

Supplementary Information for the manuscript:

Phylogeny and physiology of the candidate phylum ‘Atribacteria’ (OP9/JS1) inferred from cultivation-independent genomics

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Supplementary Information

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Supplementary Methods

Sequencing and assembly of Aarhus Bay SAGs B17 and I22.

Cell extraction and cell sorting from Aarhus Bay sediments was as described in Lloyd *et al.* (2013). Single cells were lysed and genomic DNA amplified using multiple-strand displacement amplification (MDA); after screening with bacterial 16S rRNA gene PCR, two single cells were identified as JS1 (Lloyd *et al.*, 2013) and these SAGs were re-amplified by MDA at Cardiff University. Three replicate MDA reactions were performed for each SAG, and the resulting DNA purified and pooled using Microcon centrifugal filters (Merck Millipore Ltd., Hertfordshire, UK) and eluted in 200 µl sterile TE buffer (Sigma-Aldrich, St. Louis, MO, USA). Sequencing of the SAG B17 was performed using a combination of the Roche 454 GS FLX+ System (525 bp mean read length) by CGR, University of Liverpool and Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) with 250 base paired-end reads at Edinburgh Genomics, University of Edinburgh. SAG I22 was sequenced by Illumina MiSeq platform with 250 base paired-end reads at Edinburgh Genomics, University of Edinburgh. Library preparation for Illumina MiSeq was carried out using the TruSeq DNA Sample Prep Kit following the manufacturer's recommendations and size selected to a mean insert size of approximately 300 bp PCR (SAG B17) using an E-Gel (Life Technologies, Grand Island, NY, USA) or 400 bp PCR (SAG I22) using a BluePippin instrument (Sage Science, Beverly, MA, USA). The MiSeq data were assembled for each individual SAG separately using SPAdes ver 2.5.1 (Bankevich *et al.*, 2012) with a kmer size of 87 (-k 87) and with 'single-cell', 'mismatch correction', 'rectangles graph algorithm for repeat resolution' options (--sc --careful --rectangle). The 454 data for B17 SAG was assembled using Roche GS denovo Assembler ver 2.6 with 'use read tips' and 'expected depth' options (-urt -e 500). B17 contigs from SPAdes and Newbler were further merged with minimus2 (Somer *et al.*, 2007) if contig overlap was greater than 40bp with a minimum percentage identity of 96 (options OVERLAP=40 and MINID=96) to generate the combined B17 assembly.

References for Supplementary Methods

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, *et al.* (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* **19**:455-477.

Lloyd KG, Schreiber L, Petersen DG, Kjeldsen KU, Lever MA, Steen AD, *et al.* (2013). Predominant archaea in marine sediments degrade detrital proteins. *Nature* **496**:215–218.

Somer DD, Delcher AL, Salzberg SL, Pop M. (2007). Minimus: a fast, lightweight genome assembler. *BMC Bioinform* **8**:64.

Table S1. SAG and metagenome datasets used or generated in this study.

Dataset	Type ¹	RAST ID ²	JGI IMG taxon ID	NCBI accession	Reference
Sakinaw Lake SAG co-assembly	SAG	6666666.54176	2527291521	AWNT000000000	Rinke <i>et al.</i> (2013)
Sakinaw Lake SAG 124	SAG	6666666.100175	2264867201	ASLT000000000	Rinke <i>et al.</i> (2013)
Sakinaw Lake SAG 130	SAG	6666666.100178	2264867202	ASPA000000000	Rinke <i>et al.</i> (2013)
Sakinaw Lake SAG 136	SAG	6666666.100183	2264867203	ASPC000000000	Rinke <i>et al.</i> (2013)
Sakinaw Lake SAG 217	SAG	6666666.54177	2264867208	AQRY000000000	Rinke <i>et al.</i> (2013)
Sakinaw Lake SAG 219	SAG	6666666.100187	2264867210	AQSW000000000	Rinke <i>et al.</i> (2013)
TA biofilm SAG 167	SAG	6666666.54173	2264867204	ASLS000000000	Rinke <i>et al.</i> (2013)
TA biofilm SAG 231	SAG	6666666.54174	2264867212	ASOY000000000	Rinke <i>et al.</i> (2013)
TA biofilm SAG 232	SAG	6666666.54182	2264867213	AQRR000000000	Rinke <i>et al.</i> (2013)
Etoliko Lagoon SAG 227	SAG	6666666.54175	2264867211	ASOZ000000000	Rinke <i>et al.</i> (2013)
Aarhus Bay SAG B17	SAG	6666666.54178	None	CDPL010000000	Lloyd <i>et al.</i> (2013); this study
Aarhus Bay SAG I22	SAG	6666666.94538	None	CDPM010000000	Lloyd <i>et al.</i> (2013); this study
LHC SAG co-assembly	SAG	6666666.23228	2527291510	APKF000000000	Dodsworth <i>et al.</i> (2013)
GBS 77CS MG bin	MG bin	6666666.23137	3300000106	APCU000000000	Dodsworth <i>et al.</i> (2013)
GBS 77CS MG bin, coverage-filtered bin	MG bin	6666666.94617	3300000106 ³	None	This study
TA biofilm JS1 MG bin	MG bin	6666666.54181	3300001095 ³	None	This study
Sakinaw Lake JS1 MG bin	MG bin	6666666.54179	2263328000 ³	None	This study
GBS 77CS cellulolytic enrichment 77CS	MG	None	3300000106	None	Peacock <i>et al.</i> (2013)
Terephthalate-degrading biofilm	MG	None	3300001095	None	Nobu <i>et al.</i> (2014)
Sakinaw Lake metagenomic (120m)	MG	None	2263328000	None	Unpublished
Sediment from Etoliko Lagoon, Greece	MG	None	2149837013	None	Unpublished

¹Abbreviations: MG, metagenome

²All SAG and metagenome bin datasets are publicly available on the guest account on RAST (<http://rast.nmpdr.org/>) by logging in with username and password = “guest”.

³These bins represent a subset of contigs in the corresponding metagenomes, whose accession numbers are given. Individual contig names for each of these bins are appear at the end of the supplementary information, and are also publically available via RAST (username and password “guest”) as indicated above.

Table S2. Comparison of minimum and median % identity within members of various bacterial phyla.¹

Taxon name	Number of members	Number of comparisons	Minimum % identity	Median % identity
Phylum <i>Thermodesulfobacteria</i>	7	21	88.1	93.7
Phylum <i>Chlorobi</i>	11	55	87.5	93.6
Phylum <i>Cyanobacteria</i>	7	21	86.7	88.4
Phylum <i>Chlamydiae</i>	13	78	83.7	87.65
Phylum <i>Actinobacteria</i>	2086	2135467	81.2	89.4
Phylum <i>Fusobacteria</i>	35	595	80.7	90
Phylum <i>Synergistetes</i>	15	105	79.9	83.8
Phylum <i>Crenarchaeota</i>	45	990	79.7	86.5
Phylum <i>Acidobacteria</i>	8	28	78.8	82.45
Phylum <i>Nitrospira</i>	8	28	78.7	80.55
Phylum <i>Deferribacteres</i>	11	55	78	85.1
Phylum <i>Thermi</i>	59	1711	77.8	85.9
Phylum <i>Thermotogae</i>	29	406	76.9	82.3
Phylum <i>Verrucomicrobia</i>	32	496	76	82.8
Phylum <i>Aquificae</i>	26	325	75.7	82.1
Phylum <i>Chloroflexi</i>	15	105	74.2	77.8
Candidate Phylum 'Atribacteria'	744 ²	70784 ²	74.2	80.8
Phylum <i>Planctomycetes</i>	12	66	73.7	79.5
Phylum <i>Firmicutes</i>	1592	1266268	73.1	83
Phylum <i>Spirochaetes</i>	67	2211	72.3	78.1
Phylum <i>Proteobacteria</i>	2890	4174605	72.2	81.3
Phylum <i>Tenericutes</i>	176	15400	71.2	80.3
Phylum <i>Bacteroidetes</i>	633	200023	68.5	79.8
Phylum <i>Euryarchaeota</i>	241	28920	68.3	77.3

¹Data from Yarza *et al.* 2014 Table S1 except for values for Candidate Phylum 'Atribacteria' (highlighted in yellow).

²Restricted to pairwise comparisons between OP9 (112 sequences) and JS1 (632 sequences); median % identity when including comparisons within OP9 and JS1 is higher, due to oversampling of a few taxa.

Table S3. Markers¹ associated with a diderm cell envelope structure in 'Atribacteria' genomes.

Example protein PFAM domain	BamA (YaeT)		ToIC	Secretin	Secretin- N	Secretin/ TonB	FlgH	FlgI
	PF07244	PF01103	PF02321	PF00263	PF03958	PF07660	PF02107	PF02119
SAG or MG bin²								
TAbio SAG 232								
LHC SAG coassembly	2.7E-38		6.0E-36	4.6E-43	1.4E-09	4.1E-09	4.1E-32	
GBS 77CS MG bin	2.4E-38	5.2E-51	5.3E-37	1.1E-42	1.0E-08	2.2E-09	2.3E-30	2.6E-140
TAbio SAG 167			3.4E-21	7.8E-37	9.4E-20	1.3E-07		
TAbio SAG 231	5.5E-38							
TAbio MG bin	8.2E-39	7.4E-48	2.3E-21	4.9E-36	5.5E-20			
SL SAG coassembly	1.5E-38	4.3E-53						
SL SAG 124								
SL SAG 130	2.0E-37	2.0E-42		2.0E-40	1.2E-16	4.6E-07		
SL SAG 136			2.1E-53					
SL SAG 217		1.5E-17		8.8E-39	1.3E-16	9.4E-07		
SL SAG 219				7.7E-24				
SL MG bin			6.7E-54					
Aarhus SAG I22			1.3E-18	4.7E-41	8.0E-16	2.0E-07		
Etoliko SAG 227			1.9E-18					
Aarhus SAG B17								

¹E-values of the top hit pfams (PF) associated with the presence of an outer membrane

(Sutcliffe 2010) are shown.

²Abbreviations: MG, metagenome; SL, Sakinaw Lake.

Table S4. Putative conserved, monophyletic genes in 'Atribacteria', with estimated genome coverage for each dataset shown in parentheses.

Category/classification	Predicted function	GBS 77CS meta (>99%)	OP9-1	LHC SAGco (>99%)	OP9-1	OP9-2	JS1-1	JS1-1	JS1-1	JS1-1	Other SAK SAGs (6-73%)	JS1-1	JS1-1	JS1-2	JS1-2	JS1-3	JS1-3	JS1-4
BMC cluster	BMC shell protein	X	X	X	X	X						X	X	X				X
BMC cluster	BMC shell protein	X	X	X	X	X							X	X	X			X
BMC cluster	BMC shell protein	X	X	X	X	X							X	X	X			X
BMC cluster	Predicted NADH:ubiquinone oxidoreductase (BMC)	X	X	X	X	X						X	X	X				X
BMC cluster	BMC shell protein	X	X	X	X	X						X	X	X				X
BMC cluster	NAD-dependent aldehyde dehydrogenases (BMC)	X	X	X	X	X	X					X	X	X				X
BMC cluster	BMC shell protein	X	X	X	X	X								X	X			X
BMC cluster	BMC shell protein	X	X	X	X	X						X		X	X			X
BMC cluster	Ribose 5-phosphate isomerase RpiB (BMC)	X	X	X	X	X						X		X				X
BMC cluster	Deoxyribose-phosphate aldolase (BMC)	X	X	X	X	X						X		X				X
BMC cluster	Propanediol utilization protein (BMC)	X	X	X	X	X						X		X				X
Regulation	Transcriptional regulator	X	X		X						X		X	X	X	X		X
Protease	Clostripain family				X						X		X	X				
C metabolism	Glucuronate isomerase	X	X		X						X		X	X				
C metabolism	Uroporphyrinogen-III decarboxylase	X	X								X							X
C metabolism	Predicted thioesterase - COG5496	X			X	X							X	X				
Redox balancing	Thioredoxin domain-containing protein				X								X	X			X	
Redox balancing	Electron bifurcating formate dehydrogenase, HyIB subunit	X									X		X					
Cell envelope	Membrane carboxypeptidase (penicillin-binding protein)	X	X		X						X	X	X	X				X
Cell envelope	Membrane proteins related to metalloendopeptidases	X	X								X	X	X	X				
Cell envelope	Outer membrane protein/protective antigen OMA87	X	X								X		X	X				
Cell envelope	S-layer homology domain	X	X	X	X						X		X	X				
Cell envelope	Organic solvent tolerance protein				X						X		X	X				
Cell envelope	integral membrane protein MviN	X	X	X					X		X	X	X	X				
Cell envelope/division	ATP-dependent metalloprotease FtsH	X			X	X					X		X	X	X			X
Transport/secretion	Predicted exporters, RND superfamily	X	X		X	X									X	X	X	
Transport/secretion	Preprotein translocase subunit SecY	X	X		X	X					X				X			X
Transport/secretion	protein-export membrane protein, SecD/SecE family	X	X		X	X					X		X	X				
Transport/secretion	ABC-type transport system, periplasmic component	X	X		X						X	X	X	X				X
Transport/secretion	Predicted ABC-type sugar transport system	X	X								X			X				
Transport/secretion	Type II secretory pathway, component PulD	X	X					X			X	X		X				X
Transport/secretion	Biopolymer transport protein	X	X	X							X			X				
Transport/secretion	ABC-type transport system, permease component	X	X								X		X	X				
Transport/secretion	Biopolymer transport proteins	X	X	X							X			X				
Transport/secretion	ABC-type sugar transport system, periplasmic component		X								X			X				
Housekeeping	DNA-directed RNA polymerase, beta' subunit	X	X		X						X			X				X
Housekeeping	DNA-directed RNA polymerase, beta subunit	X	X		X	X					X			X				
Housekeeping	ATPases involved in chromosome partitioning	X	X		X						X		X	X				X
Housekeeping	Single stranded DNA-binding protein (ssb)	X	X	X	X						X							X
Housekeeping	uracil-DNA glycosylase, family 4	X	X	X							X		X	X				X
Housekeeping	Thymidylate kinase	X	X	X	X						X	X		X				
Housekeeping	Argininosuccinate synthase	X	X	X							X			X				
Other/unknown	hypothetical protein	X	X		X						X		X			X	X	
Other/unknown	Protein of unknown function (DUF1329)	X	X		X								X	X	X	X		
Other/unknown	hypothetical protein	X	X								X		X	X				
Other/unknown	Domain of unknown function (DUF1844)	X	X		X	X	X				X		X					
Other/unknown	Uncharacterized protein (ATP-grasp superfamily)	X	X	X										X				X
Other/unknown	Transposase and inactivated derivatives				X						X					X		
Other/unknown	Protein of unknown function (DUF3048)	X									X			X				
Other/unknown	hypothetical protein		X								X							X
Other/unknown	Uncharacterized protein conserved in bacteria		X								X							X

Table S5. COGs, Pfams, and RAST protein-encoding gene (peg) numbers for genes predicted to be involved in propionate catabolism and energy conservation in JS1-1 and JS1-2 datasets.

	COG	Pfam	TA biofilm SAG 231	TA biofilm MG bin	Sakinaw Lake co-assembly	Sakinaw Lake MG bin	Sakinaw Lake SAG 124	Sakinaw Lake SAG 130	Sakinaw Lake SAG 136	Sakinaw Lake SAG 219	Aarhus Bay SAG 122
Propionate catabolism											
Methylmalonyl-CoA decarboxylase, alpha subunit	COG4799	PF01039	601	1651	2116	83	np	192	1021	np	np
Methylmalonyl-CoA decarboxylase, gamma subunit	COG4770	PF00364	603	np	2114	np	np	190	1019	np	np
methylmalonyl-CoA epimerase	None	PF13669	600	np	2117	84	np	193	1022	np	np
Biotin-(acetyl-CoA carboxylase) ligase	COG0340	PF03099	476	np	np	np	18	2	np	401	np
Methylmalonyl-CoA mutase, N-terminal domain	COG1884	PF01642	597	706	1709	np	np	196	np	np	np
Methylmalonyl-CoA mutase, C-terminal domain	COG2185	PF02310	598	707	1708	np	np	195	np	np	np
Succinyl-CoA synthetase, alpha subunit	COG0074	PF00549	510	np	858	np	np	381	447	np	1050
Succinyl-CoA synthetase, beta subunit	COG0045	PF00549	511	np	857	np	np	382	448-9	np	1049
Fumarate hydratase	COG1027	PF10415	np	651	np	np	np	np	np	656	np
Fumarate hydratase class I, C-terminal domain	COG1838	PF05683	np	1373	1764	np	np	1175	440	149	np
Fumarate hydratase class I, N-terminal domain	COG1951	PF05681	np	np	1763	np	np	1176	441	150	np
Pyruvate:ferredoxin oxidoreductase	COG0674	PF01558	394	np	np	np	np	1690	62	639	np
2-oxoacid:ferredoxin oxidoreductases, gamma subunit	COG1014	PF01558	953	1059	8	np	np	1325	450	103	403
2-oxoacid:ferredoxin oxidoreductases, beta subunit	COG1013	PF02775	954	1058	9	290	np	1326	451	104	404
2-oxoacid:ferredoxin oxidoreductases, alpha subunit	COG0674	PF01855	955	1057	10	np	np	1327	452	105	405
Succinate dehydrogenase/fumarate reductase	COG0029	PF02910	np	1020	388	np	np	1124	np	338	np
Malate dehydrogenase	COG2055	PF02615	np	602	1505	np	np	np	40	np	np
Pyruvate-formate lyase	COG1882	PF01228	188	84	927	93	np	np	802	831	323
Pyruvate-formate lyase-activating enzyme	COG1180	None	189	85	928	np	np	np	801	832	324
Electron transfer flavoprotein											
Electron transfer flavoprotein, alpha subunit	COG2086	PF01012	581	481	np	np	np	241	1156	396	719
Electron transfer flavoprotein, beta subunit	COG2025	PF00766	582	482	np	np	np	242	1157	397	718
Membrane-bound hydrogenase											
Hydrogenase nickel insertion protein HypA	COG0375	PF01155	831	528	228	np	np	1581	np	463	294
Membrane-bound hydrogenase MbhA subunit	COG1863	PF01899	830	529	229	np	np	np	np	462	3
Membrane-bound hydrogenase MbhB subunit	COG2212	PF04066	829	530	230	np	np	1584	np	461	4
Membrane-bound hydrogenase MbhC subunit	COG1320	PF03334	828	531	231	np	np	1585	1353	460	np

Membrane-bound hydrogenase MbhD subunit	None	None	827	532	232	np	np	1586	1354	459	np
Membrane-bound hydrogenase MbhE subunit	COG2111	None	826	533	233	np	np	1587	1355	458	1057
Membrane-bound hydrogenase MbhF subunit	COG2111	PF04039	825	534	234	np	np	1588	1356	457	1058
Membrane-bound hydrogenase MbhG subunit	COG1006	PF00420	824	535	235	np	np	1589	1357	456	610
Membrane-bound hydrogenase MbhH subunit	COG0651	PF00361	994	np	236	np	np	842	1358	455	611
Membrane-bound hydrogenase MbhI subunit	None	None	995	np	237	np	np	841	1360	454	612
Membrane-bound hydrogenase MbhJ subunit	COG3260	PF01058	996	1897	238	np	np	840	1361	453	613
Membrane-bound hydrogenase MbhK subunit	COG3262	PF00329	997	1896	239	np	np	839	np	452	614
Membrane-bound hydrogenase MbhL subunit	COG0649	PF00346	998	np	240	np	np	836	np	451	615
Membrane-bound hydrogenase MbhM subunit	COG0650	PF00146	999	np	241	np	np	837	np	444	616
Membrane-bound hydrogenase MbhN subunit	COG1143	PF12838	1000	np	242	np	np	838	np	445	617

Electron-bifurcating formate dehydrogenase and associated genes

Hydrogenase-like subunit HylC	COG1905	PF01257	1022, 1020	655, 1876	np	np	np	UC	1107	np	np
Hydrogenase-like subunit HylB	COG1894	PF01512	1021, 1019-18	1734	np	np	np	1485	1106	449	np
HylA-Formate dehydrogenase fusion protein	COG3383	PF00384	1017	1956	np	51	np	1484	1114	450	np
Formate efflux transporter	COG2116	PF01226	1016	np	np	52	np	1483	1113	np	np
Molybdopterin biosynthesis MobA	COG0746	PF12804	1015	279	1846	53	np	1482	1112	np	np
Formate dehydrogenase subunit D	COG1526	PF02634	1014	280	1845	54	np	1481	1111	np	np
Hydrogenase maturation factor HypB	COG0378	PF02492	1012	282	1843	55	np	1479	1109	np	618

Abbreviations: UC, uncalled orf in RAST annotation. np, not present

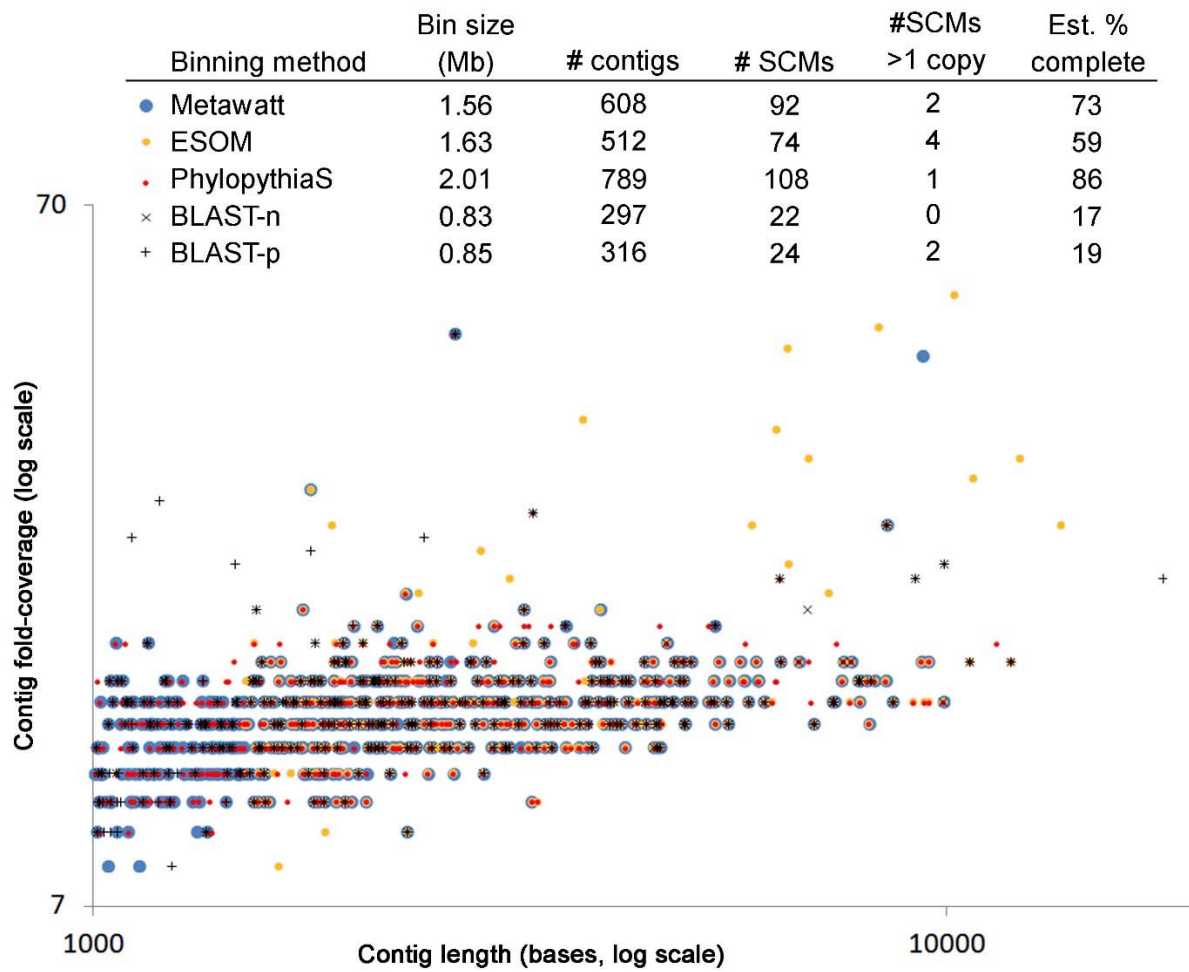


Figure S1. Comparison of several techniques for defining a JS1 bin in the TA biofilm metagenome.

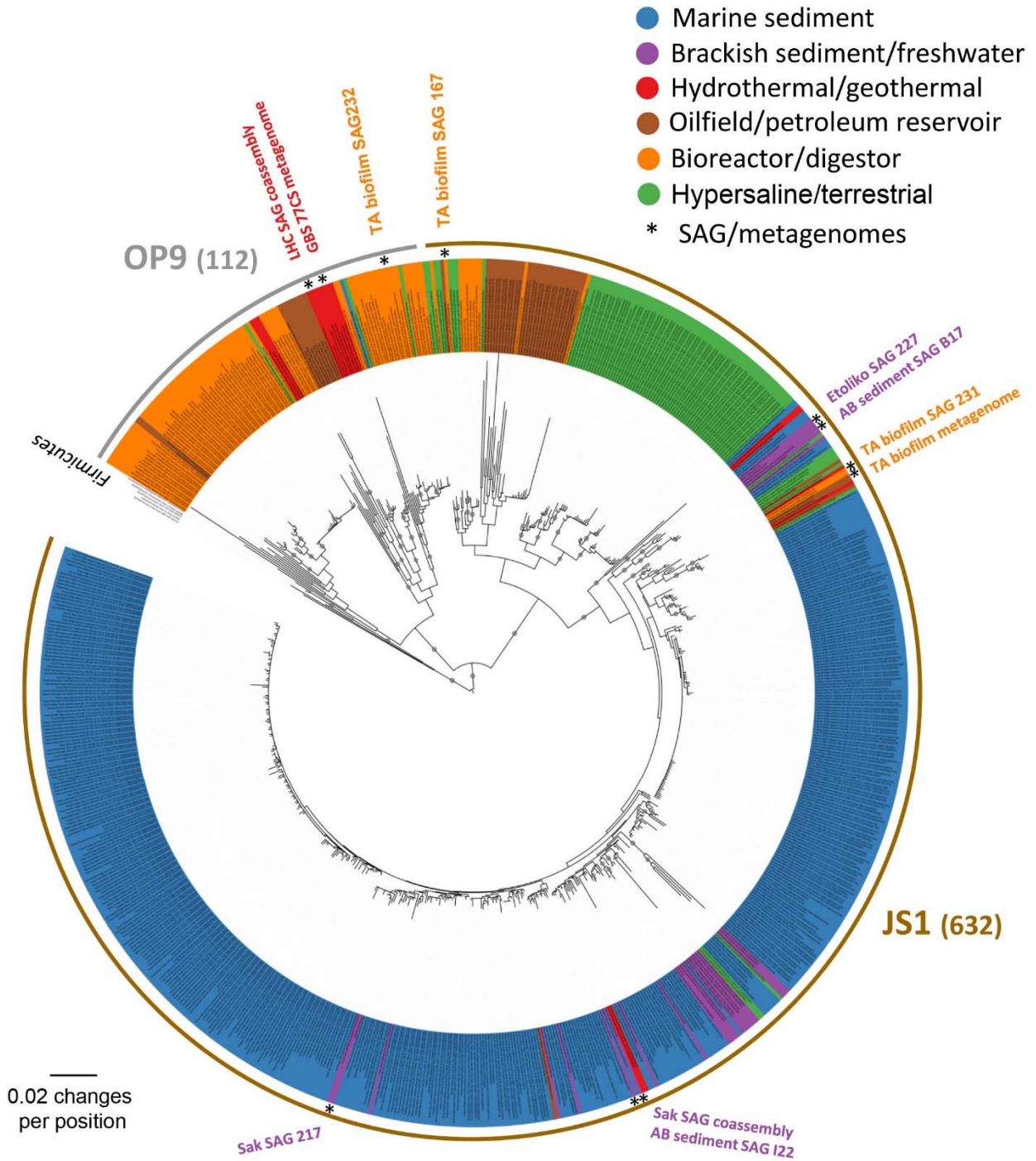


Figure S2. 16S rRNA gene phylogeny of OP9 and JS1. The number of sequences included for each lineage is indicated in parentheses.

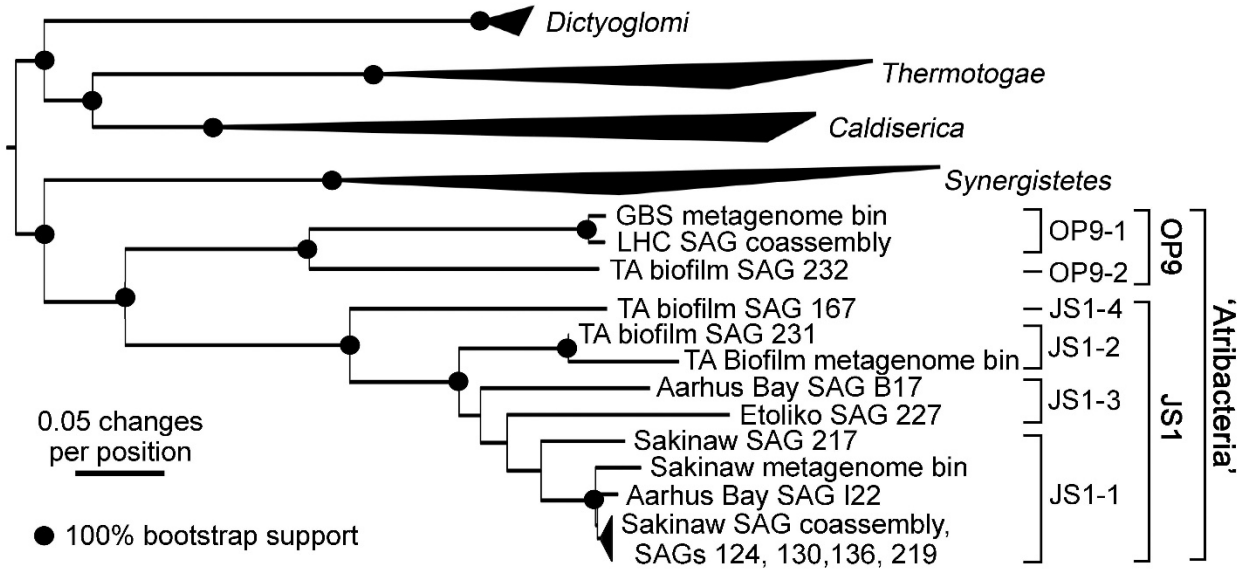


Figure S3. Phylogenomic analysis of OP9/JS1 SAGs, metagenome bin datasets, and selected bacterial phyla branching near OP9/JS1 in analyses of larger datasets. Maximum likelihood phylogeny was calculated using Fasttree based on a set of 83 SCM in Bacteria with 100 bootstrap pseudoreplicates.

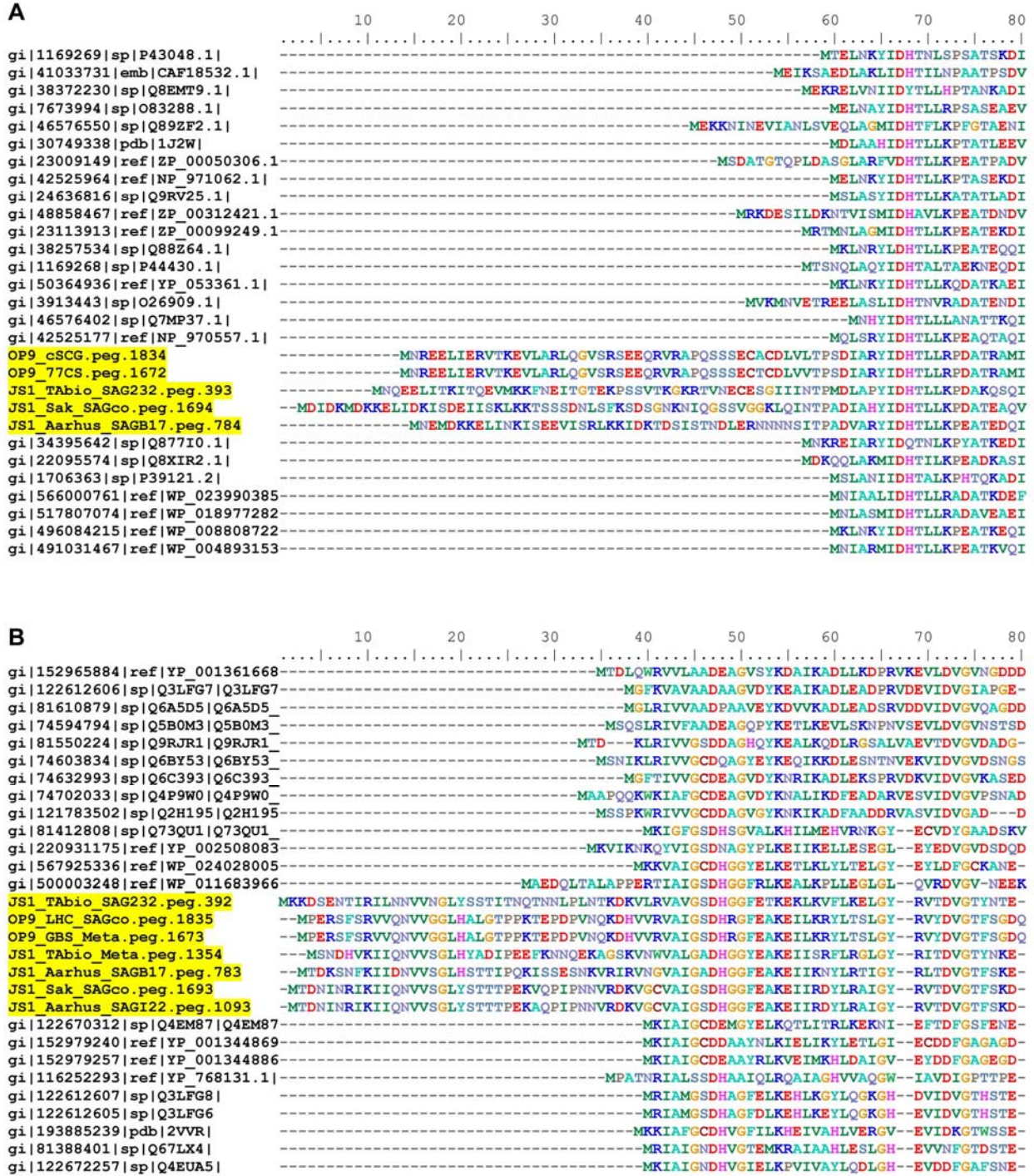


Figure S4. Multiple sequence alignment of predicted N-terminal regions of (A) DERA and (B) ribose-5-phosphate isomerase in OP9 and JS1, which are associated with BMC clusters, and closest homologs (not associated with BMC clusters). Extended N-terminal regions in BMC-

associated sequences suggest that these proteins are targeted to the inside of the BMC (Fan et al., 2010).

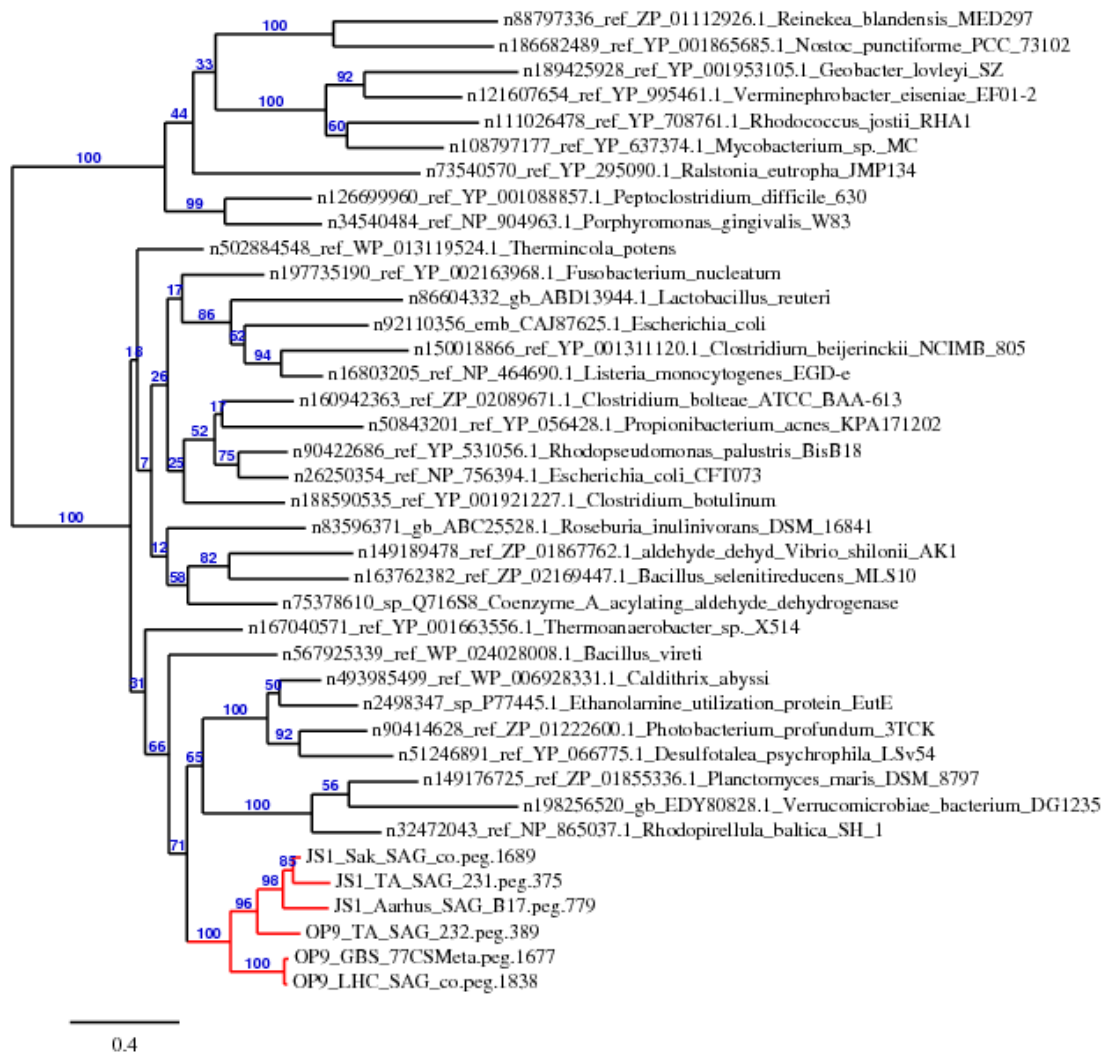


Figure S5. Phylogeny of aldehyde dehydrogenase (conserved domain cd07121) and homologs in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAXML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

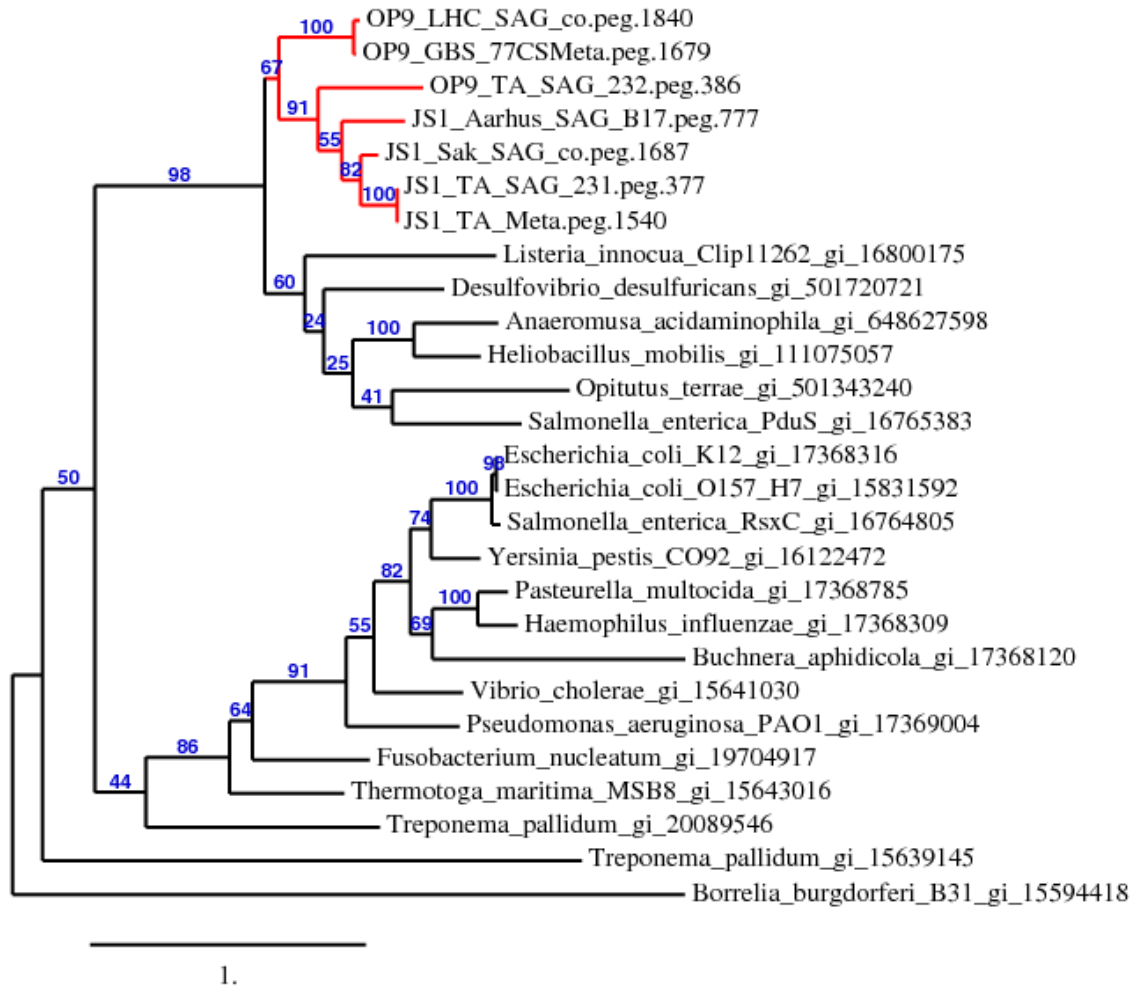


Figure S6. Phylogeny of cobalamin reductase (COG4656) and homologs in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAXML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

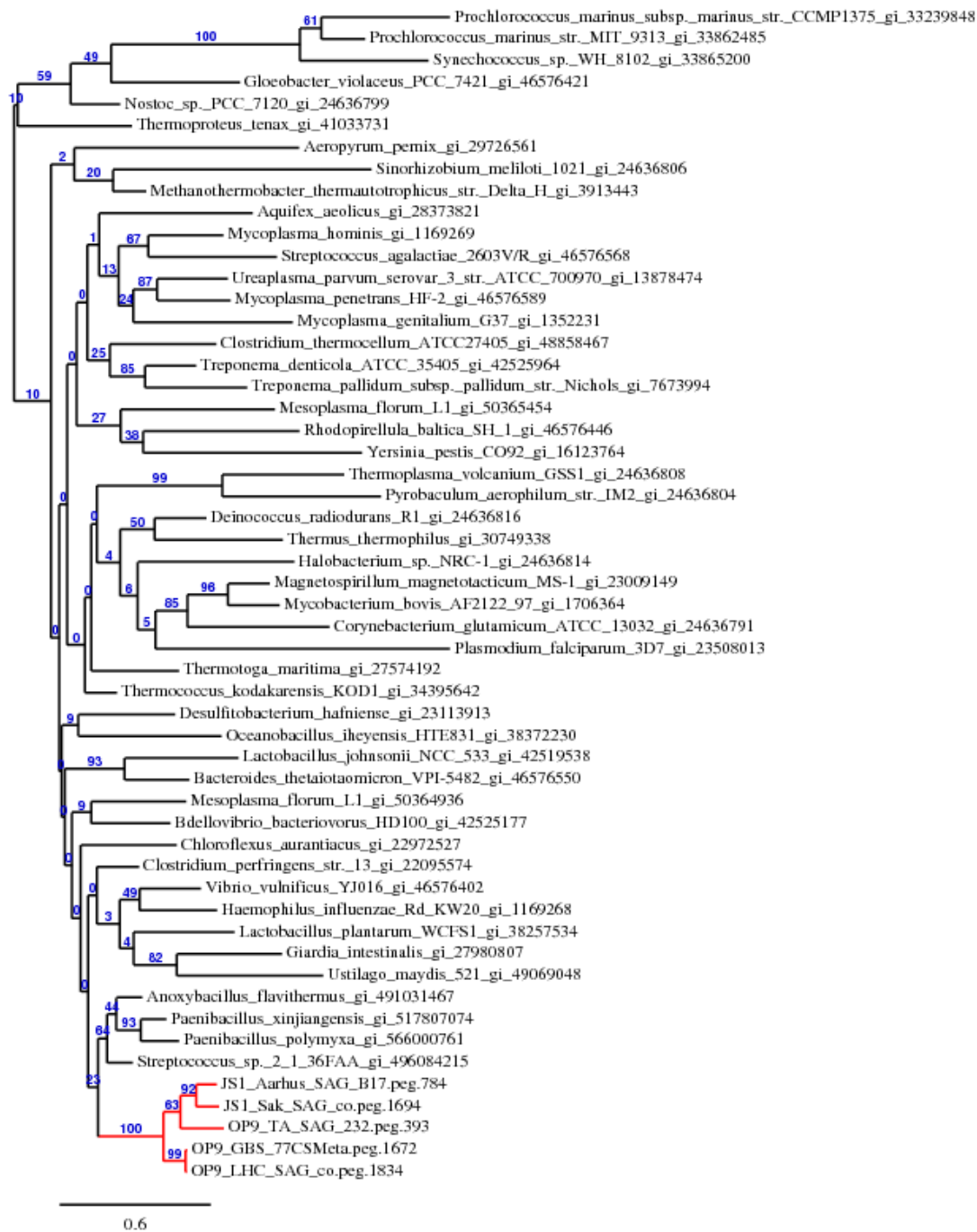


Figure S7. Phylogeny of deoxyribonucleotide aldolase (DERA, conserved domain cd00959) and homologs in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAxML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

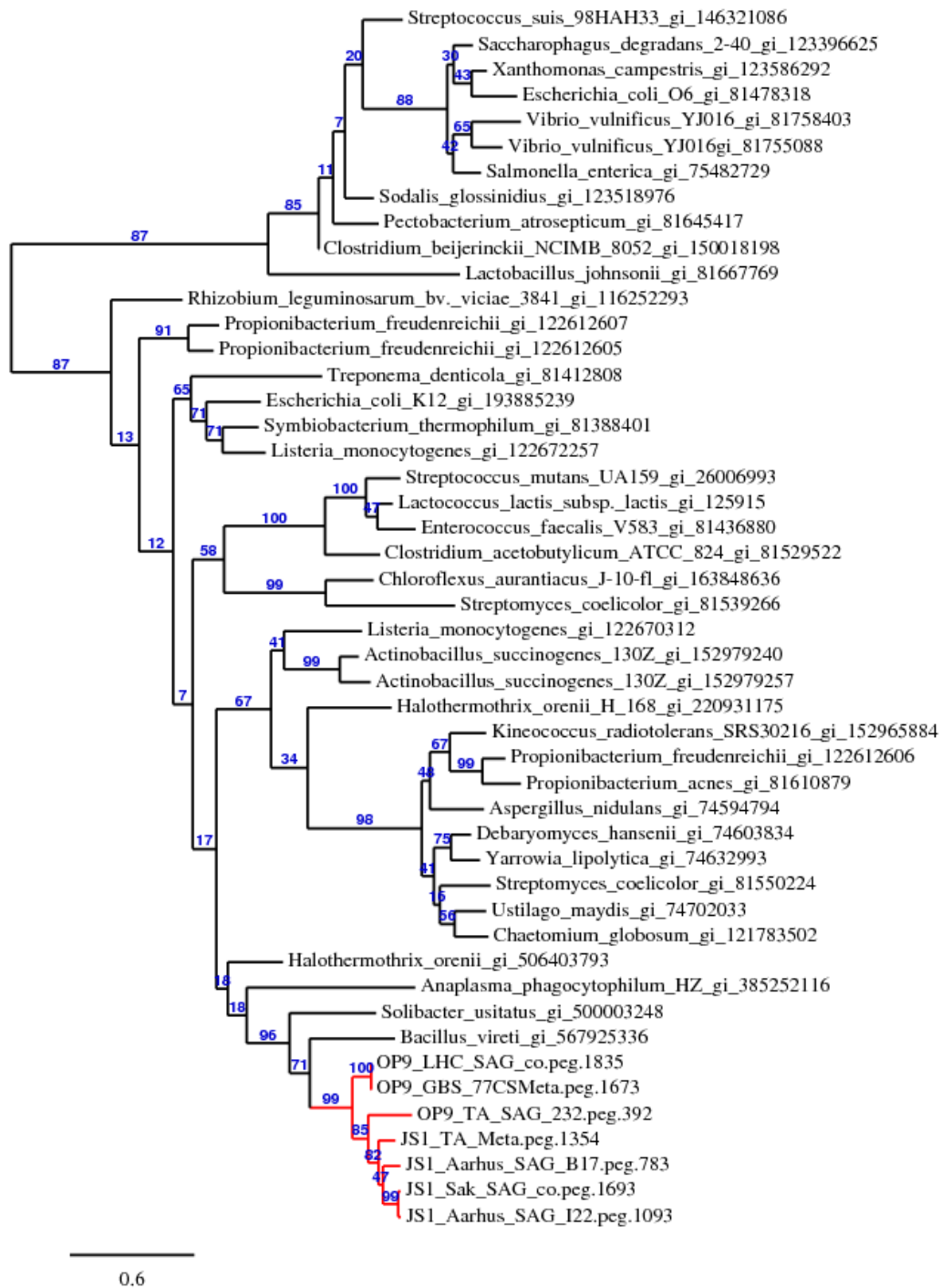


Figure S8. Phylogeny of ribose 5-phosphate isomerase (pfam02502) and homologs in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAxML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

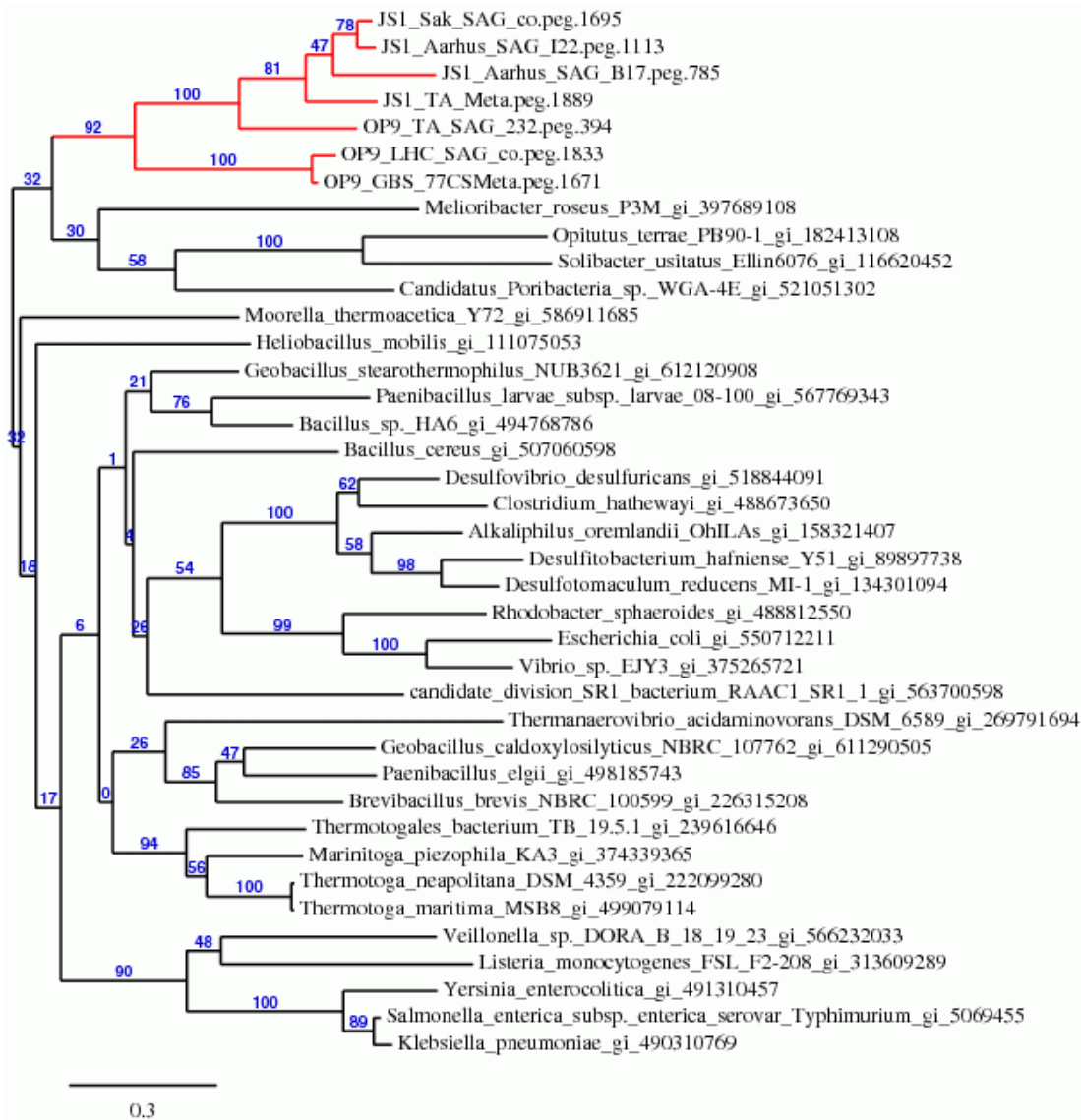


Figure S9. Phylogeny of putative phosphotransacetylase (PduL, pfam06130) and homologs in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAxML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

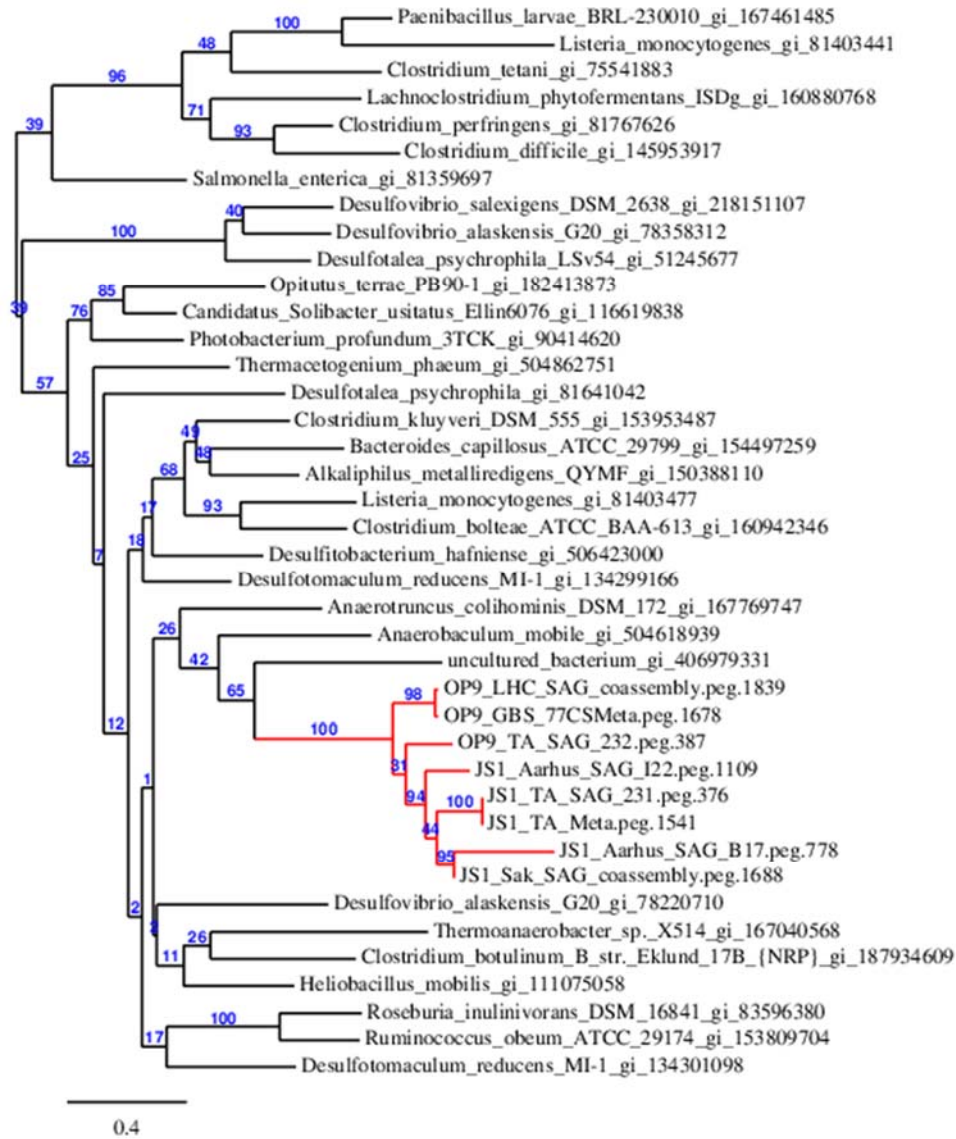


Figure S10. Phylogeny of predicted bacterial microcompartment shell proteins (conserved domains cd07053 and cd07054 in pfam00936) in ‘Atribacteria’ BMC clusters (red branches) and other organisms inferred using RAxML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

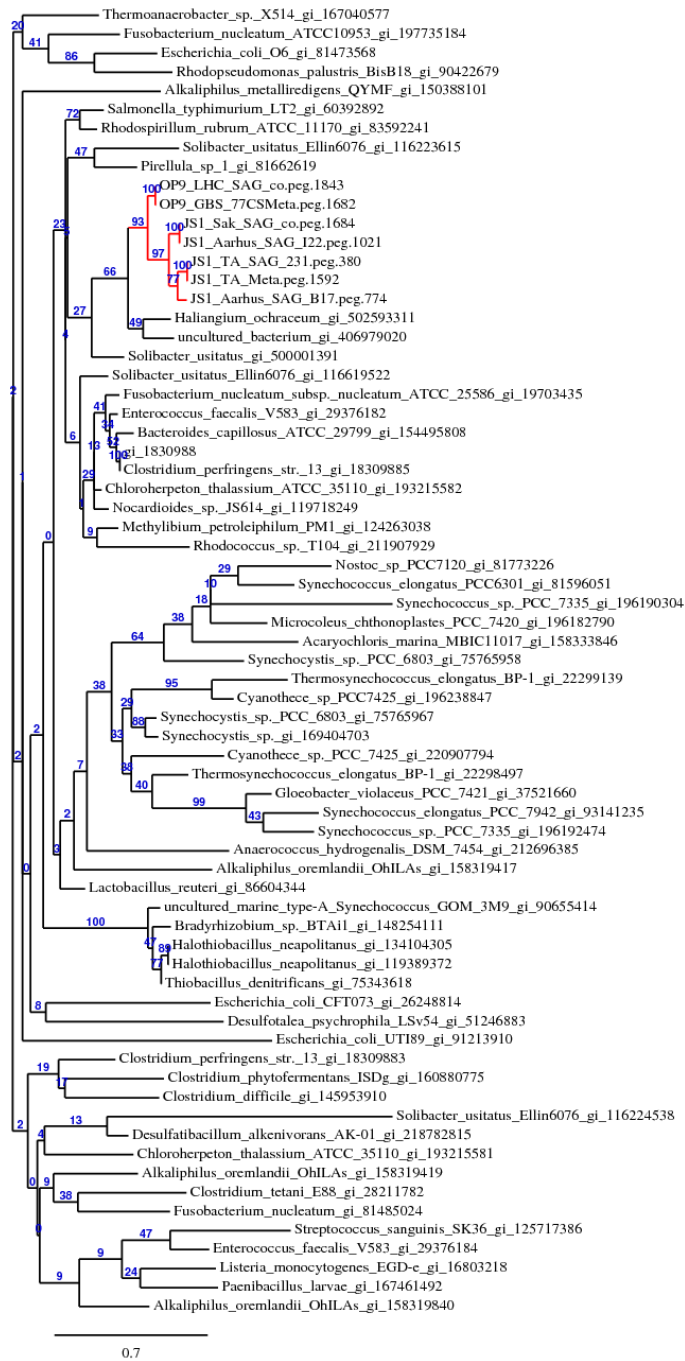


Figure S11. Phylogeny of predicted bacterial microcompartment shell proteins (conserved domain cd07045 in pfam00936) in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAXML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

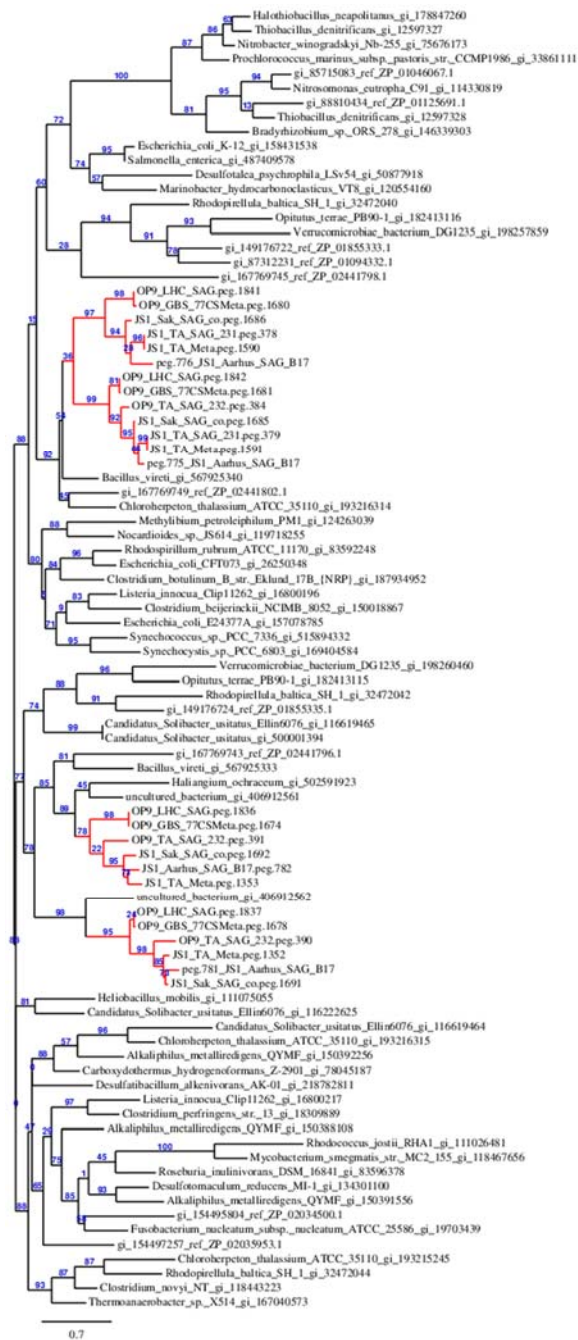


Figure S12. Phylogeny of predicted bacterial microcompartment shell proteins (conserved domain cd01614 in pfam03319) in 'Atribacteria' BMC clusters (red branches) and other organisms inferred using RAxML. The scale bar indicates the number of changes per position, and blue numbers indicate the bootstrap support (out of 100 pseudoreplicates) for a given node.

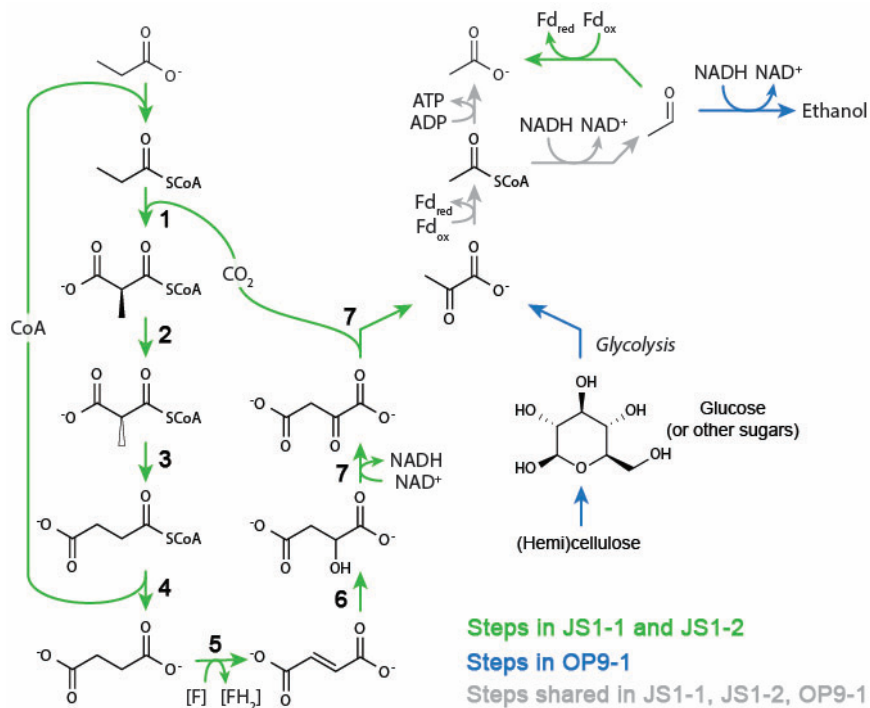


Figure S13. Predicted propionate catabolism pathway in JS1-1 and JS1-2, saccharide fermentation in OP9-1, and shared pyruvate metabolism in 'Atribacteria'. JS1 propionate catabolism (green) is predicted to converge with sugar catabolism of OP9-1 (blue) by production of pyruvate. After pyruvate is oxidatively decarboxylated to acetyl-CoA, the acetyl-CoA can either feed into substrate level phosphorylation or acetaldehyde production within the bacterial microcompartment. JS1-1 and JS1-2 reoxidize acetaldehyde to acetyl-CoA to generate reduced ferredoxin (Fd), while OP9-1 further reduces acetaldehyde to ethanol to dispose of reducing power and regenerate NAD⁺. Enzymes participating in propionate catabolism in JS1-1 and JS1-2 indicated by bold numbers are: 1, methylmalonyl-CoA decarboxylase; 2, methylmalonyl-CoA epimerase; 3, methylmalonyl-CoA mutase; 4, succinyl-CoA synthetase; 5, succinate dehydrogenase/fumarate reductase; 6, fumarate hydratase; 7, malate dehydrogenase (decarboxylating).

Contigs in metagenome bins. Metagenomes are publicly available on IMG/M.

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