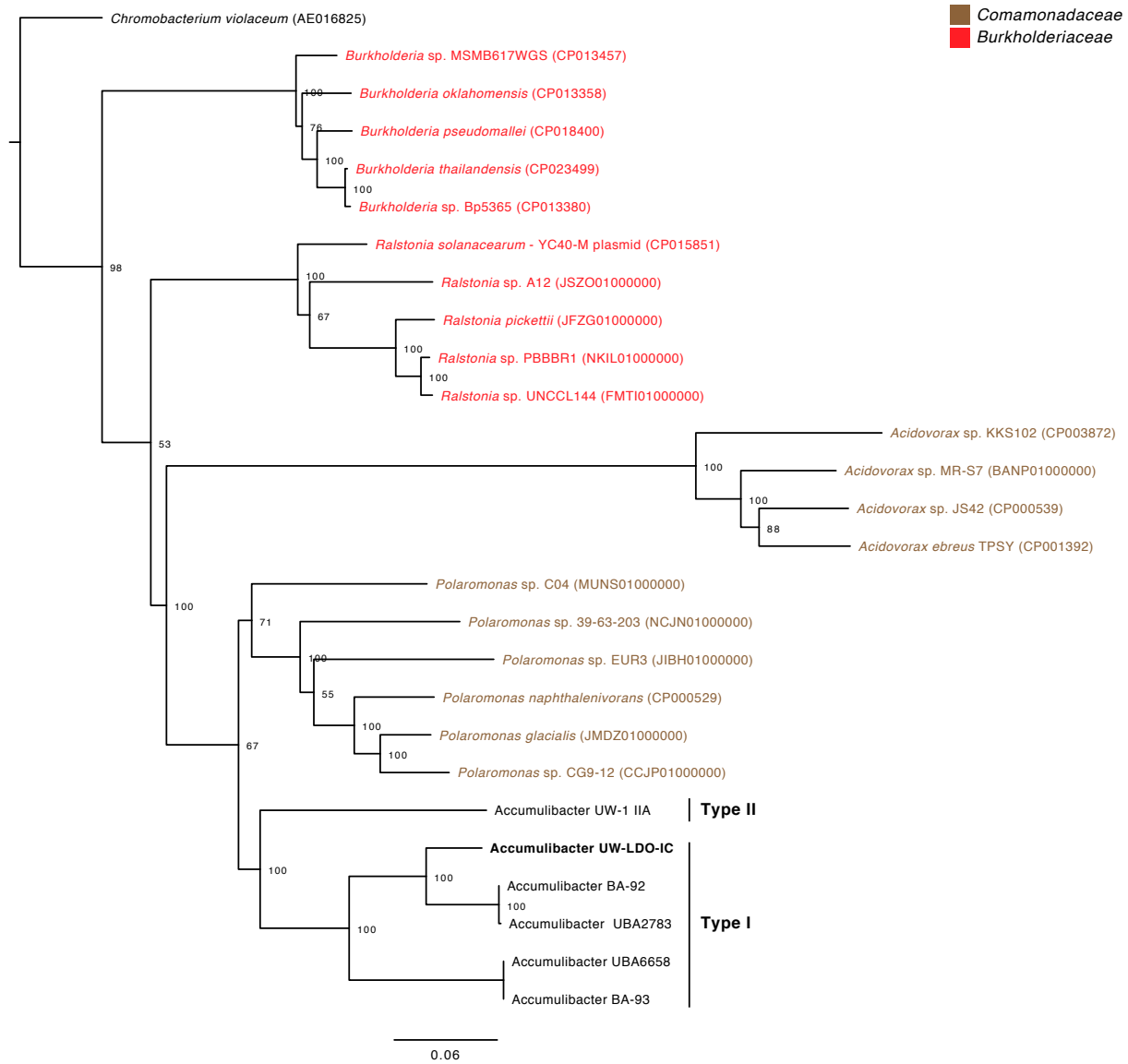


Figure S5. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to *norZ* found in the genome of *Accumulibacter* and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

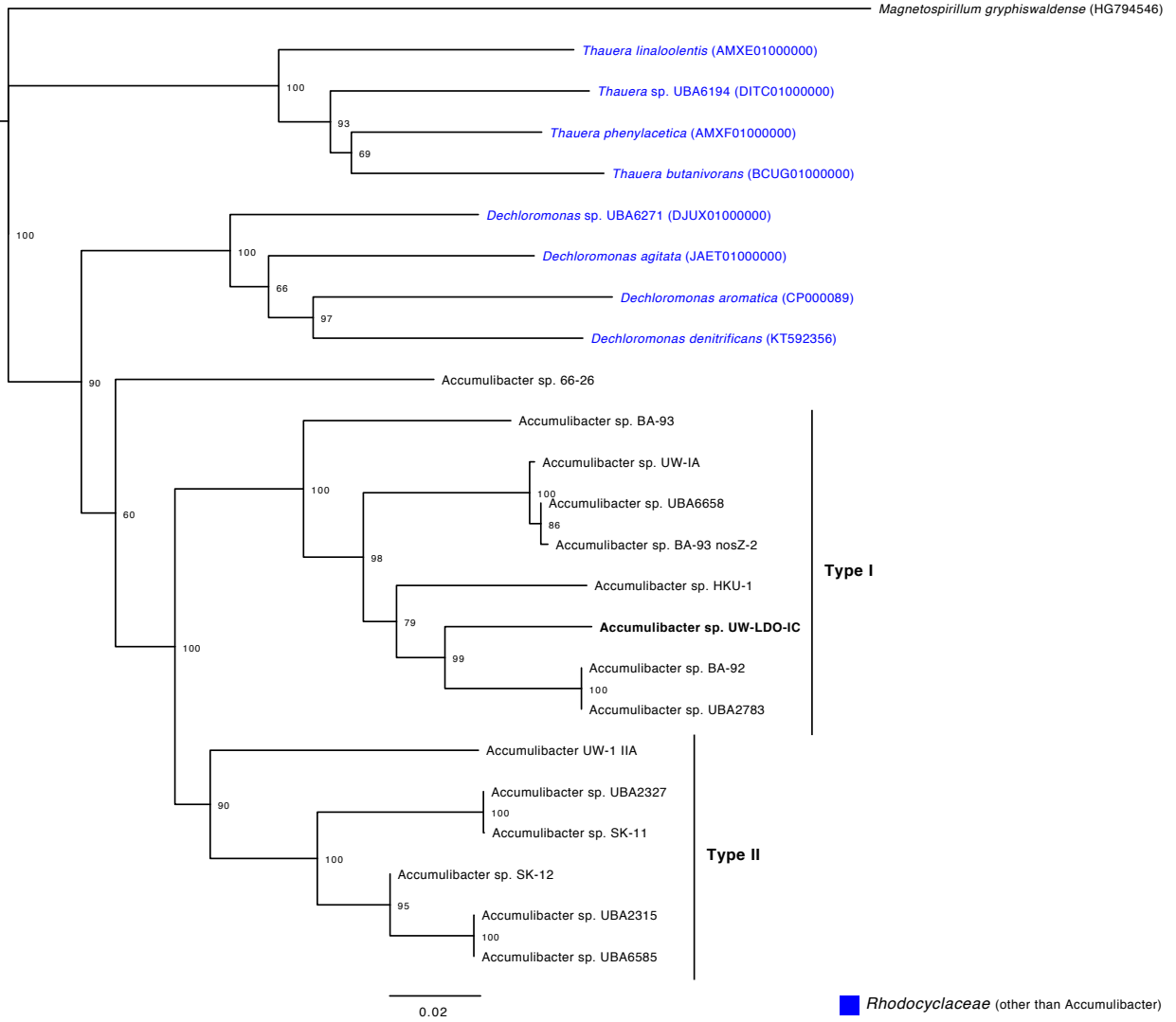


Figure S6. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to *nosZ* found in the genome of *Accumulibacter* and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

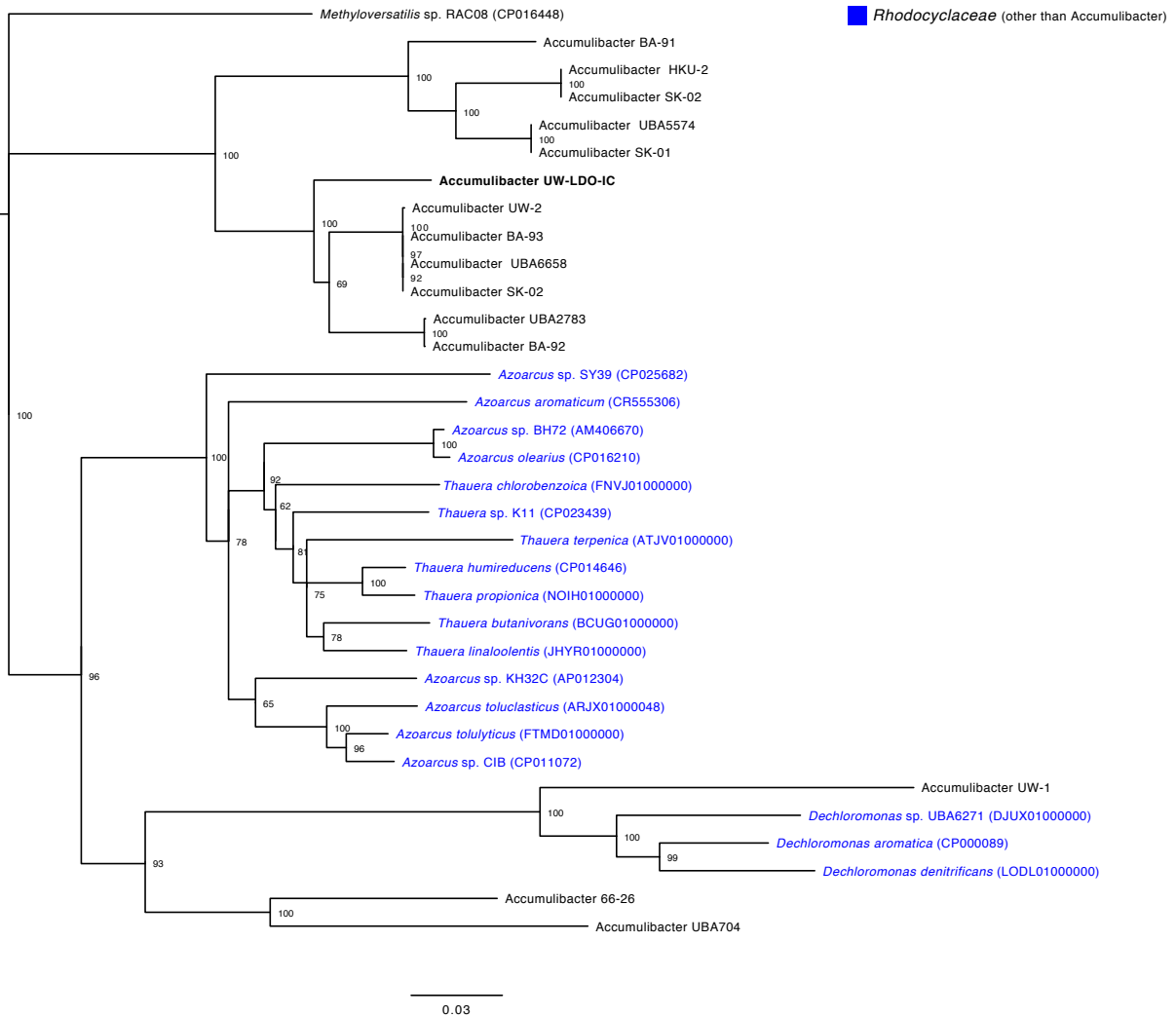


Figure S7. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to the cytochrome c oxidase *aa3* subunit I (*ctaD*), found in the genome of *Accumulibacter* and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

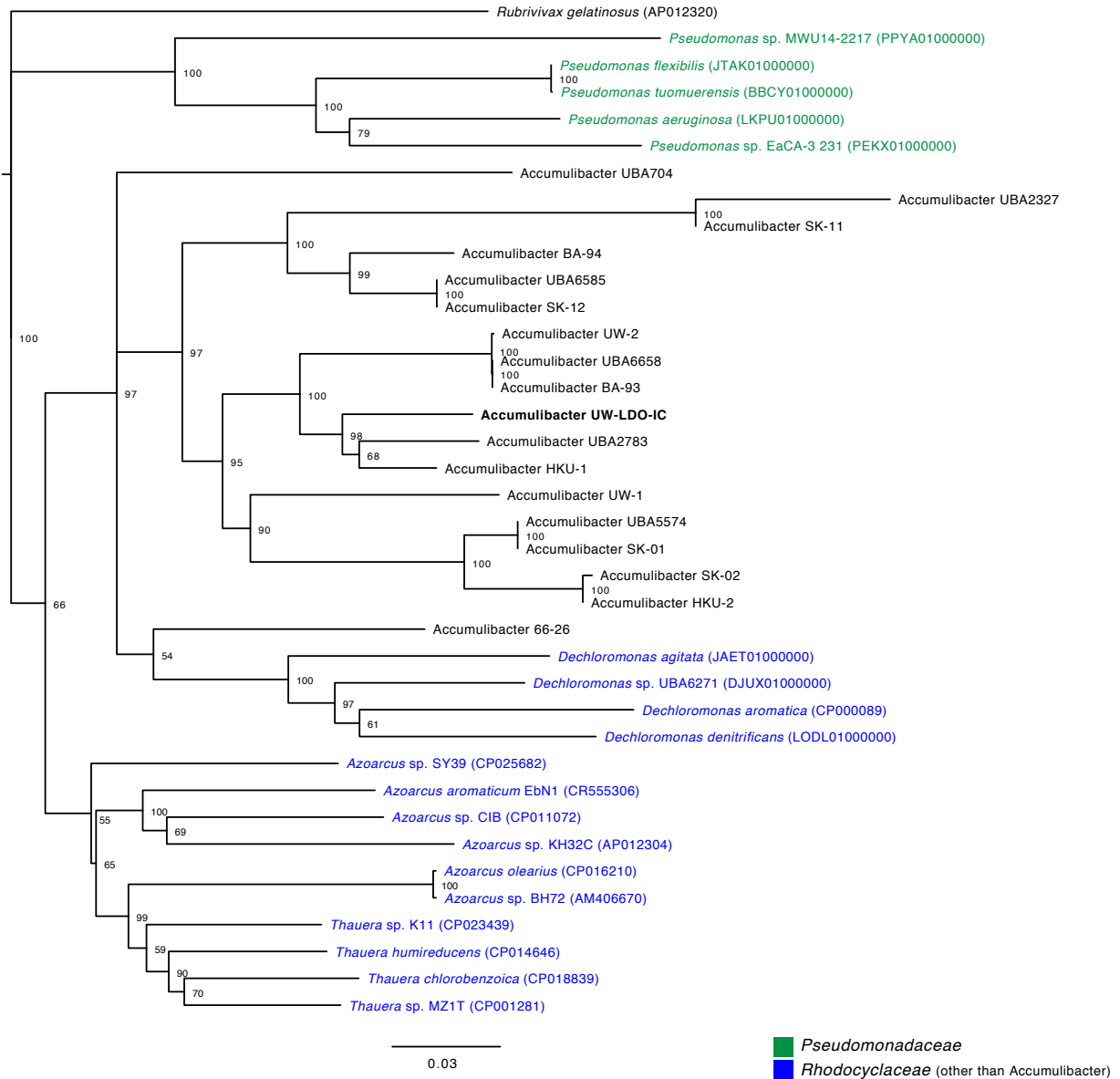


Figure S8. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to the cytochrome c oxidase *cbb3* subunit I (*ccoN*), found in the genome of *Accumulibacter* and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

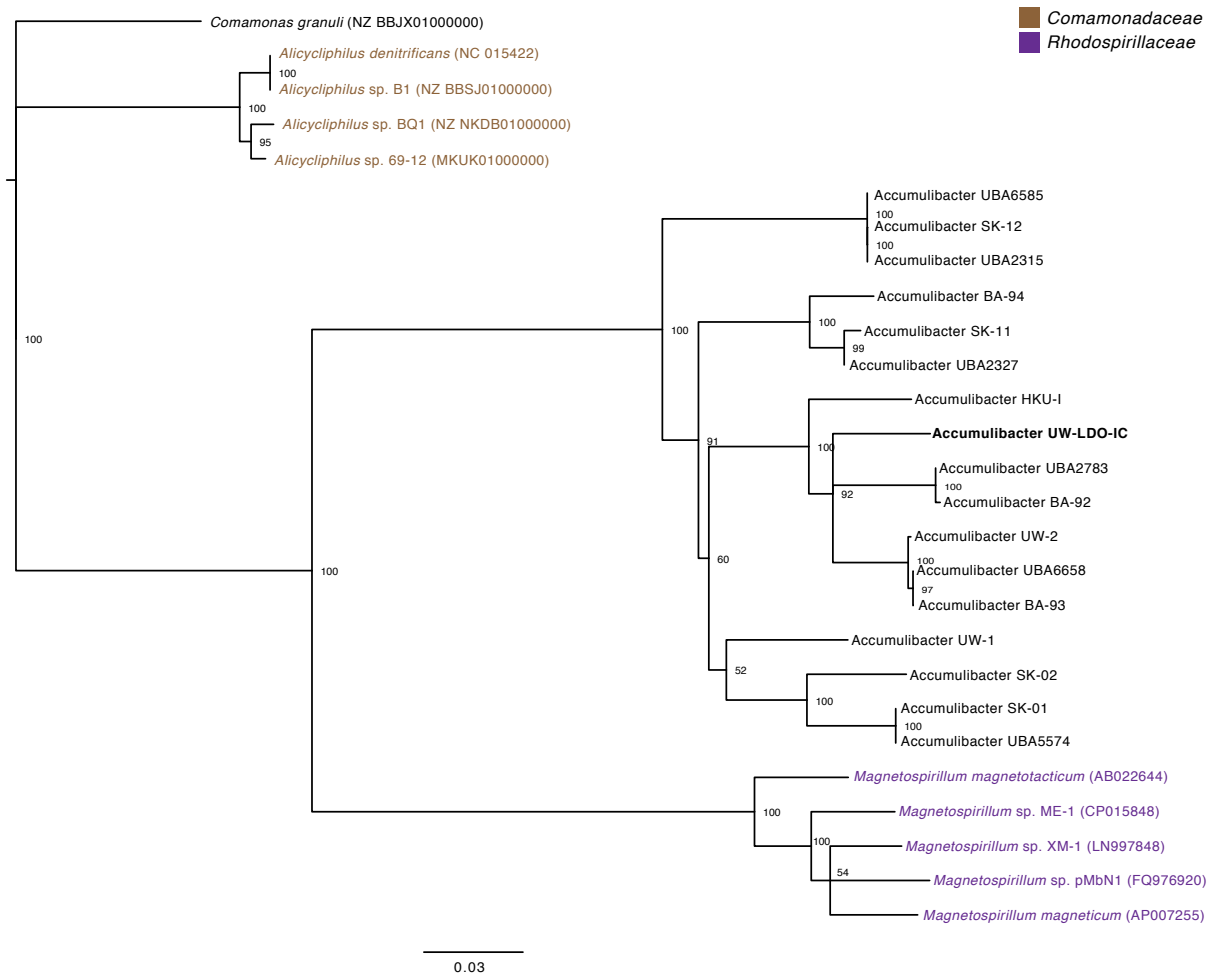


Figure S9. Neighbor-joining phylogenetic tree based on the full-length nucleic acid sequences of homologs to the cytochrome c oxidase ba_3 subunit I (*cbaA*), found in the genome of *Accumulibacter* and annotated genomes in NCBI. Bootstrap values are shown in the tree branches based on 1000 bootstrap replicates. The scale bar represents the number of nucleotide substitutions per site.

Supplementary Tables

Table S1. Bins assembled from metagenomic sample from day-522, taxonomic classification based on PhyloSift and metrics from CHECKM.

Bin Id	Lowest Assigned Taxonomy Rank	GC (%)	Genome size (Mbp)	# Scaffolds	N50	Completeness (%)	Redundancy (%)	Strain heterogeneity (%)	Mean Coverage 522-day
Bin 001	Unresolved	60.3	0.15	5	133,274	0.0	0.0	0.0	1.75
Bin 002	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae	58.1	1.88	95	32,415	70.1	0.5	0.0	7.93
Bin 003	Bacteria; Proteobacteria; Gammaproteobacteria	57.9	1.19	104	19,456	18.9	0.0	0.0	4.67
Bin 004	Unresolved	61.4	0.46	71	9,751	0.0	0.0	0.0	1.62
Bin 005	Unresolved	53.2	0.44	90	8,194	0.0	0.0	0.0	1.34
Bin 006	Unresolved	60.8	0.44	117	5,609	4.2	0.0	0.0	1.26
Bin 007	Unresolved	55.0	0.32	59	10,982	0.0	0.0	0.0	0.52
Bin 008	Bacteria; Verrucomicrobia	62.6	3.88	26	501,618	96.8	3.4	0.0	5.61
Bin 009	Unresolved	45.2	0.23	59	5,784	0.0	0.0	0.0	0.26
Bin 010	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Saprospiraceae; Lewinella	55.6	2.21	24	176,250	39.7	0.0	0.0	2.28
Bin 011	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Saprospiraceae; Lewinella	54.7	2.10	23	127,440	31.0	0.0	0.0	2.23
Bin 012	Bacteria	62.0	2.81	50	2,659,120	94.9	1.9	0.0	2.91
Bin 013	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Hyphomonadaceae	45.3	2.85	67	182,623	97.6	1.0	25.0	1.97
Bin 014	Bacteria; Proteobacteria	58.4	1.44	299	7,814	1.8	0.0	0.0	0.92
Bin 015	Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae; Caldilinea	64.3	5.77	307	38,066	92.3	1.1	0.0	3.36
Bin 016	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae	67.7	5.05	273	46,256	98.3	37.7	0.9	2.76
Bin 017	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Cryomorphaeae; Fluvicola; Fluvicola	60.8	3.85	87	78,231	98.9	0.2	0.0	1.92
Bin 018	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Dechloromonas	63.8	2.65	439	11,996	47.8	5.5	54.2	1.20
Bin 019	Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Flammeovirgaceae; Fulvivirga	42.5	4.32	79	1,137,187	98.8	2.1	11.1	1.85
Bin 020	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae	69.9	2.47	54	101,360	75.2	0.0	0.0	1.00
Bin 021	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; Beijerinckia	67.0	3.41	33	246,859	99.1	1.0	0.0	1.26
Bin 022	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	68.9	1.25	258	8,604	17.2	0.0	0.0	0.43
Bin 023	Bacteria	57.0	3.53	178	329,776	95.4	2.8	0.0	1.10
Bin 024	Bacteria	34.8	6.28	239	74,362	94.6	2.0	0.0	1.74
Bin 025	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales	57.4	5.28	158	369,154	100.0	6.9	94.1	1.30
Bin 026	Bacteria; Bacteroidetes; Flavobacteriia; Bacteroidetes	46.3	4.59	94	88,733	97.0	2.5	0.0	1.07
Bin 027	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	66.6	2.70	220	22,667	99.0	7.0	78.3	0.53
Bin 028	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	67.2	3.50	590	11,319	75.9	10.3	20.0	0.67
Bin 029	Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Flammeovirgaceae; Fulvivirga	42.1	4.68	90	246,705	99.7	1.6	0.0	0.77
Bin 030	Bacteria; Proteobacteria; Gammaproteobacteria	56.6	3.64	70	129,915	93.1	29.7	34.8	0.62
Bin 031.1	Bacteria; Proteobacteria; Gammaproteobacteria	59.6	2.81	84	166,389	72.7	2.3	0.0	0.44

Completeness, redundancy and strain heterogeneity were assessed with CHECKM 0.7.1, according to the presence of 43 single-copy reference gene. Bins designated as “Bin XXX.X” were generated using anvi'o (*See Methods*).

Table S1. Bins assembled from metagenomic sample from day-522, taxonomic classification based on PhyloSift and metrics from CHECKM (*Cont*).

Bin Id	Lowest Assigned Taxonomy Rank	GC (%)	Genome size (Mbp)	# Scaffolds	N50	Completeness (%)	Redundancy (%)	Strain heterogeneity (%)	Mean Coverage 522-day
Bin 031.2	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales	59.4	1.84	256	14,316	28.7	4.5	25.9	0.28
Bin 032	Bacteria; Ignavibacteriae; Ignavibacteria; Ignavibacteriales; Ignavibacteriaceae; Ignavibacterium	39.2	4.42	103	113,436	98.3	0.6	0.0	0.68
Bin 033	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	65.4	4.46	333	153,360	98.3	25.5	34.2	0.63
Bin 034	Bacteria; Proteobacteria; Gammaproteobacteria	62.3	4.47	586	24,795	87.9	40.3	14.4	0.64
Bin 035	Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Verrucomicrobium	58.7	8.63	310	173,586	99.3	8.8	8.7	1.03
Bin 036	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae	55.6	2.96	213	37,364	96.8	11.3	0.0	0.35
Bin 037	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Methylobacteriaceae	63.0	4.94	243	569,899	98.3	37.0	16.8	0.59
Bin 038.2	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera	69.6	1.90	252	12,628	63.2	1.8	100.0	0.30
Bin 038.3	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera	70.7	2.69	589	7,493	22.3	7.0	50.0	0.27
Bin 039	Bacteria; Proteobacteria; Betaproteobacteria; Gallionellales; Gallionellaceae; Gallionella	49.6	2.19	536	5,330	85.7	5.0	84.2	0.26
Bin 040	Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Verrucomicrobium	60.8	7.21	277	52,427	93.6	6.9	65.2	0.83
Bin 041	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Rhodospirillum	64.9	3.95	176	197,681	100.0	18.2	11.1	0.43
Bin 042	Bacteria; Proteobacteria; Deltaproteobacteria	33.1	3.29	61	629,816	96.1	0.7	0.0	0.34
Bin 043	Bacteria	51.5	3.15	90	221,518	95.3	4.8	66.7	0.32
Bin 044	Bacteria; Actinobacteria; Actinobacteria; Acidimicrobiales	72.7	5.44	558	294,835	99.2	13.3	0.0	0.54
Bin 045	Bacteria; Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae; Nitrosomonas	49.4	2.86	296	16,241	88.3	2.7	64.3	0.28
Bin 046	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Accumulibacter phosphatis	62.6	6.60	666	23,909	94.8	28.9	20.3	0.64
Bin 047	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales	63.9	4.39	782	13,343	79.7	21.9	27.9	0.39
Bin 048	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae	68.6	4.24	262	119,328	90.6	8.6	0.0	0.39
Bin 049.1	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera	67.8	0.83	180	8,572	9.3	2.7	63.2	0.07
Bin 049.2	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera	67.5	2.18	213	23,756	34.2	3.5	100.0	0.19
Bin 049.3	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera	68.1	1.50	225	14,219	40.4	3.5	100.0	0.14
Bin 049.4	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera	67.5	1.09	316	4,763	31.6	0.9	30.8	0.13
Bin 050	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter	67.1	3.68	260	32,433	96.1	5.4	19.2	0.30
Bin 051	Bacteria; Bacteroidetes; Cytophagia	44.3	4.46	211	48,531	89.1	1.2	0.0	0.35
Bin 052	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae; Phycisphaera	63.8	3.53	138	136,152	95.5	1.9	0.0	0.27
Bin 053	Bacteria; Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae; Anaerolinea	53.8	6.46	1,214	9,633	90.2	27.5	15.2	0.50
Bin 054	Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	62.3	7.57	1,074	19,131	99.3	16.9	19.1	0.55
Bin 055.1	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Saprospiraceae	52.6	1.50	721	2,272	29.3	1.7	100.0	0.06
Bin 055.2	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Saprospiraceae	50.2	6.61	293	46,761	99.5	2.5	16.7	0.56
Bin 056	Bacteria; Proteobacteria; Gammaproteobacteria	59.1	2.25	419	10,463	32.8	4.9	50.0	0.17

Completeness, redundancy and strain heterogeneity were assessed with CHECKM 0.7.1, according to the presence of 43 single-copy reference gene. Bins designated as “bin.XXX.X” were generated using anvi’o (*See Methods*).

Table S1. Bins assembled from metagenomic sample from day-522, taxonomic classification based on PhyloSift and metrics from CHECKM (*Cont*).

Bin Id	Lowest Assigned Taxonomy Rank	GC (%)	Genome size (Mbp)	# Scaffolds	N50	Completeness (%)	Redundancy (%)	Strain heterogeneity (%)	Mean Coverage 522-day
Bin 057	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales	57.9	2.43	376	10,849	61.3	15.5	84.0	0.17
Bin 058	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales	40.2	3.39	132	75,779	97.8	1.0	0.0	0.23
Bin 059	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales	60.8	4.07	834	9,247	60.3	30.2	43.5	0.27
Bin 060	Bacteria	70.1	5.83	239	51,428	97.9	6.8	9.1	0.38
Bin 061	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales	71.0	6.75	1,221	34,227	86.7	15.4	5.6	0.44
Bin 062	Bacteria; Actinobacteria; Actinobacteria; Acidimicrobiales; Acidimicrobiaceae	41.3	2.11	148	91,911	99.2	11.0	0.0	0.14
Bin 063	Bacteria; Planctomycetes; Planctomycetia; Planctomycetales; Planctomycetaceae; Planctomyces	56.4	7.39	1,206	11,128	95.5	2.3	100.0	0.47
Bin 064	Bacteria	47.4	2.14	842	2,929	15.2	0.8	0.0	0.13
Bin 065	Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Verrucomicrobium	60.8	6.31	1,166	10,868	83.5	15.6	30.6	0.36
Bin 066	Unresolved	59.7	6.64	1,127	21,161	100.0	43.7	2.7	0.38
Bin 067	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Saprospiraceae; Lewinella	39.1	7.07	982	16,257	100.0	55.3	0.0	0.40
Bin 068	Bacteria; Chloroflexi	66.0	5.63	568	17,767	97.3	8.8	25.0	0.31
Bin 069	Bacteria	62.3	2.13	378	10,706	87.9	25.9	2.9	0.12
Bin 070	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales	37.0	4.26	771	15,497	98.7	9.3	9.5	0.23
Bin 071	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales	66.4	3.39	972	4,531	49.8	34.8	53.9	0.20
Bin 072	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales	46.7	4.19	1,020	6,192	93.4	5.6	57.1	0.23
Bin 073	Bacteria; Proteobacteria; Alphaproteobacteria	41.8	3.14	983	4,183	82.3	17.1	3.2	0.17
Bin 074	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Sphingobacteriaceae; Niabella	40.4	6.78	1,798	10,294	99.5	53.7	5.5	0.35
Bin 075	Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Flammeovirgaceae; Fulvivirga	42.9	3.84	788	7,441	91.4	23.8	8.9	0.20
Bin 076	Bacteria; Actinobacteria; Actinobacteria	40.7	2.10	513	7,103	96.6	31.6	1.7	0.09
Bin 077	Bacteria	57.3	3.41	701	10,279	91.7	15.3	5.1	0.16
Bin 078	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae	35.6	6.32	1,559	7,104	92.2	46.5	4.0	0.29
Bin 079	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	65.6	4.06	1,406	3,852	71.6	55.7	26.4	0.18
Bin 080	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	69.0	3.89	1,574	2,847	46.0	28.1	25.0	0.18
Bin 081	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	69.0	6.20	2,724	2,552	77.7	65.5	16.7	0.30
Bin 082	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae	67.5	4.21	1,806	2,664	63.6	32.9	18.2	0.19
Bin 083	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae	65.3	1.33	566	2,758	32.7	5.5	37.5	0.06
Bin 084	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae	64.0	1.19	415	4,346	19.1	2.6	12.8	0.05
Bin 085	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae; Hyphomicrobium	66.0	4.85	1,315	5,676	49.7	24.1	0.0	0.21
Bin 086	Bacteria; Bacteroidetes	48.0	1.27	633	2,002	13.3	1.0	0.0	0.06
Bin 087	Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae; Caldilinea	59.3	6.80	1,633	5,903	84.0	6.6	12.5	0.29

Completeness, redundancy and strain heterogeneity were assessed with CHECKM 0.7.1, according to the presence of 43 single-copy reference gene

Bins designated as “bin.XXX.X” were generated using anvi'o (*See Methods*).

Table S1. Bins assembled from metagenomic sample from day-522, taxonomic classification based on PhyloSift and metrics from CHECKM (*Cont*).

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Bin 088	Bacteria; Bacteroidetes	44.1	1.43	561	3,129	6.0	0.0	0.0	0.06
Bin 089	Bacteria; Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bdellovibrionaceae; Bdellovibrio	53.8	4.56	1,371	6,984	94.7	64.0	2.9	0.19
Bin 090	Bacteria; Bacteroidetes	43.8	2.82	819	4,720	85.8	9.9	3.6	0.11
Bin 091	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales	67.0	8.09	3,579	2,391	65.5	59.5	3.4	0.32
Bin 092	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	65.8	0.64	333	1,995	21.0	12.3	0.0	0.03
Bin 093	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	65.5	0.51	290	1,714	23.3	23.5	9.4	0.02
Bin 094	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales	72.2	6.56	2,711	2,926	62.1	18.1	26.7	0.26
Bin 095	Bacteria; Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bdellovibrionaceae; Bdellovibrio	38.3	2.98	1,141	2,768	80.5	37.8	1.9	0.11
Bin 096	Bacteria	57.7	3.88	1,960	1,962	49.0	39.4	2.8	0.15
Bin 097.1	Bacteria; Proteobacteria; Betaproteobacteria	62.9	1.23	651	1,962	27.1	1.7	14.3	0.05
Bin 097.2	Bacteria; Proteobacteria; Gammaproteobacteria	63.4	3.13	1,645	1,941	24.3	3.4	0.0	0.11
Bin 097.3	Bacteria; Proteobacteria; Gammaproteobacteria	63.2	2.54	1,101	2,687	51.5	9.6	0.0	0.09
Bin 097.4	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Accumulibacter phosphatis	63.5	0.93	274	4,779	27.0	4.3	33.3	0.04
Bin 098	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae; Phycisphaera	69.6	6.72	2,642	3,009	88.7	56.8	1.2	0.25
Bin 099	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	70.3	4.89	2,736	1,821	62.2	51.7	6.3	0.18
Bin 100	Bacteria	45.7	1.02	466	2,321	52.7	15.3	2.7	0.04
Bin 101	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter	65.0	3.12	1,836	1,690	43.8	15.7	26.7	0.12
Bin 102	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Rhodobacter	69.2	5.11	2,623	2,055	61.2	64.7	9.5	0.18
Bin 103	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Sorangium	70.5	7.09	2,712	3,101	82.2	7.1	15.4	0.25
Bin 104	Bacteria	57.0	7.55	3,857	2,060	62.3	22.4	2.0	0.25
Bin 105	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	71.6	2.94	1,919	1,471	42.2	16.4	12.5	0.10
Bin 106	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Hyphomonadaceae	64.4	3.41	1,360	3,040	82.2	13.7	7.6	0.12
Bin 107	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	63.0	3.91	1,954	2,128	67.2	35.0	4.7	0.14
Bin 108	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae	66.2	3.43	1,770	1,972	59.1	32.4	24.9	0.12
Bin 109	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	64.7	3.68	1,845	2,106	65.5	23.3	0.0	0.13
Bin 110	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	40.6	1.48	567	3,037	88.2	3.7	50.0	0.05
Bin 111	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales	43.6	2.47	1,462	1,718	60.9	5.0	15.4	0.08
Bin 112	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales	43.2	2.51	1,144	2,465	68.4	4.9	26.7	0.08
Bin 113	Bacteria	51.5	8.28	4,346	1,986	80.8	38.7	1.6	0.26
Bin 114	Bacteria; Proteobacteria; Alphaproteobacteria	62.2	3.72	2,141	1,661	38.7	25.2	1.9	0.12
Bin 115	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	58.5	2.35	1,228	1,997	54.5	5.9	7.3	0.07

Completeness, redundancy and strain heterogeneity were assessed with CHECKM 0.7.1, according to the presence of 43 single-copy reference gene

Bins designated as “bin.XXX.X” were generated using anvi’o (*See Methods*).

Table S1. Bins assembled from metagenomic sample from day-522, taxonomic classification based on PhyloSift and metrics from CHECKM (*Cont*).

Bin Id	Lowest Assigned Taxonomy Rank	GC (%)	Genome size (Mbp)	# Scaffolds	N50	Completeness (%)	Redundancy (%)	Strain heterogeneity (%)	Mean Coverage 522-day
Bin 116	Bacteria; Bacteroidetes	66.8	2.51	1,220	2,273	65.0	8.5	4.0	0.08
Bin 117	Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales	30.6	2.17	1,293	1,580	53.1	4.7	0.0	0.07
Bin 118	Bacteria; Planctomycetes; Planctomycetia; Planctomycetales; Planctomycetaceae; Planctomyces	58.2	4.64	2,302	2,163	69.8	3.1	14.3	0.15
Bin 119.1	Bacteria	66.5	0.67	350	1,901	6.6	1.0	0.0	0.02
Bin 119.2	Bacteria; Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae; Phycisphaera	66.4	1.57	797	1,955	21.9	3.0	10.0	0.05
Bin 119.3	Bacteria	66.5	1.66	972	1,716	24.7	0.1	0.0	0.05
Bin 119.4	Bacteria; Proteobacteria	66.9	0.99	663	1,451	10.2	0.9	0.0	0.02
Bin 120	Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae; Caldilinea	60.8	4.35	2,538	1,728	59.3	16.4	0.0	0.13
Bin 121	Bacteria	69.7	4.85	2,799	1,765	57.7	17.4	0.0	0.15
Bin 122	Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Chromatiaceae	66.7	3.36	2,112	1,600	54.4	18.6	33.3	0.10
Bin 123	Bacteria; Acidobacteria	51.6	2.14	1,195	1,867	46.3	2.8	22.2	0.06
Bin 124	Bacteria	63.3	3.46	2,223	1,533	49.3	15.8	0.0	0.10

Completeness, redundancy and strain heterogeneity were assessed with CHECKM 0.7.1, according to the presence of 43 single-copy reference gene

Bins designated as “bin.XXX.X” were generated using anvi’o (*See Materials and Methods*)

Table S2. Metrics of *Accumulibacter* UW-LDO-01 after each genome refinement step.

Refinement step	GC (%)	Genome size (Mbp)	Gene count	# Scaffolds	N50	Completeness (%)	Redundancy (%)	Strain heterogeneity (%)
MaxBin (bin.046)	62.60	6.60	6,291	666	23,909	94.8	28.94	20.29
Anvio decontamination	62.49	4.55	4,097	239	36,358	94.8	0.84	100.00
Nanopore Links scaffolding	62.49	4.61	4,074	120	85,302	94.8	0.68	100.00
Gapcloser	62.48	4.70	4,166	120	85,321	95.2	0.68	100.00

Completeness, redundancy and strain heterogeneity were assessed with CHECKM 0.7.1, according to the presence of 43 single-copy reference genes.

Table S3. RNA-seq summary. Number of reads after each step of RNA reads filtering and normalization factors used for RPKM calculations, as described in Methods.

Sample	TimeA Anaerobic- 10min	TimeB Anaerobic- 32min	TimeC Aerobic- 0min	TimeD Aerobic- 22min	TimeE Aerobic- 52min	TimeF Aerobic- 292min
Total Reads	317,752,258	289,487,298	279,849,682	272,986,806	271,430,590	286,971,580
Normalization Factor from Total Reads	1.17	1.07	1.03	1.01	1.00	1.06
Quality Reads	312,696,898	285,415,418	275,951,004	270,309,846	268,730,228	283,344,794
Merged Reads	98,190,636	88,219,624	83,934,087	83,755,245	84,265,579	83,962,296
rRNA Sequences	27,374,143	21,015,585	22,055,875	17,613,784	15,900,468	21,372,211
non rRNA Sequences	70,816,493	67,204,039	61,878,212	66,141,461	68,365,111	62,590,085
Normalization Factor from Filtered Reads	1.14	1.09	1.00	1.07	1.10	1.01

Table S4. Summary of primers used for RT-qPCR.

Target Gene	Gene Symbol	Gene Length (bp)	Primer Target Site	Primer Sequence (5'-3')	Amplicon Length (bp)
Nitrite reductase	<i>nirS-1</i>	1,739	147-164 291-311	GTCGGCGATCGCGCAAT AGCGTTTTGTCCGTAGTCAGA	164
Respiratory nitrate reductase alpha chain	<i>narG</i>	3,819	336-353 403-424	CCGATGGTTCGCGGTCA CGGACTCGACGATCGATGTCC	91
Nitric oxide reductase, qNOR-like	<i>norZ</i>	2,282	1,846-1,864 2,204-2,221	CTTTGGGCTGGGTGGGT CGCGATCGGCGCTCAAAC	375
Nitrous-oxide reductase	<i>nosZ</i>	2,297	591-616 935-954	AAGAAATTCGAGCCACTAGAGGAAT AACATCCAGCGGCAATACG	363
Cytochrome c oxidase, cbb3-type, subunit I	<i>ccoN</i>	1,428	241-260 344-365	CCTGCCATACGCGACTTTT TCGGCATACTCTTTGGACTGG	124
Cytochrome c oxidase polypeptide I	<i>ctaD</i>	1,611	816-834 932-951	TCGCATGTCATCCCTGCC GGTCAGCGGAATACCGGTC	135
RNA polymerase sigma-54 factor	<i>rpoN</i>	1,440	153-170 594-611	GAGTACGCGCGGACGGA TGTGCGCTGCGAGCCCC	487

Table S5. Estimated abundance, at the genus level, of the most abundant taxonomic units in sample used for RNA-seq (522-day). Values were determined using 16S rRNA amplicon sequencing, as described in Methods.

Genus	% Relative Abundance
Candidatus Accumulibacter	34.0
Competibacteraceae CPB_C22&F32	15.9
Lewinella	10.6
unclassified Cytophagaceae	6.2
unclassified Hyphomonadaceae	4.4
Thauera	3.4
unclassified Lentisphaerae	2.4
Prostheco bacter	2.2
Saprospiraceae QEDR3BF09	1.7
Dechloromonas	1.3
Flavobacteriales PHOS-HE28	1.2
Rickettsiales SM2D12	1.0
unclassified Saprospiraceae	0.9
unclassified Chitinophagaceae	0.8
unclassified Rhodobacteraceae	0.7
Ignavibacterium	0.7
unclassified Flavobacteriaceae	0.6
Chlorobiales OPB56	0.6
Brevundimonas	0.6
Other	10.8

Table S6. Estimated abundance of each clade relative to the total *Accumulibacter* lineage in sample analyzed with RNA-seq. Values were determined using qPCR targeting the *ppk1* gene, as described in Methods.

Clade	% Relative Abundance
IA	0.0
IB	0.0
IC	74.4
ID	0.0
IE	0.1
IIA	9.2
IIB	0.1
IIC	0.0
IID	13.6
IIE	0.0
IIF	2.5
IIG	0.1

Table S7. Locus tag of genes associated with denitrification and aerobic respiration in genomes of *Accumulibacter* with published annotations.

Category	Gene	66-26	UW-1	SK-01	SK-02	BA-91	BA-94	SK-11	SK-12	BA-93	UW-2	BA-92	UW-LDO-IC
Nitrite/nitrate transport	<i>narK</i>		CAP2UW1_RS13840	CAPSK01_00973 CAPSK01_00972	AW06_03543 AW06_03542	AW09_04395 AW09_01219 AW09_01220			AW08_01696	AW11_00623	Ga0078784_16490	AW10_02293 AW10_04278 AW10_04277	DVS81_13630 DVS81_13635
Respiratory Nitrate Reduction	<i>narI</i>			CAPSK01_00968	AW06_03547	AW09_04883 AW09_04436							DVS81_13655
	<i>narJ</i>			CAPSK01_00969	AW06_03546	AW09_04435							DVS81_13650
	<i>narH</i>			CAPSK01_00970	AW06_03545	AW09_03334 AW09_04435							DVS81_13645
	<i>narG</i>			CAPSK01_00971	AW06_03544	AW09_00845							DVS81_13640
Periplasmic Nitrate Reduction	<i>napD</i>	BGO63_05850	CAP2UW1_RS19375				AW12_02777			AW11_02156	Ga0078784_16675	AW10_00993	
	<i>napA</i>	BGO63_05855	CAP2UW1_RS19380				AW12_02778			AW11_02155	Ga0078784_16676	AW10_00992	
	<i>napG</i>	BGO63_05860	CAP2UW1_RS19385					AW07_00314	AW08_03811	AW11_02154	Ga0078784_16677	AW10_00991	
	<i>napH</i>	BGO63_05865	CAP2UW1_RS19390					AW07_00315	AW08_03810	AW11_02153	Ga0078784_16678	AW10_00990	
	<i>napB</i>	BGO63_05870	CAP2UW1_RS19395						AW08_03809	AW11_02152	Ga0078784_16679	AW10_00989	
Nitrite Reduction	<i>nirS</i>	BGO63_10625 BGO63_07995	CAP2UW1_RS12415	CAPSK01_00966	AW06_03549	AW09_04881		AW07_01971	AW08_01974	AW11_03206 AW11_01110	Ga0078784_11456 Ga0078784_16169	AW10_00297 AW10_01909	DVS81_20760 DVS81_12285 DVS81_17305
	<i>nirM</i>	BGO63_10620	CAP2UW1_RS12410	CAPSK01_03625	AW06_03550	AW09_04517	AW12_00607	AW07_01106	AW08_01973	AW11_00400	Ga0078784_11457	AW10_00298	DVS81_05230
	<i>nirN</i>	BGO63_15090	CAP2UW1_RS01495	CAPSK01_04667	AW06_00888 AW06_01533	AW09_05015 AW09_02026	AW12_01837	AW07_03428	AW08_02733	AW11_00134	Ga0078784_161768	AW10_02670	DVS81_05225
	<i>nirJ</i>	BGO63_15095	CAP2UW1_RS01500	CAPSK01_04668	AW06_01532 AW06_00887	AW09_02027	AW12_01838	AW07_03429	AW08_02732	AW11_00135	Ga0078784_161769	AW10_02671	DVS81_12305
	<i>nirC</i>	BGO63_10615	CAP2UW1_RS12405	CAPSK01_02537	AW06_03944	AW09_01616		AW07_01957	AW08_01959	AW11_03210	Ga0078784_11460	AW10_00301	DVS81_16850
	<i>nirF</i>	BGO63_15190	CAP2UW1_RS16895	CAPSK01_00714	AW06_03796	AW09_02150	AW12_03398	AW07_03938	AW08_02575	AW11_03018	Ga0078784_11285	AW10_00481	DVS81_16855
	<i>nirD/ nirL</i>	BGO63_15185	CAP2UW1_RS16890	CAPSK01_00715	AW06_03795	AW09_01742 AW09_02149		AW07_03937	AW08_02576	AW11_03019	Ga0078784_11286	AW10_00481	DVS81_16860
	<i>nirG</i>	BGO63_15180	CAP2UW1_RS16885	CAPSK01_00716	AW06_03794	AW09_01741		AW07_03936	AW08_02577	AW11_03020	Ga0078784_11287	AW10_00480	DVS81_16865
<i>nirH</i>	BGO63_15175	CAP2UW1_RS16880	CAPSK01_00717	AW06_03793	AW09_01740		AW07_03935	AW08_02578	AW11_03021	Ga0078784_11288	AW10_00479	DVS81_20925	
Nitric Oxide Reduction	<i>norZ</i>	BGO63_01415 BGO63_01420	CAP2UW1_RS11595							AW11_01319		AW10_01907	DVS81_16815
Nitrous Oxide Reduction	<i>nosD</i>	BGO63_05040 BGO63_15225	CAP2UW1_RS16930 CAP2UW1_RS19520				AW12_03390	AW07_04345	AW08_03173	AW11_03011	Ga0078784_11278	AW10_01530 AW10_00489	DVS81_16795
	<i>nosZ</i>	BGO63_01150	CAP2UW1_RS16950					AW07_04349	AW08_03048	AW11_03007	Ga0078784_11274	AW10_00493	DVS81_16830
	<i>nosF</i>	BGO63_15210	CAP2UW1_RS16915					AW07_03942	AW08_02570	AW11_03014	Ga0078784_11281	AW10_00484	DVS81_16835
	<i>nosL</i>	BGO63_15205	CAP2UW1_RS16910					AW07_03941	AW08_01972	AW11_03015	Ga0078784_11282	AW10_00485	DVS81_16840
	<i>nosY</i>	BGO63_15200	CAP2UW1_RS16905					AW07_03940	AW08_02572	AW11_03016	Ga0078784_11283	AW10_00484	DVS81_13630 DVS81_13635

Table S8. Locus tag of genes associated with denitrification and aerobic respiration in genomes of *Accumulibacter* with published annotations.

Category	Gene	66-26	UW-1	SK-01	SK-02	BA-91	BA-94	SK-11	SK-12	BA-93	UW-IA	BA-92	UW-LDO-IC
Cytochrome c oxidation (<i>cbb₃</i>)	<i>ccoN</i>	BGO63_02185	CAP2UW1_RS12650	CAPSK01_02001	AW06_01080		AW12_03440	AW07_02023	AW08_01074	AW11_01636	Ga0078784_16238		DVS81_08570
	<i>ccoO</i>	BGO63_02190	CAP2UW1_RS12645	CAPSK01_02000	AW06_01081	AW09_00171	AW12_03441	AW07_02022	AW08_01075	AW11_01637	Ga0078784_16237		DVS81_08565
	<i>ccoQ</i>	BGO63_02195	CAP2UW1_RS12640	CAPSK01_01999	AW06_01082		AW12_03442	AW07_01648	AW08_01076	AW11_01638	Ga0078784_16236		DVS81_08560
	<i>ccoP</i>	BGO63_02200	CAP2UW1_RS12635	CAPSK01_01998	AW06_01083	AW09_00172	AW12_03443	AW07_02020	AW08_01077	AW11_01639	Ga0078784_16235		DVS81_08555
	<i>ccoG</i>	BGO63_02205	CAP2UW1_RS12630	CAPSK01_01997	AW06_01084	AW09_00173	AW12_03444	AW07_02019	AW08_01078	AW11_01641	Ga0078784_16233		DVS81_08545
	<i>ccoH</i>	BGO63_02210	CAP2UW1_RS12625	CAPSK01_01996	AW06_01085	AW09_00174	AW12_03445	AW07_02018	AW08_01079	AW11_01642	Ga0078784_16232		DVS81_08540
	<i>ccoI</i>	BGO63_02175	CAP2UW1_RS12660	CAPSK01_02004	AW06_01078	AW09_00469	AW12_01853	AW07_02026	AW08_01072	AW11_01634	Ga0078784_16240		DVS81_07980
	<i>ccoS</i>	BGO63_02180	CAP2UW1_RS12655	CAPSK01_02002	AW06_01079		AW12_03439		AW08_01073	AW11_01635	Ga0078784_16239		DVS81_07975
Cytochrome c oxidation (<i>ba₃</i>)	<i>cbaA</i>		CAP2UW1_RS08915	CAPSK01_02286	AW06_04048		AW12_01750	AW07_03205	AW08_03217	AW11_01056	Ga0078784_1135	AW10_02150	DVS81_11870
	<i>cbaB</i>		CAP2UW1_RS08920	CAPSK01_02287	AW06_04049	AW09_03865	AW12_01749	AW07_03206	AW08_03218	AW11_01057	Ga0078784_1136	AW10_02151	DVS81_11875
Cytochrome c oxidation (<i>aa₃</i>)	<i>ctaD</i>	BGO63_08500 BGO63_08555	CAP2UW1_RS20650	CAPSK01_02318	AW06_01187	AW09_03272				AW11_00093	Ga0078784_161727	AW10_02621	DVS81_11220
	<i>ctaC</i>	BGO63_08495 BGO63_08550	CAP2UW1_RS20655	CAPSK01_02319	AW06_01186	AW09_03273 AW09_03276		AW07_03129	AW08_03918	AW11_00092	Ga0078784_161724	AW10_02617 AW10_02620	DVS81_11215
	<i>ctaE</i>	BGO63_08505 BGO63_08565	CAP2UW1_RS20640	CAPSK01_02314	AW06_01191	AW09_03269	AW12_00507	AW07_01646	AW08_03349	AW11_00097	Ga0078784_161731	AW10_02625	DVS81_11235

Table S9. Number and percentage of unambiguous RNA filtered reads, mapping to the metagenomic assembly and Accumulibacter genomes.

Sample	TimeA Anaerobic-10min		TimeB Anaerobic-32min		TimeC Aerobic-0min		TimeD Aerobic-22min		TimeE Aerobic-52min		TimeF Aerobic-292min	
	Mapping Reads	Percentage (%)	Mapping Reads	Percentage (%)	Mapping Reads	Percentage (%)	Mapping Reads	Percentage (%)	Mapping Reads	Percentage (%)	Mapping Reads	Percentage (%)
Metagenomic Assembly	53,436,191	75.46	52,119,320	77.55	47,295,895	76.43	50,558,369	76.44	53,488,638	78.24	48,598,042	77.64
Accumulibacter Genomes												
Accumulibacter sp. UW-LDO-IC	34,153,509	48.23	33,074,188	49.21	29,322,430	47.39	30,879,766	46.69	34,022,590	49.77	31,266,884	49.96
Accumulibacter BA-94	339,200	0.48	301,935	0.45	255,777	0.41	346,896	0.52	317,890	0.46	224,432	0.36
Accumulibacter sp. 66-26	269,960	0.38	293,672	0.44	245,888	0.40	333,846	0.50	237,221	0.35	178,552	0.29
Accumulibacter HKU-1	188,368	0.27	193,623	0.29	182,887	0.30	204,697	0.31	212,465	0.31	162,371	0.26
Accumulibacter sp. SK-01	173,780	0.25	151,800	0.23	170,589	0.28	186,542	0.28	170,390	0.25	107,689	0.17
Accumulibacter UW-1	72,429	0.10	89,236	0.13	72,698	0.12	89,727	0.14	87,761	0.13	53,462	0.09
Accumulibacter UW-2	70,297	0.10	78,092	0.12	62,826	0.10	85,618	0.13	85,442	0.12	49,247	0.08
Accumulibacter sp. BA-91	60,110	0.08	58,687	0.09	46,351	0.07	58,856	0.09	56,933	0.08	48,747	0.08
Accumulibacter isolate UBA2783	50,148	0.07	49,101	0.07	41,300	0.07	50,813	0.08	56,204	0.08	44,659	0.07
Accumulibacter isolate UBA2327	49,524	0.07	44,260	0.07	37,601	0.06	48,117	0.07	49,554	0.07	41,685	0.07
Accumulibacter sp. BA-93	38,591	0.05	35,627	0.05	31,382	0.05	40,003	0.06	35,517	0.05	34,713	0.06
Accumulibacter SK-11	38,389	0.05	30,882	0.05	30,956	0.05	35,010	0.05	34,180	0.05	34,667	0.06
Accumulibacter BA-92	32,312	0.05	28,511	0.04	26,003	0.04	28,094	0.04	27,473	0.04	28,558	0.05
Accumulibacter sp. UBA704	16,636	0.02	17,642	0.03	15,813	0.03	19,947	0.03	13,896	0.02	12,744	0.02
Accumulibacter sp. SK-02	13,269	0.02	15,392	0.02	10,148	0.02	14,459	0.02	13,844	0.02	9,920	0.02
Accumulibacter isolate UBA6585	9,822	0.01	10,203	0.02	7,879	0.01	11,236	0.02	11,501	0.02	9,355	0.01
Accumulibacter isolate HKU-2	9,465	0.01	8,464	0.01	6,692	0.01	8,719	0.01	8,758	0.01	8,205	0.01
Accumulibacter isolate UBA6658	7,560	0.01	6,753	0.01	5,964	0.01	6,989	0.01	6,292	0.01	5,261	0.01
Accumulibacter isolate UBA5574	6,600	0.01	6,686	0.01	5,256	0.01	6,188	0.01	6,113	0.01	5,054	0.01
Accumulibacter isolate UBA2315	6,457	0.01	6,205	0.01	5,023	0.01	6,036	0.01	5,741	0.01	5,035	0.01
Accumulibacter SK-12	6,309	0.01	5,924	0.01	4,657	0.01	5,890	0.01	5,592	0.01	4,818	0.01