

Supplementary Table 1. Read number per library and relative abundance of reads assigned to AOM organisms (ANME-1 and HotSeep-1). Compared is the relative contribution of ANME-1 and HotSeep-1 to the active AOM community and the total community under AOM conditions (CH4), in the presence of hydrogen (H2) or hydrogen plus methane (H2CH4).

treatment library	CH4				H2			CH4H2	
	1	2	3	4	5	6	7	8	9
total reads ¹	2,376,488	2,178,329	2,073,061	2,195,275	2,112,692	1,971,533	2,345,128	2,080,255	2,017,178
total mapped to AOM organisms ²	1,368,781	1,296,564	1,263,867	1,543,374	1,488,117	1,381,823	1,646,746	1,519,801	1,412,490
% mapped to AOM organisms of total	58	60	61	70	70	70	70	73	70
HotSeep-1									
total mapped	336,297	306,961	309,485	935,073	849,066	709,035	867,875	869,395	759,486
% mapped of total	14	14	15	43	40	36	37	42	38
% mapped of AOM organisms	25	24	24	61	57	51	53	57	54
ANME-1									
total mapped	1,032,484	989,603	954,382	608,301	639,051	672,788	778,871	650,406	653,004
% mapped of total	43	45	46	28	30	34	33	31	32
% mapped of AOM organisms	75	76	76	39	43	49	47	43	46

¹ total read number after quality control

² sum of reads mapping to the draft genomes of ANME-1 and HotSeep-1

Supplementary Table 2a. C-type cytochromes identified in the thermophilic ANME-1 draft genome. Overview of protein properties including amino acid length, number of heme binding sites, protein domain annotation and predicted number of transmembrane helices and subcellular localization. Proteins discussed in the main text are indicated in bold.

ANME-1 ORF	Length (aa)	# of CXXCH motifs ¹	protein domain prediction ²			subcellular localization prediction ⁴					
			domain ID	domain accession	# of TM helices ³	Cytoplasmic_S core	Cytoplasmic Membrane_Score	Cellwall_Score	Extracellular_Score	Final_Localization	Final_Score
PROKKA_00109	263	4	Cytochrome_C7	PF14522.1	1	0.24	0.01	0.93	8.82	Extracellular	8.82
PROKKA_00110	275	4	Cytochrome_C7	PF14522.1	1	2.5	2.5	2.5	2.5	Unknown	2.5
PROKKA_00111	437	9	Cytochrome_C7	PF14522.1	1	2.2	0.01	0.99	6.8	Unknown	6.8
PROKKA_00125	186	4	Cytochrome_C7	PF14522.1	1	0	4	3.48	2.52	Unknown	4
PROKKA_00239	395	8	Cytochrom_c3_2	PF14537.1	2	0	4	3.48	2.52	Unknown	4
PROKKA_01542	203	5	Cytochrom_NNT	PF03264.9	1	7.5	1	0.87	0.63	Cytoplasmic	7.5
PROKKA_01545	248	5	Cytochrom_c3_2	PF14537.1	1	7.5	1	0.87	0.63	Cytoplasmic	7.5

¹ predicted number of heme binding sites based on identification of the CXXCH motif

² protein domain as identified by hmmscan against the pfamA database with E value cut off of 10⁻⁵; listed are the pfamA domain ID and accession of the best hit

³ predicted number of transmembrane helices using TMHMM v.2.0

⁴ subcellular localization prediction using PSORTb v.3.0; given are the PSORTb scores for each possible localization and the final predicted localization with final score

Supplementary Table 2b. Comparative analysis of c-type cytochromes identified in the thermophilic ANME-1 draft genome. Overview of results from blastp (E value cut-off of 10e-5) search of ANME-1 c-type cytochromes against the NCBI non-redundant database and the genomes of *Geobacter sulfurreducens* and *Geobacter metallireducens*. The best hit identified by highest score is shown. Proteins discussed in the main text are indicated in bold.

ANME ORF	percent identity		E value		bitscore		query coverage		subject description		subject ID	
	ncbi nt-nr	G.s.	ncbi nt-nr	G.s.	ncbi nt-nr	G.s.	ncbi nt-nr	G.s.	ncbi nt-nr	G.s.	ncbi nt-nr	G.s.
PROKKA_00109	48.05	NA	1e-63	NA	211	NA	NA	94	NA	NA	NA	NA
									hypothetical protein Areve_0081 [Archaeoglobus veneficus SNP6]	NA	NA	NA
PROKKA_00110	39.78	29.19	4e-53	7e-06	185	43.9	NA	96	49	NA	NA	NA
									hypothetical protein Areve_0080 [Archaeoglobus veneficus SNP6]	lipoprotein cytochrome c	NA	NA
PROKKA_00111	48.89	NA	2e-130	NA	397	NA	NA	96	NA	NA	NA	NA
									hypothetical protein Areve_0352 [Archaeoglobus veneficus SNP6]	NA	NA	NA
PROKKA_00125	58.73	NA	3e-56	NA	187	NA	NA	99	NA	NA	NA	NA
									hypothetical secreted protein [uncultured archaeon]	NA	NA	NA
PROKKA_00239	58.19	25.29	25.09	8e-156	457	54.3	61.2	97	39	58	NA	NA
									hypothetical protein GZ27E7_11 [uncultured archaeon GZfns27E7]	cytochrome c	NA	NA
									hypothetical membrane protein, containing NapC/NirT cytochrome c family, N-terminal region [uncultured archaeon]	NA	NA	NA
PROKKA_01542	57.64	NA	3e-70	NA	224	NA	NA	99	NA	NA	NA	NA
									hypothetical secreted protein [uncultured archaeon]	lipoprotein cytochrome c	NA	NA
PROKKA_01545	56.06	26.12	1e-73	3e-06	235	44.7	NA	79	51	NA	NA	NA
									hypothetical secreted protein [uncultured archaeon]	lipoprotein cytochrome c	NA	NA

Supplementary Table 3a. C-type cytochromes identified in the HotSeep-1 draft genome. Overview of protein properties including amino acid length, number of heme binding sites, protein domain annotation and predicted number of transmembrane helices and subcellular localization. Proteins discussed in the main text are indicated in bold.

HotSeep-1 ORF	Length (aa)	# of CXXCH motifs ¹	protein domain prediction ²		# of TM helices ³	subcellular localization prediction ⁴						Final_Score			
			domain ID	domain accession		Cytoplasmic_Score	Cytoplasmic_Membrane_Score	Periplasmic_Score	OuterMembrane_Score	Extracellular_Score	Final_Localization				
gnl 1098 HS1_001096	155	4	Cytochrome_C554	PF13435.1	0	8.96	0.51	0.26	9.84	0.01	0.26	0.14	0.26	Cytoplasmic	8.96
gnl 1188 HS1_001186	149	4	Cytochrom_CIII	PF02085.11	1	0	0.01	9.84	0.01	0.01	0.01	0.14	0.14	Periplasmic	9.84
gnl 1576 HS1_001574	169	4	Cytochrom_CIII	PF02085.11	1	0	0.12	9.76	0	0	0.06	0.11	0.11	Periplasmic	9.76
gnl 161 HS1_000160	155	4	Cytochrom_CIII	PF02085.11	1	0.33	0.06	9.44	0.06	0.06	0.06	0.11	0.11	Periplasmic	9.44
gnl 162 HS1_001619	348	7	Paired_CXXCH_1	PF09699.5	1	0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	Unknown	2.5
gnl 1635 HS1_001633	444	5	Cytochrome_C554	PF13435.1	1	0	4.9	2.5	2.5	0.1	2.5	2.5	2.5	Unknown	4.9
gnl 1638 HS1_001636	425	8	Multi-haem_cyto	PF13447.1	1	5.41	0.06	4.48	0	0	0.05	0.05	0.05	Unknown	5.41
gnl 1799 HS1_001797	131	4	Cytochrome_C554	PF13435.1	0	0	0.12	9.76	0	0.11	0.11	0.11	0.11	Periplasmic	9.76
gnl 1881 HS1_001879	148	4	Cytochrome_C554	PF13435.1	0	0	4.9	2.5	2.5	0.1	2.5	2.5	2.5	Unknown	4.9
gnl 1992 HS1_001990	339	10	Paired_CXXCH_1	PF09699.5	1	8.96	0.51	0.26	9.84	0.01	0.26	0.26	0.26	Cytoplasmic	8.96
gnl 1995 HS1_001993	266	12	Cytochrom_c3_2	PF14537.1	1	0.33	0.02	9.51	0	0	0.14	0.14	0.14	Periplasmic	9.51
gnl 1996 HS1_001994	287	12	Cytochrom_c3_2	PF14537.1	1	0	4.9	2.5	2.5	0.1	2.5	2.5	2.5	Unknown	4.9
gnl 1997 HS1_001995	340	7	Paired_CXXCH_1	PF09699.5	0	2	2	2	2	2	2	2	2	Unknown	2
gnl 2000 HS1_001998	370	7	Cytochrome_C7	PF14522.1	1	0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	Unknown	2.5
gnl 2007 HS1_002005	640	26	Paired_CXXCH_1	PF09699.5	1	8.96	0.51	0.26	9.84	0.01	0.26	0.26	0.26	Cytoplasmic	8.96
gnl 2008 HS1_002006	427	16	Paired_CXXCH_1	PF09699.5	1	0	4.9	2.5	2.5	0.1	2.5	2.5	2.5	Unknown	4.9
gnl 2101 HS1_002099	393	6	Paired_CXXCH_1	PF09699.5	1	0.01	0.01	0.11	0.23	0.23	0.64	0.64	0.64	Extracellular	9.64
gnl 2102 HS1_002100	380	5	Cytochrome_C554	PF13435.1	1	0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	Unknown	2.5
gnl 2130 HS1_002128	190	5	Cytochrome_C7	PF14522.1	1	0.04	9.82	0.12	0.12	0.01	0.01	0.01	0.01	CytoplasmicMembrane	9.82
gnl 662 HS1_000660	644	7	Cytochrome_C554	PF13435.1	1	0	4.9	2.5	2.5	0.1	2.5	2.5	2.5	Unknown	4.9
gnl 783 HS1_000781	149	4	Cytochrome_C554	PF13435.1	0	8.96	0.51	0.26	9.84	0.01	0.26	0.26	0.26	Cytoplasmic	8.96

¹ predicted number of heme binding sites based on identification of the CXXCH motif

² protein domain as identified by hmmscan against the pfamA database with E value cut off of 10⁻⁵; listed are the pfamA domain ID and accession of the best hit

³ predicted number of transmembrane helices using TMHMM v.2.0

⁴ subcellular localization prediction using PSORTb v.3.0; given are the PSORTb scores for each possible localization and the final predicted localization with final score

Supplementary Table 3b. Comparative analysis of c-type cytochromes identified in the HotSeep-1 draft genome. Overview of results from blastp (E value cut-off of 10e-5) search of HotSeep-1 c-type cytochromes against the NCBI non-redundant database and the genomes of Geobacter sulfurreducens and Geobacter metallireducens. The best hit identified by highest score is shown. Proteins discussed in the main text are indicated in bold.

HotSeep-1 ORF	percent identity			E value			bifscore			query coverage			subject description			subject ID		
	nebi nt-nr	G.s.	G.m.	nebi nt-nr	G.s.	G.m.	nebi nt-nr	G.s.	G.m.	nebi nt-nr	G.s.	G.m.	nebi nt-nr	G.s.	G.m.	nebi nt-nr	G.s.	G.m.
gml1098 HSL_001096	38.93	NA	28.37	5e-26	NA	1e-06	112	NA	42.7	79	NA	90	NA	complex Cbo4, cytochrome c subunit	gi 763398059 ref WP_04425034.1	NA	NA	gi 404498358 ref YP_006722464.1
gml1188 HSL_001186	42.95	NA	NA	1e-25	NA	NA	105	NA	NA	93	NA	NA	NA	hypothetical protein [Desulfocarboidianensis]	gi 902806100 ref WP_049675172.1	NA	NA	NA
gml1576 HSL_001574	56.85	NA	NA	4e-55	NA	NA	183	NA	NA	86	NA	NA	NA	hypothetical protein [Desulfonauticus sp. A7A]	gi 661274202 ref WP_029968257.1	NA	NA	NA
gml1616 HSL_001600	38.3	25.15	NA	4e-17	1e-06	NA	83.2	43.5	NA	89	85	NA	lipoprotein cytochrome c	gi 749571808 ref WP_028326057.1	gi 39995699 ref NP_951650.1	NA	NA	NA
gml1621 HSL_001619	43.46	26.55	NA	2e-79	5e-10	NA	259	57	NA	99	85	NA	cytochrome c	gi 551229413 ref WP_040201250.1	gi 39997894 ref NP_951758.1	NA	NA	NA
gml1635 HSL_001633	67.3	27.2	28.96	0	4e-32	1e-28	611	124	114	95	72	61	61	cytochrome c	gi 51229623 ref WP_022853211.1	953845.1	006719643.1	gi 404495537 ref YP_006719643.1
gml1638 HSL_001636	69.27	NA	44.55	0	NA	8e-130	638	NA	382	98	NA	99	98	cytochrome c	gi 51229623 ref WP_022853418.1	NA	NA	gi 404494995 ref YP_006719101.1
gml1799 HSL_001797	39.39	NA	NA	5e-23	NA	NA	98.6	NA	NA	96	NA	NA	NA	cytochrome c	gi 239797418 dbj BAH76407.1	NA	NA	NA
gml1881 HSL_001879	53.02	NA	NA	3e-48	NA	NA	164	NA	NA	100	NA	NA	NA	cytochrome c	gi 501539600 ref WP_012545087.1	NA	NA	NA
gml1992 HSL_001990	50.29	50.14	34.52	2e-110	5e-114	1e-53	337	336	179	100	96	98	98	lipoprotein cytochrome c	gi 506383573 ref WP_015903292.1	gi 39997301 ref NP_953252.1	006720711.1	gi 404496605 ref YP_006720711.1
gml1995 HSL_001993	55.31	46.99	45.77	6e-81	4e-80	1e-82	255	244	251	85	99	98	98	cytochrome c	gi 740213844 ref WP_038055696.1	gi 400756389 ref NP_951727.2	006721794.1	gi 404497688 ref YP_006721794.1
gml1996 HSL_001994	51.63	44.94	39.93	4e-81	3e-75	6e-74	257	233	229	85	85	98	98	cytochrome c	gi 740213842 ref WP_038055694.1	gi 400756706 ref NP_953690.2	006719790.1	gi 404495684 ref YP_006719790.1
gml1997 HSL_001995	42.12	26.84	NA	1e-73	1e-09	NA	243	55.8	NA	99	86	NA	NA	cytochrome c	gi 492843927 ref WP_005997881.1	gi 39997598 ref NP_953549.1	NA	NA
gml2000 HSL_001998	49.6	NA	NA	1e-90	NA	NA	291	NA	NA	93	NA	NA	NA	cytochrome c	gi 740213530 ref WP_038055382.1	NA	NA	NA
gml2007 HSL_002005	35.46	34.99	30.83	1e-125	5e-129	2e-28	398	395	114	99	99	99	99	cytochrome c	gi 746623261 ref WP_039648130.1	gi 39997590 ref NP_953541.1	006721856.1	gi 404497750 ref YP_006721856.1
gml2008 HSL_002006	37.75	33.07	29.05	2e-71	9e-49	4e-23	242	171	96.3	93	88	78	78	cytochrome c	gi 551229362 ref WP_022853160.1	gi 400756673 ref NP_953540.2	006721856.1	gi 404497750 ref YP_006721856.1
gml2101 HSL_002099	50.77	26.74	NA	8e-107	5e-14	NA	331	70.1	NA	95	83	NA	NA	cytochrome c	gi 551229354 ref WP_022853152.1 	gi 39997598 ref NP_953549.1 	NA	NA
gml2102 HSL_002100	45.15	25.82	NA	3e-87	5e-14	NA	280	69.7	NA	99	93	NA	93	cytochrome c	gi 551229355 ref WP_022853153.1	gi 39997598 ref NP_953549.1	NA	NA
gml2130 HSL_002128	43.5	NA	27.7	6e-48	NA	5e-16	166	NA	69.7	93	NA	78	78	complex ACIII, cytochrome c subunit AciA	gi 661271261 ref WP_029965361.1	NA	NA	gi 404496666 ref YP_006720772.1
gml662 HSL_000660	43.09	23.54	21.67	1e-165	8e-10	2e-06	504	58.2	47.8	93	59	31	31	cytochrome c	gi 328452139 gb AEB07968.1	gi 39997299 ref NP_953250.1	006722038.1	gi 404497932 ref YP_006722038.1
gml783 HSL_000781	36	NA	NA	5e-21	NA	NA	94.4	NA	NA	96	NA	NA	NA	cytochrome c	gi 505149112 ref WP_015330214.1	NA	NA	NA

Supplementary Table 4a. Type IV pili related proteins identified in the HotScep-1 draft genome. Overview of protein properties including amino acid length, protein domain annotation and predicted function, number of transmembrane helices and subcellular localization. Proteins discussed in the main text are indicated in bold.

HotScep-1 ORF	protein domain prediction ¹			predicted protein, function ²	# of TM helices ³	subcellular localization prediction ⁴									
	Length (aa)	domain ID	domain accession			Cytoplasmic _{Sc} score	Periplasmic _{Sc} score	OuterMembrane _{Sc} score	Extracellular _{Sc} score	Final _{Sc} localization	Final _{Sc} Score				
gnl 1376 HS1_001374	253	DiS_P_DiS	PF06750.8	pilD, prepilin peptidase	6	0	0	0	0	0	0	Cytoplasmic	Membrane	10	
gnl 1477 HS1_001475	195	IV_pilin_GFxxxE	TIGR02532	piIA, pilin major subunit	1	0	2.5	2.5	0	2.5	0	0	Unknown		2.5
gnl 1478 HS1_001476	383	IV_pilin_GFxxxE	TIGR02532	piIA, pilin major subunit	1	0.04	9.82	0.12	0.01	0.01	0.01	0	Cytoplasmic	Membrane	9.82
gnl 1518 HS1_001516	126	N_methyl_2	PFI3544.1	piIA, pilin major subunit	1	8.96	0.51	0.26	0.01	0.26	0.26	0	Cytoplasmic		8.96
gnl 1521 HS1_001519	418	PilN	PF05137.8	piIN, accessory protein	1	8.96	0.51	0.26	0.01	0.26	0.26	0	Cytoplasmic		8.96
gnl 1568 HS1_001566	216	IV_pilin_GFxxxE	TIGR02532	piIA, pilin major subunit	1	0.01	0.01	0.11	0.23	0.11	0.64	0	Extracellular		9.64
gnl 1574 HS1_001572	781	type_IV_pilB	TIGR02538	piB, assembly ATPase	0	9.97	0.01	0	0	0	0	0	Cytoplasmic		9.97
gnl 2052 HS1_002050	367	PilM_2	PF11104.3	piIM, accessory protein	0	9.12	0.88	0	0	0	0	0	Cytoplasmic		9.12
gnl 2053 HS1_002051	185	PilN	PF05137.8	piIN, accessory protein	1	8.96	0.51	0.26	0.01	0.26	0.26	0	Cytoplasmic		8.96
gnl 2054 HS1_002052	200	PilO	PF04350.8	piIO, accessory protein	1	2.11	7.88	0	0	0	0	0	Cytoplasmic	Membrane	7.88
gnl 2055 HS1_002053	134	PilP	PF04351.8	piIP, accessory protein	1	8.96	0.51	0.26	0.01	0.26	0.26	0	Cytoplasmic		8.96
gnl 2056 HS1_002054	736	IV_pilus_PilQ	TIGR02515	piIQ, secretin	0	4.28	0.24	0.37	4.74	0.37	0.37	0	Unknown		4.74
gnl 431 HS1_000429	360	piIT_fam	TIGR01420	piIT, retraction ATPase	0	9.97	0.01	0.01	0	0	0	0	Cytoplasmic		9.97
gnl 432 HS1_000430	389	piIT_fam	TIGR01420	piIT, retraction ATPase	0	9.97	0.01	0.01	0	0	0	0	Cytoplasmic		9.97
gnl 827 HS1_000825	408	Neisseria_PilC	PF05567.6	piY	0	0.01	0.01	0.11	0.23	0.11	9.64	0	Extracellular		9.64
gnl 921 HS1_000919	214	IV_pilin_GFxxxE	TIGR02532	piIA, pilin major subunit	1	0.04	9.82	0.12	0.01	0.12	0.01	0.01	Cytoplasmic	Membrane	9.82

¹ protein domain as identified by hmmscan against the pfamA and TIGRFAM database with E value cut off of 10⁻⁵; listed are the pfamA or TIGRFAM domain ID and accession of the best hit

² protein denotation derived from protein domain annotation with nomenclature and function description according to *M. xanthus* T4P (Friedrich et al., 2014)

³ predicted number of transmembrane helices using TMHMM v.2.0

⁴ subcellular localization prediction using PSORTb v.3.0; given are the PSORTb scores for each possible localization and the final predicted localization with final score

Supplementary Table 4b. Comparative analysis of type IV pilin related proteins identified in the *HotStep-1* draft genome. Overview of results from blastp (E value cut-off of 10e-5) search of HotStep-1 T4P related proteins against the NCBI non-redundant database and the genomes of *Geobacter sulfurreducens* and *Geobacter metallireducens*. The best hit identified by highest score is shown. Proteins discussed in the main text are indicated in bold.

HotStep-1 ORF	percent identity		E value		bit score		query coverage		subject description		subject ID			
	nbi	nt-ur	G.s.	G.m.	nbi	nt-ur	G.s.	G.m.	nbi	nt-ur	G.s.	G.m.		
gml1376 HS1_001374	56.91	53.2	53.31	6e-90	5e-85	3e-82	279	255	248	97	99	96	type IV prepilin-like proteins leader peptide processing enzyme [Geobacter sp. OR-1]	gi754576337 ref WP_04_gj3999714 ref NP_953_6719926.1
gml1477 HS1_001475	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
gml1478 HS1_001476	29.1	40.62	30.72	6e-19	3e-08	3e-07	96.3	51.6	47.8	84	16	39	type IV pilus minor pilin PHW	gi550921787 ref WP_02_gj400756590 ref NP_95_gi404495826 ref YP_00_6719932.1
gml1518 HS1_001516	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
gml1521 HS1_001519	24.15	NA	NA	1e-36	NA	NA	150	NA	NA	98	NA	NA	fimbrial assembly family protein [Synthrophobacter fumaroxidans]	gi500019737 ref WP_01_17004553.1
gml1568 HS1_001566	33.48	52.24	67.44	1e-12	7e-14	2e-10	73.9	63.5	53.9	100	31	20	geopilin [Geobacter daltonii FRC-32] pseudopilin OxpG¹	gi221565740 gb ACM2_gj308513307 ref NP_95_gi404496714 ref YP_00_6720820.1
gml1574 HS1_001572	64.02	47.08	47.17	0	3e-177	1e-174	758	525	518	74	73	73	hypothetical protein, partial [Desulfonitrospira sp. A7A]	gi737276342 ref WP_03_gj399977033 ref NP_953_gi404495720 ref YP_00_6719826.1
gml2062 HS1_002060	38.08	34.58	32.56	3e-71	6e-63	1e-59	238	205	196	93	93	93	type IV pilus assembly protein PHM [Synthrophobacter fumaroxidans MPOB]	gi500016691 ref WP_01_gj39997130 ref NP_953_gi404495832 ref YP_00_6719938.1
gml2063 HS1_002061	33.33	27.07	26.52	3e-24	7e-18	2e-18	104	75.5	77.4	96	98	98	hypothetical protein, partial [Desulfococcus decolorans]	gi752611341 ref WP_04_gj39997129 ref NP_953_gi404495833 ref YP_00_6719939.1
gml2064 HS1_002062	38.76	34.78	33.14	2e-33	1e-34	6e-30	129	121	108	88	92	85	pilus assembly protein PHO [Desulfobalacoccus bacterium BRH_c1(6a)]	gi783211440 ref WP_04_gj39997128 ref NP_953_gi404495834 ref YP_00_6719940.1
gml2065 HS1_002063	34.95	37.17	38.74	9e-16	7e-16	2e-12	80.5	68.2	58.2	75	79	76	pilus assembly protein PHP [Candidatus Nitrospira deltonii]	gi50302950 ref WP_01_gj39997127 ref NP_953_gi404495835 ref YP_00_6719941.1
gml2066 HS1_002064	32.96	31.66	31.86	9e-110	7e-101	1e-103	364	331	338	97	92	92	type IV pilus assembly lipoprotein PHQ	gi527024542 ref WP_02_gj39997126 ref NP_953_gi404495836 ref YP_00_6719942.1
gml431 HS1_000429	67.97	54.52	53.67	3e-179	2e-139	3e-138	514	402	399	99	98	98	twisting motility protein [Desulfatitanea sp. BRH_c12]	gi783161489 ref WP_04_gj39995544 ref NP_951_gi404498248 ref YP_00_6722354.1
gml432 HS1_000430	50.52	40.13	38.98	2e-136	4e-71	3e-69	407	228	223	97	80	80	twisting motility protein [Thermodesulfobacterium thiophilus]	gi655461830 ref WP_02_gj39995257 ref NP_951_gi404498503 ref YP_00_6719159.1
gml827 HS1_000825	32.8	25.65	30.27	8e-45	2e-19	6e-15	178	87.8	73.6	99	87	43	type IV pilus assembly protein PHV [Sulfolobus solfataricus subterraneus]	gi740070281 ref WP_03_gj39997136 ref NP_953_gi404495597 ref YP_00_6719703.1
gml921 HS1_000919	30.82	42.86	41.67	9e-15	2e-06	1e-06	79.7	43.1	43.5	68	23	22	type II secretion system pseudopilin OxpG	gi296936138 gb AD186_gj308513307 ref NP_95_gi404496714 ref YP_00_6720820.1

¹ note that respectively the *geopilin* of *G. sulfurreducens* (NP_952547.1) and *G. metallireducens* (YP_006720365.1) are identified as the second highest scoring hit with respective identity (%) of 74.42 and 69.57. E value of 1e-09 and 2e-08, bitscore of 50.8 and 47.4, query coverage of 20 and 21. In *G. sulfurreducens* and *G. metallireducens* pilA is split into two proteins (pilA-N and pilA-C), where pilA-N is proposed to be essential for pilus conductivity, the HotStep-1 pilA protein (not split) aligns to the N-terminus.

Supplementary Table 5a. Overview of read count data, TPM (transcripts per million) values, relative abundance and log₂ fold change of selected genes. Compared is the genes expression in the presence of hydrogen (H₂) or hydrogen plus methane (H₂CH₄) to the gene expression under standard AOM conditions (CH₄).

organism	gene	orf	length (nt)	treatment library	CH ₄				H ₂		CH ₄ H ₂		
					1	2	3	4	5	6	7	8	9
ANME-1	mcrA	PROKKA_00724	1713	counts	11,692.00	10,794.00	10,802.00	69.00	57.00	71.00	2,087.00	154.00	821.00
				TPM	12,717.04	12,269.62	12,727.33	133.41	105.86	125.31	3,098.36	280.63	1,449.19
				avg TPM		12,571.33			121.52			1,609.40	
				stdev TPM		261.34			14.16			1,415.68	
				avg TPM rel to log ₂ fold change rel					0.01 -6.69			0.13 -2.97	
ANME-1	cyt C	PROKKA_00239	1188	counts	660.00	671.00	621.00	5.00	13.00	13.00	161.00	18.00	54.00
				TPM	1,035.10	1,099.80	1,055.03	13.94	34.81	33.08	344.65	47.30	137.44
				avg TPM		1,063.31			27.28			176.46	
				stdev TPM		33.13			11.58			152.47	
				avg TPM rel to log ₂ fold change rel					0.03 -5.28			0.17 -2.59	
HotSeep-1	dsrA	HS1_00110_1	1437	counts	254.00	228.00	194.00	1,852.00	1,471.00	1,008.00	1,481.00	1,176.00	1,550.00
				TPM	782.21	751.61	643.39	1,956.57	1,754.35	1,477.33	1,730.11	1,401.87	2,068.15
				avg TPM		725.73			1,729.42			1,733.38	
				stdev TPM		72.94			240.59			333.15	
				avg TPM rel to log ₂ fold change rel					2.38 1.25			2.39 1.26	
HotSeep-1	cyt C	HS1_00209_9	1182	counts	609.00	610.00	677.00	586.00	665.00	832.00	847.00	639.00	624.00
				TPM	2,280.05	2,444.69	2,729.61	752.65	964.20	1,482.45	1,202.93	926.06	1,012.22
				avg TPM		2,484.78			1,066.43			1,047.07	
				stdev TPM		227.44			375.49			141.69	
				avg TPM rel to log ₂ fold change rel					0.43 -1.22			0.42 -1.25	
HotSeep-1	pilA	HS1_00156_6	651	counts	140.00	143.00	172.00	53.00	56.00	129.00	182.00	51.00	66.00
				TPM	951.68	1,040.56	1,259.15	123.60	147.42	417.33	469.32	134.20	194.39
				avg TPM		1,083.80			229.45			265.97	
				stdev TPM		158.23			163.15			178.66	
				avg TPM rel to CH ₄ log ₂ fold change rel to CH ₄					0.21 -2.24			0.25 -2.03	

Supplementary Table 5b. Summary of differential gene expression analysis. Pairwise tests were computed with methane supplemented samples (control) vs hydrogen or hydrogen plus methane supplemented samples (treatment). Results for selected genes are shown.

Control vs treatment	gene, organism	ORF	rel abd ¹ (control)	rel abd (treatment)	diff btw ² (log2 fold change)	effect ³	p value (glm) ⁴	corrected p value (glm)
CH4⁵ vs CH4H2⁶	dsrA, HotSeep-1	HS1_001101	8.5	9.3	0.9	4.1	0.000201	0.01143
	cyt C, HotSeep-1	HS1_002099	9.9	8.4	-1.5	-4.7	0.000144	0.01151
	pilA, HotSeep-1	HS1_001566	7.9	4.9	-2.9	-4.1	0.001391	0.03749
	mcrA, ANME-1	PROKKA_00724	12.1	11.2	-1.0	-1.9	0.030949	0.15460
	cyt C, ANME-1	PROKKA_00239	8.09	7.33	-0.76	-1.14	0.043207	0.17502
CH4 vs H2⁷	dsrA, HotSeep-1	HS1_001101	8.4	9.4	0.9	2.3	0.002328	0.04261
	cyt C, HotSeep-1	HS1_002099	9.9	8.2	-1.7	-5.1	0.000106	0.00756
	pilA, HotSeep-1	HS1_001566	7.8	4.7	-3.1	-4.0	0.000399	0.01566
	mcrA, ANME-1	PROKKA_00724	12.1	8.1	-4.0	-13.8	0.000000	0.00002
	cyt C, ANME-1	PROKKA_00239	8.0	5.5	-2.6	-3.7	0.001100	0.01145
CH4 vs H2 HotSeep-1⁸	cyt C, HotSeep-1	HS1_002099	10.3	7.4	-2.9	-9.8	0.000002	0.00022
	pilA, HotSeep-1	HS1_001566	8.2	6.1	-2.0	-3.5	0.000250	0.00508
	dsrA, HotSeep-1	HS1_001101	8.8	7.4	-1.4	-4.6	0.000088	0.00242
	recA, HotSeep-1	HS1_000399	2.6	2.7	0.1	0.0	0.487771	0.62247
	16S, HotSeep-1	HS1_r0003	18.1	18.1	0.0	0.1	0.295099	0.49161

¹ relative abundance as computed by aldex2 with centered log ratio transformed data and 128 Monte Carlo instances of sampling from a Dirichlet distribution

² difference in relative abundance of control and treatment; as relative abundance data are log₂ transformed values the difference between control and treatment represents the log₂ fold change

³ effect size of treatment

⁴ general linearized model

⁵ CH4: TAOM enrichment supplemented with methane

⁶ CH4H2: TAOM enrichment supplemented with methane plus hydrogen

⁷ H2: TAOM enrichment supplemented with hydrogen

⁸ H2 HotSeep-1: HotSeep-1 culture supplemented with hydrogen