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# **Supplementary Information for** Removal of phosphoglycolate in hyperthermophilic archaea

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#### **Supporting Information Text**

#### Results

#### Gene disruption of TK0150 and its effect on growth

5,10-Methylenetetrahydrofolate is known as a C1 donor for multiple reactions (1-4). As serine hydroxymethyltransferase reactions are reversible (1, 5), growth of  $\Delta$ TK0150 cells in the presence of serine is most likely due to the serine hydroxymethyltransferase reaction proceeding in the direction of 5,10-methylenetetrahydrofolate and glycine formation. In order to evaluate whether the serine auxotrophy observed in the  $\Delta$ TK0150 strain was due to a shortage of 5.10-methylenetetrahydrofolate and not an impairment in the serine biosynthesis pathway per se, we examined the effects of adding an alternative C1 donor to the medium. Ketopantoate is a precursor of CoA biosynthesis in T. kodakarensis, and is generated from ketoisovalerate by ketopantoate hydroxymethyltransferase (KPHMT) (SI Appendix, Fig. S4B) (6, 7). The addition of ketopantoate should be able to provide 5,10-methylenetetrahydrofolate through the reverse reaction catalyzed by KPHMT. When ketopantoate was supplemented into ASW-AA- $S^0$  without serine, growth of  $\Delta TK0150$  was partially restored (*SI Appendix*, Fig. S5B and Fig. S7A), suggesting that 5,10-methylenetetrahydrofolate was generated from ketopantoate by the KPHMT reaction proceeding in the direction of ketoisovalerate formation. The lack of complete complementation is most likely due to insufficient levels of KPHMT activity to provide adequate levels of serine. To confirm that the KPHMT reaction was actually proceeding in the direction of ketoisovalerate generation, we examined valine auxotrophy. Wild-type T. kodakarensis does not harbor a pathway for valine biosynthesis, and displays valine auxotrophy. However, valine can be synthesized by an aminotransferase reaction with ketoisovalerate as an amino group acceptor. The TK0186 protein, described above, displays aminotransferase activity on ketoisovalerate. We can thus confirm the generation of ketoisovalerate through KPHMT activity by supplementing ketopantoate to the medium in the absence of valine. As shown in SI Appendix, Fig. S7B, the addition of ketopantoate, although with a decrease in growth rate, complemented the valine auxotrophy of *T. kodakarensis* KU216, indicating that KPHMT can function to generate ketoisovalerate from ketopantoate (SI Appendix, Fig. S5C). Interestingly, KU216 cells displayed higher growth rates when serine was additionally removed from the medium (SI Appendix, Fig. S7C). This can be explained by the fact that serine is also a C1 5,10-methylenetetrahydrofolate donor leading to generation via serine hydroxymethyltransferase. Removal of serine would result in a decrease in 5,10methylenetetrahydrofolate, pulling the ketopantoate hydroxymethyltransferase reaction towards 5,10-methylenetetrahydrofolate and ketoisovalerate generation (SI Appendix, Fig. **S5D**). When the  $\Delta$ TK0150 strain was grown under this condition, a decrease in cell yield was observed, similar to the results of the strain grown in medium without serine (SI Appendix, Fig. S7A). This is due to the fact that 5,10-methylenetetrahydrofolate can no longer be produced from glycine, and serine biosynthesis relies solely on the production of 5,10methylenetetrahydrofolate from ketopantoate (SI Appendix, Fig. S5D).

#### Methods

**Composition of growth medium.** ASW-YT-S<sup>0</sup> and ASW-YT-Pyr medium were composed of 0.8×ASW (8), 5.0 g L<sup>-1</sup> yeast extract, 5.0 g L<sup>-1</sup> tryptone, and 0.8 mg L<sup>-1</sup> resazurin with 2.0 g L<sup>-1</sup> elemental sulfur or 5.0 g L<sup>-1</sup> sodium pyruvate, respectively. ASW-AA-S<sup>0</sup> medium was composed of 0.8×ASW, a mixture of 20 amino acids, minerals, a mixture of vitamins (8), 0.8 mg L<sup>-1</sup> resazurin, and 2.0 g L<sup>-1</sup> elemental sulfur. Modified versions of these media, ASW-YT-m1 medium (*SI Appendix*, Table S3) and ASW-AA-m1 medium (*SI Appendix*, Table S4), were also used. Solid medium used to isolate transformants were based on ASW-AA-S<sup>0</sup> medium and supplemented with 10 g L<sup>-1</sup> Gelrite, 7.5 g L<sup>-1</sup> 5-fluoroorotic acid (5-FOA), 10 mg L<sup>-1</sup> uracil and 4.5 mL of 1 M NaOH. Elemental sulfur was replaced with 0.2% (v/v) polysulfide solution (8).

**Construction of gene expression plasmids.** The TK0683 and TK1734 coding regions with extensions homologous to the plasmid ends were amplified by PCR using *T. kodakarensis* KOD1 genomic DNA as a template and inserted into pET21a(+) expression plasmid (Merck KGaA, Darmstadt, Germany) by infusion cloning. TK0186, TK0551, TK1094 and TK2301 coding regions were amplified so that additional restriction sites were incorporated. After digestion with Ndel and EcoRI for TK0186 or BamHI for TK0551, TK1094 and TK2301, DNA fragments were ligated with pET21a(+) digested with the same enzymes. All plasmids were

sequenced, and respectively named pET-TK0683, pET-TK1734, pET-TK0186, pET-TK0551, pET-TK1094 and pET-TK2301. Primers used for plasmid construction are listed in *SI Appendix*, **Table S5**.

**Gene expression in** *E. coli* and purification of the recombinant proteins. *E. coli* BL21-CodonPlus(DE3)-RIL was transformed with pET-TK0683, pET-TK1734, pET-TK0186, pET-TK0551, pET-TK1094 or pET-TK2301. Transformants were cultivated in LB medium until OD<sub>660</sub> reached 0.4-0.8. Isopropyl- $\beta$ -D-1-thiogalactopyranoside (0.1 mM) was added, and cultures were continued for another 4 h. As TK1094 protein was insoluble, cells harboring pET-TK1094 were cultivated at 37°C for 18 h without the addition of isopropyl- $\beta$ -D-1-thiogalactopyranoside. Cells were collected by centrifugation (4°C, 5,000×*g*, 15 min), washed with 50 mM Tris-HCI (pH 8.0) containing 1% NaCl, and centrifuged again (4°C, 5,000×*g*, 15 min). Cells were resuspended with 50 mM Tris-HCI (pH 7.5) and sonicated with TOMY UD-201 (TOMY SEIKO, Tokyo, Japan) (OUTPUT:4, DUTY:50, 20-30 min). After centrifugation (4°C, 20,400×*g*, 15 min), the supernatant was treated for 10 min at 90°C (TK1734 and TK2301) or 85°C (TK0186, TK0551, TK0683 and TK1094) and centrifuged (4°C, 15,000×*g*, 15 min) to remove thermolabile proteins.

For TK1734 and TK2301 proteins, supernatants were applied to a ResourceQ column (GE Healthcare, Little Chalfont, Buckinghamshire, UK) equilibrated with 50 mM Tris-HCI (pH 8.0 at 80°C). Proteins were eluted with a linear gradient of 0 to 1.0 M NaCl. Fractions including TK1734 or TK2301 protein were collected and centrifuged with an Amicon Ultra centrifugal filter unit (MWCO 3,000) (EMD Millipore, Billerica, MA, USA). Samples were then mixed with approximately equal volumes of 3 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and separated by hydrophobic interaction chromatography (Resource ISO, GE Healthcare) equilibrated with 50 mM Tris-HCI (pH 8.0 at 80°C) containing 1.5 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Proteins were eluted with a linear gradient of 1.5 M to 0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Fractions containing TK1734 or TK2301 protein were collected and centrifuged with an Amicon Ultra centrifugal filter unit (MWCO 3,000). Finally, proteins were applied to gelfiltration chromatography (Superdex 200 Increase 10/300 GL, GE Healthcare) and separated with a mobile phase of 50 mM Tris-HCI (pH 8.0 at 80°C) containing 0.15 M NaCI. TK0551 and TK0683 proteins were purified with the same procedures without the use of hydrophobic interaction chromatography. Different mobile phases were used for the anion exchange chromatography (50 mM Tris-HCl, pH 7.5 at room temperature) and gel-filtration chromatography (0.15 M NaCl, 50 mM Tris-HCl, pH 7.5 at room temperature). TK0186 and TK1094 proteins were purified with anion exchange chromatography and gel-filtration chromatography with the procedures described for the TK1734 and TK2301 proteins. Protein concentrations were determined with the Protein Assay System (Bio-Rad, Hercules, CA, USA) using bovine serum albumin (Thermo Fisher Scientific, Waltham, MA, USA) as a standard.

**Construction of gene disruption plasmids.** Coding regions of TK0150, TK0186, TK0551, TK0683, TK1734, and TK2301 with approximately 1,000 bps each of 5'- and 3'-flanking regions were amplified by PCR using *T. kodakarensis* KU216 genomic DNA as a template. Each fragment was ligated with HinclI-digested pUD3 plasmid, containing a *pyrF* marker cassette. Inverse PCR was carried out to remove the regions to be deleted, and the amplified fragments were self-ligated. Sequences of the resulting six disruption plasmids were confirmed. Primers used for construction of the disruption plasmids are listed in *SI Appendix*, Table S6.

**Transformation of** *T. kodakarensis.* For gene disruption, *T. kodakarensis* KU216 ( $\Delta pyrF$ ) was used as the host strain. KU216 cells grown in ASW-YT-S<sup>0</sup> medium for 12 h at 85°C were harvested (4°C, 15,000×*g*, 5 min) from 3 mL culture. Cells were resuspended in 200 µL 0.8×ASW and kept on ice for 30 min. Three micrograms of the disruption plasmid were added to the cells and the mixture was kept on ice for 1 h. The mixture was subjected to heat shock for 45 s at 85°C and kept on ice for 10 min. The mixture was inoculated into uracil-free synthetic medium (ASW-AA-S<sup>0</sup> ( $\Delta$ TK0150,  $\Delta$ TK0551,  $\Delta$ TK0683) or ASW-AA-m1-S<sup>0</sup> ( $\Delta$ TK0186,  $\Delta$ TK1734,  $\Delta$ TK2301)), and incubated at 85°C for 2 d. Cells were cultivated in the same medium for another 2 d to enrich transformants harboring the *pyrF* gene via single crossover insertion. Cells were spread onto solid medium (ASW-AA ( $\Delta$ TK0150,  $\Delta$ TK0551,  $\Delta$ TK0551,  $\Delta$ TK0683) or ASW-AA-m1 ( $\Delta$ TK0186,  $\Delta$ TK1734,  $\Delta$ TK2301)) with 0.75% 5-FOA and 10 µg mL<sup>-1</sup> uracil and incubated for 24 h to select cells whose *pyrF* gene was removed via a second recombination event. Genotypes of the obtained colonies were confirmed by PCR and DNA sequencing analysis. Primers used for analyses of deletion mutants are listed in *SI Appendix*, Table S6.

**Tracer-based metabolomic analysis with** <sup>13</sup>**C-glycolate.** *T. kodakarensis* KU216 was grown in 10 mL ASW-YT-m1-Pyr supplemented with 0.08 mM Na<sub>2</sub>S and 0.01% (w/v) 1,2-<sup>13</sup>C-labeled sodium glycolate. Cells were grown for 20 h, collected by centrifugation and washed with 5 mL 0.8×ASW. Preparation of protein-derived amino acids from the cells and the conditions of subsequent analysis using Microfluidic capillary electrophoresis-mass spectrometry (CE–MS) are described in Fukuyama et al. (9). Briefly, protein-derived amino acids were prepared by hydrolyzation with 12N HCI. The purified protein-derived amino acids were analyzed by using a ZipChip CE system (908devices, Boston, MA, USA) coupled with an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). The obtained MS data was analyzed using Qual Browser in Xcalibur version 4.3.73.11.

Glycolate measurement in the culture medium using LC–MS/MS. T. kodakarensis KU216 was grown in 10 mL ASW-YT-m1-Pyr for 20 h under the microaerobic condition defined above. After cultivation, cells were removed by centrifugation (4°C,  $5000 \times g$ , 15 min) followed by filtration with a 0.2 µm filter (Steradisc S-2502S; Kurabo Industries, Osaka, Japan). Glycolate concentrations were measured after derivatization with 3-nitrophenylhydrazine referring to a previous report (10). LC-MS/MS measurement was performed on an ExionLC liquidchromatograph coupled with a quadrupole time-of flight mass spectrometer X500R QTOF system (SCIEX, Toronto, Canada) with an electrospray ionization source in negative ion mode. The samples were separated with an ACQUITY UPLC BEH C18 column (2.1×100 mm, 1.7 μm; Waters, Milford, MA) kept at 40°C using a mobile phase combination of water (A) and 0.01% formic acid in methanol (B). The gradient was started from 18% B, and the percentage of B was linearly raised to 90% in 15 min after injection at a flow rate of 0.2 mL min<sup>-1</sup>. During the analysis, samples were kept at 4°C in the auto sampler. The injection volume was 10 µL. MS/MS data of glycolate were collected in multiple reaction monitoring (MRM) mode as the mass transition pair of m/z 210.05 $\rightarrow$ 152.05. The capillary voltage and the collision energy voltage were set at 4000V and -30V, respectively. Quantitative analyses of glycolate based on MRM chromatograms were performed using SCIEX OS software ver 1.7.

Glycolate detection in the culture medium using LC-high resolution MS (HRMS). Glycolate in the culture medium was prepared as described previously with some modifications (11). Briefly, the culture medium was filtered with a 0.2- $\mu$ m filter and then acidified with 12 N HCl to a final concentration of 0.1 M. To concentrate glycolate, 2 mL of ethyl acetate was added to 12 mL of the sample. After shaking and phase separation, the organic solvent fraction was transferred to a 17-mL glass test tube, with repeated extraction of glycolate from the remaining aqueous fraction using another 2 mL of ethyl acetate. Glycolate was concentrated by evaporation under a N<sub>2</sub> stream from the organic solvent fractions at room temperature. Measurement of exact mass of glycolate using LC-HRMS was performed with an UltiMate 3000RS liquid chromatography (Thermo Fisher Scientific) coupled to electrospray ionization high resolution mass spectrometry analysis on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). Sample separations were performed with an Intrada Organic Acid column 3 μm 2.0 × 150 mm, (Imtakt, Kyoto, Japan) with a linear gradient of 5%-95% solvent B (90% 100 mM ammonium formate, 10% acetonitrile, 0.1% formic acid) over 20 min with a constant flow of 200 µL/min; solvent A (10% acetonitrile, 0.1% formic acid). The column oven was set at 40°C. Full scan MS spectra were acquired in the Orbitrap mass analyzer (polarity: Negative, m/z range: 70-210, resolution:120,000 full width at half maximum (FWHM)) using internal calibration (EASY-IC). MS/MS spectra were acquired in the Orbitrap mass analyzer (mass range: Normal, scan range mode: Auto, resolution:15,000 FWHM) and Stepped Higherenergy collisional dissociation MS/MS fragmentation with normalized collision energy of 15%, 35% and 45%

|               | Genus                                  | Organism<br>Mothonocoldococcus ionnocobii  | Rubisco PG |
|---------------|--|--|------------|
|               |  | Methanocaldococcus jannaschii<br>Methanocaldococcus fervens  |            |
|               |  | Methanocaldococcus rervens<br>Methanocaldococcus vulcanius   |            |
|               | Mathematic                             | Methanocaldococcus sp. FS406-22  |            |
|               | Methanocaldococcus                     | Methanocaldococcus infernus  |            |
|               |  | Methanocaldococcus bathoardescens  |            |
|               |  | Methanocaldococcus lauensis SG7  |            |
|               | Methanotorris                          | Methanocaldococcus lauensis SG1<br>Methanotorris igneus  |            |
|               | Methanotoms                            | Methanococcus maripaludis S2   |            |
|               |  | Methanococcus maripaludis C5   |            |
|               |  | Methanococcus maripaludis C6   |            |
|               |  | Methanococcus maripaludis C7   |            |
|               |  | Methanococcus maripaludis X1   |            |
|               | Methanococcus                          | Methanococcus maripaludis KA1  |            |
|               |  | Methanococcus maripaludis OS7  |            |
|               |  | Methanococcus maripaludis DSM 2067   |            |
|               |  | Methanococcus aeolicus<br>Methanococcus vannielii  |            |
|               |  | Methanococcus voltae   |            |
|               | Methanothermococcus                    | Methanothermococcus okinawensis  |            |
|               | Methanofervidicoccus                   | Methanofervidicoccus sp. A16   |            |
|               |  | Methanothermobacter thermautotrophicus   |            |
|               |  | Methanothermobacter marburgensis   |            |
|               |  | Methanothermobacter wolfeii  |            |
|               |  | Methanothermobacter tenebrarum   |            |
|               | Methanothermobacter                    | Methanothermobacter sp. CaT2   |            |
|               |  | Methanothermobacter sp. EMTCatA1<br>Methanothermobacter sp. MT-2   |            |
|               |  | Methanothermobacter sp. KEPCO-1  |            |
|               |  | Methanothermobacter sp. THM-1  |            |
|               |  | Methanothermobacter sp. THM-2  |            |
|               |  | Methanosphaera stadtmanae  |            |
|               | Methanosphaera                         | Methanosphaera sp. BMS   |            |
|               |  | Methanosphaera sp. ISO3-F5   |            |
|               |  | Methanobrevibacter ruminantium   |            |
|               |  | Methanobrevibacter smithii<br>Methanobrevibacter sp. AbM4  |            |
|               |  | Methanobrevibacter sp. AbM4<br>Methanobrevibacter millerae   |            |
|               | Methanobrevibacter                     | Methanobrevibacter sp. YE315   |            |
|               |  | Methanobrevibacter olleyae   |            |
|               |  | Methanobrevibacter arboriphilus  |            |
|               |  | Methanobrevibacter sp. TLL-48-HuF1   |            |
|               |  | Methanobacterium lacus   |            |
|               |  | Methanobacterium paludis   |            |
|               |  | Methanobacterium sp. MB1   |            |
|               |  | Methanobacterium formicicum BRM9   |            |
|               |  | Methanobacterium formicicum DSM1535<br>Methanobacterium congolense   |            |
|               | Methanobacterium                       | Methanobacterium subterraneum  |            |
|               |  | Methanobacterium sp. MZ-A1   |            |
|               |  | Methanobacterium sp. BRmetb2   |            |
| Euryarchaeota |  | Methanobacterium sp. BAmetb5   |            |
| -             |  | Methanobacterium alkalithermotolerans  |            |
|               |  | Methanobacterium ferruginis  |            |
|               | Methanothermus                         | Methanothermus fervidus  |            |
|               | Methanopyrus                           | Methanopyrus kandleri  |            |
|               |  | Archaeoglobus fulgidus DSM 4304  |            |
|               |  |  |            |
|               | Archaeoglobus                          | Archaeoglobus fulgidus DSM 8774<br>Archaeoglobus profundus   |            |
|               | Archaeoglobus                          | Archaeoglobus profundus  |            |
|               | Archaeoglobus                          |  |            |
|               | Archaeoglobus<br>Ferroglobus           | Archaeoglobus profundus<br>Archaeoglobus veneficus   |            |
|               | Ferroglobus                            | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus acetivorans   |            |
|               |  | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus acetivorans<br>Geoglobus atangari   |            |
|               | Ferroglobus                            | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sultaticallidus<br>Ferroglobus placidus<br>Geoglobus acetivorans<br>Geoglobus ahangari<br>Pyrococcus furiosus DSM 3638   |            |
|               | Ferroglobus                            | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus activorans<br>Geoglobus activorans<br>Geoglobus activorans<br>Pyrococcus furiosus DSM 3638<br>Pyrococcus furiosus COM1  |            |
|               | Ferroglobus                            | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus acetivorans<br>Geoglobus atangari<br>Pyrococcus furiosus DSM 3638<br>Pyrococcus furiosus COM1<br>Pyrococcus horikoshi   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus acetivorans<br>Geoglobus acetivorans<br>Geoglobus atengari<br>Pyrococcus furiosus DSM 3638<br>Pyrococcus furiosus COM1<br>Pyrococcus fonkoshi<br>Pyrococcus abyssi<br>Pyrococcus abyssi   |            |
|               | Ferroglobus                            | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus activorans<br>Geoglobus anterivorans<br>Pyrococcus furiosus DSM 3638<br>Pyrococcus horikoshti<br>Pyrococcus horikoshti<br>Pyrococcus abyssi<br>Pyrococcus abyssi<br>Pyrococcus abyssi   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus acetivorans<br>Geoglobus acetivorans<br>Geoglobus atengari<br>Pyrococcus furiosus DSM 3638<br>Pyrococcus furiosus COM1<br>Pyrococcus fonkoshi<br>Pyrococcus abyssi<br>Pyrococcus abyssi   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sultaticaliidus         Ferroglobus placidus         Geoglobus activarans         Geoglobus atangari         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus abysis         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus sp. ST04         Pyrococcus kukulkanii   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus placidus         Ferroglobus placidus         Geoglobus acetivorans         Geoglobus acetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus abyssi         Pyrococcus abyssi         Pyrococcus sp. NA2         Pyrococcus sp. ST04         Pyrococcus sukuklanii         Pyrococcus sukuklanii   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus aetivorans         Geoglobus aetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus shorikoshii         Pyrococcus sous Activation         Pyrococcus suisus COM1         Pyrococcus spring         Pyrococus spring   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus sulfaticaliidus         Ferroglobus placidus         Geoglobus acetivorans         Geoglobus acetivorans         Geoglobus acetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus abysis         Pyrococcus spirsiosus COM1         Pyrococcus spirsiosus COM1         Pyrococcus abysi         Pyrococcus spirsion  |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus acetivorans         Geoglobus acetivorans         Geoglobus acetivorans         Pyrococcus furiosus COM1         Pyrococcus struiosus COM1         Pyrococcus struiosus COM1         Pyrococcus struiosus COM1         Pyrococcus spissi         Pyrococcus spissi         Pyrococcus spissi         Pyrococcus spissi         Pyrococcus kuklikanii         Pyrococcus kodakarensis         Thermococcus gammatolerans   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfatcelilidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus solutiosus COM1         Pyrococcus solutionsus         Thermococcus solutionsus         Thermococcus solutionsus         Thermococcus solutiorsus         Thermococcus solutiorsus         Thermococcus solutionsus  |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus<br>Archaeoglobus veneficus<br>Archaeoglobus sulfaticallidus<br>Ferroglobus placidus<br>Geoglobus activorans<br>Geoglobus activorans<br>Pyrococcus furiosus DSM 3638<br>Pyrococcus furiosus COM1<br>Pyrococcus horikoshi<br>Pyrococcus spison<br>Pyrococcus spison<br>Pyrococcus spison<br>Pyrococcus spison<br>Pyrococcus spison<br>Pyrococcus spison<br>Pyrococcus kukulkanii<br>Pyrococcus kukulkanii<br>Pyrococcus kukulkanii<br>Pyrococcus kukulkanii<br>Thermococcus kodakarensis<br>Thermococcus spinotoes<br>Thermococcus spinotoes<br>Thermococcus barophilus  |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfatcelilidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus solutiosus COM1         Pyrococcus solutiosus COM1         Pyrococcus solutiosus COM1         Pyrococcus solution         Pyrococcus soliticus   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus sopyssi         Pyrococcus sopyssi         Pyrococcus spensil         Pyrococcus spensil         Pyrococcus spensil         Pyrococcus kukukanii         Pyrococcus kodaarensis         Thermococcus sibinicus         Thermococcus spensibilicus         Thermococcus spensibilicus         Thermococcus spensibilicus         Thermococcus spensibilicus         Thermococcus spensibilicus         Thermococcus spensibilicus         Thermococcus spensetilicus         Thermococcus  |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus placidus         Ferroglobus placidus         Geoglobus acetivorans         Geoglobus acetivorans         Geoglobus acetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus solitosus Thermococcus childerans         Thermococcus solitosus         Thermococcus solitosus         Thermococcus solitosus         Thermococcus solitosus         Thermococcus solitorals         Thermococcus solitorals         Thermococcus solitorals         Thermococcus Solitorals         Theremococcus solitorals <td< td=""><td></td></td<> |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus furiosus COM1         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus sp. ST04         Pyrococcus kukikanii         Pyrococcus kukikanii         Pyrococcus kukikanii         Pyrococcus kodakarensis         Thermococcus barophilus         Thermococcus barophilus         Thermococcus barophilus         Thermococcus p. Sp. SM4         Thermococcus activolerans                                     |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus spanso         Thermococcus gammatolerans         Thermococcus spanso         Thermococcus spansuti  |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus sulfatcallidus         Ferraglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus solus occus         Pyrococcus solus         Pyrococcus solution         Thermococcus coldarensis         Thermococcus solution         Thermococcus solutions   |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfatcallidus         Ferroglobus placidus         Geoglobus activorans         Pyrococcus hrikoshii         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus kukulkanii         Pyrococcus kukulkanii         Pyrococcus kukulkanii         Pyrococcus sp. STO4         Pyrococcus sp. Molerans         Thermococcus garmatolerans         Thermococcus garphilus         Thermococcus sp. AS57         Thermococcus sloralis         Thermococcus sp. AM4         Thermococcus sp. AM4         Thermococcus agranivalelae         Thermococcu  |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus aetivorans         Geoglobus aetivorans         Geoglobus aetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus shorikoshii         Pyrococcus spession         Thermococcus loralis         Thermococcus loralis         Thermococcus spess  |            |
|               | Ferroglobus<br>Geoglobus               | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus sulfaticallidus         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus sp. ST04         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus sp. ST04         Pyrococcus kukukanii         Pyrococcus sp. ST04         Pyrococcus kukukanii         Pyrococcus sp. ST04         Thermococcus garmatolerans         Thermococcus garmatolerans         Thermococcus garphilus         Thermococcus sp. 4557         Thermococcus cleftensis         Thermococcus cleftensis         Thermococcus garativinellae         Thermococcus garativinellae         Thermococcus garativinellae         Thermococcus guythermalis  |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus aetivorans         Geoglobus aetivorans         Geoglobus aetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus shorikoshii         Pyrococcus spession         Thermococcus loralis         Thermococcus loralis         Thermococcus loral  |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus COM1         Pyrococcus horikoshi         Pyrococcus sprissus COM1         Pyrococcus sprissus         Thermococcus sprissus         Thermococcus sprissus         Thermococcus sprissus         Thermococcus analting         Thermococcus analting         Thermococcus analting         Thermococcus analting         Thermococcus analting         Thermococcus analting         Theremococcus priscophilus   |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus veneficus         Archaeoglobus placidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus soprisus COM1         Pyrococcus spaysonsii         Pyrococcus sholophagus         Thermococcus spainteus   |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus sulfaticallidus         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshi         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus sp. ST04         Pyrococcus kukikanii         Pyrococcus kukikanii         Pyrococcus kukikanii         Pyrococcus kukikanii         Pyrococcus sp. ST04         Pyrococcus kukikanii         Thermococcus barophilus         Thermococcus paralyinellee         Thermococcus selorophilus   |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfatcallidus         Ferroglobus placidus         Geoglobus activorans         Pyrococcus furiosus COM1         Pyrococcus sophilis         Pyrococcus sophilis         Pyrococcus sophilis         Pyrococcus sophilis         Pyrococcus kukulkanii         Pyrococcus kodakarensis         Thermococcus garmatolerans         Thermococcus subiricus         Thermococcus subiricus         Thermococcus suborphilus         Thermococcus suborphilus         Thermococcus suborphilus         Thermococcus suborphilus         Thermococcus suborphilus         Thermococcus paralyinellae         Thermococcus adulti         Thermococcus paralying         Thermococcus pertophilus         Thermococcus pertophilus         Thermococcus gregonarius         Thermococcus gregonarius         Thermococcus pertophilus         Thermococcus perto  |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus aetivorans         Geoglobus aetivorans         Geoglobus aetivorans         Geoglobus aetivorans         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshii         Pyrococcus solvisus COM1         Thermococcus solvisus COM2         Thermococcus solvisus COM2         Thermococcus solvisus COM2         Thermococcus solvi                    |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfatcallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus sulfatcallidus         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus suniosus COM1         Pyrococcus horikoshii         Pyrococcus sp. NA2         Pyrococcus sp. NA2         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus sp. ST04         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus sp. ST04         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus sp. ST04         Pyrococcus sp. ST04         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus sp. ST04         Thermococcus garmatolerans         Thermococcus garphinules         Thermococcus garphylinuls         Thermococcus paralylinellae         Thermococcus garphinules         Thermococcus garphinuls  |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Pyrococcus brikoshi         Pyrococcus spysis         Thermococcus gammatolerans         Thermococcus spysis         Thermococcus s  |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfaticalilidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus sulfaticalilidus         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus horikoshi         Pyrococcus spinsus DSM 3638         Pyrococcus spinsus COM1         Pyrococcus spinsus         Pyrococcus spinsus         Pyrococcus spinsus         Pyrococcus kukilkanii         Pyrococcus kukilkanii         Pyrococcus kinophagus         Thermococcus barophilus         Thermococcus barophilus         Thermococcus paralyinellae         Thermococcus activatis         Thermococcus paralyinellae         Thermococcus spinsus         Thermococcus spinsus         Thermococcus spinsus         Thermococcus sporgonarius   |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfatcallidus         Ferroglobus placidus         Geoglobus activorans         Pyrococcus hrikoshi         Pyrococcus spinsus COM1         Pyrococcus spinsus         Pyrococcus spinsus         Pyrococcus spinsus         Pyrococcus spinsus         Pyrococcus kukukani         Thermococcus subrophilus         Thermococcus periophilus         Thermococcus pelenophilus         Thermococcus pelenophilus   |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfaticallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Geoglobus activorans         Pyrococcus furiosus DSM 3638         Pyrococcus brikoshii         Pyrococcus solves COM1         Pyrococcus solves         Pyrococcus kukulkanii         Pyrococcus kodatarensis         Thermococcus solves         Thermococcus solves         Thermococcus solves         Pyremoccus solves         Pyremococcus solves         Thermococcus solves <t< td=""><td></td></t<>   |            |
|               | Ferroqlobus<br>Geoglobus<br>Pyrococcus | Archaeoglobus profundus         Archaeoglobus sulfatcallidus         Ferroglobus placidus         Geoglobus activorans         Geoglobus activorans         Geoglobus sulfatcallidus         Pyrococcus furiosus DSM 3638         Pyrococcus furiosus COM1         Pyrococcus shrikoshi         Pyrococcus shrikoshi         Pyrococcus spanosii         Pyrococcus spanosii         Pyrococcus spanosii         Pyrococcus kukukanii         Pyrococcus kukukanii         Pyrococcus kodakarensis         Thermococcus spanosii         Thermococcus shorthonghagus         Thermococcus shorthius         Thermococcus shortholius         Thermococcus sepophilus         Thermococcus plexophilus         Thermococcus plexophilus         Thermococcus gelere         Thermococcus shortholus         Thermococcus shortholus         Thermococcus shortholus         Thermococcus shortholus <td></td>                       |            |

Table S1. Presence or absence of gene homologs of Rubisco and 2-PG phosphatase on various archaeal genomes.

| Page         Output         Output         Output         Page           Main surgery and page         Additional page of page         Additional page         Additional page           Additional page         Additional page  |               |                      |   |          |     |
|---|---------------|----------------------|---|----------|-----|
| Englands         Mathematical solution         Mathematical solution           Mathematical solution         Mathematical solution         Mathematical solution           Mathematical solution         Mathematical solution         Mathematical solution         Mathematical solution           Mathematical solution         Mathematical solution         Mathematical solution         Mathematical solution         Mathematical solution           Mathematical solution         Mathematic   | Phylum        | Genus                | Organism<br>Methanosarcina barkeri Fusaro | Rubisco  | PGP |
|   |               |                      |   |          |     |
| Expendence         Methodocals along 3         Image: 1           Methodocals along 3         Image: 1         Image: 1         Image: 1           Methodocals along 3         Image: 1         Image: 1         Image: 1         Image: 1           Methodocals along 3         Image: 1   |               |                      |   |          |     |
| Environment         Environment         Environment         Environment           Hallwaren and Part         Enviro  |               |                      |   |          |     |
| Ergenden         Methodologi generation and fund <ul> <li>Methodologi generation and fund</li> <li>Methodologi generation and fu</li></ul>  |               |                      | Methanosarcina acetivorans                |          |     |
| Engeneration and processing and procesing and procesing and processing and processing and processing an                       |               |                      |   |          |     |
| Egeneration of the second of the se                       |               |                      |   |          |     |
| Europerational solution as for the solution                       |               |                      | Methanosarcina mazei C16                  |          |     |
| Eugenetation <ul> <li>Methodocation as UP14</li> <li>Methodocatin as UP14</li> <li>Methodocation as UP14</li></ul>   |               | Methanosarcina       |   |          |     |
| Environment         Methological as DPTA           Methological as DPTA         Methological as DPTA           Methological as DPTA         Methological as DPTA           Methological as Detrocols 10:0         Methological as Detrocols 10:0           Methological as Detroc   |               | methanosa cina       |   |          |     |
| Eurorentes         Medinacionales (2000)         Initial (2000)           Medinacionales (2000)         Medinacionales (2000)         Initial (2000)  |               |                      |   |          |     |
| Englished Solution (Solution (Sol                       |               |                      |   |          |     |
| Eugenback         Methonological point         Methonological point           Methonological point         M  |               |                      |   |          |     |
| Europendant Ph.1 <ul> <li>Methological provides of the second provid</li></ul>   |               |                      |   |          |     |
| Eugenome         Methonogeneration         Image: Section Sec   |               |                      |   |          |     |
| Euryordnots         Methonococide control           Methonococide         Methonococide   |               |                      | Methanosarcina thermophila CHTI-55        |          |     |
| Ensemble         Methanococoles burken           Methanococoles of methanococ         Methanococoles methanococ           Methanococoles methanococ         Methanococoles methanococ           Methanocococ         Methanocococ           Methanocococ         Methanocococo           Methanocococo         Methanocococo </td <td></td> <td></td> <td></td> <td></td> <td></td>   |               |                      |   |          |     |
| Burgersback         Methanococcides methy dens           Methanococcides methy dens         Methanococcides methy dens           Methanoches methy dens         Methanoches methy dens           Methanoches methy dens         Me  |               |                      |   |          |     |
| Image: stand                        |               | Methanococcoides     |   |          |     |
| International problem         Methanological methodsharm         International methodsharm           Methanological methodsharm         Methanological methodsharm         International methodsharm           Methanological methodsharmethodsharm         Methanological methodsha  |               |                      |   |          |     |
| Mefnanciskum         Mefnanciskum         Mefnanciskum           Mefnanciskum         Mefnanciskum Palnos         Image: Status Palnos           Mefnanciskum         Mefnanciskum Palnos         Image: Status Palnos           Mefnanciskum Palnos         Mefnanciskum Palnos         Image: Status Palnos           Mefnanciskum Palnos         Image: Status Palnos         Image: Status Palnos           Mefnanciskum Palnos         Image: Status Palnos         Image: Status Palnos           Mefnanciskum Palnos         Mefnanciskum Palnos         Image: Status Paln   |               | Methanohalophilus    |   |          |     |
| Methanologia         Methanologia zalogia         Impanzioalia zalogia           Methanologia         Methanologia         Impanzioalia           Methanologia         Methanologia         Methanologia           Methanologia         Methanologia <td></td> <td></td> <td>Methanohalobium evestigatum</td> <td></td> <td></td>  |               |                      | Methanohalobium evestigatum               |          |     |
| Methanolous         Methanolous andrah           Methanolous         Methanolous marginou           Methanolous         Methanolous           Metha   |               | Methanosalsum        |   | لكري     |     |
| Indining         Methanology and setures           Methanology and setures         Methanology and setures           Methanology and se   |               | Mathematichus        | Methanolobus psychrophilds                |          |     |
| Metrasonsthjovoras         Metrasonsthjovoras         Aufinizione           Metrasonsthjovoras         Metrasonsthjovoras         Metrasonsthjovoras           Metrasonsthjovoras         Metrasonsthjovoras         Metrasonsthjovoras <t< td=""><td></td><td>wethanolobus</td><td>Methanolobus mangrovi</td><td></td><td></td></t<>  |               | wethanolobus         | Methanolobus mangrovi                     |          |     |
| Methammorococcus p. H6         Methammorococcus p. H6           Methammorococcus p. H6         Methammorococcus p. H6           Methamothrix         Methamothrix frammorococcus p. H6           Methamothrix         Methamothrix frammorococcus p. H6           Methamothrix         Methamothrix frammorococus p. H6           Methamothrix frammorococus p. H6         Methamothrix frammorococus p. H6           Methamothrix frammorococus p. H6         Methamothrix frammorococus p. H6           Methamothrix frammorococus p. H6         Methamothrix frammorococus p. H6           Methamothrix         Methamothrix           Met   |               | Methanomethylovorans |   |          |     |
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| Methanolivi:         Methanologi           Methanologi Rium         Methanologi Rium           Methanologi Rium         <   |               | Methanimicrococcus   | Methanimicrococcus sp. Es2                |          |     |
| Methanoprillum         Methanoprillum 19, 13.6 147 2.7.3           Methanoprillum 19, 14.6 148, 14.6 14.6 14.6 14.6 14.6 14.6 14.6 14.6  |               | Methanothrix         |   |          |     |
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| Methanocorpusculum         Methano   |               | Methanospirillum     |   |          |     |
| Methanoculae maringin           Methanoculae upgensis MS2           Methanoculae upgensis MAB1           Methanoclas upgensis           Methanoclas upgensis <td></td> <td></td> <td></td> <td></td> <td></td>  |               |                      |   |          |     |
| Methanoculleus         Methanoculleus bourgenis MAB1           Methanoculleus submarinus         Methanoculleus submarinus           Methanoculleus submarinus         Methanoculleus submarinus           Methanoculleus submarinus         Methanoculleus submarinus           Methanoculleus submarinus         Methanoculleus submarinus           Methanoculleus         Methanoculleus constantis           Methanoculleus constantis         Methanoculleus constantis           Methanoculleus constantis <td></td> <td>Methanocolpusculum</td> <td></td> <td></td> <td></td>   |               | Methanocolpusculum   |   |          |     |
| Methanocliles achiogensis           Methanocliles achiogensis <td></td> <td></td> <td></td> <td></td> <td></td>   |               |                      |   |          |     |
| Methanociclia: submanus           Methanociclia: submanus           Methanociclia: submanus           Methanopianus   |               | Methanoculleus       |   |          |     |
| Methanoldanus         Methanoldanus         Methanoldanus           Euryarcheota         Methanoldanus         Methanoldanus         Methanoldanus           Methanoldanus         Methanoldanus  |               |                      | Methanoculleus submarinus                 |          |     |
| Methanoplanus         Methanoplanus         methanoplanus           Methanoplanus         Methanoplanus         Methanoplanus           Methanoplanus         Methanoplanus         Methanoplanus           Methanoplanus         Methanoplanus         Methanoplanus           Methanonegula         Methanoregula boorel         Methanoregula boorel           Methanoregula         Methanoregula boorel         Methanoregula boorel           Methanoregula boorel         Methanoregula boorel         Methanoregula boorel           Methanoregula boorel         Methanoregula boorel         Methanorel           Methanorella consolita anuarun RIC-11         Methanorella consolita anuarun RIC-11         Methanorella consolita anuarun RIC-11           Halobacterium SDL 51         Halobacterium SDL 54         Methanorella consolita anuarun RIC-11         Methanorella consolita anuarun RIC-11           Halobacterium         Halobacterium SDL 54         Halobacterium SDL 54         Methanorella consolita anuarun RIC-11           Halobacterium         Halobacterium SDL 54         Methanorella consolita anuarun RIC-11  |               | Mathematica          |   |          |     |
| Euryarchaeda Metinanofalia Methanogenium Methanogenium Methanogenium Methanogenium Methanogenium ogs.348F Methanogenium ogs.348F Methanogenium ogs.348F Methanogenium ogs.348F Methanogenium Methanogenium ogs.348F Methanogenium Halobacterium |               |                      |   |          |     |
| Methanogenium     Methanogenium op. SABF       Methanoregula     Methanoregula bonei       Methanoregula     Methanoregula bonei       Methanosphaerula     Methanoregula formicica       Methanosphaerula     Methanoregula formicica       Methanosphaerula     Methanosphaerula paludicula       Methanosphaerula     Methanosphaerula paludicula       Methanosphaerula     Methanosphaerula       Halobacterum     Salurchaerula       Halobacterum     Falobacterula       Halobacterum     Falobacte   | Euryarchaeota |                      | Methanoplanus sp. FWC-SCC4                |          |     |
| Methanogenium sp. S48F           Methanoneropula         Methanoregula bororis           Methanoregula         Methanoregula formicica           Methanoregula         Methanoregula formicica           Methanoregula         Methanoregula formicica           Methanocegula formicica         Methanocegula formicica           Methanocella         Methanocella aronzae           Halobacterium salinarum NRC-1         Halobacterium salinarum NRC-1           Halobacterium sp. DL1         Halobacterium sp. DL1           Halobacterium sp. DL1         Halobacterium noticum HSR1           Halobacterium noticum sp. DL1-2         Halobacterium noticum HSR1           Halobacterium noticum BRR         Halobacterium noticum HSR1           Halobacterium noticum BRR         Halaneroarcheaum sp. HSR-20           Salarcheaum         Salarcheaum sp. DSR-20           Halabacterium sp. DSR-20         Halabacterium sp. HSR-20           Halaraeroarchaeum sp. HSR-20         Halaraeroarcheaum sp. HSR-20           Halaraeroarchaeum sp. HSR-20         Halaraeroarchaeum sp. HSR-20           Halaraeroarchaeum sp. HSR-20         Halaraeroarchaeum sp. HSR   |               | Methanofollis        |   |          |     |
| Methanomicrobium     Methanomicrobium antiguum       Methanoregula     Methanoregula boonei       Methanosphaerula     Methanoregula formicica       Methanosphaerula     Methanocella palutoria       Methanocella     Methanocella corradi       Methanocella     Methanocella corradi       Methanocella avorazia     Methanocella corradi       Methanocella avorazia     Methanocella corradi       Methanocella avorazia     Methanocella corradi       Methanocella avorazia     Methanocella corradi       Halobacterium spinoni     Methanocella avorazia       Halobacterium spinoni     Methanocella spinoni       Halobacterium     Halobacterium spinoni       Halobacterium     Halobacterium spinoni       Halobacterium     Halobacterium spinoni       Halobacterium     Halobacterium spinonicum HTSR1       Halobacterium     Halobacterium spinonicum HTSR1<  |               | Methanogenium        |   |          |     |
| Methanosphaerula         Methanosphaerula palvstris           Methanosphaerula jalvstris         Methanosphaerula palvstris           Methanosella ometaliuciola         Methanosella ometaliuciola           Methanosella ometaliuciona         Methanosella ometaliuciona           Methanosella ometaliuciona         Methanosella ometaliuciona           Methanosella ometaliuciona         Methanosella ometaliuciona           Methanosella ometaliuciona         Methanosella ometaliuciona           Halobacterium subienziona         Methanosella ometaliuciona           Halobacterium sp. DL1         Halobacterium sp. GSL:19           Halobacterium sp. GSL:19         Halobacterium sp. GSL:19           Halobacterium sp. GSL:19         Halobacterium sp. GSL:19           Halobacterium sp. GSL:10         Halobacterium sp. GSL:10           Halobacterium sp. GSL:10         Halobacterium sp. GSL:10           Halobacterium Sp. GSL:10         Halobacterium sp. GSL:11           Halobacterium Sp. GSL:11         Halobacterium sp. GSL:11           Halobacterium Sp. Harch-Bakt         Saliarchaeum Sp. Harch-Bakt           Salinarchaeum Sp. Harch-Bakt   |               | Methanomicrobium     | Methanomicrobium antiquum                 |          |     |
| Methanosphaerula         Methanosphaerula palustris           Methanocella palustris         Methanocella palustris           Methanocella aronzate         Methanocella aronzate           Methanocella aronzate         Methanocella aronzate           Halobacterum salinarum NRC-1         Halobacterum salinarum NRC-4001           Halobacterum sulinarum NRC-3001         Halobacterum sp. 1           Halobacterum sp. 1         Halobacterum sp. 1           Halobacterum sp. 201-19         Halobacterum sp. 201-19           Halobacterum sp. 201-2         Halobacterum sp. 201-2           Halobacterum sp. 201-42         Halobacterum sp. 201-43           Halobacterum Torense         Halobacterum Torense           Halobacterum Torense         Halobacterum Torense           Halobacterum Torense         Halobacterum Torense           Halaneeroarchaeum         Halobacterum sp. 20R-1           Halaneeroarchaeum         Halobacterum sp. 20R-1           Halaneeroarchaeum         Halobaccus dombrowskii           Halarchaeum         Halakalicoccus dorbrowskii           Halobaccus         Halobaccus dorbrowskii           Halorussus walis         Salinarchaeum sp. Harch-Bsk1           Salinarchaeum         Salinarchaeum sp. Harch-Bsk1           Salinarchaeum         Salinarchaeum sp. Harch-Bsk1 <tr< td=""><td></td><td>Methanoregula</td><td></td><td></td><td></td></tr<>   |               | Methanoregula        |   |          |     |
| Methanocella         Methanocella conradii           Methanocella conradii         Methanocella conradii           Halobacterium salinarum NRC1         Halobacterium salinarum NRC1           Halobacterium salinarum NRC34001         Halobacterium salinarum NRC34001           Halobacterium salinarum NRC34001         Halobacterium solicarum NRC34001           Halobacterium solicarum NRC34001         Halobacterium NRC34001           Halobacterium solicarum NRC34001         Halobacterium NRC34001           Halabacterium Salurireducens NRC3         Halabacterium NRC3400           Halabacterium Salurireducens NRC340         Halabacterium NRC3400           Halabacocus         Halabacterium Salurireducens NRC3400           Halabacocus         Halabacocus soluritricus           Halabacocus         Halabacocus soluritricus           Halabacocus         Halabacocus soluritricus           Halabalacocus soluritricus soluritricus         Halabalabacocus soluritricus           Halabalabacocus soluritricus         Halabalabacocus soluritricus           Halabalabacocus soluritritricus         Halabalabacocus soluritricus </td <td></td> <td>Methanosphaerula</td> <td>Methanosphaerula palustris</td> <td></td> <td></td>   |               | Methanosphaerula     | Methanosphaerula palustris                |          |     |
| Methanocella rvorzae           Hałobacterum salinarum NRC-1           Hałobacterum salinarum NRC-34001           Hałobacterum salinarum RRC-34001           Hałobacterum sp. DL1           Hałobacterum NRC-34001           Hałobacterum noricense           Hałobacterum Inorincum HTSR1           Hałobacterum Inorincum HTSR1           Hałobacterum Inorincum HTSR6           Hałaneroarchaeum sufturieducens MZ-SA2           Hałaneroarchaeum sufturieducens MZ-SA2           Hałaneroarchaeum sp. HSR-CO           Salarchaeum           Hałarchaeura sp. USR-CO           Hałarchaeura Sp. CBA 120   |               |                      | Methanocella paludicola                   |          |     |
| Halobacterium sainarum RC1           Halobacterium sainarum RC34001           Halobacterium sainarum NRC34001           Halobacterium sainarum NRC34001           Halobacterium sainarum NRC34001           Halobacterium sp. 50.11           Halobacterium sp. 50.14           Halaraeroarchaeum sp. 50.14           Halaraeroarchaeum sp. 50.14           Halaraeroarchaeum sp. 50.14           Halarchaeum sp. 50.14           Haloraeroarchaeum sp. 140000000   |               | Methanocella         |   |          |     |
| Halobacterium sol       Image: Constraint of the second seco  |               |                      | Halobacterium salinarum NRC-1             |          |     |
| Halobacterium sp. DL1       Imalobacterium sp. DL1         Halobacterium sp. GSL-19       Imalobacterium sp. BOL4-2         Halobacterium sp. BOL4-2       Imalobacterium sp. BOL4-2         Halobacterium ilioreum       Halobacterium sp. BOL4-2         Halobacterium ilioreum       Halobacterium ilioreum         Halobacterium ilioreum       Halobacterium ilioreum         Halobacterium ilioreum       Halobacterium ilioreum         Halobacterium ilioreum SR       Imalobacterium ilioreum         Halanaeroarchaeum multimeducens NSR2       Imalobacterium ilioreum SR         Halanaeroarchaeum sulturireducens NSP       Imalobacterium ilioreum SR         Halanaeroarchaeum sp. JOR-1       Halanaeroarchaeum sp. JOR-1         Halarchaeum Sp. JOR-1       Halarchaeum Sp. CBA120         Halarchaeum Sp. CBA120       Imalorobrowskii         Halorussus Jimi       Imalorobrowskii         Halorussus gallinuktus       Imalorussus gallinuktus         Halorussus sallincus       Imalorussus vallis         Halorussus sallincus       Imalorus sp. IM2453         Halorussus sallincus       Imalorussus vallis         Halorussus vallis       Imalorus sp. IM2453         Halorussus sallincus       Imalorussus vallis         Halorussus vallis       Imalorussus vallis         Halorussus sallincu  |               |                      |   |          |     |
| Halobacterium       Halobacterium sp. CSL-19         Halobacterium sp. CSL-2       Halobacterium         Halobacterium sp. BCL-2.       Halobacterium         Halobacterium itoreum       Halobacterium itoreum         Halobacterium itoreum       Halobacterium         Halobacterium itoreum       Halobacterium         Halobacterium itoreum       Halobacterium         Halobacterium   |               |                      |   |          |     |
| Halobacterium sp. BOL-2:       Halobacterium noricense         Halobacterium iltoreum       Halobacterium iltoreum         Halobacterium iltoreum       Halobacterium iltoreum         Halobacterium iltoreum       Halodesulfurachaeum formicicum HTSR1         Halodesulfurachaeum sulfurfeducens HSR2       Halanaeroarchaeum sulfurfeducens HSR2         Halanaeroarchaeum sulfurfeducens HSR2       Halanaeroarchaeum sulfurfeducens HSR2         Halanaeroarchaeum sulfurfeducens HSR2       Halanaeroarchaeum sulfurfeducens HSR2         Halakalicoccus       Halanaeroarchaeum sulfurfeducens HSR2         Halakalicoccus       Halakalicoccus jeotgali         Halobaccus geldgali       Halakalicocus         Halorussus gelafinilyficus       Halorussus gelafinilyficus         Halorussus valila       Halorussus valila         Halorussus valila       Halorussus valila         Halorussus valila       Halorussus valila         Halorusus valila       Halorusus valila         Halorusus valila       <   |               | Halobacterium        | Halobacterium hubeiense                   |          |     |
| Halobacterium moreuns       Halobacterium moreuns         Halodesulfurarchaeum       Halodesulfurarchaeum formicicum HTSR1         Halanaeroarchaeum       Halanaeroarchaeum sulfuireducens HSR2         Halanaeroarchaeum       Halanaeroarchaeum sulfuireducens HSR2         Halanaeroarchaeum sp. USR-1       Halanaeroarchaeum sp. USR-1         Halanaeroarchaeum sp. USR-1       Halanaeroarchaeum sp. USR-1         Halarchaeum       Salarchaeum sp. USR-1         Halakalicoccus       Halakalicoccus jeotgali         Halorchaeum       Halorchaeum sp. USR-1         Halorchaeum       Halorchaeum sp. USR-1         Halorcussus       Indicoccus jeotgali         Halorussus       Halorussus gelatinityticus         Halorussus       Salinarchaeum sp. Harcht-Bsk1         Salinarchaeum       Salinarchaeum sp. Harcht-Bsk1         Salinarchaeum       Salinarchaeum sp. Harcht-Bsk1         Halorusa       Halorusaus valiis         Haloracula       Haloracula Arizaeroarchaeum sp. Harcht-Bsk1         Haloracula Sinalensis       Haloracula Sinalensis         Haloracula Sinalensis   |               |                      |   | <u> </u> |     |
| Halodesulfurarchaeum       Halodesulfurarchaeum formicicum HTSR1         Halodesulfurarchaeum       Halanaeroarchaeum sulfuirieducens MSR4         Halanaeroarchaeum       Halanaeroarchaeum sulfuirieducens MSR2         Halanaeroarchaeum       Halanaeroarchaeum sulfuirieducens MSR2         Halanaeroarchaeum sulfuirieducens MS7-SA2         Halanaeroarchaeum sulfuirieducens MS7-SA2         Halanaeroarchaeum sulfuirieducens MS7-SA2         Halarchaeum       Halarchaeum sp. CBA1220         Halakalicoccus       Halakalicoccus gelgali         Halocscus       Halarchaeum         Halocscus       Halarchaeum         Halorussus       Halorussus limi         Halorussus       Halorussus gelatinlyticus         Halorussus valiis       Halorussus valiis         Salinarchaeum       Salinarchaeum sp. Hacht-Bsk1         Salinarchaeum       Salinarchaeum sp. Hacht-Bsk1         Salinarchaeum       Salinarchaeum sp. H263         Natranaeroarchaeum       Halorussus valiis         Haloarcula       Halorus sp. CBA115         Halorus sp. JPL23       Halorus sp. JPL23         Halorus sp. Japanica ATICC 33960       Halorus sp. Japanica ATIC 33960         Halorus sp. Japanica Signanica Attiona       Halorus sp. Japanica Signanica Attiona         Halorarula sp. JPL23  |               |                      | Hadaba ata dana wa dana a                 |          |     |
| Halodesulfurarchaeum     Halonaeur formicicum HSR6       Halanaeroarchaeum sulfurireducens HSR2       Halanaeroarchaeum sulfurireducens M27-SA2       Halanaeroarchaeum sp. JOR-1       Salarchaeum       Salarchaeum       Halarchaeum sp. JOR-1       Halorussus       Halorussus felatinilyticus       Halorussus gelatinilyticus       Halorussus vallis       Salinarchaeum sp. Harcht-Bsk1       Salinarchaeum       Salinarchaeum sp. JUR453       Natranaeroarchaeum       Haloruslu hispanica NG01       Haloarcula hispanica NG01       Haloarcula asinainstui       Haloarcula asinainstis       Haloarcula asinainstis       Haloarcula asinainstis       Haloarcula asinainstis       Haloarcula sinainstis       Haloarcula sinainstis       Haloarcula sinainstis       Haloarcula sinainstis       Haloarcula sinainstis       Halorarcula sinainstis       Halorarcul  |               |                      | Halobacterium litoreum                    |          |     |
| Halanaeroarchaeum       Halanaeroarchaeum sulfurireducens M27-SA2         Halanaeroarchaeum sulfurireducens M27-SA2         Halarchaeum       Salarchaeum sp. USR-CO         Halarchaeum       Halarchaeum sp. CBA1220         Halarchaeum       Halorussus limi         Halorussus limi       Halorussus limi         Halorussus sulis       Halorussus salilacus         Halorussus valis       Halorussus valis         Salinarchaeum       Salinarchaeum sp. Harch-Bsk1         Salinarchaeum       Salinarchaeum sp. Harch-Bsk1         Natranaeroarchaeum       Natranaeroarchaeum sulfidigenes         Haloarcula       Haloarcula inspanica ATCC 33960         Haloarcula sp. CBA1115       Haloarcula sp. CBA1115         Haloarcula sp. CBA1115       Haloarcula sp. PL23         Haloarcula sinainensis       Natronomonas moolapensis         Natronomonas       Natronomonas moolapensis         Natronomonas salinien       Halorhabdus uahensis  |               | Halodesulfurarchaeum |   |          |     |
| Halanaeroarchaeum       Halanaeroarchaeum sp. HSR-CO         Salarchaeum       Salarchaeum sp. JOR-1         Halarchaeum       Halarchaeum sp. JOR-1         Halarchaeum       Halarchaeum sp. CBA1220         Halarchaeum       Halarchaeum sp. GBA1220         Halarchaeum       Halarchaeum sp. GBA1220         Halarchaeum       Halarchaeum sp. CBA1220         Halarchaeum       Halorcocus jeotgali         Halorussus       Halorussus gelatinilyticus         Halorussus       Halorussus gelatinilyticus         Halorussus vallis       Halorussus vallis         Salinarchaeum       Salinarchaeum sp. Harcht-Bsk1         Salinarchaeum       Salinarchaeum sull/digenes         Haloarcula hispanica N601       Haloarcula hispanica N601         Haloarcula hispanica N601       Haloarcula signalica         Haloarcula signalicalis       Haloarcula signalicalis         Natronomonas       JP-23         Haloarcula signalicalis       Haloarcula signalicalis         Natronomonas salina       Haloranomas shalophila         Haloranda signalicalis       Haloranda signalicalis         Haloranda signalicalis       Haloranda signalicalis         Haloarcula signalicalis       Haloarcula signalicalis         Halorandu signalicalis       Halorandi sign  |               |                      |   |          |     |
| Salarchaeum       Salarchaeum sp. JOR-1         Halarchaeum       Halarchaeum sp. GBA1220         Halarchaeum       Halarchaeum sp. GBA1220         Halarchaeum       Halarchaeum sp. GBA1220         Halarchaeum       Halorcoccus dombrowskii         Halococcus       Halorussus limi         Halorussus       Halorussus gelatinilyticus         Halorussus       Halorussus gelatinilyticus         Halorussus vallis       Salinarchaeum         Salinarchaeum       Salinarchaeum sp. Hacht-Bsk1         Salinarchaeum       Salinarchaeum sp. Hacht-Bsk1         Natranaeroarchaeum       Natranaeroarchaeum sulfidigenes         Haloarcula nispanica ATCC 39600       Haloarcula nispanica ATCC         Haloarcula a nispanica N601       Haloarcula a nispanica N601         Haloarcula a sinaliensis       Haloarcula a sinaliensis         Natronomonas       Natronomonas pharaonis         Natronomonas salina       Natronomonas salina         Halorhabdus       Halorhabdus sp. CBA1104         Halorhabdus       Halorhabdus sp. CBA1104   |               | Halanaeroarchaeum    | Halanaeroarchaeum sulfurireducens M27-SA2 |          |     |
| Halarchaeum       Halarchaeum sp. CBA1220         Halalkalicoccus       Halarchaeum sp. CBA120         Halarchaeum       Halorussus gelatinilyicus         Halorussus       Halorussus gelatinilyicus         Halorussus       Halorussus gelatinilyicus         Halorussus       Halorussus gelatinilyicus         Halorussus       Halorussus gelatinilyicus         Halorussus vallis       Halorussus vallis         Salinarchaeum       Salinarchaeum sp. Ma263         Natranaeroarchaeum       Natranaeroarchaeum sp. M2453         Haloarcula hispanica ATCC 33960       Haloarcula hispanica ATCC 33960         Haloarcula hispanica ATCC 33960       Haloarcula apincina Sis         Haloarcula apincina Kof01       Haloarcula apincina Sis         Haloarcula apincina Sis       Haloarcula apincina Sis         Haloarcula apincina Sis       Haloarcula sinainensis         Natronomonas       Natronomonas shalophila         Natronomonas salina       Halorabdus subaniss         Halorhabdus       Halorhabdus sp. CBA1104         Halorhabdus sp. SC BA1104       Halorhabdus sp. SC BA1104  |               | Salarchaeum          |   |          |     |
| Halalkalicoccus     Halakalicoccus jeotgali       Halococcus     Halorussus dombrowskii       Halorussus Ilmi     Halorussus silmi       Halorussus     Halorussus salilacus       Halorussus salilacus     Halorussus salilacus       Salinarchaeum     Salinarchaeum sp. Harcht-Bsk1       Salinarchaeum     Salinarchaeum sp. IM2453       Natranaeroarchaeum     Natraneroarchaeum sulfidigenes       Haloarcula inspanica ATCC 33960     Haloarcula nispanica ATCC 33960       Haloarcula pispanica N601     Haloarcula sp. CBA1115       Haloarcula sinaitensis     Haloarcula sinaitensis       Natronomonas     Natronomonas salina       Natronomonas salina     Haloarcula sinaitensis       Halorhabdus     Halorhabdus sp. CBA1104  |               |                      |   |          |     |
| Halorussus limi       Halorussus selia         Halorussus seliacus       Halorussus seliacus         Halorussus seliacus       Halorussus seliacus         Salinarchaeum       Salinarchaeum sp. Harcht-Bsk1         Salinarchaeum       Salinarchaeum sp. Harcht-Bsk1         Natranaeroarchaeum       Natraneeroarchaeum sulfidigenés         Haloarcula narismortui       Haloarcula narismortui         Haloarcula appinca NG01       Haloarcula spinca NG01         Haloarcula spinca NG01       Haloarcula sinaliensis         Haloarcula sinaliensis       Haloarcula sinaliensis         Natronomonas       Natronomonas selane         Halorhabdus       Halorhabdus sp. CBA1104         Halorhabdus       Halorhabdus sp. CBA1104   |               | Halalkalicoccus      | Halalkalicoccus jeotgali                  |          |     |
| Halorussus     Halorussus salilacius       Halorussus valiis  |               | rialococcus          |   |          |     |
| Halorussus alliacus       Halorussus alliacus         Halorussus vallis       Salinarchaeum sp. Harcht-Bsk1         Salinarchaeum       Salinarchaeum sp. M2453         Natranaeroarchaeum       Natranaeroarchaeum sulfidigenes         Haloarcula nispanica ATCC 33960       Haloarcula nispanica ATCC 33960         Haloarcula nispanica ATCC 33960       Haloarcula nispanica N601         Haloarcula nispanica ATCC 33960       Haloarcula nispanica N601         Haloarcula nispanica Solo       Haloarcula nispanica N601         Haloarcula nispanica N601       Haloarcula nispanica N601         Halornaborus nispanica N601       Haloarcula nispanica N601         Halornabous solonas       Natronomonas nolapensis         Natronomonas nalophila       Halorhabdus sutahensis         Halorhabdus sutahensis       Halorhabdus sp. SVX81   |               | Halorussus           | Halorussus gelatinilyticus                |          |     |
| Salinarchaeum       Salinarchaeum sp. Harcht-Bsk1         Salinarchaeum sp. IM2453         Natranaeroarchaeum       Natranaeroarchaeum sulfidigenes         Haloarcula nispanica ATCC 33960         Haloarcula nispanica ATCC 33960         Haloarcula nispanica N601         Haloarcula spenica N601         Haloarcula nispanica N601         Haloarcula nispanica N601         Haloarcula spenica N601         Haloarcula sinaliensis         Natronomonas pharaonis         Natronomonas salina         Natronomonas salina         Halorhabdus utahensis         Halorhabdus utahensis         Halorhabdus sp. CBA1104         Halorhabdus sp. SVX81   |               |                      | Halorussus salilacus                      |          |     |
| Salinarchaeum     Salinarchaeum sul/Idigenes       Natranaeroarchaeum     Natranaeroarchaeum sul/Idigenes       Haloarcula marismortui     Haloarcula marismortui       Haloarcula hispanica NC0C 33960     Haloarcula hispanica NC0C 33960       Haloarcula sp. CBA1115     Haloarcula sp. CBA1115       Haloarcula sp. J.P-L23     Haloarcula sinaliensis       Natronomonas     Natronomonas plaraonis       Natronomonas salapatia     Haloarcula sinaliensis       Halorhabdus     Halorhabdus sp. CBA1104   |               |                      |   |          |     |
| Haloarcula marismortui       Haloarcula mispenica ATCC 33960       Haloarcula hispenica N601       Haloarcula nispenica N601       Haloarcula sp. CBA1115       Haloarcula sp. JP-L23       Haloarcula sp. JP-L23       Natronomonas pharaonis       Natronomonas halophila       Natronomonas salina       Halorhabdus sp. CBA1104       Halorhabdus sp. SVX81   |               |                      | Salinarchaeum sp. IM2453                  |          |     |
| Haloarcula hispanica ATCC 33960         Haloarcula hispanica NG01         Haloarcula spinica NG01         Natronomonas moolapensis         Natronomonas saliophila         Natronomonas saliophila         Halorhabdus utahensis         Halorhabdus sp. CBA1104         Halorhabdus sp. SVX81  |               | Natranaeroarchaeum   |   |          |     |
| Haloarcula ispanica N601     Haloarcula sp. CBA1115       Haloarcula sp. CBA1115     Haloarcula sp. CBA1115       Haloarcula sp. CBA1115     Haloarcula sp. CBA1115       Haloarcula sp. CBA1104     Haloarcula sp. CBA1104       Haloarcula sp. CBA1104     Haloarcula sp. CBA1104   |               |                      |   |          |     |
| Haloarcula sinailensis       Haloarcula sinailensis       Natronomonas pharaonis       Natronomonas moolapensis       Natronomonas salina       Haloarcula sinailensis       Haloarcula sinailensis       Natronomonas moolapensis       Haloarcula sinailensis       Haloarcula sinailensis       Halorhabdus utahensis       Halorhabdus sp. CBA1104       Halorhabdus sp. SVX81  |               |                      | Haloarcula hispanica N601                 |          |     |
| Haloarcula sp. JP-L23       Haloarcula sp. JP-L23       Natronomonas pharaonis       Natronomonas moolapensis       Natronomonas moolapensis       Natronomonas salina       Halorhabdus utahensis       Halorhabdus simatea       Halorhabdus sp. SVX81  |               | Haloarcula           |   |          |     |
| Haloarcula siniensis     Haloarcula siniensis       Natronomonas pharaonis     Natronomonas pharaonis       Natronomonas moolagensis     Natronomonas halophila       Natronomonas salina     Halorhabdus sutahensis       Halorhabdus sp. CBA1104     Halorhabdus sp. SVX81  |               |                      |   |          |     |
| Natronomonas     Natronomonas moolapensis       Natronomonas naturonomonas moolapensis     Image: Comparison of C   |               |                      | Haloarcula sinaiiensis                    |          |     |
| Natronomonas     Natronomonas halophila       Natronomonas salophila       Natronomonas salophila       Halorhabdus utahensis       Halorhabdus sp. CBA1104       Halorhabdus sp. SVX81   |               |                      |   |          |     |
| Natronomonas salina       Halorhabdus utahensis       Halorhabdus sp. CBA1104       Halorhabdus sp. SVX81   |               | Natronomonas         |   |          |     |
| Halorhabdus sp. CBA1104<br>Halorhabdus sp. CBA1104<br>Halorhabdus sp. SVX81   |               |                      | Natronomonas salina                       |          |     |
| Halorhabdus Halorhabdus sp. CBA1104<br>Halorhabdus sp. SVX81  |               |                      |   | ┞───┤    |     |
| Halorhabdus sp. SVX81   |               | Halorhabdus          |   |          |     |
| Halorhabdus sp. BNX81   |               |                      | Halorhabdus sp. SVX81                     |          |     |
|   |               |                      | Halorhabdus sp. BNX81                     |          |     |

| Phylum           | Genus  | Organism  | Rubisco | PGP |
|------------------|--|---|---------|-----|
|                  |  | Halomicrobium mukohataei  |         |     |
|                  |  | Halomicrobium mukohataei JP60   |         |     |
|                  | Halomicrobium  | Halomicrobium sp. LC1Hm   |         |     |
|                  |  |   |         |     |
|                  |  | Halomicrobium sp. ZPS1  |         |     |
|                  | Halorientalis  | Halorientalis sp. IM1011  |         |     |
|                  |  | Halapricum salinum  |         |     |
|                  | Halapricum   |   |         |     |
|                  |  | Halapricum desulfuricans  |         |     |
|                  |  | Halosimplex rubrum  |         |     |
|                  | Halosimplex  | Halosimplex pelagicum   |         |     |
|                  |  | Halosimplex litoreum  |         |     |
|                  | Heleseesidee   |   |         |     |
|                  | Halococcoides  | Halococcoides cellulosivorans   |         |     |
|                  | Salinirubellus   | Salinirubellus salinus  |         |     |
|                  | Halocatena   | Halocatena salina   |         |     |
|                  |  | Haloquadratum walsbyi DSM 16790   |         |     |
|                  | Haloquadratum  |   |         |     |
|                  | · · · · · · · · · · · · · · · · · · ·  | Haloquadratum walsbyi C23   |         |     |
|                  |  | Haloferax volcanii  |         |     |
|                  |  | Haloferax mediterranei  |         |     |
|                  |  | Haloferax gibbonsii   |         |     |
|                  | Haloferax  |   |         |     |
|                  |  | Haloferax alexandrinus  |         |     |
|                  |  | Haloferax larsenii  |         |     |
|                  |  | Haloferax lucentense  |         |     |
|                  | Halogeometricum  | Halogeometricum borinquense   |         |     |
|                  | Thalogeomethoam  |   |         |     |
|                  |  | Haloplanus rubicundus CBA1112   |         |     |
|                  | Haloplanus   | Haloplanus rubicundus CBA1113   |         |     |
|                  | . aopianas   | Haloplanus aerogenes  |         |     |
|                  |  | Haloplanus rallus   |         |     |
|                  | Halobellus   |   |         |     |
|                  | Halobellus   | Halobellus limi   |         |     |
|                  | Haloprofundus  | Haloprofundus sp. MHR1  |         |     |
|                  |  | Halorubrum lacusprofundi  |         |     |
|                  |  | Halorubrum sp. PV6  |         |     |
|                  |  | Halorubrum sp. BOL3-1   |         |     |
|                  |  |   |         |     |
|                  | Halorubrum   | Halorubrum ezzemoulense   |         |     |
|                  |  | Halorubrum sp. CBA1229  |         |     |
|                  |  | Halorubrum sodomense  |         |     |
|                  |  | Halorubrum salinarum  |         |     |
|                  |  | Halorubrum sp. 2020YC2  |         |     |
|                  | C-lizizzzzu  |   |         |     |
|                  | Salinigranum   | Salinigranum rubrum   |         |     |
|                  | Halohasta  | Halohasta litchfieldiae   |         |     |
|                  | Halolamina   | Halolamina sp. CBA1230  |         |     |
| Euryarchaeota    |  | Halobaculum halophilum  |         |     |
| Ediyaronacota    |  |   |         |     |
|                  |  | Halobaculum salinum   |         |     |
|                  | Halobaculum  | Halobaculum magnesiiphilum  |         |     |
|                  |  | Halobaculum roseum  |         |     |
|                  |  | Halobaculum rubrum  |         |     |
|                  |  | Halobaculum sp. CBA1158   |         |     |
|                  |  | Halalkaliarchaeum desulfuricum  |         |     |
|                  | Halalkaliarchaeum  |   |         |     |
|                  |  | Halalkaliarchaeum sp. AArc-CO   |         |     |
|                  | unclassified Haloferacales   | Halophilic archaeon DL31  |         |     |
|                  |  | Haloterrigena turkmenica  |         |     |
|                  | Haloterrigena  | Haloterrigena salifodinae   |         |     |
|                  | ··   |   |         |     |
|                  |  | Haloterrigena alkaliphila   |         |     |
|                  | Natrialba  | Natrialba magadii   |         |     |
|                  | Halopiger  | Halopiger xanaduensis   |         |     |
|                  |  | Natrinema sp. J7-2  |         |     |
|                  |  | Natrinema pellirubrum   |         |     |
|                  |  |   |         |     |
|                  |  | Natrinema versiforme  |         |     |
|                  | Natrinema  | Natrinema pallidum  |         |     |
|                  | Natimenta  | Natrinema zhouii  |         |     |
|                  |  | Natrinema halophilum  |         |     |
|                  |  | Natrinema thermotolerans  |         |     |
|                  |  |   |         |     |
|                  |  | Natrinema longum  |         |     |
|                  | Natronobacterium   | Natronobacterium gregoryi   |         |     |
|                  | Halovivax  | Halovivax ruber   |         |     |
|                  |  | Natronosalvus rutilus   |         |     |
|                  | Natronosalvus  | Natronosalvus halobius  |         |     |
|                  | Neterran   |   |         |     |
|                  | Natronococcus  | Natronococcus occultus  |         |     |
|                  | Halostagnicola   |   | 1       |     |
|                  |  | Halostagnicola larsenii   |         |     |
|                  | Halobiforma  | Halostagnicola larsenii<br>Halobiforma lacisalsi  |         |     |
|                  |  | Halobiforma lacisalsi   |         |     |
|                  | Halobiforma  | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum   |         |     |
|                  |  | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg   |         |     |
|                  | Halobiforma  | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1   |         |     |
|                  | Halobiforma  | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1<br>Natrialbaceae archaeon AArc-T1-2   |         |     |
|                  | Halobiforma  | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum  | Halobiforma lacisalsi<br>Natrarchaeobaculum sagyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1<br>Natrialbaceae archaeon AArc-T1-2<br>Natrionorubrum bangense  |         |     |
|                  | Halobiforma  | Halobiforma lacisalsi<br>Natrarchaeobaculum sagyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc-1<br>Natrialbaceae archaeon AArc-1-2<br>Natronorubrum bangense<br>Natronorubrum abiense  |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum   | Halobforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1<br>Natraibaceaa archaeon AArc-T1-2<br>Natronorubrum bangense<br>Natronorubrum albiense<br>Natronorubrum algingense   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea   | Halobiforma lacisalsi<br>Natrarchaeobaculum segyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1<br>Natrialbaceae archaeon AArc-T1-2<br>Natronorubrum abiense<br>Natronorubrum abiense<br>Natronorubrum daqingense<br>Natronorubrum daqingense<br>Natonohaloarchaea archaeon SG9   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum   | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc-1<br>Natrialbaceae archaeon AArc-11-2<br>Natronorubrum bangense<br>Natronorubrum dagingense<br>Natronorubrum dagingense<br>Natonoloarchaea archaeon SG9<br>Candidatus Methanoliparum sp. LAM-1   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum   | Halobiforma lacisalsi     Natrarchaeobaculum segyptiacum     Natrarchaeobaculum sulfurireducens AArc-Mg     Natrarchaeobaculum sulfurireducens AArc1     Natriarbaeobaculum sulfurireducens AArc2     Natronorubrum ablense     Natronorubrum ablense     Natronorubrum algingense     Natronorubrum algingense     Nanohaloarchaea archaeon SG9     Candidatus Methanoliparum sp. LAM-1     Methanonatronarchaeum sp. AMET6-2  |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea   | Halobiforma lacisalsi<br>Natrarchaeobaculum aegyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc-1<br>Natrialbaceae archaeon AArc-11-2<br>Natronorubrum bangense<br>Natronorubrum dagingense<br>Natronorubrum dagingense<br>Natonoloarchaea archaeon SG9<br>Candidatus Methanoliparum sp. LAM-1   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum<br>Methanonatronarchaeum  | Halobiforma lacisalsi<br>Natrarchaeobaculum segyptiacum<br>Natrarchaeobaculum sulfurireducens AArc-Mg<br>Natrarchaeobaculum sulfurireducens AArc1<br>Natrialbaceae archaeon AArc-T1-2<br>Natronorubrum abigense<br>Natronorubrum abigense<br>Natronorubrum dagingense<br>Natronorubrum dagingense<br>Natonohaloarchaea archaeon SG9<br>Candidatus Methanoliparum sp. LAM-1<br>Methanonatronarchaeum sp. AMET-2<br>Methanonatronarchaeum sp. AMET-S1   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum   | Halobforma lacisalsi     Natrarchaeobaculum sagyptiacum     Natrarchaeobaculum sulfurireducens AArc-Mg     Natrarchaeobaculum sulfurireducens AArc1     Natraibaceaa archaeon AArc-T1-2     Natronorubrum bangense     Natronorubrum albiense     Natronorubrum algingense     Nanohaloarchaea archaeon SG9     Candidatus Methanoliparum sp. LAM-1     Methanonatronarchaeum sp. AMET-S1     Thermoplasma acidophium   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum<br>Methanonatronarchaeum<br>Thermoplasma  | Halobiforma lacisalsi     Natrarchaeobaculum segyptiacum     Natrarchaeobaculum sulfurireducens AArc-Mg     Natrarchaeobaculum sulfurireducens AArc1     Natrarchaeobaculum sulfurireducens AArc2     Natronorubrum alogense     Natronorubrum sp. LAM-1     Methanonatronarchaeum sp. AMET6-2     Methanonatronarchaeum sp. AMET-SI     Thermoplasma acidophilum     Thermoplasma volcanium   |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum<br>Methanonatronarchaeum  | Halobiforma lacisalsi         Natrarchaeobaculum segyptiacum         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natroncubrum abigense         Natronorubrum abigense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum bettense         Natronorubrum dagingense         Natronorubrum barbanatronarchaeum sp. AMET-S1         Thermoplasma volcanium         Picrophilus oshimae  |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum<br>Methanonatronarchaeum<br>Thermoplasma<br>Picrophilus   | Halobforma lacisalsi     Natrarchaeobaculum segyptiacum     Natrarchaeobaculum sulfurireducens AArc-Mg     Natrarchaeobaculum sulfurireducens AArc1     Natranchaeobaculum sulfurireducens AArc2     Natronorubrum bangense     Natronorubrum albiense     Natronorubrum algingense     Secongelsense acidamanus |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum<br>Methanonatronarchaeum<br>Thermoplasma  | Halobiforma lacisalsi         Natrarchaeobaculum segyptiacum         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natroncubrum abigense         Natronorubrum abigense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum bettense         Natronorubrum dagingense         Natronorubrum barbanatronarchaeum sp. AMET-S1         Thermoplasma volcanium         Picrophilus oshimae  |         |     |
|                  | Halobiforma<br>Natrarchaeobaculum<br>Natronorubrum<br>unclassified Nanohaloarchaea<br>Methanoliparum<br>Methanonatronarchaeum<br>Thermoplasma<br>Picrophilus   | Halobiforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc2         Natronorubrum abiense         Natronorubrum abiense         Natronorubrum daqingense         Natronorubrum daqingense         Nanohaloarchaea archaeon SG9         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET6-2         Methanonatronarchaeum sp. AMET-SI         Thermoplasma acidophilum         Thermoplasma acidophilum         Feroplasma acidiphilum         Feroplasma acidiphilum  |         |     |
|                  | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanoliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma   | Halobforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natriabacea archaeon AArc-T1-2         Natronorubrum bangense         Natronorubrum albiense         Natronorubrum algingense         Nanohaloarchaea archaeon SG9         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET-S1         Thermoplasma acidophilum         Thermoplasma acidophilum         Picropilus oshimae         Ferroplasma acidipmaus         Ferroplasma acidiputum         Cuniculiplasma divulgatum  |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanoliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         unclassified Thermoplasmatales  | Halobiforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natronorubrum abiense         Natrononatronarchaeum sp. LAM-1         Methanonatronarchaeum sp. AMETe-SI         Thermoplasma acidamium         Thermoplasma volcanium         Picrophilus oshimae         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Thermoplasma acidare archaeon BRNA1  |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanoliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         Unclassified Thermoplasmatales         Methanomethylophilus                               | Halobforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natriabacea archaeon AArc-T1-2         Natronorubrum bangense         Natronorubrum bangense         Natronorubrum abiense         Natronorubrum abiense         Natronorubrum abiense         Natronorubrum abiense         Natronadatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMETe-2         Methanonatronarchaeum sp. AMETe-S1         Thermoplasma acidophlum         Picrophlus oshimae         Ferroplasma acidarmanus         Ferroplasma acidiphilum         Cunclulplasma divulgatum         Thermoplasmateles archaeon BRNA1         Methanomethylophilus alvi   |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanoliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         unclassified Thermoplasmatales         Methanomethylophilus         Methanometsiliooccus  | Halobforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natronorubrum bangense         Natronorubrum albiense         Natronorubrum algingense         Natronorubrum algingense         Nanohaloarchaea archaeon SG9         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET-2         Methanonatronarchaeum sp. AMET-51         Thermoplasma acidophilum         Picrophilus oshimae         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Thermoplasmaatiles archaeon BRNA1         Methanomatromassiliacoccus intestinalis Issoire-Mx1   |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanoliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         Unclassified Thermoplasmatales         Methanomethylophilus                               | Halobforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natronorubrum bangense         Natronorubrum albiense         Natronorubrum algingense         Natronorubrum algingense         Nanohaloarchaea archaeon SG9         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET-2         Methanonatronarchaeum sp. AMET-51         Thermoplasma acidophilum         Picrophilus oshimae         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Thermoplasmaatiles archaeon BRNA1         Methanomatromassiliacoccus intestinalis Issoire-Mx1   |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanonilparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         unclassified Thermoplasmatales         Methanomethylophilus         Methanomethylophilus | Halobiforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc2         Natrarchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc2         Natronorubrum albiense         Natronorubrum aloignense         Natronorubrum daignense         Natronorubrum daignense         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET6-2         Methanonatronarchaeum sp. AMET-SI         Thermoplasma acidophilum         Thermoplasma acidarmanus         Ferroplasma acidaphilum         Thermoplasma acidaphilum         Thermoplasma acidaphilum         Thermoplasma acidaphilum         Thermoplasma acidaphilum         Thermoplasma acidaphilum         Cuniculiplasma divulgatum         Thermoplasma divulgatum         Thermoplasma acidaphilum         Cuniculiplasma divulgatum         Thermoplasma lacidaphilus alvi         Candidatus Methanomassilliocccus intestinalis Issoire-Mx1         Candidatus Methanomasonalisticoccus intestinalis Issoire-Mx1         Candidatus Methanomassilinoccus in   |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanonliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         unclassified Thermoplasmatales         Methanomethylophilus         Methanongsmum        | Halobforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natronorubrum bangense         Natronorubrum albiense         Natronorubrum alpiense         Natronorubrum alpiense         Nanohaloarchaea archaeon SG9         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET-S1         Thermoplasma acidophilum         Thermoplasma acidophilum         Picrophilus oshimae         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Cuniculplasma divulgatum         Thermoplasmatales archaeon BRNA1         Methanomethylophilus akri         Candidatus Methanomassiliicoccus intestinalis Issoire-Mx1         Candidatus Methanoquarant se U3.2.1   |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanonilparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         unclassified Thermoplasmatales         Methanomethylophilus         Methanomethylophilus | Halobiforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natronorubrum bangense         Natronorubrum alienses         Natronorubrum dagingense         Natronorubrum dagingense         Natronorubrum alienses         Methanonatronarchaeum sp. AMET-6-2         Methanonatronarchaeum sp. AMET-5-2         Methanonatronarchaeum sp. AMET-5-1         Thermoplasma acidaphilum         Thermoplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Thermoplasmatiles archaeon BRNA1         Methanomethylophilus alvi         Candidatus Methanoplas   |         |     |
| Thermoplasmatota | Halobiforma         Natrarchaeobaculum         Natronorubrum         unclassified Nanohaloarchaea         Methanonliparum         Methanonatronarchaeum         Thermoplasma         Picrophilus         Ferroplasma         Cuniculiplasma         unclassified Thermoplasmatales         Methanomethylophilus         Methanongsmum        | Halobforma lacisalsi         Natrarchaeobaculum sulfurireducens AArc-Mg         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natrarchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natranchaeobaculum sulfurireducens AArc1         Natronorubrum bangense         Natronorubrum albiense         Natronorubrum alpiense         Natronorubrum alpiense         Nanohaloarchaea archaeon SG9         Candidatus Methanoliparum sp. LAM-1         Methanonatronarchaeum sp. AMET-S1         Thermoplasma acidophilum         Thermoplasma acidophilum         Picrophilus oshimae         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Ferroplasma acidarmanus         Cuniculplasma divulgatum         Thermoplasmatales archaeon BRNA1         Methanomethylophilus akri         Candidatus Methanomassiliicoccus intestinalis Issoire-Mx1         Candidatus Methanoquarant se U3.2.1   |         |     |

| Dhudum         | 0.000                     | Orranian  | Dubing  | DCD      |
|----------------|---------------------------|---|---------|----------|
| Phylum         | Genus                     | Organism<br>Aeropyrum pernix  | Rubisco | PGP      |
|                | Aeropyrum                 | Aeropyrum camini  |         |          |
|                |                           | Staphylothermus marinus   |         |          |
|                | Staphylothermus           | Staphylothermus hellenicus  |         |          |
|                |                           | Ignicoccus hospitalis   |         |          |
|                | Ignicoccus                | Ignicoccus islandicus   |         |          |
|                | -                         | Ignicoccus pacificus  |         |          |
|                |                           | Desulfurococcus amylolyticus 1221n                                      |         |          |
|                | Desulfurococcus           | Desulfurococcus amylolyticus DSM 16532                                  |         |          |
|                |                           | Desulfurococcus mucosus   |         |          |
|                | Thermosphaera             | Thermosphaera aggregans   |         |          |
|                | Ignisphaera               | Ignisphaera aggregans   |         |          |
|                | Thermogladius             | Thermogladius calderae  |         |          |
|                | Hyperthermus              | Hyperthermus butylicus  |         | i        |
|                | Pyrolobus                 | Pyrolobus fumarii   |         | <u> </u> |
|                | Pyrodictium               | Pyrodictium delaneyi  |         |          |
|                |                           | Pyrodictium abyssi  |         |          |
|                | Pyrofollis                | Pyrofollis japonicus  |         |          |
|                | Sulfurisphaera            | Sulfurisphaera tokodaii<br>Sulfurisphaera ohwakuensis                   |         |          |
|                |                           | Saccharolobus solfataricus P2   |         |          |
|                |                           |   |         |          |
|                |                           | Saccharolobus solfataricus 98/2<br>Saccharolobus solfataricus SULA      |         |          |
|                | Saccharolobus             | Saccharolobus solfataricus SARC-B                                       |         |          |
|                | Gaoonaroiobus             | Saccharolobus soliataricus SARC-D<br>Saccharolobus solfataricus SARC-C  |         |          |
|                |                           | Saccharolobus soliataricus SARC-C<br>Saccharolobus shibatae             |         |          |
|                |                           | Saccharolobus shibatae<br>Saccharolobus caldissimus                     |         |          |
|                |                           | Salcharolobus caldasimus<br>Sulfolobus acidocaldarius DSM 639           |         |          |
|                |                           | Sulfolobus acidocaldarius DSN 639<br>Sulfolobus acidocaldarius N8       |         |          |
|                |                           | Sulfolobus acidocaldarius No<br>Sulfolobus acidocaldarius Ron12/I       |         |          |
|                |                           | Sulfolobus acidocaldarius SUSAZ   |         |          |
|                |                           | Sulfolobus islandicus L.S.2.15  |         |          |
|                |                           | Sulfolobus islandicus E.O.2.10<br>Sulfolobus islandicus M.14.25         |         |          |
|                |                           | Sulfolobus islandicus M.16.27   |         |          |
|                |                           | Sulfolobus islandicus M.16.4  |         |          |
|                |                           | Sulfolobus islandicus Y.G.57.14   |         |          |
|                | Sulfolobus                | Sulfolobus islandicus Y.N.15.51   | -       |          |
|                |                           | Sulfolobus islandicus L.D.8.5   |         |          |
|                |                           | Sulfolobus islandicus HVE10/4   |         |          |
|                |                           | Sulfolobus islandicus REY15A  |         |          |
|                |                           | Sulfolobus islandicus LAL14/1   |         |          |
|                |                           | Sulfolobus sp. A20  |         |          |
|                |                           | Sulfolobus sp. E5-1-F   |         |          |
| Thermoproteota |                           | Sulfolobus sp. E11-6  |         |          |
|                |                           | Sulfolobus sp. S-194  |         |          |
|                |                           | Metallosphaera sedula DSM 5348  |         |          |
|                |                           | Metallosphaera sedula MJ1HA   |         |          |
|                |                           | Metallosphaera cuprina  |         |          |
|                | Metallosphaera            | Metallosphaera hakonensis JCM 8857 = DSM 7519                           |         |          |
|                |                           | Metallosphaera prunae   |         |          |
|                |                           | Metallosphaera tengchongensis   |         |          |
|                |                           | Metallosphaera javensis   |         |          |
|                |                           | Acidianus hospitalis  |         |          |
|                |                           | Acidianus manzaensis  |         |          |
|                | Acidianus                 | Acidianus brierleyi   |         |          |
|                |                           | Acidianus sulfidivorans   |         |          |
|                |                           | Acidianus ambivalens  |         |          |
|                | Sulfadiiaaaaua            | Acidianus sp. HS-5<br>Sulfadiiseasus acidiabilus                        |         |          |
|                | Sulfodiicoccus            | Sulfodiicoccus acidiphilus  |         |          |
|                | Stygiolobus               | Stygiolobus azoricus  |         |          |
|                |                           | Stygiolobus caldivivus<br>Sulfuracidifex tepidarius                     |         |          |
|                | Sulfuracidifex            | Sulfuracidifex metallicus   |         |          |
|                | unclassified Sulfolobales | Sulfolobales archaeon HS-7  |         |          |
|                |                           | Pyrobaculum aerophilum  |         |          |
|                |                           | Pyrobaculum islandicum  |         |          |
|                |                           | Pyrobaculum ralidifontis  |         | i        |
|                |                           | Pyrobaculum arsenaticum   |         |          |
|                | Pyrobaculum               | Pyrobaculum ferrireducens   |         |          |
|                |                           | Pyrobaculum oguniense   |         |          |
|                |                           | Pyrobaculum neutrophilum  |         |          |
|                |                           | Pyrobaculum sp. WP30  |         |          |
|                | Caldivirga                | Caldivirga maquilingensis   |         |          |
|                | Thormoprotouo             | Thermoproteus tenax   |         |          |
|                | Thermoproteus             | Thermoproteus uzoniensis  |         |          |
|                |                           | Vulcanisaeta distributa   |         |          |
|                | Vulcanisaeta              | Vulcanisaeta moutnovskia  |         |          |
|                |                           | Vulcanisaeta souniana   |         |          |
|                |                           | Thermofilum pendens   |         |          |
|                | Thermofilum               | Thermofilum adornatum 1910b   |         |          |
|                | 1                         | Thermofilum adornatum 1505  |         |          |
|                |                           |   |         |          |
|                | Infirmifilum              | Infirmifilum uzonense   |         |          |
|                | Infirmifilum              | Infirmifilum lucidum  |         |          |
|                |                           | Infirmifilum lucidum<br>Acidilobus saccharovorans                       |         |          |
|                | Acidilobus                | Infirmifilium lucidum<br>Acidilobus saccharovorans<br>Acidilobus sp. 7A |         |          |
|                |                           | Infirmifilum lucidum<br>Acidilobus saccharovorans                       |         |          |

| Phylum   | Genus                       | Organism                                   | Rubisco | PGP                                     |
|--|-----------------------------|--|---------|---|
|  |                             | Nitrosopumilus maritimus                   |         | Í                                       |
|  |                             | Candidatus Nitrosopumilus sediminis        |         | Í                                       |
|  |                             | Candidatus Nitrosopumilus koreensis        |         | Í                                       |
|  |                             | Nitrosopumilus piranensis                  |         |   |
|  |                             | Nitrosopumilus adriaticus                  |         | Í                                       |
|  | Nitrosopumilus              | Candidatus Nitrosopumilus sp. SW           |         | ĺ                                       |
|  |                             | Nitrosopumilus cobalaminigenes             |         | ĺ                                       |
|  |                             | Nitrosopumilus oxyclinae                   |         | Í                                       |
|  |                             | Nitrosopumilus ureiphilus                  |         | (                                       |
|  |                             | Nitrosopumilus sp. K4                      |         | ĺ                                       |
|  |                             | Nitrosopumilus zosterae                    |         | Í                                       |
|  | Nitrosomarinus              | Candidatus Nitrosomarinus catalina         |         | ĺ                                       |
|  | Nitrosarchaeum              | Nitrosarchaeum sp. AC2                     |         | Í                                       |
| Nitrososphaerota   |                             | Candidatus Nitrososphaera gargensis        |         | ĺ                                       |
|  | Nitrososphaera              | Nitrososphaera viennensis                  |         |   |
|  |                             | Candidatus Nitrososphaera evergladensis    |         |   |
|  | A 194                       | Candidatus Nitrosocosmicus oleophilus      |         |   |
|  | Nitrosocosmicus             | Candidatus Nitrosocosmicus franklandus     |         |   |
|  | Nitrosocaldus               | Candidatus Nitrosocaldus cavascurensis     |         | í – – – – – – – – – – – – – – – – – – – |
|  | Cenarchaeum                 | Cenarchaeum symbiosum                      |         |   |
|  | Caldarchaeum                | Candidatus Caldarchaeum subterraneum       |         |   |
|  | Nitrosopelagicus            | Candidatus Nitrosopelagicus brevis         |         |   |
|  |                             | Candidatus Nitrosotenuis cloacae           |         |   |
|  | Nitrosotenuis               | Candidatus Nitrosotenuis sp. DW1           |         |   |
|  |                             | Candidatus Nitrosotenuis chungbukensis     |         | (                                       |
|  | Nitrosotalea                | Candidatus Nitrosotalea devanaterra        |         |   |
|  | Conexivisphaera             | Conexivisphaera calida                     |         | í – – – – – – – – – – – – – – – – – – – |
| Korarchaeota   | Korarchaeum                 | Candidatus Korarchaeum cryptofilum         |         |   |
|  |                             | Candidatus Bathvarchaeota archaeon BA1     |         |   |
| Bathyarchaeota   | unclassified Bathyarchaeota | Candidatus Bathyarchaeota archaeon BA2     |         | í – – – – – – – – – – – – – – – – – – – |
|  | Nanoarchaeum                | Nanoarchaeum equitans                      |         | í – – – – – – – – – – – – – – – – – – – |
| Nanoarchaeota  | Nanopusillus                | Candidatus Nanopusillus acidilobi          |         |   |
|  | Nanobdella                  | Nanobdella aerobiophila                    |         | í – – – – – – – – – – – – – – – – – – – |
|  | Mancarchaeum                | Candidatus Mancarchaeum acidiphilum Mia14  |         | í – – – – – – – – – – – – – – – – – – – |
| Micrarchaeota  | Fermentimicrarchaeum        | Candidatus Fermentimicrarchaeum limneticum |         |   |
|  | Micrarchaeum                | Candidatus Micrarchaeum sp. A DKE          |         |   |
| Nanohaloarchaeota  | Nanohalobium                | Candidatus Nanohalobium constans           |         |   |
|  | La Manaka anna              | Candidatus Lokiarchaeum sp. GC14 75        |         |   |
| Lokiarchaeota  | Lokiarchaeum                | Candidatus Lokiarchaeum sp. B-35           |         |   |
|  | Prometheoarchaeum           | Candidatus Prometheoarchaeum syntrophicum  |         |   |
| and the state of t |                             | Archaeon GW2011 AR10                       |         |   |
| unclassified Archaea   |                             | Archaeon GW2011 AR20                       |         |   |

The presence of a gene homolog is indicated in black.

| Strain or plasmid         | Relevant characteristic(s)  | Source or reference      |
|---------------------------|---|--------------------------|
| Strains                   |   |                          |
| Escherichia coli          |   |                          |
| DH5a                      | supE44 $\Delta$ lacU169 ( $\Phi$ 80 lacZ $\Delta$ M15) hsdR17 recA1 endA1 gyrA96 thi-1 relAl  | Stratagene (La Jolla, CA |
| BL21-CodonPlus(DE3)-RIL   | $\textit{E. coli B F}^{-}\textit{ompT}\textit{hsdS}(r_{B}^{-}m_{B}^{-})\textit{dcm}^{+}\textit{Tet}^{'}\textit{gal}\lambda(DE3)\textit{endA}\textit{Hte}(\textit{argUileY}\textit{leuW}\textit{Cam}^{'})$ | Stratagene               |
| Thermococcus kodakarensis |   |                          |
| KOD1                      | Wild-type   | (12)                     |
| KU216*                    | KOD1 <i>ApyrF</i>   | (13)                     |
| ∆TK0150-3*                | KU216 ATK0150   | This study               |
| ∆TK0150-26                | KU216 ATK0150   | This study               |
| ∆TK0186-17*               | KU216 ATK0186   | This study               |
| ∆TK0186-25                | KU216 ATK0186   | This study               |
| ∆TK0186-28                | KU216 ATK0186   | This study               |
| ΔTK0186-33                | KU216 <b>ΔTK0186</b>  | This study               |
| ΔTK0548*                  | KU216 ATK0548   | (14)                     |
| ∆TK0551-1*                | KU216 ATK0551   | This study               |
| ΔTK0551-2                 | KU216 ATK0551   | This study               |
| ∆TK0683-13*               | KU216 ATK0683   | This study               |
| ∆TK0683-15                | KU216 ATK0683   | This study               |
| ∆TK1094*                  | KU216 <b>ΔTK1094</b>  | (15)                     |
| ∆TK1734-2*                | KU216 <b>ΔTK1734</b>  | This study               |
| ∆TK1734-4                 | KU216 <b>ΔTK1734</b>  | This study               |
| ∆TK1734-5                 | KU216 <b>ΔTK1734</b>  | This study               |
| ∆TK1734-6                 | KU216 ΔTK1734   | This study               |
| ∆TK2268*                  | KU216 ATK2268   | (14)                     |
| ∆TK2301-1*                | KU216 ATK2301   | This study               |
| ∆TK2301-2                 | KU216 <b>ΔTK2301</b>  | This study               |
| ∆TK2301-3                 | KU216 <b>ΔTK2301</b>  | This study               |
| ∆TK2301-4                 | KU216 ATK2301   | This study               |
| Plasmids                  |   |                          |
| pUC118                    | Amp <sup>r</sup> general cloning vector   | Takara (Kyoto, Japan)    |
| pUD3                      | pUC118 derivative; <i>pyrF</i> marker cassette (P <sub>pyrF</sub> :: <i>pyrF</i> )  | (16)                     |
| pUDTK0150                 | pUD3 derivative; TK0150 disruption vector   | This study               |
| pUDTK0186                 | pUD3 derivative; TK0186 disruption vector   | This study               |
| pUDTK0551                 | pUD3 derivative; TK0551 disruption vector   | (17)                     |
| pUDTK0683                 | pUD3 derivative; TK0683 disruption vector   | (17)                     |
| pUDTK1734                 | pUD3 derivative; TK1734 disruption vector   | This study               |
| pUDTK2301                 | pUD3 derivative; TK2301 disruption vector   | This study               |
| pET-21a(+)                | Amp <sup>r</sup> general expression vector  | Novagen (Madison, Wis    |
| DET-TK0186                | pET-21a(+) derivative; TK0186   | This study               |
| pET-TK0551                | pET-21a(+) derivative; TK0551   | This study               |
| pET-TK0683                | pET-21a(+) derivative; TK0683   | This study               |
| pET-TK1734                | pET-21a(+) derivative; TK1734   | This study               |
| pET-TK2301                | pET-21a(+) derivative; TK2301   | This study               |

# Table S2. List of strains and plasmids.

*T. kodakarensis* mutant strains with asterisk were used for activity measurement of cell extracts or growth experiments.

| Component                            | Concentration (mg L <sup>-1</sup> ) |
|--------------------------------------|-------------------------------------|
| NaCl                                 | 16000                               |
| MgCl₂ • 6H₂O                         | 2400                                |
| MgSO₄ · 7H₂O                         | 4800                                |
| $(NH_4)_2SO_4$                       | 800                                 |
| NaHCO <sub>3</sub>                   | 160                                 |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 240                                 |
| KCI                                  | 400                                 |
| $KH_2PO_4$                           | 336                                 |
| NaBr                                 | 40                                  |
| SrCl <sub>2</sub> ·6H <sub>2</sub> O | 16                                  |
| Fe(NH <sub>4</sub> )citrate          | 8                                   |
| KI                                   | 3.32                                |
| $H_3BO_3$                            | 1.236                               |
| NiCl <sub>2</sub> ·6H <sub>2</sub> O | 2.38                                |
| Yeast extract                        | 5000                                |
| Tryptone                             | 5000                                |
| Resazurin                            | 0.8                                 |

| Component                                       | Concentration (mg L <sup>-1</sup> ) | Component                            | Concentration ( $\mu$ g L <sup>-1</sup> ) |
|---|-------------------------------------|--------------------------------------|---|
| NaCl  | 16000                               | MnSO <sub>4</sub> ·5H <sub>2</sub> O | 2500                                      |
| MgCl <sub>2</sub> ·6H <sub>2</sub> O            | 2400                                | CoCl <sub>2</sub>                    | 500                                       |
| MgSO₄·7H₂O                                      | 4800                                | ZnSO <sub>4</sub>                    | 500                                       |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 800                                 | $CuSO_4 \cdot 5H_2O$                 | 50  |
| NaHCO <sub>3</sub>                              | 160                                 | AIK(SO <sub>4</sub> ) <sub>2</sub>   | 50  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O            | 240                                 | H <sub>3</sub> BO <sub>3</sub>       | 50  |
| KCI   | 400                                 | Na₂MoO₄ · 2H₂O                       | 50  |
| KH <sub>2</sub> PO <sub>4</sub>                 | 336                                 | Nicotinic acid                       | 400                                       |
| NaBr  | 40                                  | Biotin                               | 160                                       |
| SrCl <sub>2</sub> ·6H <sub>2</sub> O            | 16                                  | Calcium pantothenate                 | 400                                       |
| Fe(NH <sub>4</sub> )citrate                     | 8                                   | Lipoic acid                          | 400                                       |
| KI  | 3.32                                | Folic acid                           | 160                                       |
| H <sub>3</sub> BO <sub>3</sub>                  | 1.236                               | p-Aminobenzoic acid                  | 400                                       |
| NiCl <sub>2</sub> •6H <sub>2</sub> O            | 2.38                                | Thiamine                             | 400                                       |
| Resazurin                                       | 0.8                                 | Riboflavin                           | 400                                       |
| ∟-Alanine                                       | 75                                  | Pyridoxine                           | 400                                       |
| ∟-Cysteine · HCl · H₂O                          | 250                                 | Cobalamin                            | 400                                       |
| ∟-Aspartic acid                                 | 50                                  |                                      |   |
| ∟-Glutamic acid                                 | 200                                 |                                      |   |
| ∟-Phenylalanine                                 | 75                                  |                                      |   |
| Glycine   | 200                                 |                                      |   |
| $\bot$ -Histidine · HCl · H <sub>2</sub> O      | 100                                 |                                      |   |
| L-Isoleucine                                    | 100                                 |                                      |   |
| ∟-Lysine • HCl                                  | 100                                 |                                      |   |
| ∟-Leucine                                       | 100                                 |                                      |   |
| ∟-Methionine                                    | 75                                  |                                      |   |
| ∟-Asparagine • H₂O                              | 100                                 |                                      |   |
| ∟-Proline                                       | 125                                 |                                      |   |
| L-Glutamine                                     | 50                                  |                                      |   |
| L-Arginine • HCl                                | 250                                 |                                      |   |
| L-Serine  | 75                                  |                                      |   |
| L-Threonine                                     | 100                                 |                                      |   |
| ∟-Valine  | 200                                 |                                      |   |
| ∟-Tryptophan                                    | 75                                  |                                      |   |
| ∟-Tyrosine                                      | 100                                 |                                      |   |

| Use                                  | Gene      | Туре | Primer name       | 5'-sequence-3'                                   |
|--------------------------------------|-----------|------|-------------------|--|
|                                      |           |      | TK0186-f          | TTTTGGATCC <u>CATATG</u> CAGGAAAAGCTTGAGAACAA    |
|                                      | TK0186    | A    | TK0186-r          | TTT <u>GAATTC</u> TCACTTGAGCTTCTTTATCTCCTCCTC    |
|                                      | THOFFI    |      | ldhA1-F           | GGG <u>CATATG</u> AGGCCGAGAGTTCTTGTGACATTTAAG    |
|                                      | TK0551    | A    | ldhA1-R           | GGG <u>GGATCC</u> TCACAGCATCTTAACTTCTTCCGGCGGGCG |
| For expression vector                | TKOOOO    |      | IdhA2-infusion-F  | GAAGGAGATATACATATGAGGCCTAAGGTTTTCATAACCCGTGCC    |
| construction                         | TK0683    | A    | ldhA2-infusion-R  | GCTCGAATTCGGATCTCAGAAGCCGGGTTTTCTAACCTTCACGAC    |
|                                      | TI(470.4  |      | TK1734-infusion-f | AAGGAGATATACATATGACGAGAAAAATCGGCATTATCTTCG       |
|                                      | TK1734    | A    | TK1734-infusion-r | GCTCGAATTCGGATCCCTAAAGGGCAGCCTCCAGATACCTT        |
|                                      | TK0004    |      | TK2301-f          | GGG <u>CATATG</u> ACAATAAAGGCAATATCTGTCG         |
|                                      | TK2301    | A    | TK2301-r          | GGG <u>GGATCC</u> TCAGGTCTTTTCTTCGGGGGAGATAT     |
|                                      |           |      | TK0186seqF1       | TACGGAATCCCAATGAGCAAG                            |
|                                      | TK0186    | в    | TK0186seqF2       | ATGAGCCTCGAGAGGAGAAAGC                           |
|                                      |           |      | TK0186seqF3       | GAAGACGGCCACCTCGACAG                             |
|                                      | TK0551    |      | TK0551seqF1       | AAGTACGCCGACGTTGATTT                             |
|                                      |           |      | TK0551seqF2       | AAGCTCATCAGGAGGGGTGA                             |
|                                      |           | в    | TK0551seqF3       | GAAAGAATTAAGCTCCTTGA                             |
|                                      |           |      | TK0551seqR1       | TTCCCTGTTCACGAGGTCTT                             |
|                                      |           |      | TK0551seqR2       | CTCGTTGATGATATGGTAGG                             |
|                                      |           |      | TK0551seqR3       | GTCGGTGTAAACGAACTTCC                             |
|                                      |           |      | TK0683seqF1       | GAGCACTTTGAGGTTGAGGT                             |
| For DNA sequencing of<br>target gene |           |      | TK0683seqF2       | GAGGCCGACCACTTCACCCG                             |
| target gene                          | TKOCOO    |      | TK0683seqF3       | ATGATAAACGAGGAAAGGCT                             |
|                                      | TK0683    | В    | TK0683seqR1       | GAGGGTCGGAGGTACTTCAC                             |
|                                      |           |      | TK0683seqR2       | GTACTGGGTTTCCTTAGTCA                             |
|                                      |           |      | TK0683seqR3       | TATCAGCCTCCTGGCCGTTG                             |
|                                      | TK1734    | В    | TK1734seqF        | CGAGAAGCTCAAGTACGGAACGCTCG                       |
|                                      | 1K1/34    |      | TK1734seqR        | CGAGCGTTCCGTACTTGAGCTTCTCGTAGGTTAAC              |
|                                      | TK2301    | В    | TK2301seqF        | AGCTGAAAAAGAGGTATCCCGAGGCTC                      |
|                                      | 11/2301   |      | TK2301seqR        | GAGTATCCACTCCTCGTCCATGTTGGTCAGGTAGA              |
|                                      | universel | 6    | T7Pmodified       | CGCGAAATTAATACGACTCACTATAGG                      |
|                                      | universal | С    | T7Tmodified       | CCAAGGGGTTATGCTAGTTATTGCT                        |

### Table S5. List of primers used for expression vector construction in this study.

A: Primers for amplifying target gene to be overexpressed in *E. coli* with appropriate restriction sites. Underlined sequences indicate the restriction sites. B: Primers annealing within the target genes.

C: Primers annealing outside of multi cloning region including NdeI, EcoRI and BamHI sites and used for sequencing target genes.

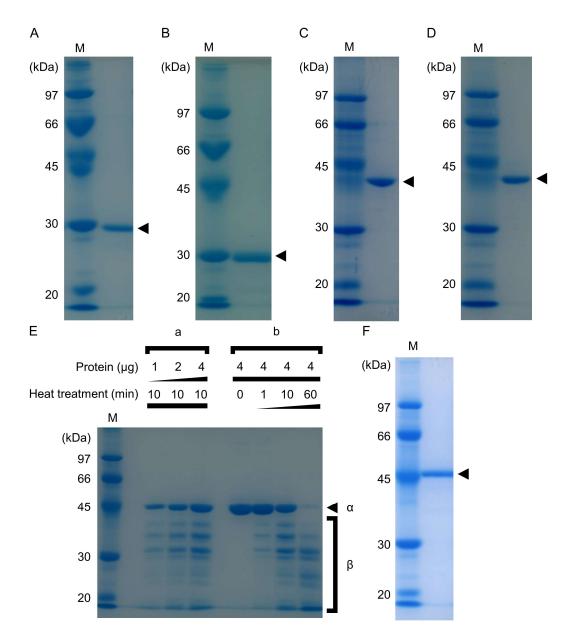
| Use   | Gene             | Туре              | Primer name   | 5'-sequence-3'  |
|---|------------------|-------------------|---|---|
|   |                  |                   | pTK0150F  | ATACACTTTGGTGAGAAAAGAAAAGTTCA   |
|   | TK0150           |                   | pTK0150R  | ACCAGCTCCGCAGTCCTCACGGCCTCAT  |
|   |                  | E                 | D1fTK0150<br>U1rTK0150  | TGGGCTTTTTCTTCTTCATTATTTCCTTAT<br>GAACATCACCGTCCATGGTTGCAACTT   |
|   |                  |                   | dTK0186F1   | GACCTCCGTTGGATCGCGCATC  |
|   |                  |                   | dTK0186R1   | GCTACTACTACCTCCCAAAGG   |
|   | TK0186           |                   | dTK0186F2   | AGTTCTACAAGCCGAGGAGG  |
| d' d' d d'  |                  | E                 | dTK0186R2   | CGACTATCATTACCTCGACCTTGC  |
| or disruption vector construction                                   |                  | D                 | dTK1734F1   | CCAGCGCCGTTATCCCGAAAC   |
|   | TK1734           | D                 | dTK1734R1   | AGCGAGAACACGGAGTGCGTTG  |
|   | 11(17)4          | E                 | dTK1734F2   | ATGGACGCAGGAGAGCTCATAAAGAAG   |
|   |                  | -                 | dTK1734R2   | GAGGGAAAAGAGGAAGAGGG  |
|   |                  |                   | dTK2301F1   | AGGGTTGAGCAGGTGAGTATC   |
|   | TK2301           |                   | dTK2301R1   | CCACAACTGGCTGATAGCG   |
|   |                  | E                 | dTK2301F2<br>dTK2301R2  | AGGGTGGTTGTCATGTTCAAG<br>TTATCTCACCCGGAAAATGAGGG  |
|   |                  |                   | TK0150sqf1  | CCTATAAAAGCTCTCTTTG   |
|   |                  |                   | TK0150sqf2  | ATCCAGAATCGTCGCCCTTG  |
|   | TK0150           | F                 | TK0150sqr1  | AATAACAGGAGGAGAGTATA  |
|   |                  |                   | TK0150sqr2  | AGGACAACTGCAAGGAGCGT  |
|   |                  |                   | dTK0186seqF1  | CGGAGGAGAAGATTAGGGAAG   |
|   |                  |                   | dTK0186seqF2  | GTGCTATTCAGCCCTGAGGGAGATC   |
|   |                  |                   | dTK0186seqF3  | GAAATTCCGAAAGCCATAAAAC  |
|   |                  |                   | dTK0186seqF4  | GCATTGAGTCCACCGACGAG  |
|   | TK0186           | F                 | dTK0186seqF5  | GATGAAGGCTTCTGAGGTTAGGG   |
|   |                  |                   | dTK0186seqR1  | CCCTAACCTCAGAAGCCTTCATC   |
|   |                  |                   | dTK0186seqR2  | TTCCAAGCGTTAATGTACAAAAAG  |
|   |                  |                   | dTK0186seqR3<br>dTK0186seqR4  | CGAGTTCTGGAAAGAGATG<br>CGACGTCATAATGACTCAC  |
| ł   |                  | + +               | SEQDIdhA1-F1  | CGACGTCATAATGACTCAC   |
|   |                  |                   | SEQDIdhA1-F2  | AACCCTGATGAGCGGCCTCG  |
|   |                  |                   | SEQIdhA1-F3   | AGGGCCGGGAAGAGAACCTC  |
|   |                  |                   | SEQIdhA1-F4   | TTTCCCGGTTTCTCCAAAAG  |
|   | TK0551           | F                 | SEQIdhA1-R1   | GGTTTGTGTTCTCGTGTTCA  |
|   |                  |                   | SEQldhA1-R2   | ATCGTCAATTCCAATGTGGA  |
| or DNA convencing of 51 and 21                                      |                  |                   | SEQldhA1-R3   | CCCCCTTGCCAGCTTCAGCA  |
| or DNA sequencing of 5'- and 3'-<br>flanking regions of target gene |                  |                   | SEQldhA1-R4   | AAGACCGTAGGGTTCGAGGA  |
| hanking regions of larger gene                                      |                  |                   | SEQIdhA2-F1   | CCCTAATGAGCTTCGGTGCG  |
|   |                  |                   | SEQIdhA2-F2   | CCTCTCGGGCCTTTGGAGCA  |
|   |                  |                   | SEQIdhA2-F3   | GGAAACCTGATACCGCTCCT  |
|   | TK0683           | F                 | SEQIdhA2-F4   | AACACAGAAAGTGAGCGCTT  |
|   |                  |                   | SEQIdhA2-R1   | ATTTCCCCTTCAATTTCACG  |
|   |                  |                   | SEQIdhA2-R2<br>SEQIdhA2-R3  | CCTCGCCGGTCAGCTTCCCT<br>GTCTCGGGTGAATCGTATGA  |
|   |                  |                   | SEQIdhA2-R3   | ATCAGTCCTTATAAAACTGA  |
|   |                  |                   | dTK1734seqF1  | AAACATGCGTACGAACTCTACAC   |
|   |                  |                   | dTK1734seqF2  | GTTTATCAATTATGACAGGGGTG   |
|   | TK1734           | F                 | dTK1734seqF3  | CGCTTCCAGTTGCCAACTCT  |
|   |                  |                   | dTK1734seqR1  | GTTTCAAGATTTACTCAAAAAC  |
|   |                  |                   | dTK1734seqR2  | AGAGTTGGCAACTGGAAGCG  |
| 1   |                  |                   | dTK2301seqF1  | CTGTAACGGTTCTCGCCATC  |
|   |                  |                   | dTK2301seqF2  | CCCTCTGGCCACGGGTATC   |
|   | TK2301           | F                 | dTK2301seqF3  | GTTTCAGGTCCGCTCTTTGGGTTCG   |
|   |                  |                   | dTK2301seqR1  | GCGTTCTTTCATACCTCAG   |
| -   |                  | +                 | dTK2301seqR2  | CGAACCCAAAGAGCGGACCTGAAAC   |
|   | universal        | G                 | M4S   | CTGGCGAAAGGGGGATGTGC<br>ACACTTTATGCTTCCGGCTC  |
|   |                  | +                 | RVS<br>dTK0150outE  | AAAOTOOAOTOAAOTAOOAOOTOAOOTT  |
|   |                  | н                 | dTK0150outF<br>dTK0150outR  |   |
|   | TK0150           |                   | dTK01500ull<br>dTK0150inF   | ATGATCGAAGTCGGGGGAATACAAGGTCA   |
|   |                  |                   | dTK0150inR  | TCAAAGGCTCCTCAGGTATTCGGCGTAGG   |
| İ   |                  |                   | dTK0186outF   | TACTCCGGTGGAGTAACGTCAAAGGC  |
|   | TICCO            | н                 | dTK0186outR   | TCCACCCTCAAGCTCAGGCCCGACTC  |
|   | TK0186           |                   | dTK0186inF  | CAGGAAGCCAGCAGGCGCT   |
|   | 1K0100           |                   | UTK010000   | 0400440004004000001   |
|   | 1K0100           |                   | dTK0186inR  | CGATGACCCTTGGAATGTGC  |
|   | 1K0100           |                   | dTK0186inR<br>CHDldhA1-F  | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACGAGCATAGGCGAAAACG  |
|   |                  | I<br>H            | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R  | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACGAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT  |
|   | TK0186           |                   | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R<br>CHIdhA1-F   | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACGAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA  |
| For PCR analysis of gene  |                  | н                 | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R<br>CHIdhA1-F<br>CHldhA1-R  | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT  |
| For PCR analysis of gene<br>disruptants                             |                  | н                 | dTK0186inR<br>CHDIdhA1-F<br>CHDIdhA1-R<br>CHIdhA1-F<br>CHIdhA1-R<br>CHIdhA1-R<br>CHIDhA2-F  | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGGGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGGCTGGA  |
|   |                  | н —               | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R<br>CHIdhA1-F<br>CHIdhA1-R<br>CHIdhA1-R<br>CHDldhA2-F<br>CHDldhA2-R   | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACGAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCCTTGGAACGCAGATAGGGGCTGGA<br>TTTGAAAGCTCCTCTCTGAGCTTTGGAAGG   |
|   | TK0551           | н —               | dTK0186inR<br>CHDIdhA1-F<br>CHDidhA1-R<br>CHIdhA1-R<br>CHIdhA1-R<br>CHIdhA1-R<br>CHDIdhA2-F<br>CHDIdhA2-R<br>CHIdhA2-F  | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGCTTGGAA<br>TTTGAAAGCTCCTCTCTGAGCTTTGGAAGG<br>AGGTTTTCATAACCCGTGCCATTCCCGAGA  |
|   | TK0551           | H —<br>I —<br>H — | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R<br>CHldhA1-R<br>CHldhA1-R<br>CHDldhA2-F<br>CHDldhA2-R<br>CHDldhA2-R<br>CHldhA2-R<br>CHldhA2-R  | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGGCTGGA<br>TTTGAAAGCTCCTCTCGAGCTTTGGAAGG<br>AGGTTTTCATAACCCGTGCCATTCCCGAGA<br>GGTTTTCTAACCTTCACGACTTCCTTATTC   |
|   | TK0551<br>TK0683 | H -               | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R<br>CHldhA1-R<br>CHldhA1-R<br>CHDldhA2-F<br>CHDldhA2-R<br>CHDldhA2-R<br>CHldhA2-R<br>CHldhA2-R<br>dTK1734outF                             | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGGTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGGA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGGCTGGA<br>TTTGAAAGCTCCTCTCTGAGCTTGGAAGG<br>AGGTTTTCTAACCTGCCATTCCCTAGA<br>GGTTTTCTAACCTTCACGACTTCCTTATTC<br>ATCCCAATCGTAGTTCCTGGCATAC   |
|   | TK0551           | H                 | dTK0186inR<br>CHDIdhA1-F<br>CHIdhA1-F<br>CHIdhA1-F<br>CHIdhA1-F<br>CHDIdhA2-F<br>CHDIdhA2-R<br>CHIdhA2-R<br>CHIdhA2-R<br>dTK1734outF<br>dTK1734outF                             | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGGGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGGCTGGA<br>TTTGAAAGCTCCTCTCTGAGCTTTGGAAGG<br>AGGTTTTCATAACCCGTGCCATTCCCGAGA<br>GGTTTTCATAACCTCACGACTTCCCGAGA<br>ACCTTCCCGTCGTACTCACGACTAC<br>AACTTTCCGTCGTACTCATCG  |
|   | TK0551<br>TK0683 | H —<br>I —<br>H — | dTK0186inR<br>CHDldhA1-F<br>CHDldhA1-R<br>CHldhA1-R<br>CHldhA1-R<br>CHDldhA2-F<br>CHDldhA2-R<br>CHDldhA2-R<br>CHldhA2-R<br>CHldhA2-R<br>dTK1734outF                             | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACCAGCATAGGCGAAAACG<br>ACTCCCGGTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGGA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGGCTGGA<br>TTTGAAAGCTCCTCTCTGAGCTTGGAAGG<br>AGGTTTTCTAACCTGCCATTCCCTAGA<br>GGTTTTCTAACCTTCACGACTTCCTTATTC<br>ATCCCAATCGTAGTTCCTGGCATAC   |
|   | TK0551<br>TK0683 | H                 | dTK0186inR<br>CHDIdhA1-F<br>CHDIdhA1-R<br>CHIdhA1-R<br>CHIdhA1-R<br>CHDIdhA2-F<br>CHDIdhA2-R<br>CHIdhA2-R<br>CHIdhA2-R<br>CHIdhA2-R<br>dTK1734outF<br>dTK1734outF<br>dTK1734inF | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACGAGCATAGGCGAAAACG<br>ACTCCCGCTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGCCTGGA<br>TTTGAAAGCTCCTCTCGAGCTTTGGAAGG<br>AGGTTTTCATAACCCGTGCCATTCCCGAGA<br>GGTTTTCTAACCTCAGACTTCCTTATTC<br>ATCCCAATCGTAGTTCCTGGCATACCATAC<br>AAGCTTCCGTGGTACTCATCG<br>ATGACGAGAAAAATCGGCATTATCTTC                    |
|   | TK0551<br>TK0683 | H                 | dTK0186inR<br>CHDIdhA1-F<br>CHDIdhA1-R<br>CHIdhA1-R<br>CHIdhA1-R<br>CHIdhA2-F<br>CHDIdhA2-R<br>CHIdhA2-R<br>CHIdhA2-R<br>dTK1734outF<br>dTK1734outR<br>dTK1734inF<br>dTK1734inR | CGATGACCCTTGGAATGTGC<br>TCGGCTATCTCACGAGCATAGGCGAAAACG<br>ACTCCCGGTCCCGGTAGGCCAGAAGCTCAT<br>GAGTTCTTGTGACATTTAAGATGAAGAGCA<br>TTCCGGCGGGCGAACCTTCACGACTTCCCT<br>ATACTCGTTGGAACGCAGATAGGGGCTGGA<br>TTTGAAGCTCCTCTCTGAGCTTTGGAAGG<br>AGGTTTTCATAACCCGTGCCATTCCCGAGA<br>GGTTTTCTAACCTCACGACTTCCTTATTC<br>ATCCCATCGTAGTACCTACCG<br>AACTTTCCGTCGTACTCATCG<br>ATGACGAGAAAAATCGGCATTATCTTC<br>CTAAAGGGCAGCCTCCAGATAC |

## Table S6. List of primers used for genetic analysis in this study.

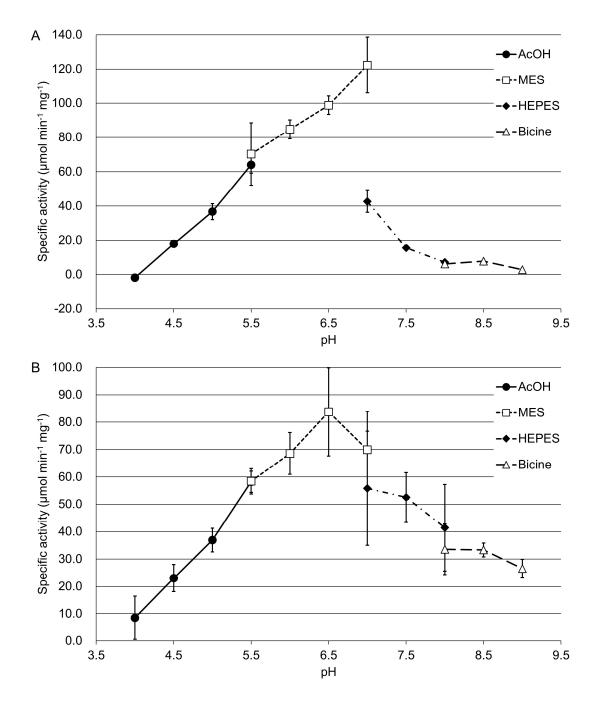
D: Primers for amplifying target genes and their 5'- and 3'-flanking regions. E: Primers for inverse PCR amplifying 5'- and 3'-flanking regions and entire plasmid to exclude target genes. F: Primers for sequencing 5'- or 3'-flanking region of target genes.

G: Primers annealing outside of multi cloning site of pUD3.

H: Primers annealing outside of homologous regions for homologous recombination. I: Primers annealing within the target genes.



**Fig. S1. SDS-PAGE analysis of the purified recombinant proteins.** (*A* to *D* and *F*) The genes TK1734 (*A*), TK2301 (*B*), TK0551 (*C*), TK0683 (*D*) and TK1094 (*F*) were expressed in *E. coli* and the recombinant proteins were purified with procedures described in the Methods section. The homogeneity of each sample was analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue. M represents molecular weight marker. (*E*) The recombinant protein of TK0186 expressed in *E. coli* was purified as described in the Methods section and analyzed by SDS-PAGE/Coomassie Brilliant Blue staining. M represents molecular weight marker. (a) Different amounts of protein were incubated at 98°C for 10 min in SDS-PAGE buffer. (b) Protein samples mixed with SDS-PAGE buffer were incubated at 98°C for various periods of time. (*α*) Main protein band corresponding to intact TK0186 protein, (β) low molecular weight proteins corresponding to fragments of the TK0186 protein.



**Fig. S2. Effect of pH on the 2-PG phosphatase activity of the TK1734 and TK2301 proteins.** The phosphatase activities of the TK1734 (*A*) and TK2301 (*B*) recombinant proteins were measured in mixtures based on various buffers with concentrations of 50 mM. Symbols: closed circles, acetate-NaOH (pH 4.0-5.5); open squares, MES-NaOH (pH 5.5-7.0); closed diamonds, HEPES-NaOH (pH 7.0-8.0); open triangles, Bicine-NaOH (pH 8.0-9.0). The data represent the average of three independent experiments and are shown with the SD values.

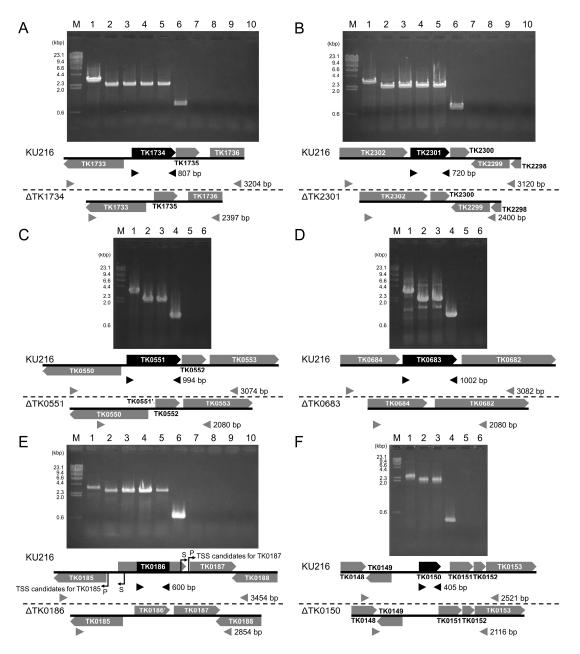


Fig. S3. PCR analysis of the gene disruption strains. The genotypes of individual gene disruption strains were analyzed by PCR using primer sets annealing outside of the 5'- and 3'homologous regions for homologous recombination (outside pair, indicated by gray arrowheads) and the 5'- and 3'-terminal regions of the coding region of the target genes (inside pair, indicated by black arrowheads). (A) Lanes 1-5: outside, lanes 6-10: inside. Lanes 1, 6: T. kodakarensis KU216, lanes 2, 7: ΔTK1734-2, lanes 3, 8: ΔTK1734-4, lanes 4, 9: ΔTK1734-5, lanes 5, 10:  $\Delta$ TK1734-6. (B) Lanes 1-5: outside, lanes 6-10: inside. Lanes 1, 6: T. kodakarensis KU216, lanes 2, 7:  $\Delta$ TK2301-1, lanes 3, 8:  $\Delta$ TK2301-2, lanes 4, 9:  $\Delta$ TK2301-3, lanes 5, 10: ∆TK2301-4. (C) Lanes 1-3: outside, lanes 4-6: inside. Lanes 1, 4: T. kodakarensis KU216, lanes 2, 5: ΔTK0551-1, lanes 3, 6: ΔTK0551-2. (D) Lanes 1-3: outside, lanes 4-6: inside. Lanes 1, 4: T. kodakarensis KU216, lanes 2, 5: ΔTK0683-13, lanes 3, 6: ΔTK0683-15. (E) Lanes 1-5: outside, lanes 6-10: inside. Lanes 1, 6: T. kodakarensis KU216, lanes 2, 7: ATK0186-17, lanes 3, 8: ΔTK0186-25, lanes 4, 9: ΔTK0186-28, lanes 5, 10: ΔTK0186-33. Arrows P and S represent primary and secondary transcription initiation sites, respectively (18). (F) Lanes 1-3: outside, lanes 4-6: inside. Lanes 1, 4: T. kodakarensis KU216, lanes 2, 5: ∆TK0150-3, lanes 3, 6:  $\Delta$ TK0150-26.  $\lambda$ DNA digested with HindIII was used as a DNA marker (M).

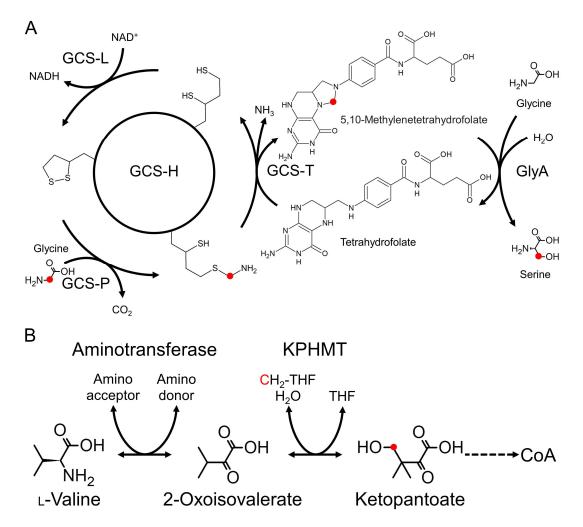


Fig. S4. C1 transfer reactions in T. kodakarensis. (A) The glycine cleavage system and C1 transfer. The glycine cleavage system is composed of four components: T-protein (aminomethyltransferase), P-protein (glycine decarboxylase), L-protein (dihydrolipoyl dehydrogenase) and H-protein, a protein modified with lipoic acid that interacts with all other components. In the system, P-protein first transfers an aminomethyl group from glycine onto the lipoic acid, which is attached to the H-protein linked with decarboxylation. Next, T-protein transfers the methylene carbon onto a tetrahydrofolate (THF) molecule, releasing ammonia and generating 5,10-methylenetetrahydrofolate (CH<sub>2</sub>-THF). The reduced lipoic acid through the reactions of P- and T-protein is re-oxidized by L-protein utilizing NAD<sup>+</sup>. The produced CH<sub>2</sub>-THF is used as a C1 carrier for various C1 transfer reactions such as the serine hydroxymethyl transferase (GlyA) reaction. (B) Metabolism linking valine and coenzyme A biosynthesis. T. kodakarensis does not harbor a de novo synthesis pathway of 2-oxoisovalerate. Therefore, coenzyme A (CoA) biosynthesis in T. kodakarensis is dependent on 2-oxoisovalerate deneration from valine. Valine is converted to 2-oxoisovalerate via transamination catalyzed by aminotransferases such as the TK0186 protein which was confirmed to display activity in this study. The produced 2-oxoisovalerate is next converted to ketopantoate by ketopantoate hydroxymethyltransferase (KPHMT). The reaction is known to utilize CH<sub>2</sub>-THF as a C1 donor. The growth measurements of ∆TK0150 suggested that the KPHMT reaction shares the same C1 carrier with the GlyA reaction in T. kodakarensis.



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Ketopantoate

L-Serine

HoNed

GlyA

КРНМТ

CH<sub>2</sub>-THF

Ketopantoate

HaN

GlyA

КРНМТ

CH2-THF



L-Threonine

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H<sub>2</sub>N

∟-Valine

∟-Threonine

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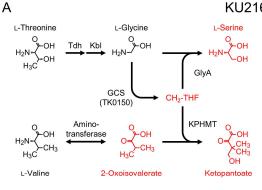
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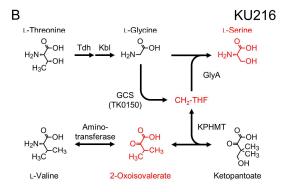
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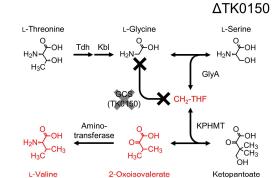


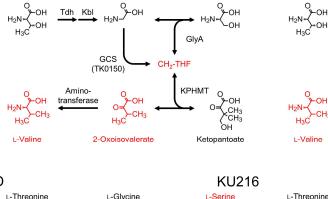


L-Glycine

С

L-Threonine





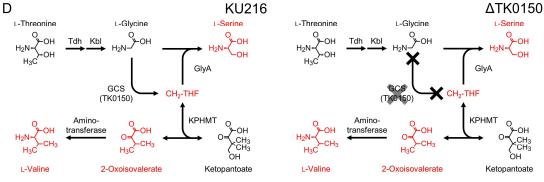


Fig. S5. Metabolic links for 5,10-methylenetetrahydrofolate generation and utilization. Compounds colored in black indicate the components present in the medium. Compounds colored in red (or gray) indicate those expected to be (or not to be) synthesized in the corresponding cells (KU216/∆TK0150). (A) ASW-AA-Sº-Ura⁺-Ser⁻ medium. (B) ASW-AA-Sº-

Ura<sup>+</sup>-Ser<sup>-</sup> medium with ketopantoate. (*C*) ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Val<sup>-</sup> medium with ketopantoate. (*D*) ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Ser<sup>-</sup>-Val<sup>-</sup> medium with ketopantoate.

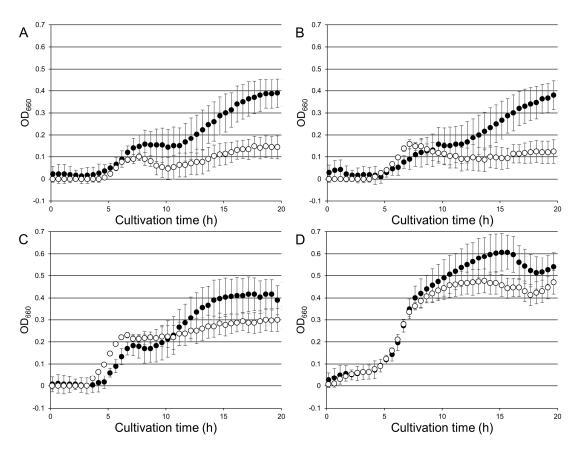
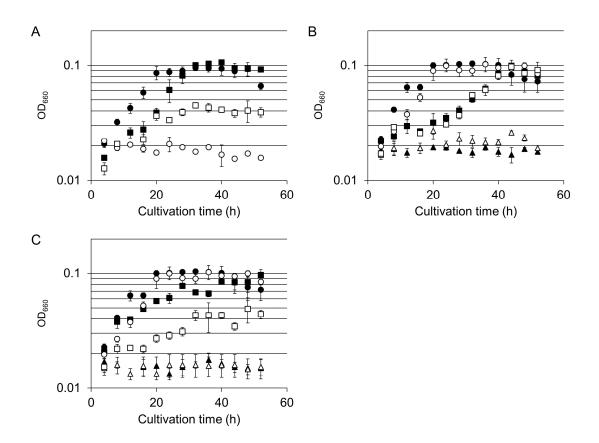


Fig. S6. Growth properties of *T. kodakarensis* KU216 and  $\Delta$ TK1734 strains under microaerobic conditions. KU216 (closed circles) and  $\Delta$ TK1734 (open circles) strains were cultivated in 10 mL ASW-YT-m1-Pyr medium in 20-mL vials supplemented with 25 g L<sup>-1</sup> uridine. All media were left in an anaerobic box for 24 h prior to inoculation to allow dissolved gases to equilibrate with the atmosphere in the anaerobic box (oxygen concentration: ~0.1%). Media for *A* were prepared without Na<sub>2</sub>S (microaerobic conditions), and the concentration of Na<sub>2</sub>S in *B*, *C* and *D* were 0.08 mM, 0.16 mM and 0.32 mM, respectively. Error bars indicate the SD values of three independent culture experiments.



**Fig. S7. Growth properties of** *T. kodakarensis* **KU216** and  $\Delta$ **TK0150** strains. (*A*) Effects of ketopantoate on serine auxotrophy were examined by cultivating cells in ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Ser-medium with or without 2 mM ketopantoate. Symbols: KU216 with (closed squares) or without ketopantoate (closed circles),  $\Delta$ TK0150 with (open squares) or without ketopantoate (open circles). (*B*) Effects of ketopantoate on valine auxotrophy was examined by cultivating cells in ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Val<sup>-</sup> medium with or without 2 mM ketopantoate. Symbols: KU216 with valine (closed circles), without valine and with (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with valine (open circles), without valine and with (open squares) or without (open triangles) ketopantoate. (*C*) Effects of ketopantoate in ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Ser-Val<sup>-</sup> medium with or without 2 mM ketopantoate in ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Ser-Val<sup>-</sup> medium with or without 2 mM ketopantoate in ASW-AA-S<sup>0</sup>-Ura<sup>+</sup>-Ser-Val<sup>-</sup> medium with or without 2 mM ketopantoate. Symbols: KU216 with serine and valine (closed circles), without serine and valine, and with (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with serine and valine (open circles), without serine and valine (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with serine and valine (open circles), without serine and valine (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with serine and valine (open circles), without serine and valine (be no circles), without serine and valine, and with (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with serine and valine (open circles), without serine and valine, and with (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with serine and valine (open circles), without serine and valine, and with (closed squares) or without (closed triangles) ketopantoate,  $\Delta$ TK0150 with serine and valine) (open circles), without serine and valine, and with (open squ

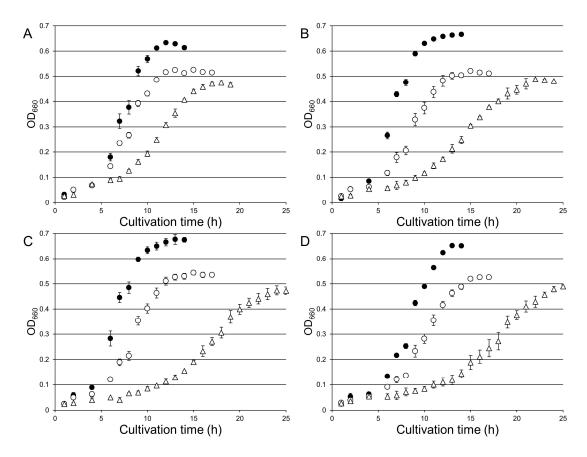
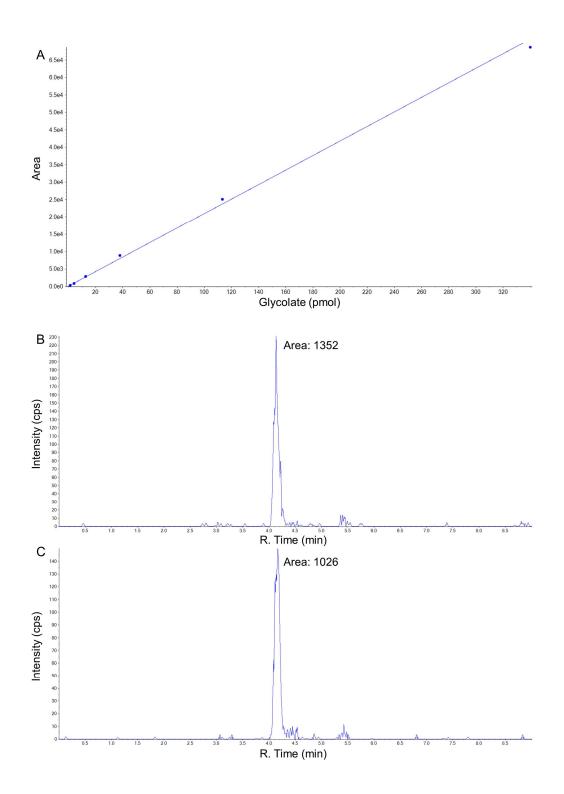


Fig. S8. Growth properties of *T. kodakarensis* KU216,  $\Delta$ TK0683 and  $\Delta$ TK0150 strains under microaerobic conditions. KU216 (closed circles),  $\Delta$ TK0683 (open circles) and  $\Delta$ TK0150 (open triangles) strains were cultivated in 10 mL ASW-YT-m1-Pyr medium in 30-mL test tubes. All media were left in an anaerobic box for 24 h prior to inoculation to allow dissolved gases to equilibrate with the atmosphere in the anaerobic box (oxygen concentration: ~0.1%). Media for *A* was prepared without Na<sub>2</sub>S (microaerobic conditions), and the concentration of Na<sub>2</sub>S in *B*, *C* and *D* were 0.08 mM, 0.16 mM and 0.32 mM, respectively. Error bars indicate the SD values of three independent culture experiments.



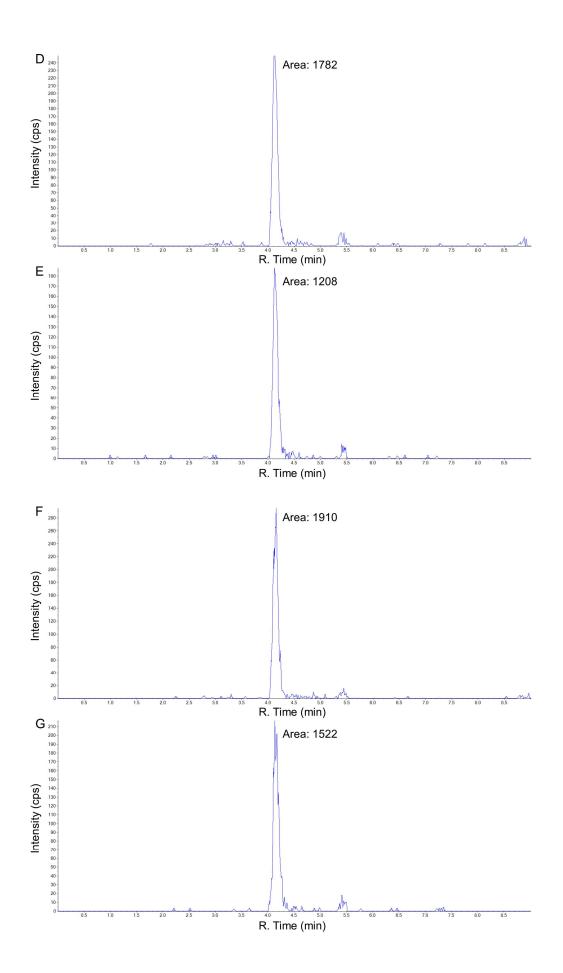


Fig. S9. Detection of glycolate in the culture supernatant after growth of *T. kodakarensis* KU216 under microaerobic conditions. The concentration of glycolate in the medium was examined by LC–MS/MS as described in the Methods section included in *SI Appendix. A* Calibration curve of amount of glycolate with peak area. Three sets of experiments were performed (*B/C*), (*D/E*) and (*F/G*). Each set represents data from medium with (*B*, *D*, *F*) or without (*C*, *E*, *G*) cell inoculation. Media in each set, including that used for pre-culture, were prepared together. Based on peak area, concentrations of glycolate deriving from *T. kodakarensis* were calculated as follows: B (24.414  $\mu$ M) – C (18.163  $\mu$ M) = 6.251  $\mu$ M, D (32.652  $\mu$ M) – E (21.656  $\mu$ M) = 10.996  $\mu$ M, F (35.112  $\mu$ M) – G (27.677  $\mu$ M) = 7.435  $\mu$ M.

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