

Chasing the elusive *Euryarchaeota* class WSA2:
genomes reveal a uniquely fastidious methyl-reducing methanogen

- Supplementary Information -

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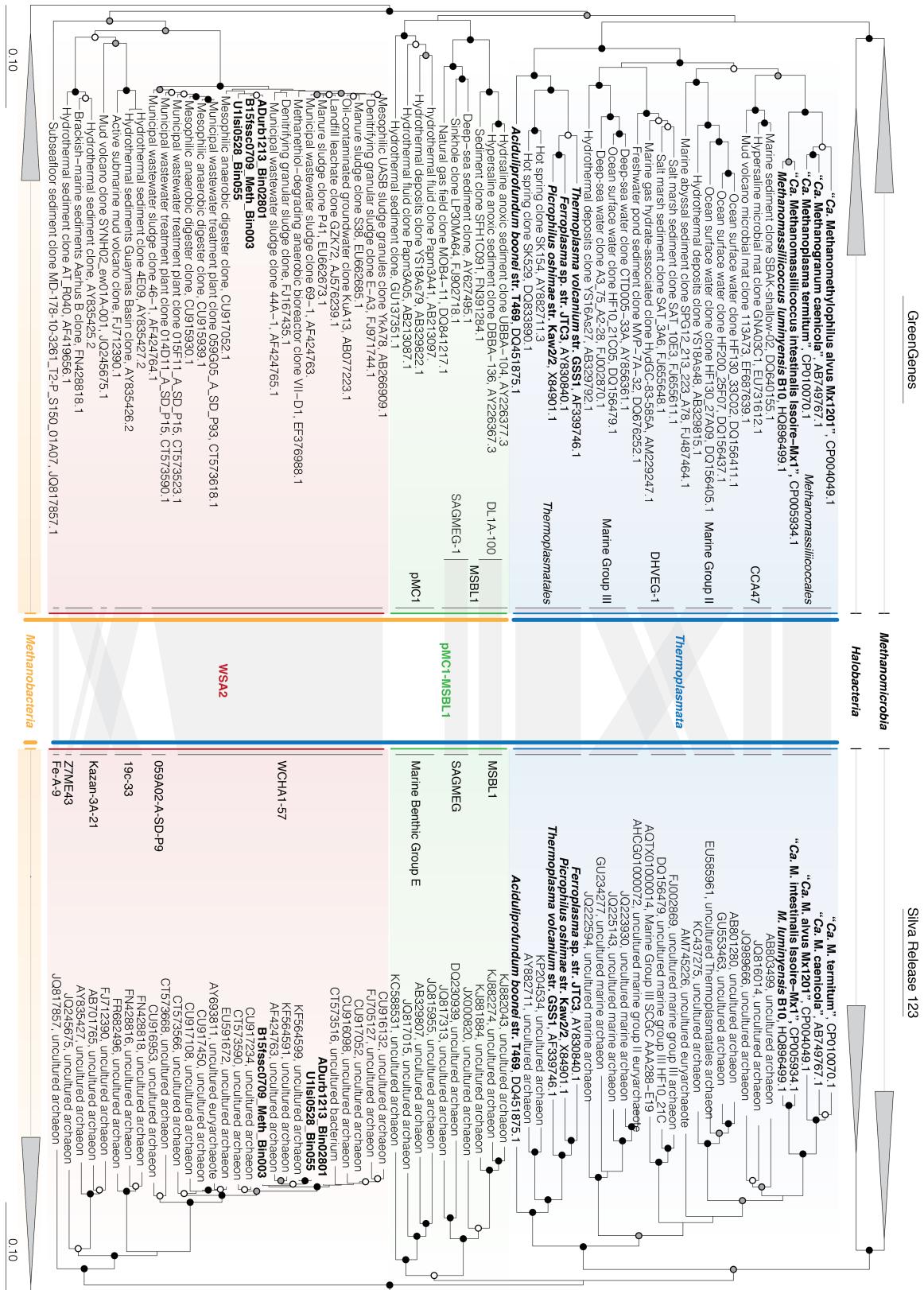
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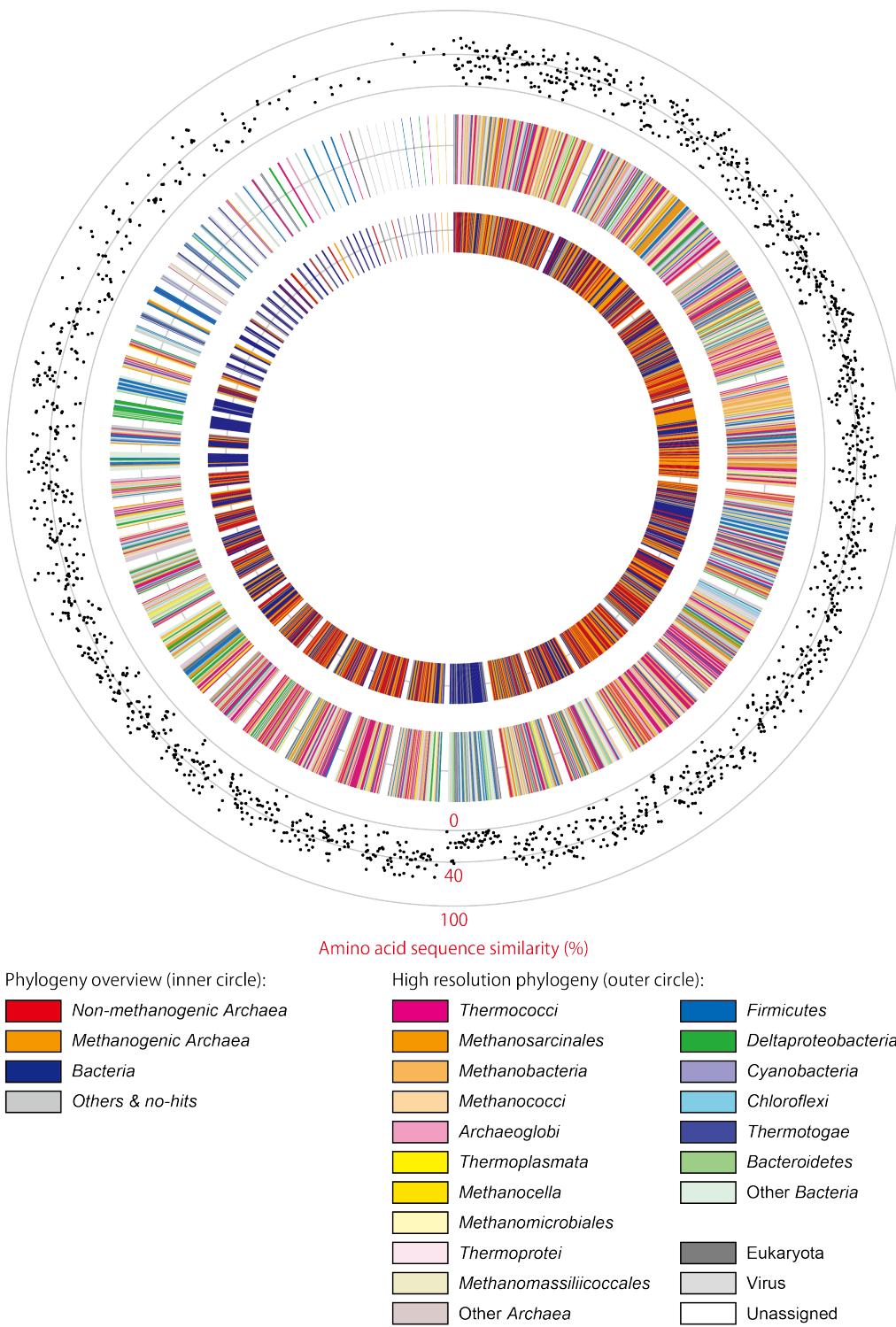
Supplementary Figure 1. WSA2 phylogeny based on methyl coenzyme M reductase subunit A. The tree was calculated using maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP). The bootstrap values for all methods are shown. The bar indicates 10% base substitution.

Supplementary Figures



Supplementary Figure 2. (see caption on next page)

Supplementary Figure 2. 16S rRNA-based phylogeny of WSA2 (red) compared to closely related *Euryarchaeota* classes and clusters (pMC1 and MSBL1) using both Greengenes (left) and Silva v123 (right) databases. The trees were constructed using ARB neighbor-joining algorithm with 5000 bootstrap replications, sequences at least 1200bp in length, and *Methanobacteria* as the outgroup. Bootstrap values greater than 90% (black), 75% (gray), and 50% (white) are shown. Clades corresponding to each other in the two databases are indicated. The current classification of each clade to *Thermoplasmata* (blue) and *Methanobacteria* (orange) classes are shown at the center with thick vertical bars. The renewed classification is depicted as thin adjacent lines, categorizing the sequences as *Thermoplasmata*, pMC1-MSBL1, WSA2, or *Methanobacteria*.



Supplementary Figure 3. The phylogeny of genes in the anaerobic digester WSA2 genome based on blastp against the current GenBank non-redundant protein sequence database. Contigs are shown in order of decreasing length from the top in the clock-wise direction. Genes are colored individually based on their closest phylogenetic relative using warmer and cooler colors for *Archaea* and *Bacteria* respectively at low-(domain; inner circle) and high- (ranging between phylum and order) resolution phylogenetic classification. The outer circle indicates the blastp-based similarity of the corresponding WSA2 genome to their top hit.

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Supplementary Figure 4. Phylogeny of methylated thiol Coenzyme M methyltransferase (Mts) against associated corrinoid proteins and methanol- (MtaC) and methylamine- (MtmC, MtbC, and MttC) specific methyltransferase corrinoid proteins. The tree was constructed using Clustalw with default parameters for alignment and neighbor-joining clustering method for tree construction with 5000 bootstrapped replications. For Mts families associated with *Methanomassiliicoccales* (blue), *Methanomicrobium* (orange), and WSA2 (red), lines are drawn connecting neighboring methyltransferases and corrinoid proteins. For Mts families that have discernable association with specific corrinoid proteins, backgrounds are provided behind these lines. Nodes are marked for bootstrap values greater than 90% (black), 75% (gray with outline), and 50% (gray).

Supplementary Table 1. Genome characteristics of WSA2 extracted from metagenomes of an anaerobic digester (AD) in Urbana, Illinois and full-scale and lab-scale purified terephthalic acid (PTA) process wastewater-treating methanogenic bioreactors. Genomes were identified for four WSA2 populations and one representative genome was selected for each WSA2 population (bolded). Bins (or strains) from the same population in different samples (*i.e.*, time points for Urbana AD and reactor depths for Full-scale PTA) (marked with *) are shown with estimated genome completeness calculated using 40 marker genes. (abbreviations: CDS - coding sequence)

	ADurb1213_Bin02801	ADurb1013_Bin02101*	ADurb1113_Bin01801*	B15fssc0709_Meth_Bin003	B03fssc0709_Meth_Bin005*	BMIXfssc0709_Meth_Bin006*	U1lsi0528_Bin055	U1lsi0528_Bin089
Source	Urbana AD			Full-scale PTA			Lab-scale PTA	Lab-scale PTA
Accession Number	LNJE00000000	LNJB00000000	LNJD00000000	LNGF00000000	LNGE00000000	LNJC00000000	LNGC00000000	LNGD00000000
Locus Tag	APG09	AN188	APG08	APG11	APG10	APG12	AMQ22	AMQ74
Genome Size (Mb)	1482299	1495432	1497132	1659905	1610745	1557731	1994208	1794529
G+C (%)	32.9	33.2	33.2	34.6	34.3	34.5	35.4	34.1
Contigs	66	77	60	76	87	61	306	313
Max Contig Length (kb)	133838	211347	177638	97942	72872	118394	54809	52116
N50	80261	51722	67697	34825	27886	34208	11053	10190
N90	17554	11451	17402	10145	10054	13090	2665	2281
Gene	1632	1635	1644	1887	1838	1769	2328	2000
CDS	1585	1591	1597	1845	1802	1732	2291	1964
tRNA	44	41	43	39	34	36	35	34
rRNA	3	3	4	3	2	3	2	2
Est. Completeness (%)	92.5	92.5	92.5	92.5	92.5	92.5	90	85
Genome Overlap (Mb)	n/a	1328761	1354711	n/a	1470664	1498838	n/a	n/a
Overlapping Contigs	n/a	55	47	n/a	79	55	n/a	n/a
Overlapping CDS	n/a	1375 (88%)	1396 (89%)	n/a	1679 (90%)	1668 (90%)	n/a	n/a

Supplementary Table 2. Methanogenesis-related catabolic genes of the WSA2 genomes, including core genes (black), genes found in only some genomes (blue), and genes missing from all genomes

	Function	Abbr	ADurb1213_Bin02801	B15fssc0709	U1lsi0528_Bin055	U1lsi0528_Bin089
Mcr complex	Methyl-CoM reductase I subunit beta	mcrB	APG09_00777	APG11_01871	AMQ22_00305	#N/A
	Methyl-CoM reductase I operon protein D	mcrD	APG09_00776	APG11_00758	AMQ22_00304	AMQ74_00970
	Methyl-CoM reductase I subunit gamma	mcrG	APG09_00775	APG11_00759	AMQ22_00303	AMQ74_00969
	Methyl-CoM reductase I subunit alpha	mcrA	APG09_00774	APG11_00760	AMQ22_00302	AMQ74_00968
	Methyl-CoM reductase I operon protein C	mcrC	APG09_00807	#N/A	#N/A	#N/A
Methylthiol metabolism	Methylthiol-coenzyme M methyltransferase - corrinoid fusion protein	MtsA	APG09_00949	APG11_00978	AMQ22_01897	#N/A
	Methyltransferase corrinoid protein reductive activase	MtlD	APG09_00713	APG11_01859	AMQ22_01391	AMQ74_01087
	MtaA-related methylcobamide/CoM methyltransferase	MtlB	APG09_00714	APG11_01858	AMQ22_01392	AMQ74_01088
	Methylthiol-coenzyme M methyltransferase	MtlA	APG09_00715	APG11_01477	AMQ22_01393	AMQ74_01089
	Corrinoid protein	MtlC	APG09_00716	APG11_01835	AMQ22_01394	AMQ74_01090
HdrABC-MvhDGA hydrogenase	Heterodisulfide reductase subunit A	HdrA	APG09_00680	APG11_01207	AMQ22_00755	#N/A
	Heterodisulfide reductase subunit C	HdrC	APG09_00679	APG11_01208	AMQ22_00754	AMQ74_01710
	Heterodisulfide reductase subunit B	HdrB	APG09_00678	APG11_01209	AMQ22_00753	AMQ74_01709
	F420-non-reducing hydrogenase iron-sulfur subunit D	MvhD	APG09_00677	APG11_01210	AMQ22_00752	AMQ74_01708
	Hydrogenase 2 maturation endopeptidase		APG09_00676	APG11_01211	AMQ22_00751	AMQ74_01707
HdrABC-MvhDGA hydrogenase	F420-non-reducing hydrogenase iron-sulfur subunit A	MvhA	APG09_01330	APG11_00305	AMQ22_00322	#N/A
	F420-non-reducing hydrogenase iron-sulfur subunit G	MvhG	APG09_01329	APG11_00306	AMQ22_00321	#N/A
	Heterodisulfide reductase subunit A	HdrA	#N/A	#N/A	AMQ22_00324	#N/A
	F420-non-reducing hydrogenase iron-sulfur subunit D	MvhD	#N/A	#N/A	AMQ22_00323	#N/A
	F420-non-reducing hydrogenase iron-sulfur subunit G	MvhG	#N/A	#N/A	AMQ22_00322	#N/A
Other Hdr	F420-non-reducing hydrogenase iron-sulfur subunit A	MvhA	#N/A	#N/A	AMQ22_00321	#N/A
	Heterodisulfide reductase subunit C	HdrC	#N/A	#N/A	AMQ22_00320	AMQ74_01220
	Heterodisulfide reductase subunit B	HdrB	#N/A	#N/A	AMQ22_00319	AMQ74_01221
	Heterodisulfide reductase subunit A	HdrA	APG09_00907	#N/A	AMQ22_01655	#N/A
	Heterodisulfide reductase subunit A	HdrA	APG09_00225	#N/A	#N/A	#N/A
Envergy-converting hydrogenase Ehb	Heterodisulfide reductase subunit D	HdrD	APG09_01421	#N/A	#N/A	#N/A
	energy-converting hydrogenase B subunit A	EhbA	APG09_00460	APG11_00234	AMQ22_01802	#N/A
	energy-converting hydrogenase B subunit B	EhbB	APG09_00459	APG11_00233	AMQ22_01803	#N/A
	energy-converting hydrogenase B subunit C	EhbC	APG09_00458	APG11_00232	AMQ22_01804	#N/A
	energy-converting hydrogenase B subunit D	EhbD	APG09_00457	APG11_00231	AMQ22_01805	#N/A
	energy-converting hydrogenase B subunit E	EhbE	APG09_00456	APG11_00230	AMQ22_01806	#N/A
	energy-converting hydrogenase B subunit F	EhbF	APG09_00455	APG11_00229	#N/A	#N/A
	energy-converting hydrogenase B subunit G	EhbG	APG09_00454	APG11_00228	AMQ22_01964	#N/A
	energy-converting hydrogenase B subunits H and I fusion protein	EhbHI	APG09_00453	APG11_00227	AMQ22_01965	AMQ74_01530
	energy-converting hydrogenase B subunit J	EhbJ	APG09_00452	APG11_00226	AMQ22_01966	AMQ74_01529
	energy-converting hydrogenase B subunit K, polyferredoxin	EhbK	APG09_00451	APG11_00225	AMQ22_01967	AMQ74_01528
	energy-converting hydrogenase B subunit K, polyferredoxin	EhbK	APG09_00450	APG11_00224	AMQ22_01968	AMQ74_01527
	energy-converting hydrogenase B subunit L	EhbL	APG09_00449	APG11_00223	AMQ22_01969	AMQ74_01526
	energy-converting hydrogenase B subunit M	EhbM	APG09_00448	APG11_00222	#N/A	AMQ74_01525
	energy-converting hydrogenase B subunit N, hydrogenase large sunbunit	EhbN	APG09_00447	APG11_00221	#N/A	#N/A
	energy-converting hydrogenase B subunit O	EhbO	APG09_00446	APG11_00220	AMQ22_00985	#N/A
	energy-converting hydrogenase B subunit P	EhbP	APG09_00445	APG11_00219	AMQ22_00984	AMQ74_01647
F420 hydrogenase	Coenzyme F420 hydrogenase subunit alpha	FrhA	APG09_00532	APG11_00938	AMQ22_01241	AMQ74_00801
	Hydrogenase 3 maturation protease		APG09_00531	APG11_00939	AMQ22_01242	AMQ74_00802
	Coenzyme F420 hydrogenase subunit beta	FrhB	APG09_00530	APG11_00940	AMQ22_01243	AMQ74_00803

Supplementary Table 3. Biosynthesis pathways of the WSA2 genomes, including core genes (black), genes found in only some genomes (blue), and genes missing from all genomes

	Function	Abbr	ADurb1213_Bin02801	B15fsc0709	U1lsi0528_Bin055	U1lsi0528_Bin089
Methylmalonyl-CoA pathway	methylmalonyl-CoA carboxyltransferase		APG09_00568	APG11_01543	AMQ22_01538	AMQ74_01179
	sodium ion-translocating decarboxylase, gamma subunit		APG09_00569	APG11_01544	AMQ22_01537	AMQ74_01180
	decarboxylase biotin/lipoyl attachment domain-containing protein		APG09_00570	APG11_01545	AMQ22_01536	AMQ74_01181
	sodium ion-translocating decarboxylase, beta subunit		APG09_00571	APG11_01546	AMQ22_01535	AMQ74_01182
	decarboxylase biotin/lipoyl attachment domain-containing protein		APG09_00572	APG11_01547	AMQ22_01534	AMQ74_01183
	methylmalonyl-CoA mutase		APG09_01502	APG11_01040	AMQ22_00728	AMQ74_01184
	methylmalonyl-CoA epimerase		APG09_01504	APG11_01232	AMQ22_00730	AMQ74_01095
	malonate-semialdehyde dehydrogenase (acetylating)	mmsA	APG09_01420	APG11_00891	AMQ22_02031	AMQ74_00935
	citrate synthase	CS	APG09_00144	APG11_01242	AMQ22_00720	AMQ74_00795
	aconitate hydratase		APG09_00880	APG11_00984	AMQ22_00836	AMQ74_01300
Tricarboxylic acid cycle	isocitrate dehydrogenase		APG09_00881	APG11_00983	#N/A	AMQ74_01301
	2-oxoglutarate ferredoxin oxidoreductase	korA	AN188_01327	APG11_00418	AMQ22_01047	#N/A
	2-oxoglutarate ferredoxin oxidoreductase	korB	AN188_01326	APG11_00419	AMQ22_01048	AMQ74_01919
	2-oxoglutarate ferredoxin oxidoreductase	korC	AN188_01325	APG11_00420	AMQ22_01049	AMQ74_01918
	succinyl-CoA synthase		APG09_00860	APG11_01090	#N/A	AMQ74_00411
	fumarate hydratase	fumA	APG09_01332	APG11_00302	AMQ22_00326	AMQ74_01058
	fumarate hydratase	fumB	APG09_01333	APG11_00301	AMQ22_00327	AMQ74_01057
	fumarate reductase		APG09_00204	APG11_00310	AMQ22_00317	AMQ74_01223
	malate dehydrogenase		APG09_01334	APG11_00300	AMQ22_00328	AMQ74_01056
	pyruvate carboxylase	pyc	APG09_01380	APG11_00441	AMQ22_01678	AMQ74_00644
Malonate	acetyl-CoA carboxylase [Meiothermus taiwanensis]		APG09_00590	APG11_01289	AMQ22_00133	AMQ74_00454
	acetyl-CoA carboxylase biotin carboxylase subunit [Brevibacillus sp. BC25]		APG09_00588	#N/A	AMQ22_00135	AMQ74_01139
	malonate decarboxylase acyl carrier protein [Enterorhabdus caecimuris]		APG09_00587	APG11_00171	AMQ22_00136	AMQ74_01140
	malonate decarboxylase, beta subunit [Enterorhabdus caecimuris]		APG09_00586	#N/A	AMQ22_00137	AMQ74_01141
	malonate decarboxylase gamma subunit [Phascolarctobacterium sp. CAG:207]		APG09_00585	#N/A	AMQ22_00138	AMQ74_01142
Glycolysis (incomplete)	holo-ACP synthase, malonate decarboxylase-specific [Pelosinus fermentans]		APG09_00583	APG11_01561	AMQ22_00140	AMQ74_01144
	fructose-1,6-bisphosphatase		APG09_00327	APG11_01410	AMQ22_01108	AMQ74_00432
	fructose-1,6-bisphosphate aldolase		#N/A	#N/A	#N/A	#N/A
	glyceraldehyde-3-phosphate dehydrogenase [Thermococcus kodakarensis]		APG09_01155	APG11_01358	AMQ22_00415	AMQ74_01415
	phosphoglyceromutase		APG09_00160	APG11_01107	AMQ22_00835	AMQ74_00427
Pentose phosphate pathway (some incomplete)	phosphoenolpyruvate synthase		APG09_00111	APG11_00966	AMQ22_00917	AMQ74_00017
	transketolase		#N/A	APG11_01152	#N/A	#N/A
	ribulose-phosphate 3-epimerase		#N/A	APG11_01155	#N/A	#N/A
	transaldolase		#N/A	APG11_01154	#N/A	#N/A
	3-hexulose-6-phosphate synthase [Thermoplasmatales archaeon SCGC AB-540-F20]		APG09_00091	APG11_00349	AMQ22_00405	AMQ74_00037
Aspartate & Asparagine	ribose-5-phosphate isomerase		APG09_01390	APG11_00430	AMQ22_02156	AMQ74_00833
	aminotransferase [Thermococcus barophilus]		APG09_00020	APG11_01170	AMQ22_00338	AMQ74_00212
Glutamate / Glutamine / Arginine	asparagine synthase		APG09_01393	APG11_00427	AMQ22_01057	AMQ74_00830
	aminotransferase [Thermococcus barophilus]		APG09_00020	APG11_01170	AMQ22_00338	AMQ74_00212
	glutamine synthetase [Methanothermobacter thermautotrophicus CaT2]	glnA	APG09_00477	APG11_00258	AMQ22_00506	AMQ74_01080
	glutamate N-acetyltransferase / amino-acid N-acetyltransferase	argJ	APG09_00363	APG11_00551	AMQ22_01506	AMQ74_00055
	acetylglutamate/LysW-gamma-L-alpha-amino adipate kinase	lysZ	APG09_00663	APG11_01224	AMQ22_00738	AMQ74_00719
	N-acetyl-gamma-glutamyl-phosphate reductase	argC	APG09_00664	APG11_01223	AMQ22_00739	AMQ74_00718
	acetylornithine aminotransferase [Herpetosiphon aurantiacus]	argD	APG09_00662	APG11_01225	AMQ22_00737	AMQ74_00720
	acetylornithine deacetylase	argE	APG09_00661	APG11_01226	AMQ22_00736	AMQ74_00721
	argininosuccinate synthase		APG09_00171	#N/A	#N/A	#N/A
	argininosuccinate lyase		APG09_00170	#N/A	#N/A	#N/A
Proline (incomplete)	arginase		APG09_00700	#N/A	#N/A	#N/A
	ornithine cyclodeaminase		#N/A	#N/A	#N/A	#N/A
Lysine	pyrrolidine-5-carboxylate reductase		#N/A	APG11_00064	#N/A	#N/A
	ornithine carbamoyltransferase	argF	APG09_00790	APG11_00179	AMQ22_00526	AMQ74_00950
	argininosuccinate synthase	argG	APG09_01034	APG11_01054	AMQ22_00895	AMQ74_00917
	aminotransferase		AN188_00355	APG11_00956	AMQ22_01308	AMQ74_00509
	alpha-aminoadipate-LysW ligase	lysX	APG09_00665	APG11_01222	AMQ22_00740	AMQ74_00717
	acetylglutamate/LysW-gamma-L-alpha-aminoadipate kinase	lysY	APG09_00663	APG11_01224	AMQ22_00738	AMQ74_00719
	N-acetyl-gamma-glutamyl-phosphate/LysW-gamma-L-alpha-aminoadipyl-6-phosphate reductase	lysZ	APG09_00664	APG11_01223	AMQ22_00739	AMQ74_00718
	acetylornithine/LysW-gamma-L-lysine aminotransferase	lysJ	APG09_00662	APG11_01225	AMQ22_00737	AMQ74_00720
	LysW-gamma-L-lysine carboxypeptidase	lysK	APG09_00661	APG11_01226	AMQ22_00736	AMQ74_00721
	aspartate kinase		#N/A	#N/A	#N/A	#N/A
Homoserine & Threonine (incomplete)	aspartate-semialdehyde dehydrogenase		APG09_00102	APG11_00338	AMQ22_01145	AMQ74_00026
	homoserine dehydrogenase		APG09_00104	APG11_00336	AMQ22_01143	AMQ74_00024
	homoserine kinase		#N/A	#N/A	#N/A	#N/A
Serine	threonine synthase	thrC	APG09_00533	APG11_00937	AMQ22_01240	AMQ74_00804
	2-hydroxyacid dehydrogenase		APG09_00174	APG11_01674	AMQ22_02272	#N/A
Tyrosine & Phenylalanine (some incomplete)	phosphoserine phosphatase		APG09_01032	APG11_01052	AMQ22_00897	AMQ74_00915
	Transketolase		#N/A	APG11_01152	#N/A	#N/A
	3-deoxy-7-phosphoheptulonate synthase	aroF	APG09_01016	APG11_01688	AMQ22_01758	AMQ74_01878
	3-dehydroquinate synthase	aroB	APG09_01015	APG11_01687	AMQ22_01757	AMQ74_01652
	3-dehydroquinate dehydratase / shikimate dehydrogenase	aroDE	APG09_01014	APG11_01686	AMQ22_01756	AMQ74_01653
	shikimate kinase	aroK	APG09_01012	APG11_01684	AMQ22_01754	AMQ74_01958
	3-phosphoshikimate 1-carboxyvinyltransferase	aroA	APG09_01013	APG11_01685	AMQ22_01755	#N/A
	chorismate synthase	aroC	APG09_01011	APG11_01683	#N/A	#N/A
	chorismate mutase		APG09_01018	APG11_01690	AMQ22_01822	AMQ74_01843
	prephenate dehydrogenase		APG09_01010	APG11_01682	AMQ22_02004	AMQ74_00756

		pheA2	APG09_01007	APG11_01681	AMQ22_02003	AMQ74_00755
Histidine	prephenate dehydratase		AN188_00029	APG11_00975	#N/A	AMQ74_00714
	ribose-phosphate pyrophosphokinase	hisG	APG09_00872	APG11_00996	AMQ22_00846	AMQ74_00597
	ATP phosphoribosyltransferase		hisIE	APG09_01055	APG11_01075	AMQ22_01025
	phosphoribosyl-ATP pyrophosphohydrolase / phosphoribosyl-AMP cyclohydrolase	hisA	APG09_01058	APG11_01078	AMQ22_01022	AMQ74_00110
	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase		APG09_01059	APG11_01079	AMQ22_01021	AMQ74_00109
	imidazole glycerol phosphate synthase [Methanofollis liminatans]	hisF	APG09_01056	APG11_01076	AMQ22_01024	AMQ74_00112
	imidazole glycerol phosphate synthase	hisC	APG09_01060	APG11_01080	AMQ22_01020	#N/A
	histidinol-phosphate aminotransferase	hisB	APG09_01057	APG11_01077	AMQ22_01023	AMQ74_00111
	imidazoleglycerol-phosphate dehydratase / histidinol phosphatase	hisD	APG09_01061	APG11_01081	AMQ22_01019	AMQ74_00107
	histidinol dehydrogenase	ilvB	APG09_00658	APG11_00407	AMQ22_00446	AMQ74_00724
Isoleucine, Leucine, & Valine (some incomplete)	Acetolactate synthase	ilvC	#N/A	APG11_00405	AMQ22_00448	AMQ74_00726
	ketol-acid reductoisomerase	ilvD	APG09_00654	APG11_00400	AMQ22_00453	AMQ74_01386
	dihydroxy-acid dehydratase		APG09_00655	APG11_00401	AMQ22_00452	AMQ74_01385
	2-isopropylmalate synthase		#N/A	APG11_00403	AMQ22_00450	AMQ74_00728
	2-isopropylmalate isomerase		APG09_00667	APG11_01220	AMQ22_00742	AMQ74_01276
	3-isopropylmalate dehydratase large subunit		APG09_00668	APG11_01219	AMQ22_00743	AMQ74_01277
	3-isopropylmalate dehydratase small subunit		#N/A	APG11_01221	#N/A	#N/A
	isocitrate/isopropylmalate dehydrogenase		#N/A	APG11_01221	#N/A	#N/A
	ammonium transporter		#N/A	APG11_00473	#N/A	AMQ74_00135
	ribose ABC transporter ATP-binding protein		#N/A	APG11_00519	AMQ22_01098	#N/A
Transporters	sugar transporter		#N/A	APG11_00883	AMQ22_02115	AMQ74_00342
	arabinose ABC transporter permease		#N/A	#N/A	#N/A	AMQ74_00888
	sugar ABC transporter ATPase		#N/A	#N/A	#N/A	AMQ74_01640
	citrate transporter	APG09_00856	APG11_01087	AMQ22_00815	AMQ74_00408	
	polysaccharide ABC transporter ATP-binding protein		#N/A	APG11_01568	#N/A	#N/A
	C4-dicarboxylate ABC transporter subunit		#N/A	#N/A	AMQ22_00588	#N/A
	branched-chain amino acid ABC transporter	APG09_01366	APG11_00784	AMQ22_00277	AMQ74_00327	
	amino acid ABC transporter substrate-binding protein	APG09_01367	APG11_00783	AMQ22_00278	#N/A	
	peptide ABC transporter ATP-binding protein		#N/A	APG11_01024	AMQ22_01596	#N/A
	glutamine ABC transporter permease		#N/A	APG11_01025	AMQ22_01595	AMQ74_01832
	ABC transporter substrate-binding protein		#N/A	APG11_01026	AMQ22_01593	AMQ74_01833
	cation transporter		#N/A	APG11_01029	#N/A	#N/A
	peptide ABC transporter substrate-binding protein	APG09_00231	#N/A	#N/A	#N/A	#N/A
	peptide ABC transporter permease	APG09_00233	#N/A	AMQ22_02103	AMQ74_00634	
	peptide ABC transporter permease	APG09_00234	#N/A	#N/A	AMQ74_00633	
	oligopeptide/dipeptide transporter, ATP-binding protein	APG09_00235	#N/A	#N/A	AMQ74_00632	
	peptide ABC transporter, ATP-binding protein	APG09_00236	#N/A	#N/A	AMQ74_00631	
	peptide ABC transporter substrate-binding protein		#N/A	#N/A	#N/A	AMQ74_01962
	glutamine ABC transporter ATP-binding protein		#N/A	#N/A	#N/A	#N/A
	glutamine ABC transporter permease		#N/A	#N/A	#N/A	AMQ74_01832
	sulfonate transporter	APG09_00054	APG11_01537	AMQ22_00745	AMQ74_01279	
	sulfonate transporter	APG09_00055	APG11_01538	AMQ22_01913	AMQ74_00234	
	sulfonate transporter	APG09_00670	APG11_01217	#N/A	AMQ74_00235	

Supplementary Table 4. Methanogenesis cofactor biosynthesis of the WSA2 genomes, including core genes (black), genes found in only some genomes (blue), and genes missing from all genomes

	Function	Abbr	ADurb1213_Bin02801	B15fssc0709	U1lsi0528_Bin055	U1lsi0528_Bin089
Cobamide / F430 synthesis	Glutamyl-tRNA reductase	HemA	APG09_00239	APG11_00898	AMQ22_00477	AMQ74_00686
	glutamate-1-semialdehyde 2,1-aminomutase	HemL	APG09_00440	APG11_00214	AMQ22_00979	AMQ74_01568
	delta-aminolevulinic acid dehydratase	HemB	APG09_00240	APG11_00899	AMQ22_00478	AMQ74_00687
	porphobilinogen deaminase	HemC	APG09_00441	APG11_00215	AMQ22_00980	AMQ74_01643
	Uroporphyrinogen-III C-methyltransferase	CobA	APG09_00442	APG11_00216	AMQ22_00981	AMQ74_01644
	precorrin-2 dehydrogenase	CysG	APG09_00238	APG11_00897	AMQ22_00476	AMQ74_00685
	Sirohydrochlorin cobaltochelatase	CbiX	APG09_00244	APG11_00903	AMQ22_00482	AMQ74_00691
Coenzyme B synthesis	cobyricin acid a,c-diamide synthase	CbiA	APG09_00248	APG11_00907	AMQ22_00486	#N/A
	Cob(I)alamin adenosyltransferase	CobO	APG09_00030	APG11_01177	#N/A	AMQ74_00180
	Homoaconitase large subunit	aksD	APG09_00668	APG11_01219	AMQ22_00743	AMQ74_01277
F420 modification	Homoaconitase small subunit	aksE	APG09_00667	APG11_01220	AMQ22_00742	AMQ74_01276
	homoisocitrate dehydrogenase	aksF	APG09_00669	APG11_01221	AMQ22_00741	#N/A
	homoisocitrate synthase	aksA	APG09_00167	APG11_01809	#N/A	#N/A
F420 pathway	F420 alpha-L-glutamate ligase	cofF	APG09_01181	APG11_00877	AMQ22_01190	AMQ74_01207
	F420 gamma-L-glutamate ligase	cofE	APG09_00852	APG11_01498	#N/A	AMQ74_00403
	F420-0-gamma-glutamyl ligase		APG09_01382	APG11_00439	AMQ22_01674	AMQ74_00639
F420 usage	7,8-didemethyl-8-hydroxy-5-deazariboflavin synthase	CofGH	#N/A	#N/A	#N/A	#N/A
	LPPG:Fo 2-phospho-L-lactate transferase	CofD	APG09_00851	APG11_01499	AMQ22_02011	AMQ74_00402
	2-phospho-L-lactate guanylyltransferase	CofC	APG09_00850	APG11_01500	AMQ22_02012	#N/A
	transporter		APG09_00853	APG11_01497	AMQ22_00811	AMQ74_00404
NADPH usage	Coenzyme F420:NADPH oxidoreductase		APG09_00948	APG11_00979	AMQ22_01896	AMQ74_01307
	Malonyl-CoA reductase (NADP)		APG09_00664	APG11_01223	AMQ22_00739	AMQ74_00718
	Isocitrate dehydrogenase (NADP)		APG09_00881	APG11_00983	#N/A	AMQ74_01301
S layer	S layer protein		APG09_00651	APG11_00625	AMQ22_01410	AMQ74_00995
	S layer protein (peptidoglycan anchored)		APG09_01383	APG11_00438	AMQ22_01673	AMQ74_00638
	S-layer-related duplication domain-containing protein		#N/A	APG11_01121	#N/A	#N/A
	S-layer structural protein		APG09_00793	APG11_00176	AMQ22_00529	AMQ74_00947