Supporting Information

King 10.1073/pnas.1424989112

SI Materials and Methods

A CO-oxidizing halophile was enriched from BSF-1 sediment slurries in neoprene-stoppered 160-cm^3 serum bottles containing about 100-ppm headspace CO concentrations and CM1 medium (1) with a sodium chloride concentration of 3.8 M and sodium pyruvate as a carbon and energy source (25 mM). Serum bottles were shaken at 100 rpm on a rotary shaker at 40 °C. Enrichments positive for CO uptake were transferred through several rounds of liquid culture; isolates were obtained from colonies that grew on CM1 plates solidified with 1.5% (wt/vol) agar. Genomic DNA extracted from purified isolates with an UltraClean Microbial DNA Kit (MO BIO) was used to amplify 16S rRNA genes with primers 21f and 1492r and standard amplification protocols (e.g., 2, 3). Amplicons were sequenced by the Louisiana State University Core Genome Facility (3). The 16S rRNA gene sequence of isolate BSF-1 was subjected to BLAST (4) analysis and classification using the SINA aligner (5); both indicated that isolate BSF-1 was a member of the Euryarchaeota extreme halophiles in the genus *Halorubrum* and most closely related to *H. lipolyticum*. A phylogenetic analysis of aligned 16S rRNA gene sequences in the ARB database from validated members of *Halorubrum* and *Halobacterium* was conducted using MEGA6.06 (6) and a maximum-likelihood algorithm.

1. Burns DG, et al. (2007) *Haloquadratum walsbyi* gen. nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. *Int J Syst Evol Microbiol* 57(Pt 2):387–392.
2. Miyashita A, et al. (2009) Development of 16S rRNA gene-targeted primers for detection of archaeal anaerobic methanotrophs (ANMEs). *FEMS Microbiol Lett* 297(1): 31–37.
3. King CE, King GM (2014) *Thermomicrobium carboxidovorans* KI3^T sp. nov., and *Thermorudis peleae* KI4^T gen. nov., sp. nov., carbon monoxide-oxidizing bacteria from geothermally-heated biofilms. *Int J Syst Evol Microbiol* 64(Pt 8):2586–2592.
4. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215(3):403–410.
5. Pruesse E, Peplies J, Glöckner FO (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28(14):1823–1829.
6. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729.

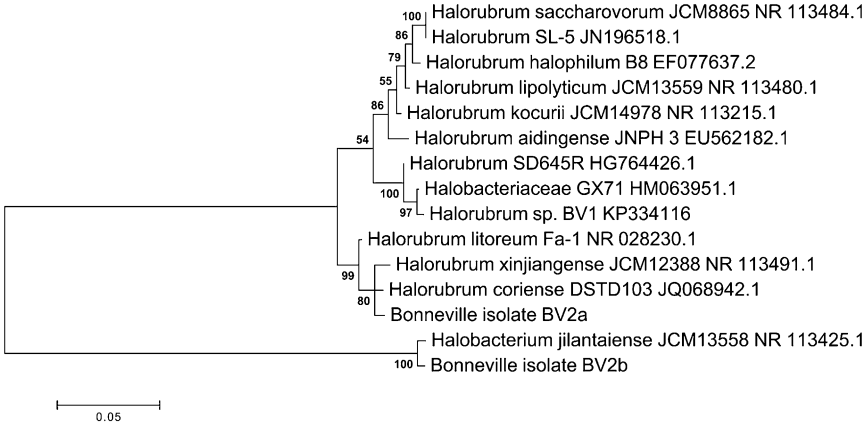

Fig. S1. Phylogenetic analysis of partial 16S rRNA gene sequences for isolate *Halorubrum* sp. BV1 and other select members of the genus. A sequence from *Halobacterium jilantaiense* JCM-13558 was used as an outgroup. Phylogenetic relationships were inferred using maximum likelihood with a GTR (general time-reversible) model. Branch support is indicated for 100 bootstrap replicates.

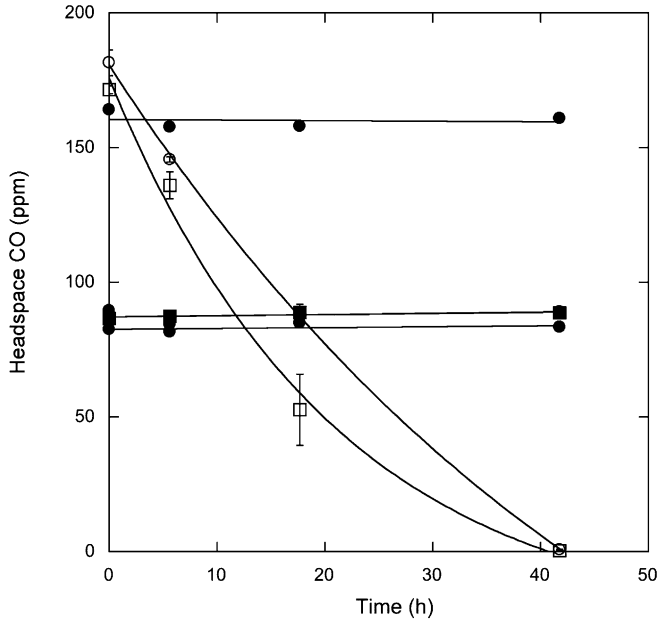

Fig. S2. Headspace CO concentrations (parts per million; ppm) for four treatments with *Alkalilimnicola ehrlichii* MLHE-1: oxic (○); oxic + 50 mM magnesium perchlorate (□); anoxic (●); and anoxic + 50 mM magnesium perchlorate (■). Oxic treatments were initiated with ~170–180 ppm CO; values shown are means of triplicates ± 1 SE. Two anoxic replicates were initiated with ~80–90 ppm CO, and a third replicate was initiated with about 160 ppm; CO concentrations for each anoxic replicate at each time point are shown. The anoxic + perchlorate treatment was initiated with ~85 ppm; values shown are means of triplicates ± 1 SE.

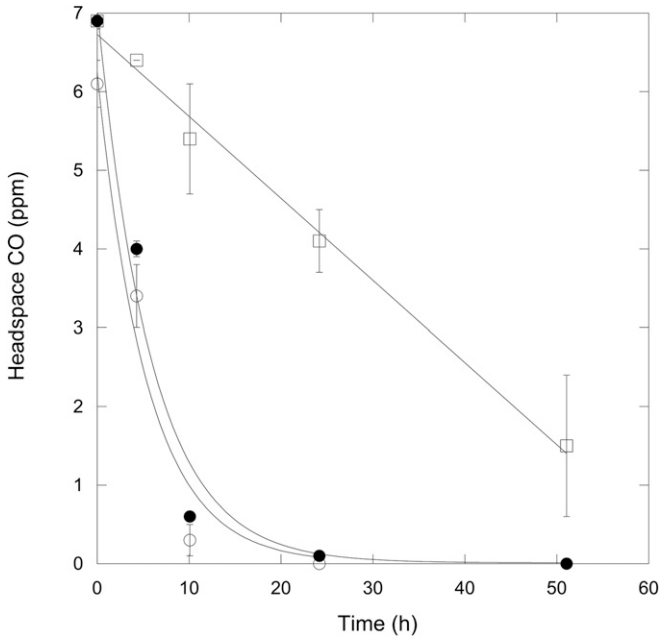

Fig. S3. Headspace CO concentrations (ppm; values shown are means of triplicates ± 1 SE) for incubations of *A. ehrlichii* MLHE-1 in oxic (○), 0.2% oxygen (●), and anoxic + 5 mM sodium nitrate treatments (□).

Table S1. Sample locations, sampling dates, water potentials, and apparent maximum CO uptake rates

Site	GPS position	Date	H_2O potential, MPa	CO uptake rate, nmol $gfw^{-1}{\cdot}h^{-1}$
MacKenzie State Park, HI				
1.1	19.439389 x 155.862472	9 April 2014	−3.2	22.7
1.2	19.439389 x 155.862472	9 April 2014	−3.7	16.2
1.3	19.439389 x 155.862472	9 April 2014	−13.2	ND
2.1	19.439389 x 155.862472	9 April 2014	−39.1	ND
2.2	19.439389 x 155.862472	9 April 2014	−39.7	ND
2.3	19.439389 x 155.862472	9 April 2014	−28.5	0.40
3.1	19.355878 x 155.862220	9 April 2014	−3.1	2.70
3.2	19.355878 x 155.862220	9 April 2014	−2.4	ND
3.3	19.355878 x 155.862220	9 April 2014	−5.3	3.5
2.1	19.439389 x 155.862472	7 July 2014	−0.8	4.8
2.2	19.439389 x 155.862472	7 July 2014	−0.7	5.8
2.3	19.439389 x 155.862472	7 July 2014	−2.1	0.79
2.4	19.439389 x 155.862472	7 July 2014	−1.3	0.48
2.5	19.439389 x 155.862472	7 July 2014	−0.5	0.14
2.6	19.439389 x 155.862472	7 July 2014	−0.6	0.09
3.1	19.355878 x 155.862220	7 July 2014	−1.6	1.3
3.2	19.355878 x 155.862220	7 July 2014	−1.0	1.8
3.3	19.355878 x 155.862220	7 July 2014	−0.6	0.43
Holei Sea Arch, HI				
HSA1	19.389361 x 155.249028	8 July 2014	−42.7	0.58
HSA2	19.389361 x 155.249028	8 July 2014	−33.3	0.36
HSA3	19.389361 x 155.249028	8 July 2014	−43.0	ND
HSA4	19.389361 x 155.249028	8 July 2014	−3.6	0.32
HSA5	19.389361 x 155.249028	8 July 2014	−44.2	0.23
HSA6	19.389361 x 155.249028	8 July 2014	−9.7	ND
HSA7	19.389361 x 155.249028	8 July 2014	−6.0	ND
HSA8	19.389361 x 155.249028	8 July 2014	−117.4	0.26
Bonneville Salt Flats, UT				
Wendover	40.515472 x 114.044917	22 July 2013	−41.2	0.23
BSF-1*	40.737722 x 113.858694	13 August 2013	−41.5	0.10
Atacama Desert, Chile†				
LL2A	−23.062444 x 68.215278	13 August 2013	−10.4	0.16
LL3	−23.062444 x 68.215278	13 August 2013	−11.5	0.17
LL4	−23.062444 x 68.215278	13 August 2013	−11.8	0.06
LL8	−23.062444 x 68.215278	13 August 2013	−9.3	0.16
Cejar 3	−21.266667 x 69.616667	13 August 2013	−7.5	0.10
LL2A	−23.062444 x 68.215278	28 April 2014	−10.4	0.12
LL3	−23.062444 x 68.215278	28 April 2014	−11.5	0.12
LL4	−23.062444 x 68.215278	28 April 2014	−11.8	0.12
LL8	−23.062444 x 68.215278	28 April 2014	−9.3	NA
Cejar 3	−21.266667 x 69.616667	28 April 2014	−7.5	0.11

Water potential and CO uptake rates were measured as described in the text. NA, not assayed; ND, not detectable.
*BSF-1 was collected on 25 July 2013; the assay date is given in the table.
†LL and Cejar samples were collected on 22 and 23 May 2013, respectively; the assay dates are given in the table.

Table S2. CO uptake rates [nmol (mg cell dry weight)$^{-1}{\cdot}h^{-1} \pm 1$ SE, $n = 3$] observed for *A. ehrlichii* MLHE-1 incubated under conditions of varied water potential and temperature

Treatment	−8.3 MPa		−18.1 MPa	
Temperature	6 °C	30 °C	6 °C	30 °C
Rate, $nmol^{-1}{\cdot}h^{-1}$	5.95 ± 0.12	19.32 ± 2.16	0.14 ± 0.08	1.55 ± 0.07

Rates for treatments with varied water potential and temperature were obtained for cells incubated with starting headspace CO concentrations of ~70 ppm and were based on initial linear uptake.