

1 **Supplemental Materials**

2 **Adaptations of Atribacteria to life in methane hydrates: hot traits for cold life**

3 **Authors:** Jennifer B. Glass^{1*}, Piyush Ranjan^{2*}, Cecilia B. Kretz^{1#}, Brook L. Nunn³, Abigail M. Johnson¹, James McManus⁴, Frank J. Stewart²

4 **Affiliations:**

5 ¹School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA

6 ²School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

7 ³Department of Genome Sciences, University of Washington, Seattle, WA

8 ⁴Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA

9

10 [#]Now at: Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention,
11 Atlanta, Georgia, USA

12 *Correspondence to: jennifer.glass@eas.gatech.edu

13 **Dedication:** To Katrina Edwards

14

15 **Table S1.** Additional geochemical data for ODP Site 1244 at Hydrate Ridge drilled on IODP Leg 204.

16 See **Fig. 1** for methane, sulfate, manganese, iron, and iodide concentrations.

17

Hole	Depth (mbsf)	%TC	%TN	%TS	%TIC	%TOC	C:N	%CaCO ₃	reactive Fe (%)	reactive Mn (%)
C1H2	1.95/2.25	2.07	0.2	0.42	0.17	1.9	11.05	1.46	0.38	0.002
C1H3	3.45/3.75	1.88	0.14	0.55	0.70	1.2	9.85	5.81	0.45	0.004
F2H4	8.6	1.54	0.17	0.39	0.44	1.1	7.55	3.66	0.57	0.005
F3H4	18.1	1.55	0.24	0.64	0.08	1.5	7.14	0.68	0.80	0.004
C3H4	20.69	1.22	0.18	0.22	0.08	1.1	7.40	0.65	1.10	0.004
E10H5	68.55	1.71	0.22	0.42	0.13	1.6	8.38	1.08	0.51	0.003
E19H5	138.89	1.42	0.20	0.07	0.51	0.9	5.32	4.23	1.41	0.012

18

19
20

Table S2. Atribacteria OTUs in E10-H5 sediment sample (69 mbsf, in the GHSZ) that comprised >0.1% of total Atribacteria OTUs.

OTU Name	Representative Sequence	GenBank ID	Top Hit Sampling Location	Absolute Abundance	Relative Abundance (Atribacteria)
E10H5-AtriRepOTU-007	E10H5_60	AB804573.1	Ocean drilling sample, offshore Shimokita Peninsula, Japan	197,371	68.6
E10H5-AtriRepOTU-023	E10H5_1989	JF495341.1	Anoxic fjord sediments	6861	2.4
E10H5-AtriRepOTU-027	E10H5_2961	KM269692.1	Marine sediment	1096	0.4
E10H5-AtriRepOTU-015	E10H5_3277	AB805815.1	Ocean drilling sample, offshore Shimokita Peninsula, Japan	1083	0.4
E10H5-AtriRepOTU-024	E10H5_2423	JF495354.1	Anoxic fjord sediments	939	0.3
E10H5-AtriRepOTU-021	E10H5_24232	JF495353.1	Anoxic fjord sediments	833	0.2
E10H5-AtriRepOTU-010	E10H5_4352	EF687467.1	Sediment underneath an iron-oxidizing mat, Chefren mud volcano, Nile Deep Sea Fan, Eastern Mediterranean	668	0.2
E10H5-AtriRepOTU-016	E10H5_1096	GU553715.1	Subseafloor sediment, Yung-An Ridge, gas hydrate potential field, Taiwan	536	0.2
E10H5-AtriRepOTU-039	E10H5_13741	JX001059.1	Subseafloor sediment at the deformation front, gas hydrate potential field, Taiwan	450	0.2
E10H5-AtriRepOTU-043	E10H5_16732	KM356487.1	Hydrate Ridge, Oregon	442	0.2
E10H5-AtriRepOTU-037	E10H5_3220	JQ816843.1	Subseafloor sediment, Yung-An Ridge, gas hydrate potential field, Taiwan	313	0.1
E10H5-AtriRepOTU-031	E10H5_11380	KM356401.1	Hydrate Ridge, Oregon	212	0.1

21
22
23
24
25

26 **Table S3.** Metagenome sequence statistics. All statistics are based on contigs of size ≥ 500 bp, unless otherwise noted (e.g., "#
 27 contigs (≥ 0 bp)" and "Total length (≥ 0 bp)" include all contigs).

	C1H2-contigs	C1H3-contigs	C3H4-contigs	E10H5-contigs	E19H5-contigs	E5H5-contigs	F2H4-contigs	F3H4-contigs
# contigs (≥ 0 bp)	95150	360783	191608	50209	27713	210768	127188	63852
# contigs (≥ 1000 bp)	20047	61637	37768	12138	5361	42010	21629	14467
Total length (≥ 0 bp)	86071151	271255728	168175282	48985505	30387546	175406954	99776174	69472077
Total length (≥ 1000 bp)	55876587	150001556	105618381	33498337	21662818	105488664	57427902	49557755
# contigs	35768	126717	71801	21052	9651	82530	43665	25258
Largest contig	73528	47315	349313	40997	241892	43171	103744	232470
Total length	66908509	195416623	129426606	39796035	24667348	133920418	72810367	57141798
GC (%)	43.14	45.35	44.21	41.27	44.79	45.97	40.15	38.38
N50	2605	2041	2638	2777	5514	2209	2330	3953
N75	1337	1051	1242	1366	1815	1114	1127	1600
L50	6125	24423	11911	3865	946	15482	7511	3324
L75	15123	58282	30099	9002	2977	37226	18969	9170
# N's per 100 kbp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
# predicted genes (unique)	91393	277467	174749	53499	26195	186583	100958	73188
# predicted genes (≥ 0 bp)	91882	277905	175191	53668	26316	186919	101224	73764
# predicted genes (≥ 300 bp)	58546	194181	125284	36792	15914	131069	71029	52112
# predicted genes (≥ 1500 bp)	2253	10170	7458	2313	1332	7451	3757	3971
# predicted genes (≥ 3000 bp)	109	533	508	133	95	396	189	324

28 **Table S4. Hun-encoding genes in *Atribacteria* MAG B2 with percent identity to homologous genes in other MAGs.** Hun genes were also
 29 present in other MAGs from Site 1244 including *Planctomycetes* C1H3-B36 (contigs 839, 2603, and 27309) and *Firmicutes* E5B5-B3 (contigs 32
 30 and 225).
 31

Gene	Contig	Gene	<i>Actinobacteria</i> RBG_13_35_12	<i>Atribacteria</i> JGI 0000014-F07	<i>Atribacteria</i> CG2_30_33_13	<i>Omnitrophica</i> WOR_2 SM23_29	<i>Omnitrophica</i> RBG_13_46_9	<i>Omnitrophica</i> RIFCSPHIGHO2_ 02_FULL_45_28
<i>hunR</i>	E10H5 C341	RXG63122.1	OFW53501.1 (92%)	WP 090598640.1 (89%)	N/A	N/A	N/A	N/A
<i>hunA</i>	E10H5 C341	RXG63123.1	OFW53497.1 (91%)	WP 090598643.1 (89%)	OIP66900.1 (85%)	KPK38155.1 (68%)	OGW74721.1 (62%)	OGW81410.1 (63%)
<i>hunB</i>	E10H5 C341	RXG63124.1	OFW53500.1 (91%)	WP 090598656.1 (93%)	OIP66908.1 (89%)	KPK38154.1 (77%)	OGW74722.1 (72%)	OGW81411.1 (69%)
<i>hunC</i>	E10H5 C341	RXG63125.1	OFW53499.1 (95%)	WP 090598644.1 (94%)	OIP66907.1 (91%)	KPK38158.1 (80%)	OGW74723.1 (78%)	OGW81412.1 (68%)
<i>hunD</i>	E10H5 C341	RXG63126.1	OFW53496.1 (93%)	WP 090598646.1 (95%)	OIP66899.1 (92%)	KPK38153.1 (81%)	OGW74724.1 (79%)	OGW81413.1 (70%)
<i>hunE</i>	E10H5 C341	RXG63127.1	OFW53495.1 (96%)	WP 090598648.1 (95%)	OIP66906.1 (95%)	KPK39520.1 (90%)	OGW74725.1 (89%)	OGW81414.1 (85%)
<i>hunF</i>	E10H5 C341	RXG63128.1	OFW53494.1 (85%)	WP 090598650.1 (83%)	OIP66898.1 (87%)	KPK39524.1 (73%)	OGW74727.1 (73%)	OGW81415.1 (68%)
<i>hunG</i>	E10H5 C341	RXG63129.1	OFW53493.1 (97%)	WP 090598653.1 (94%)	OIP66897.1 (94%)	KPK39519.1 (85%)	OGW74726.1 (87%)	OGW81416.1 (78%)
<i>hunH</i>	E10H5 C341	RXG63130.1	OFW53492.1 (88%)	WP 090599466.1 (88%)	OIP66896.1 (89%)	N/A	N/A	OGW81417.1 (77%)
<i>hunI</i>	E10H5 C1989	RXG66691.1	OFW53491.1 (89%)	WP 090599464.1 (89%)	OIP66895.1 (80%)	KPK39517.1 (83%)	OGW74728.1 (81%)	OGW81418.1 (72%)
<i>hunJ</i>	E10H5 C1989	RXG66692.1	OFW53490.1 (95%)	WP 090599461.1 (85%)	OIP66894.1 (88%)	KPK40785.1 (85%)	OGW74729.1 (84%)	OGW81419.1 (80%)
<i>hunK</i>	E10H5 C1989	RXG66693.1	OFW53489.1 (91%)	WP 090599460.1 (90%)	OIP66893.1 (90%)	KPK40786.1 (80%)	N/A	OGW81420.1 (76%)
<i>hunL</i>	E10H5 C1989	RXG66694.1	OFW53498.1 (87%)	WP 090599470.1 (82%)	OIP66892.1 (86%)	N/A	OGW74730.1 (76%)	OGW81421.1 (74%)
<i>hunM</i>	E10H5 C1989	RXG66695.1	OFW53488.1 (87%)	WP 090599458.1 (86%)	OIP66891.1 (86%)	KPK40787.1 (73%)	OGW74758.1 (73%)	OGW81422.1 (65%)
<i>hunN</i>	E10H5 C1989	RXG66696.1	N/A	WP 090599455.1 (95%)	OIP66905.1 (91%)	KPK40788.1 (85%)	OGW74759.1 (85%)	OGW81430.1 (70%)
<i>hunO</i>	E10H5 C5669	RXG63005.1	OFW53487.1 (91%)	WP 090599453.1 (88%)	OIP66890.1 (91%)	KPK40789.1 (77%)	OGW74731.1 (74%)	OGW81423.1 (70%)
<i>hunP</i>	E10H5 C5669	RXG63006.1	N/A	WP 090599451.1 (91%)	OIP66889.1 (88%)	KPK40790.1 (81%)	OGW74732.1 (76%)	OGW81424.1 (75%)

32
 33

34 **Table S5. Acyl carrier protein (*acpP*) genes in E10-H5-B2 *Atribacteria* genomic bin with percent identity to *acpP* genes in five other**
 35 ***Atribacteria* genomes (*Atribacteria* 34_128, 4572_76, HGW-1, RBG_19FT_COMBO_35_14, and CG2_30_33_13).**
 36 *indicates a partial gene sequence.
 37

Contig	Gene	Amino acids	34_128	4572_76	HGW-1	RBG_19FT_COMBO_35_14	CG2_30_33_13
Accession number:			KUK56008.1	OQY41085.1	PKP59192.1	OGD14223.1	OIP70495.1
E10H5_C2	RXG66890.1	74	74	72	73	73	69
E10H5_C194	RXG65326.1	74	81	80	81	81	74
E10H5_C207	RXG66930.1	74	78	76	78	77	73
E10H5_C233	RXG62688.1	71	83	82	82	82	79
E10H5_C307	RXG65598.1	82	85	80	81	80	76
E10H5_C434	RXG65685.1	74	81	80	81	81	74
E10H5_C481	RXG65420.1	74	89	84	85	84	80
E10H5_C779	RXG65278.1	74	91	85	86	85	80
E10H5_C907	RXG66987.1	74	80	77	78	77	76
E10H5_C1000	RXG65256.1	74	86	82	82	82	77
E10H5_C2175	RXG64430.1	64*	77	74	81	84	81
E10H5_C2325	RXG64010.1	74	81	78	81	80	76
E10H5_C3768	RXG63887.1	74	80	80	78	78	73
E10H5_C4452	RXG64281.1	74	80	78	76	73	72
E10H5_C5892	RXG66594.1	74	93	91	89	84	81
E10H5_C6352	RXG66200.1	74	76	73	74	73	68
E10H5_C1912	RXG63329.1	54*	87	85	85	83	83

38 **Table S6. Microcompartment genes in E10-H5-B2 *Atribacteria* genomic bin.**

Gene product	Contig	Gene	Top hit (% identity)	Top hit
D-sugar isomerase	E10H5_	RXG65472.1	PKP61549.1 (94%)	<i>Atribacteria</i> HGW-1
EutM	C2169	RXG65473.1	PKP61548.1 (90%)	
EutN		RXG65474.1	PKP61539.1 (97%)	
EutN		RXG65475.1	PKP61537.1 (88%)	
RnfC		RXG65476.1	PKP55925.1 (93%)	
PduT		RXG65477.1	PKP55924.1 (93%)	
EutE	E10H5_	RXG62592.1	OGD15515.1 (98%)	
EutN	C5048	RXG62591.1	OGD15517.1 (94%)	
EutN		RXG62590.1	OGD15517.1 (94%)	
RpiB		RXG62589.1	OGD15527.1 (94%)	

39
40

Table S7. Glycosyltransferase contigs in E10-H5-B2 *Atribacteria* genomic bin, and percent identity to homologous genes in other genomes.

Annotation	Contig	Gene	Top hit (% identity)	Top hit
Glycosyltransferase 2	E10H5_C38	N/A	OFW63513.1 (72%)	<i>Actinobacteria</i> RBG_19FT_COMBO_36_27
Glycosyltransferase 1		RXG64781.1	OFW63514.1 (72%)	<i>Actinobacteria</i> RBG_19FT_COMBO_36_27
Glycosyltransferase 2		RXG64782.1	OGD31966.1 (66%)	<i>Atribacteria</i> RBG_16_35_8
Glycosyltransferase 2		RXG64783.1	WP_104083790.1 (34%)	<i>Cryobacterium</i> Y11
Glycosyltransferase 2		RXG64791.1	GBC98638.1 (43%)	<i>Bacterium</i> HR17
NAD-dependent epimerase/dehydratase		RXG64792.1	OFW55242.1 (82%)	<i>Actinobacteria</i> RBG_13_35_12
NDP-sugar synthase		RXG64793.1	OFW55241.1 (82%)	<i>Actinobacteria</i> RBG_13_35_12
GDP-mannose 4,6-dehydratase		RXG64794.1	OFW55240.1 (88%)	<i>Actinobacteria</i> RBG_13_35_12
Glycosyltransferase 1		E10H5_C81	RXG64335.1	WP_093394743.1 (45%)
Glycosyltransferase 1	RXG64336.1		WP_093394743.1 (53%)	<i>Thermodesulforhabdus norvegica</i>
Glycosyltransferase 1	RXG64337.1		OGI06781.1 (40%)	<i>Melainabacteria</i> RIFCSPLOWO2_12_FULL_35_11
Glycosyltransferase 1	RXG64338.1		SPE32180.1 (46%)	<i>Solibacteres</i> SbA2
O-antigen ligase	RXG64339.1		OGD19921.1 (30%)	<i>Aminicenantes</i> RBG_13_64_14
Glycosyltransferase 1	RXG64340.1		OGD19922.1 (55%)	<i>Aminicenantes</i> RBG_13_64_14
Polysaccharide (de)acetylase	E10H5_C230	RXG63410.1	WP_019599125.1 (28%)	<i>Rhodonellum</i> spp.
Exopolysaccharide polyprenyl glycosylphosphotransferase		RXG63411.1	PKP61696.1 (88%)	<i>Atribacteria</i> HGW-1
Sugar epimerase		RXG63412.1	PKP61697.1 (77%)	<i>Atribacteria</i> HGW-1
Glycosyltransferase 1 (Cap1E-like)		RXG63413.1	WP_071120025.1 (57%)	<i>Romboutsia timonensis</i>
UDP-N-acetylglucosamine 4-epimerase		RXG63414.1	WP_036938033.1 (71%)	<i>Pseudobacteroides cellulosolvans</i>
Vi polysaccharide biosynthesis protein		RXG63415.1	WP_071120026.1 (68%)	<i>Romboutsia timonensis</i>
Phospholipid carrier-dependent glycosyltransferase		RXG63416.1	WP_036935155.1 (48%)	<i>Pseudobacteroides cellulosolvans</i>
Glycosyltransferase		RXG63417.1	PJE73714.1 (51%)	<i>Terrybacteria</i> CG10_big_fil_rev_8_21_14_0_10_41_10
Phospholipid carrier-dependent glycosyltransferase		RXG63418.1	WP_036935155.1 (43%)	<i>Pseudobacteroides cellulosolvans</i>
Glycosyltransferase 1	E10H5_C3323	RXG63206.1	PKP61698.1 (81%)	<i>Atribacteria</i> HGW-1
GDP-fucose synthetase		RXG63207.1	PKP61699.1 (85%)	<i>Atribacteria</i> HGW-1
GDP-mannose 4,6-dehydratase		(2,613-2,990)	PKP61700.1 (82%)	<i>Atribacteria</i> HGW-1
UDP-glucose 6-dehydrogenase (Ugd)	E10H5_C266	RXG63573.1	PKP61722.1 (77%)	<i>Atribacteria</i> HGW-1
NAD-dependent epimerase/dehydratase		RXG63566.1	PKP59007.1 (93%)	<i>Atribacteria</i> HGW-1
Aminotransferase		RXG63567.1	PKP59008.1 (93%)	<i>Atribacteria</i> HGW-1
Formyltransferase (non-ribosomal peptide synthetase-like)		RXG63568.1	PKP59009.1 (90%)	<i>Atribacteria</i> HGW-1
Acetyltransferase		RXG63569.1	PKP59010.1 (87%)	<i>Atribacteria</i> HGW-1
Deacetylase		RXG63570.1	RME50047.1 (61%)	<i>Deltaproteobacteria</i> bacterium
7-keto-8-aminopelargonate synthetase	E10H5_C306	RXG65589.1	OGD15025.1 (91%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14
UDP-glucose 4-epimerase		RXG65590.1	OGD15024.1 (84%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14
UDP-glucose 4-epimerase	E10H5_C441	RXG65590.1	OGD15024.1 (87%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14
Radical SAM P-methyltransferase	E10H5_C558	RXG63508.1	OGD16377.1 (81%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14
Glycosyltransferase 1	E10H5_C1163	RXG62499.1	WP_090599978.1 (92%)	<i>Atribacteria</i> JGI_0000014-F07
Glycosyltransferase 1 (Cap1E-like)	E10H5_C3994	RXG63236.1	WP_071605078.1 (53%)	<i>Anaeroarcus burkinensis</i>
Exopolysaccharide polyprenyl glycosylphosphotransferase		RXG63237.1	PKP62045.1 (76%)	<i>Atribacteria</i> HGW-1
Acetyltransferase		RXG63239.1	PKP62044.1 (84%)	<i>Atribacteria</i> HGW-1
Polysaccharide biosynthesis protein		RXG63238.1	PKP62043.1 (87%)	<i>Atribacteria</i> HGW-1
Glycosyltransferase 4	E10H5_C4124	RXG64087.1	PKP55791.1 (95%)	<i>Atribacteria</i> HGW-1
Cyclic nucleotide-binding domain-containing protein		RXG64088.1	PKP55792.1 (93%)	<i>Atribacteria</i> HGW-1
NAD-dependent epimerase/dehydratase	E10H5_C4631	RXG66927.1	PKP58948.1 (97%)	<i>Atribacteria</i> HGW-1
Glycosyltransferase 4		RXG66928.1	PKP58071.1 (83%)	<i>Atribacteria</i> HGW-1
Bacillithiol biosynthesis deacetylase	E10H5_C4676	RXG65226.1	OGD13744.1 (93%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14
Glycosyltransferase 2		RXG65225.1	OGW00676.1 (55%)	<i>Nitrospinae</i> RIFCSPLOWO2_01_FULL_39_10

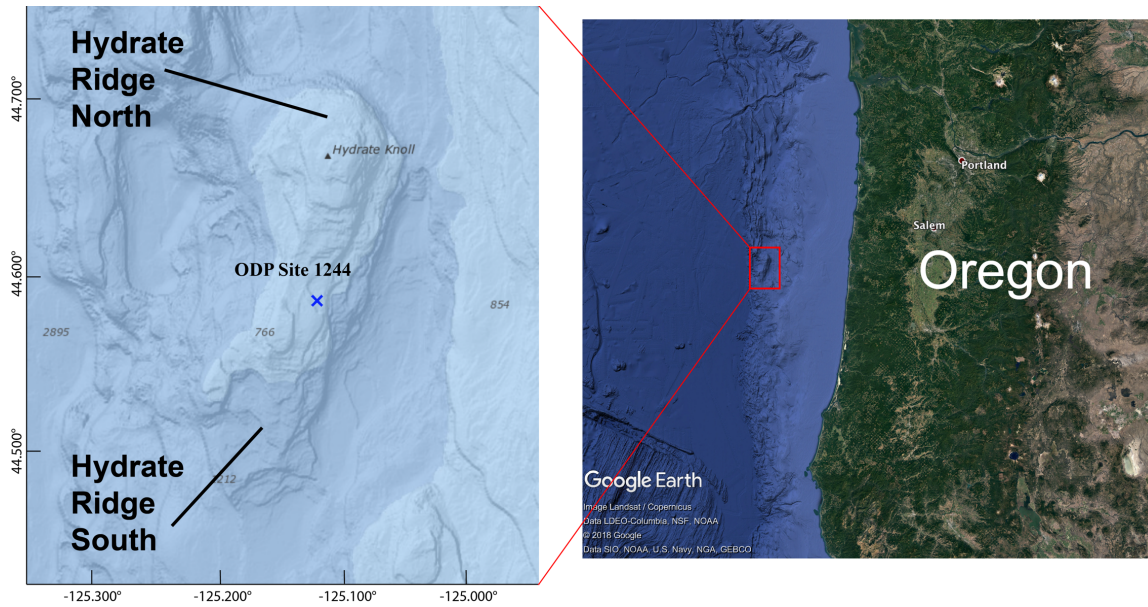
42 **Table S8. Genes encoded on contigs containing the HTH-XRE transcriptional regulator/antitoxin AtiR in E10-H5-B2 *Atribacteria* genomic**
 43 **bin, as shown in Figure 5, and percent identity to homologous genes in other genomes. Bolded genes were expressed in the metaproteome.**
 44 ***truncated protein; **contain conserved arginine residue for carboxylate transport.**
 45

Annotation	Gene	Contig	Gene	Top hit (% identity)	Top hit
HTH-XRE regulator	<i>atiR</i>	E10H5_C107	RXG62479.1	EGC82411.1 (49%)	<i>Anaerococcus prevotii</i>
		E10H5_C473	RXG64192.1	EGC82411.1 (48%)	<i>Anaerococcus prevotii</i>
		E10H5_C194	RXG65323.1	EGC82411.1 (44%)	<i>Anaerococcus prevotii</i>
		E10H5_C1742	RXG64293.1	EGC82411.1 (41%)	<i>Anaerococcus prevotii</i>
		E10H5_C95	RXG62928.1*	EGC82411.1 (44%)	<i>Anaerococcus prevotii</i>
		E10H5_C238	RXG62729.1	EGC82411.1 (46%)	<i>Anaerococcus prevotii</i>
		E10H5_C2	RXG66887.1	EGC82411.1 (47%)	<i>Anaerococcus prevotii</i>
		E10H5_C486	RXG66788.1	EGC82411.1 (43%)	<i>Anaerococcus prevotii</i>
		E10H5_C687	RXG66393.1	EGC82411.1 (46%)	<i>Anaerococcus prevotii</i>
		E10H5_C103	RXG62795.1	WP_053481761.1 (39%)	<i>Lysinibacillus</i> FJAT-14745
		E10H5_C85	RXG66363.1	WP_053481761.1 (43%)	<i>Lysinibacillus</i> FJAT-14745
		E10H5_C4712	RXG63519.1	EGC82411.1 (46%)	<i>Anaerococcus prevotii</i>
		E10H5_C1316	RXG65641.1	WP_067697725.1 (43%)	<i>Nocardia jejuensis</i>
		E10H5_C5280	RXG63000.1	WP_067697725.1 (47%)	<i>Nocardia jejuensis</i>
		E10H5_C2922	RXG65842.1	EGC82411.1 (48%)	<i>Anaerococcus prevotii</i>
Tripartite tricarboxylate transporter	<i>tctC</i> <i>tctB</i> <i>tctA</i>	E10H5_C95	RXG62929.1	WP_066240574.1 (53%)	<i>Anaerospromusa subterranea</i>
			RXG62930.1	WP_118245178.1 (35%)	<i>Clostridium</i> AM58-1XD
			RXG62931.1	WP_093692001.1 (54%)	<i>Sporolituus thermophilus</i>
	<i>tctC</i> <i>tctB</i> <i>tctA</i>	E10H5_C107	RXG62483.1	WP_052514908.1 (51%)	<i>Dethiosulfatarculus sandiegensis</i>
			RXG62484.1	WP_044347447.1 (39%)	<i>Dethiosulfatarculus sandiegensis</i>
			RXG62485.1	WP_044347445.1 (56%)	<i>Dethiosulfatarculus sandiegensis</i>
C4-dicarboxylate transporter	<i>dctP</i> <i>dctQ</i> <i>dctM</i>	E10H5_C95	RXG62935.1**	PKL21426.1 (55%)	<i>Spirochaetae</i> HGW-1
			RXG62936.1	AEG13811.1 (34%)	<i>Desulfofundulus kuznetsovii</i> DSM 6115
			RXG62937.1	PKL21182.1 (57%)	<i>Spirochaetae</i> HGW-4
	<i>dctP</i> <i>dctQ</i> <i>dctM</i>	E10H5_C238	RXG62728.1	PIX34871.1 (81%)	<i>Atribacteria</i> CG_4_8_14_3_um_filter_34_18
			RXG62727.1	PIX34872.1 (77%)	<i>Atribacteria</i> CG_4_8_14_3_um_filter_34_18
			RXG62726.1	PIX34873.1 (75%)	<i>Atribacteria</i> CG_4_8_14_3_um_filter_34_18
<i>dctP</i> <i>dctQ</i> <i>dctM</i>	E10H5_C486	RXG66786.1**	RLC38797.1 (85%)	<i>Nealsonbacteria</i> bacterium	
		RXG66785.1	OGD14926.1 (73%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14	
		RXG66784.1	OGD14925.1 (82%)	<i>Atribacteria</i> RBG_19FT_COMBO_35_14	
	<i>dctP</i> <i>dctQ</i> <i>dctM</i>	E10H5_C664	RXG63168.1	WP_044348842.1 (36%)	<i>Dethiosulfatarculus sandiegensis</i>
			RXG63170.1	WP_052515103.1 (32%)	<i>Dethiosulfatarculus sandiegensis</i>
			RXG63171.1	WP_044348841.1 (57%)	<i>Dethiosulfatarculus sandiegensis</i>
Glycerol-3-phosphate transporter	<i>ugpB</i> <i>ugpA</i> <i>ugpE</i>	E10H5_C85	RXG66368.1	KPJ85459.1 (68%)	<i>Spirochaetes</i> DG_61
			RXG66369.1	KPJ85458.1 (67%)	<i>Spirochaetes</i> DG_61
			RXG66370.1	KPJ85457.1 (71%)	<i>Spirochaetes</i> DG_61
	<i>ugpB</i> <i>ugpA</i> <i>ugpE</i>	E10H5_C103	RXG62790.1	WP_068137292.1 (47%)	<i>Limnochorda pilosa</i>
			RXG62789.1	WP_082726097.1 (46%)	<i>Limnochorda pilosa</i>
			RXG62788.1	WP_068137283.1 (46%)	<i>Limnochorda pilosa</i>
Branched chain amino acid transporters	<i>livH</i> <i>livM</i> <i>livG</i> <i>livF</i> <i>livK</i>	E10H5_C2	RXG66901.1	PKP61938.1 (90%)	<i>Atribacteria</i> HGW-1
			RXG66900.1	PKP61939.1 (85%)	<i>Atribacteria</i> HGW-1
			RXG66899.1	PKP61948.1 (85%)	<i>Atribacteria</i> HGW-1
			RXG66898.1	PKP61940.1 (80%)	<i>Atribacteria</i> HGW-1
			RXG66897.1	PKP61941.1 (89%)	<i>Atribacteria</i> HGW-1

	<i>livK</i> <i>livH</i> <i>livM</i> <i>livG</i> <i>livF</i>	E10H5_C238	RXG62734.1 RXG62732.1 RXG62736.1 RXG62731.1 RXG62730.1	WP_068748291.1 (42%) RLE68614.1 (60%) RLE68615.1 (55%) RLE68616.1 (57%) RLE68617.1 (56%)	<i>Thermovenabulum gondwanense</i> <i>Thermoprotei</i> archaeon <i>Thermoprotei</i> archaeon <i>Thermoprotei</i> archaeon <i>Thermoprotei</i> archaeon
	<i>livK</i> <i>livH</i> <i>livM</i> <i>livG</i> <i>livF</i>	E10H5_C687	RXG66394.1 RXG66395.1 RXG66396.1 RXG66397.1 RXG66398.1	OQY40502.1 (43%) OQY40503.1 (41%) OQY40504.1 (42%) OGP70799.1 (50%) OQY40505.1 (55%)	<i>Atribacteria</i> 4572_76 <i>Atribacteria</i> 4572_76 <i>Atribacteria</i> 4572_76 <i>Deltaproteobacteria</i> RBG_16_50_11 <i>Atribacteria</i> 4572_76
	<i>livH</i> <i>livM</i>	E10H5_C8009	30420 30421	OQY40503.1 (95%) OQY40504.1 (93%)	<i>Atribacteria</i> 4572_76 <i>Atribacteria</i> 4572_76
Purine transporter	<i>bmpA</i>	E10H5_C473	RXG64193.1 RXG64197.1 RXG64194.1 RXG64195.1	PKP58720.1 (94%) PKP60518.1 (88%) PKP60517.1 (94%) PKP60516.1 (96%)	<i>Atribacteria</i> HGW-1 <i>Atribacteria</i> HGW-1 <i>Atribacteria</i> HGW-1 <i>Atribacteria</i> HGW-1
Other genes	M20 <i>gabT</i> C69 RTX RTX	E10H5_C194 E10H5_C194 E10H5_C194 E10H5_C4712 E10H5_C2795 E10H5_C1316 E10H5_C2922	RXG65322.1 RXG65321.1 RXG65329.1 RXG63518.1 RXG64595.1 RXG65639.1 RXG65844.1	WP_034420537.1 (72%) WP_034420536.1 (73%) WP_034420535.1 (75%) PKP55816.1 (87%) WP_093794159.1 (43%) OGD35967.1 (42%) OGD35967.1 (43%)	<i>Clostridiales</i> DRI-13 <i>Clostridiales</i> DRI-13 <i>Clostridiales</i> DRI-13 <i>Atribacteria</i> HGW-1 <i>Sporomusa acidovorans</i> <i>Atribacteria</i> RBG_16_35_8 <i>Atribacteria</i> RBG_16_35_8

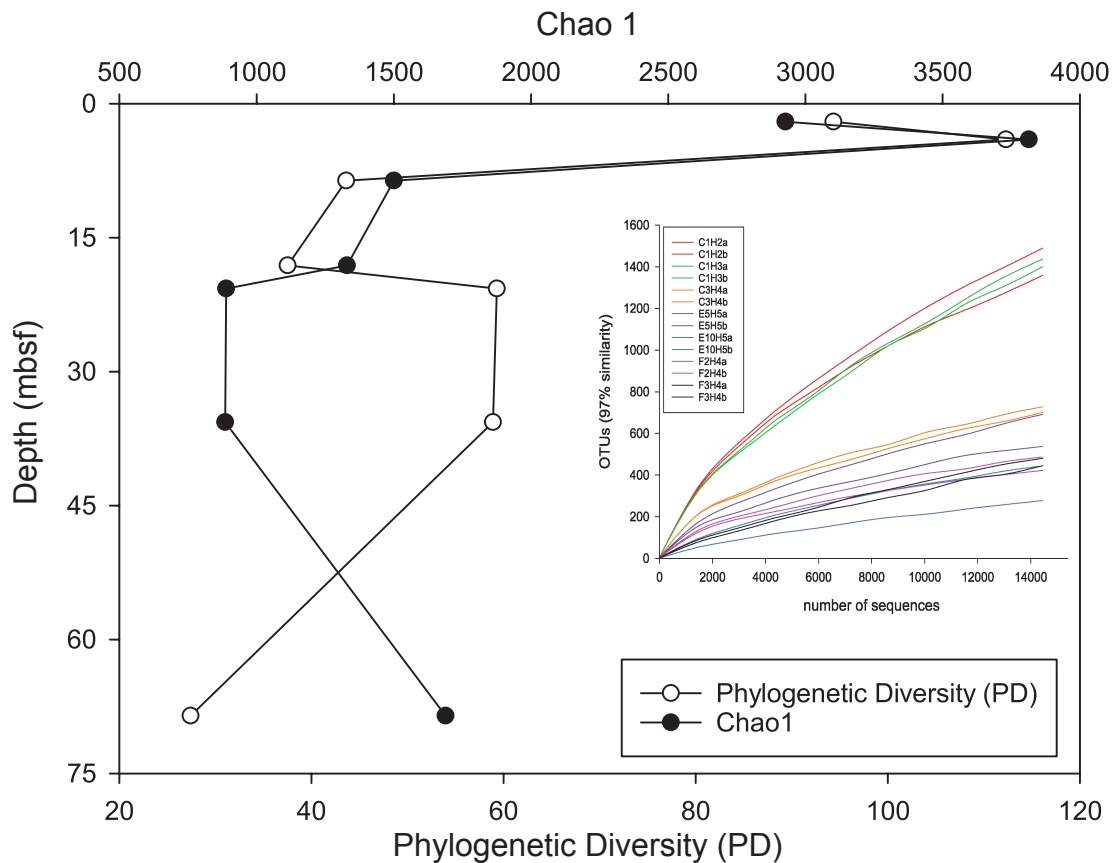
47 **Table S9. Putative toxin-antitoxin systems in *Atribacteria* E10H5-B2, and percent identity to homologous genes in other genomes.**

Annotation	Gene	Contig	Gene	Top hit (% identity)	Top hit
Antitoxin Toxin	MazE-like MazF-like	E10H5_C26	RXG62841.1	PIU28774.1 (82%)	<i>Actinobacteria</i> CG08_land_8_20_14_0_20_35_9
			RXG62840.1	PIU28775.1 (88%)	<i>Actinobacteria</i> CG08_land_8_20_14_0_20_35_9
Antitoxin Toxin	NikR-like MazF-like	E10H5_C33	RXG63986.1	PIU25819.1 (95%)	<i>Atribacteria</i> CG08_land_8_20_14_0_20_33_29
			RXG63985.1	PIU25820.1 (83%)	<i>Atribacteria</i> CG08_land_8_20_14_0_20_33_29
Antitoxin Toxin	MazE-like MazF-like	E10H5_C68	RXG64905.1	PKP54844.1 (89%)	<i>Atribacteria</i> HGW-1
			RXG64906.1	PKP54843.1 (84%)	<i>Atribacteria</i> HGW-1
Antitoxin Toxin	VapC-like MazE-like	E10H5_C81	RXG64104.1	PKP54729.1 (90%)	<i>Atribacteria</i> HGW-1
			RXG64105.1	PKP54728.1 (81%)	<i>Atribacteria</i> HGW-1
Antitoxin Toxin	MazE-like MazF-like	E10H5_C81	RXG64330.1	PIY33044.1 (92%)	<i>Atribacteria</i> CG_4_10_14_3_um_filter_34_13
			RXG64331.1	PIY33045.1 (92%)	<i>Atribacteria</i> CG_4_10_14_3_um_filter_34_13
Antitoxin Toxin	MazE-like MazF-like	E10H5_C184	RXG64099.1	OQY39409.1 (97%)	<i>Atribacteria</i> 4572_76
			RXG64098.1	OQY39408.1 (98%)	<i>Atribacteria</i> 4572_76
Antitoxin Toxin	MazE-like MazF-like	E10H5_C1146	RXG64860.1	WP_090599534.1 (91%)	<i>Atribacteria</i> JGI 0000014-F07
			RXG64859.1	WP_090599536.1 (89%)	<i>Atribacteria</i> JGI 0000014-F07
Antitoxin Toxin	MazE-like MazF-like	E10H5_C1351	RXG65145.1	PKP54770.1 (94%)	<i>Atribacteria</i> HGW-1
			RXG65146.1	PKP54769.1 (89%)	<i>Atribacteria</i> HGW-1
Antitoxin Toxin	MazE-like MazF-like	E10H5_C5010	RXG67035.1	WP_078128337.1 (59%)	<i>Leptospira alexanderi</i>
			RXG67036.1	WP_010577027.1 (74%)	<i>Leptospira alexanderi</i>



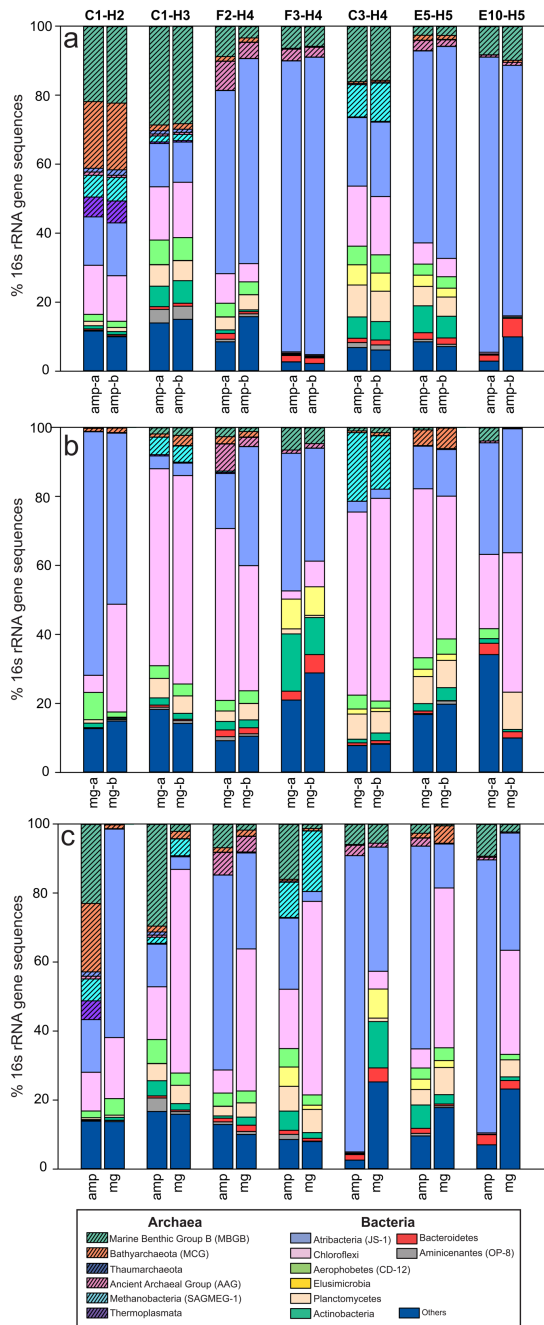
48

49 **Figure S1.** Map of Site ODP 1244 (44°35.1784'N; 125°7.1902'W) on Hydrate Ridge, drilled on IODP Leg 204. The site is located 80 km west of
 50 Oregon, in the accretionary complex of the Cascadia subduction zone, on the eastern flank of Hydrate Ridge, ~3 km northeast of the southern summit.



52

53 **Figure S2.** Chao1 and phylogenetic diversity (PD) as a function of sediment depth. Data points are
 54 mean values based on rarefaction at a standardized sequence count ($n=14,390$) per sample. The
 55 inset shows the number of observed OTUs (97% similarity clusters) as a function of sequencing
 56 depth based on rarefaction of OTU counts for replicate samples.



57

58 **Figure S3.** Comparison of the 16S rRNA gene amplicon sequencing vs. 16S rRNA metagenomic

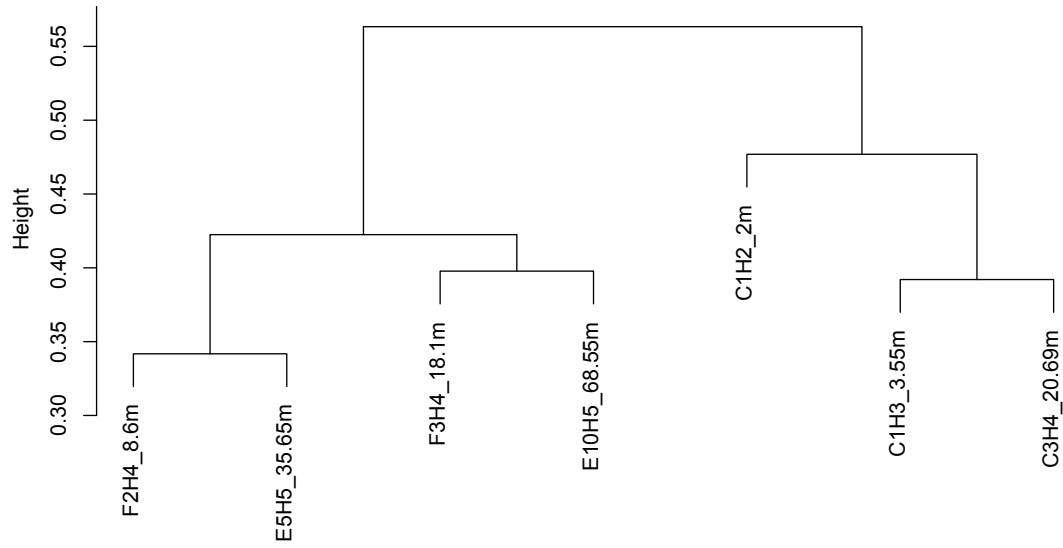
59 sequences, and replicates of each for: (a) amplicon sequence (technical replicates “a” and “b”), (b)

60 metagenomic sequences (technical replicates “a” and “b”), and (c) average amplicon (“amp”, left

61 bar) and metagenome (“mg”, right bar). Hatched and solid bars are archaeal and bacterial clades,

62 respectively. The “others” category contains phyla with a total abundance of <2% for any given

63 sample.



64

65 **Figure S4.** Hierarchical clustering dendrogram showing sample groupings for Atribacteria
 66 communities at different depths. OTU abundances were log transformed and OTUs with 0
 67 abundance (absence) were ignored.