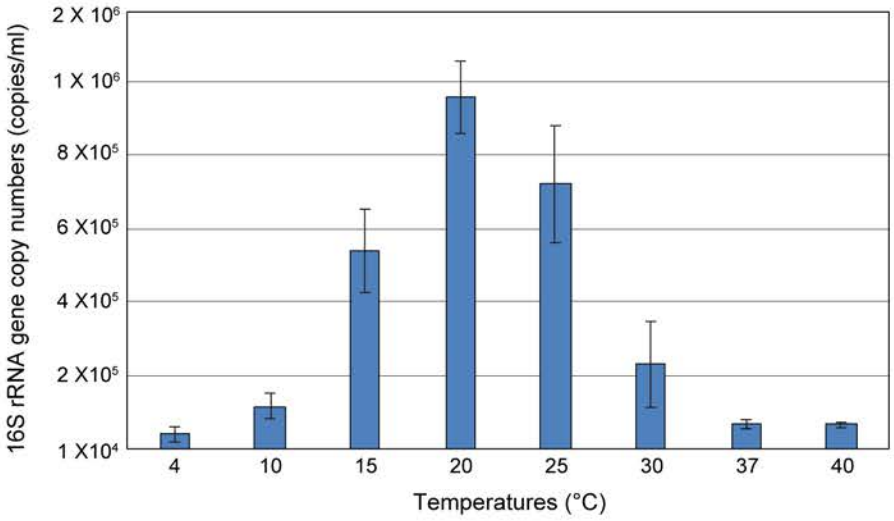
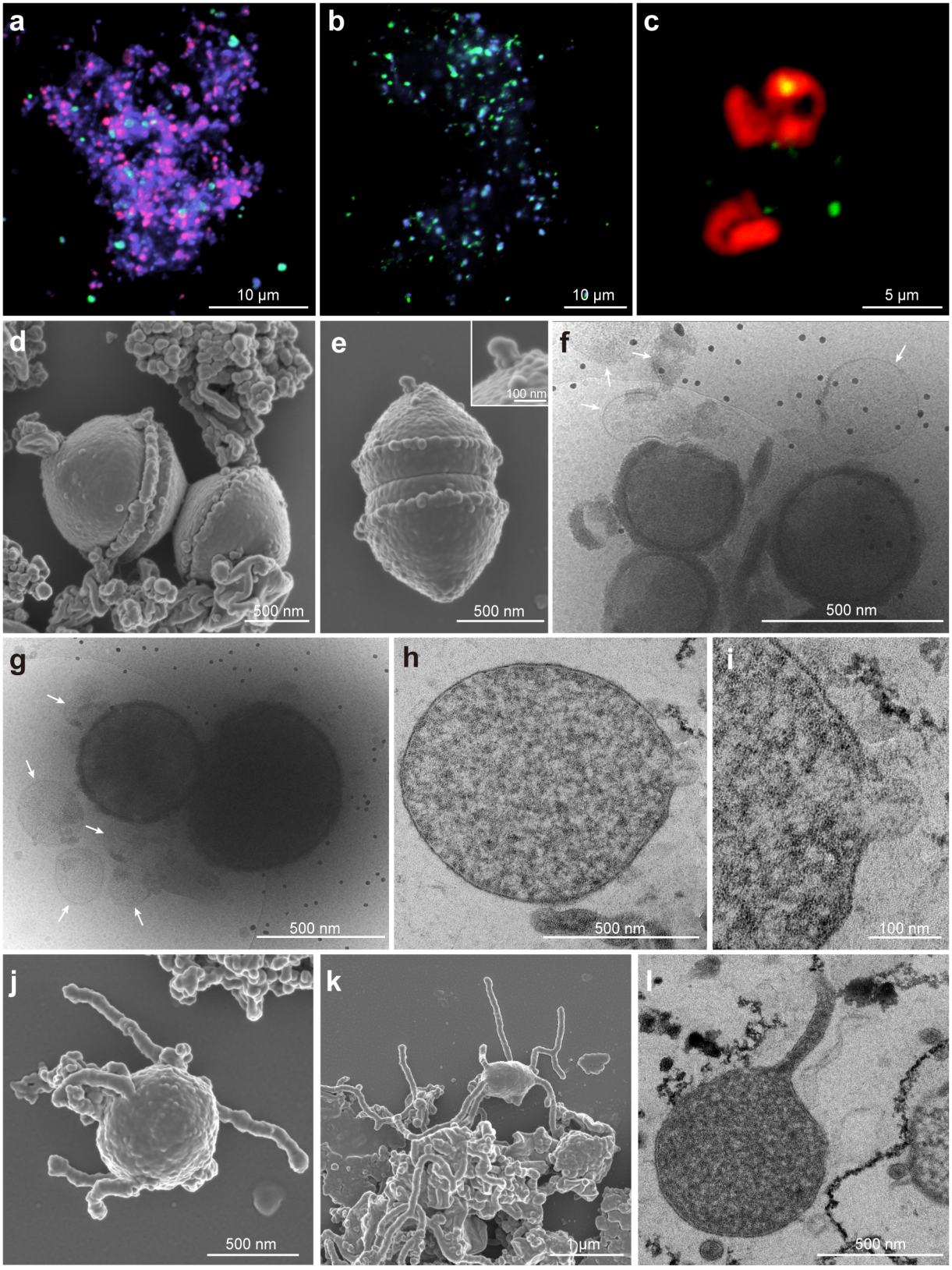
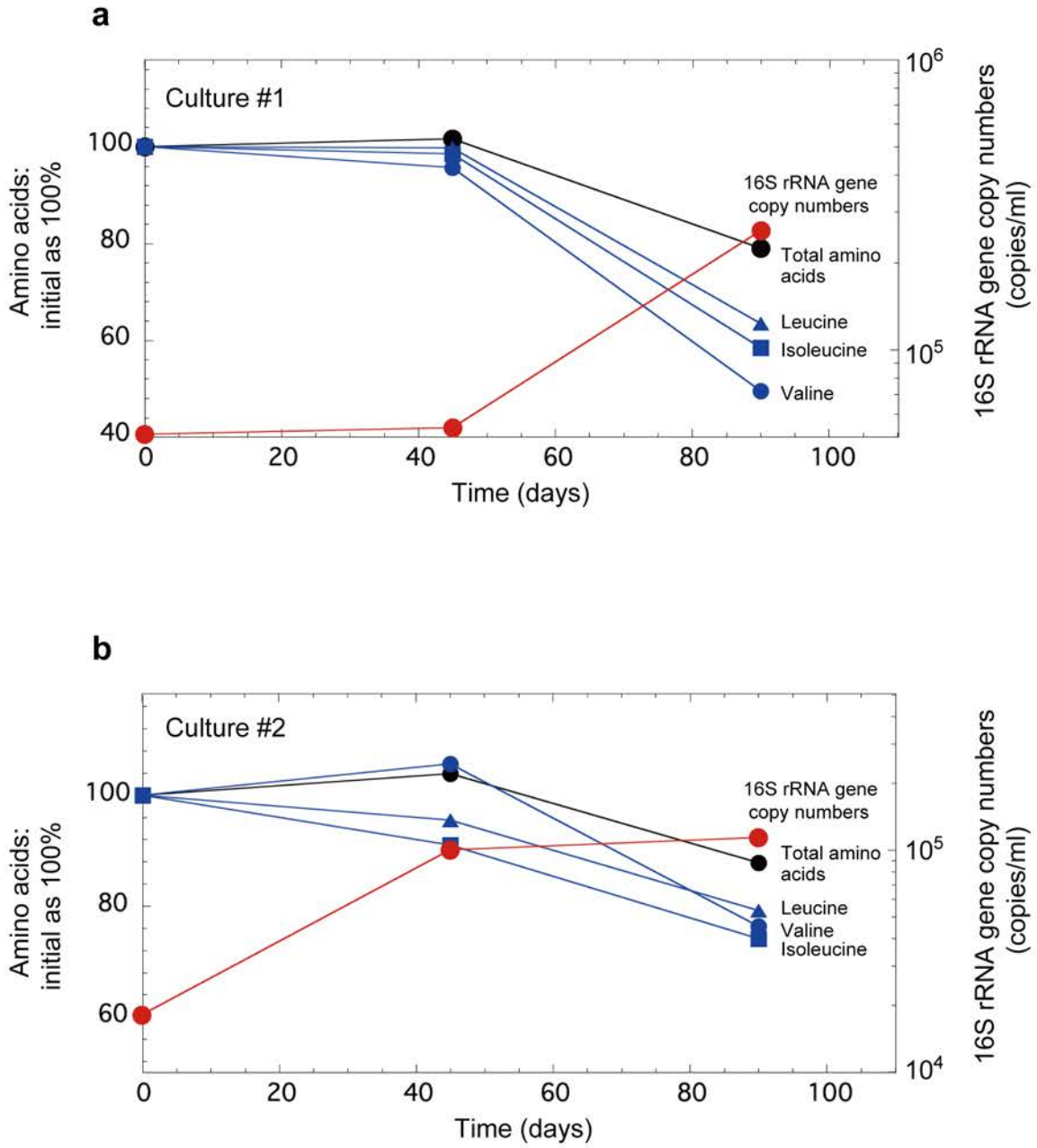


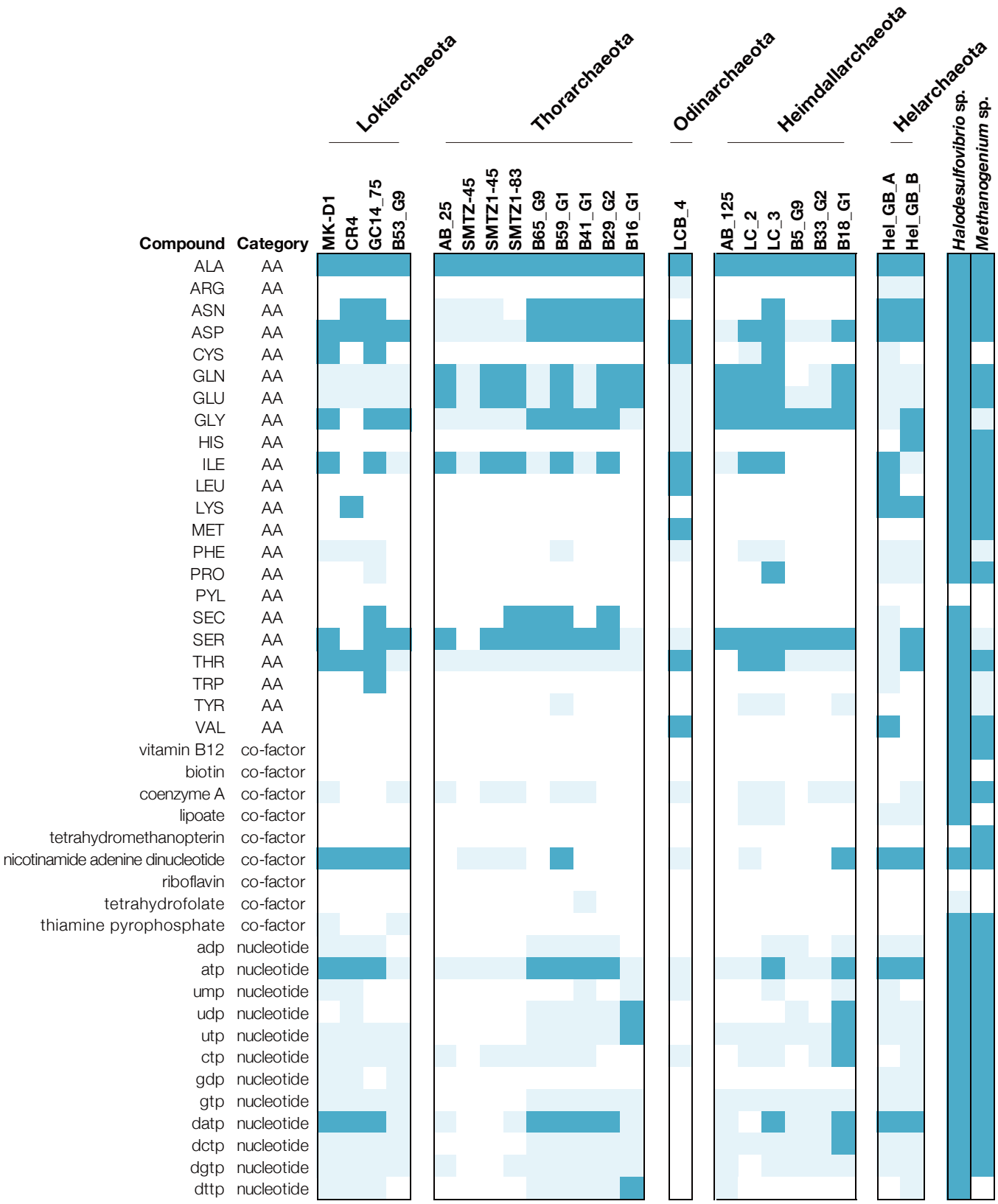
Extended Data Fig. 1 | Effect of temperature on growth of MK-D1. Error bars indicate standard deviations of triplicate determinations. The temperature range test was performed twice, and both results displayed similar results.



Extended Data Fig. 2 | Other representative photomicrographs of MK-D1. a, b, Fluorescence images of cells from enrichment cultures after eight (**a**) and eleven (**b**) transfers stained with DAPI (violet) and hybridized with nucleotide probes targeting MK-D1 (green) and *Bacteria* (red). The images are other fields of view, which were taken at the same time for the Figs. 1b and c images. **c**, A fluorescence image of cells in the enrichments after eleven transfers hybridized with nucleotide probes targeting MK-D1 (green) and *Archaea* (but with one mismatch against MK-D1; red). (Large and irregular coccoid-shaped cells stained by ARC915 only are likely *Methanogenium*.) **d**, **e**, Dividing cells of MK-D1 with a bleb. The upper-right inset image in **e** shows close-up of the bleb. f, g, Cryo-EM images of MK-D1 cells and large MVs (white arrows). **h**, **i**, Ultrathin sections of MK-D1 cells with an MV. The image **i** shows a close-up image of **h**. **j**, **k**, SEM images of MK-D1 cells with protrusions. **l**, Ultrathin section of a MK-D1 cell with a protrusion. Detailed iTAG analyses of cultures are shown in Supplementary Table S2.



Extended Data Fig. 3 | The amino acid concentrations and growth curves of MK-D1 in pure co-cultures. a, b, Results from cultures #1 (**a**) and #2 (**b**) are shown. Please note that the initial concentrations of amino acids were normalized as 100%. Total amino acids and several representative amino acids (valine, leucine and isoleucine) are independently shown for the duplicate culture samples. Detailed iTAG-based community compositions of the cultures are shown in Supplementary Table S2.



Extended Data Fig. 4 | AA, co-factor, and nucleotide biosynthesis capacities of MK-D1 and other Asgard archaea. Genomes encoding genes for synthesis from pyruvate or acetyl-CoA (dark blue) and synthesis from other intermediates (light blue) are indicated. Those without complete pathways for either are indicated white. *Halodesulfovibrio* sp. strain MK-HDV and *Methanogenium* sp. strain MK-MG isolated in this study are also shown.

Extended Data Table 1 | SSU rRNA gene clones obtained from the primary and six successive transferred enrichment culture

<Primary enrichment culture>

Clone library using universal primers (530F/907R)

Phylotype name	No. of clones	Accession no.	Sequence length (bp)	Closest cultured species or clone (accession number)	Sequence identity (%)	Phylogenetic affiliation	Identical or almost identical clones detected from the AOM bioreactor enrichment (accession number, sequence identity %)[a]
111_U1	40	LC490621	374	*Halodesulfovibrio aestuarii* strain Sylt 3 (NR_116770)	99	genus *Halodesulfovibrio*	—
111_U2	3	—	377	*Methylobacter marinus* strain A45 (NR_025132)	100	genus *Methylobacter*	MK903D_B19 (AB831411, 100%)
111_U3	2	—	374	*Photobacterium indicum* strain NBRC 14233 (NR_113657)	100	genus *Photobacterium*	MK903D_B9 (AB831402, 100%)
111_U4	1	LC490622	377	subseafloor sediment clone ODP1251B13.14 (AB177314)	99	subgroup 21 within the phylum *Acidobacteria*	—
111_U5[b]	1	LC490623	377	hydrothermal seep sediment BAC_OTU_13 (KP091106)	100	GIF9 group within the class *Dehalococcoidia*	MK0D_B60 (AB831337, 99.5%)
111_U6	1	LC490624	374	*Roseovarius gaetbuli* strain YM-20 (NR_134163)	99	genus *Roseovarius*	—

Clone library using archaeal primers (340F/932R)

Phylotype name	No. of clones	Accession no.	Sequence length (bp)	Closest cultured species or clone (accession number)	Sequence identity (%)	Phylogenetic affiliation	Identical or almost identical clones detected from the AOM bioreactor enrichment (accession bumber, sequence identity %)[a]
111_A1	6	—	535	*Methanococcoides burtonii* strain DSM 6242 (NR_074242)	99	genus *Methanococcoides*	MK903D_A2 (AB831282, 100%)
111_A2	5	LC490620	513	*Methanogenium cariaci* strain JR1 (NR_104730)	99	genus *Methanogenium*	—
111_A3	2	—	534	methane seep clone AN_5119N_arc_E4_T3 (KM356859)	99	ANME-2a	MK0D_A9 (AB831268, 100%)
111_A4	2	LC490619	516	methane seep clone AC_5120_arc_D2_T3 (KM356804)	99	Lokiarchaeota (*Ca*. P. syntrophicum strain MK-D1)	MK903R_A35 (AB831305, 99.0%)

<Six successive transferred enrichment culture>

Clone library using universal primers (530F/907R)

Phylotype name	No. of clones	Accession no.	Sequence length (bp)	Closest cultured species or clone (accession number)	Sequence identity (%)	Phylogenetic affiliation	Identical or almost identical clones detected from the AOM bioreactor enrichment (accession bumber, sequence identity %)[a]
111-5_U1	40	—	374	*Halodesulfovibrio oceani* strain I.8.1 (NR_116768)	100	genus *Halodesulfovibrio*	—
111-5_U2	6	—	380	methane seep clone AC_5120_arc_D2_T3 (KM356804)	100	Lokiarchaeota (*Ca*. P. syntrophicum strain MK-D1)	—
111-5_U3	1	—	380	*Methanogenium boonei* strain AK-7 (NR_115706)	99	genus *Methanogenium*	—

[a]The clone sequences have been reported in our previous study[15].

[b]An anaerobic bacterium strain MK-GIF9, which has the identical 16S rRNA gene sequence of the OTU, has successfully been isolated from the enrichment culture (Nakahara *et al*. Cultivation of previously uncultured *Chloroflexi*, a bacterial dark matter in subseafloor biosphere. 16th International Symposiumon Microbial Ecology [ISME16], Montreal, Canada [2016]). Detailed infomation about the cultivation, and physiological and genomic properties of the bacterium will be reported in the near future.

Extended Data Table 2 | Carbon isotope fractionation values in MK-D1 cultures after 120 days incubation with and without stable isotope labeled amino acids

Culture ID	$\delta^{13}C$-CO_2 (‰ VPDB)[a]	$\delta^{13}C$-CH_4 (‰ VPDB)[a]
Co-cultures with Methanobacterium		
No.1 with stable istope labeled AAs	-12.3	4094.8
No.2 with stable istope labeled AAs	-9.3	6990.7
No.3 w/o stable istope labeled AAs	-23.1	-36.7
No.4 w/o stable istope labeled AAs	-23.1	-33.1
Tri-cultures with Halodesulfovibrio and Methanogenium		
No.5 with stable istope labeled AAs	318.5	86.0
No.6 with stable istope labeled AAs	309.3	87.8
No.7 w/o stable istope labeled AAs	-22.6	-95.5
No.8 w/o stable istope labeled AAs	-22.8	-97.8

[a]‰ versus the Vienna Pee Dee Belemnite.

Extended Data Table 3 | Growth of *Ca.* P. syntrophicum strain MK-D1 for 120 days incubation with a range of substrates

Culture name	Substrate	Initial MK-D1 16S rRNA gene copies per ml of culture	Final MK-D1 16S rRNA gene copies per ml of culture	No. of MK-D1 16S rRNA gene copies relative to initial culture	Community compositions evaluated by iTAG analysis (%)[a]			
					MK-D1	*Methanogenium* sp.	*Methenobacterium* sp. strain MO-MB1	Others
Inoculum	Casamino acids (CA)[b] + 20 amino acids mixture (AAs)[c] + powdered milk (PM)[d]	—	5.91E+05	—	39.8	36.8	23.3	0.01
Control-1	CA + 20 AAs + PM	1.42E+04	1.62E+05	11.36	76.7	21.8	1.4	0.03
Control-2	CA + 20 AAs + PM	4.67E+03	6.55E+04	14.03	60.3	38.0	1.6	0.04
H2-1	CA + 20 AAs + PM + 1.5 kPa H_2[e] + 10 mM 2-bromoethane sulfonate (2-BES)[f]	9.46E+03	4.35E+03	0.46	—	—	—	—
H2-2	CA + 20 AAs + PM + 1.5 kPa H_2 + 10 mM 2-BES	1.37E+04	3.28E+03	0.24	—	—	—	—
H2-3	CA + 20 AAs + PM + 1.5 kPa H_2 + 10 mM 2-BES	3.10E+04	8.27E+03	0.27	—	—	—	—
Formate-1	CA + 20 AAs + PM + 1 mM Formate + 10 mM 2-BES	2.76E+04	2.00E+03	0.07	—	—	—	—
Formate-2	CA + 20 AAs + PM + 1 mM Formate + 10 mM 2-BES	1.46E+04	9.49E+03	0.65	—	—	—	—
Nitrate-1	CA + 20 AAs + PM + 500 µM Nitrate[g]	2.13E+04	8.43E+03	0.40	—	—	—	—
Nitrate-2	CA + 20 AAs + PM + 500 µM Nitrate	1.47E+04	5.19E+03	0.35	—	—	—	—
Sulfate-1	CA + 20 AAs + PM + 500 µM Sulfate	5.28E+03	9.21E+04	17.42	79.5	19.5	1.0	0.03
Sulfate-2	CA + 20 AAs + PM + 500 µM Sulfate	3.39E+04	5.28E+04	1.56	—	—	—	—
Thiosulfate-1	CA + 20 AAs + PM + 500 µM Thiosulfate	1.23E+04	5.00E+04	4.05	—	—	—	—
Thiosulfate-2	CA + 20 AAs + PM + 500 µM Thiosulfate	2.29E+04	6.09E+04	2.66	—	—	—	—
Lactate-1	CA + 20 AAs + PM + 1 mM Lactate	5.31E+03	1.31E+04	2.46	—	—	—	—
Lactate-2	CA + 20 AAs + PM + 1 mM Lactate	1.53E+04	1.91E+04	1.25	—	—	—	—
Acetate-1	CA + 20 AAs + PM + 1 mM Acetate	2.63E+04	9.17E+04	3.48	—	—	—	—
Acetate-2	CA + 20 AAs + PM + 1 mM Acetate	1.56E+04	2.13E+04	1.36	—	—	—	—
Glucose-1	CA + 20 AAs + PM + 1 mM Glucose	1.12E+04	1.16E+05	10.33	73.8	24.3	1.9	0.03
Glucose-2	CA + 20 AAs + PM + 1 mM Glucose	1.06E+04	1.06E+05	10.01	70.3	28.0	1.7	Not detected
Fructose-1	CA + 20 AAs + PM + 1 mM Fructose	3.18E+04	3.31E+04	1.04	—	—	—	—
Fructose-2	CA + 20 AAs + PM + 1 mM Fructose	1.79E+04	1.44E+05	8.08	—	—	—	—
Xylose-1	CA + 20 AAs + PM + 1 mM Xylose	2.82E+04	6.79E+03	0.24	—	—	—	—
Xylose-2	CA + 20 AAs + PM + 1 mM Xylose	9.25E+03	1.18E+05	12.73	61.4	36.5	2.1	0.01
Ribose-1	CA + 20 AAs + PM + 1 mM Ribose	1.42E+04	2.88E+04	2.02	—	—	—	—
Ribose-2	CA + 20 AAs + PM + 1 mM Ribose	7.34E+03	2.29E+04	3.13	—	—	—	—
Maltose-1	CA + 20 AAs + PM + 1 mM Maltose	2.84E+04	1.21E+05	4.25	—	—	—	—
Maltose-2	CA + 20 AAs + PM + 1 mM Maltose	2.17E+04	4.55E+04	2.09	—	—	—	—
Citrate-1	CA + 20 AAs + PM + 1 mM Citrate	3.36E+04	1.20E+05	3.56	—	—	—	—
Citrate-2	CA + 20 AAs + PM + 1 mM Citrate	1.82E+04	5.73E+04	3.15	—	—	—	—
Pyruvate-1	CA + 20 AAs + PM + 1 mM Pyruvate	1.73E+04	9.37E+04	5.42	—	—	—	—
Pyruvate-2	CA + 20 AAs + PM+ 1 mM Pyruvate	2.22E+04	4.86E+03	0.22	—	—	—	—
Fumarate-1	CA + 20 AAs + PM + 1 mM Fumarate	3.16E+04	7.20E+04	2.28	—	—	—	—
Fumarate-2	CA + 20 AAs + PM + 1 mM Fumarate	1.94E+04	2.35E+04	1.21	—	—	—	—
Archaeal cell-1	CA + 20 AAs + PM + archaeal cell membrane components[h]	1.53E+04	1.42E+05	9.27	81.5	17.5	0.8	0.3
Archaeal cell-2	CA + 20 AAs + PM + archaeal cell membrane components	4.17E+04	1.05E+05	2.52	—	—	—	—

A dash indicates that data were not taken for that sample.

[a]The iTAG analysis was performed on the samples in which an increase of 16S rRNA gene copy numbers of MK-D1 about 10 times or more after incubation was observed by the qPCR assay. The detailed results are shown in Supplementary Table S2.

[b]Final concentration of Casamino acids was 0.05% (w/v).

[c]Final concentration of each amino acid was 0.1 mM.

[d]A powdered milk for baby (Hohoemi, Meiji Co., Ltd., Tokyo, Japan) was used at a final concentation of 0.1 % (w/v).

[e]The concentration of hydrogen gas was in the head space of the culture bottle.

[f]2-BES was added to inhibit methanogens.

[g]Addition of nitrate completely supressed growth of the *Ca*. P. syntrophicum. This is probably bacause nitrate inhibited fomate dehydrogenase activity of *Ca*. P. syntrophicum[78].

[h]Archaeal cell membrane components were mixture of phytol, intact polar lipid (IPL)-glycerol-dialkyl-glycerol tetraesthers (GDGTs), and core lipid (CL)-GDGTs (each at a final concentration 50 ng/ml). The reason for using the archaeal membrane components is that these have positive effect on the growth for some archaeal species: (i) archaeal cell extract including membrane lipids stimulates growth of an extremely thermophilic archaeon *Thermocaldium modestius*[79], and (ii) a hyperthermophilic archaeon *Thermofilum pendes* requires the polar lipids for the growth, which was obtained from an archaeal species of *Thermoproteus tenax*[80].