Genome Sequence of *Silicibacter pomeroyi* Reveals Unique Adaptations to the Marine Environment

Supplementary Information

Supplementary Figure S1.

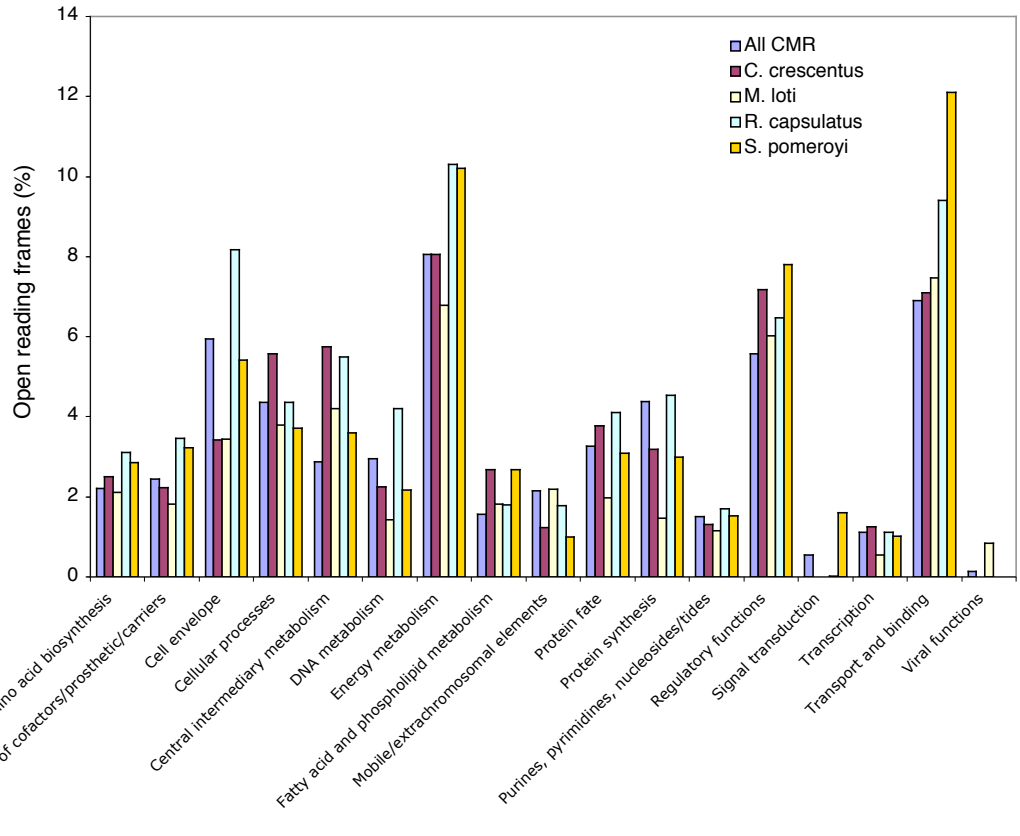

Figure S1. Percentage of *S. pomeroyi* open reading frames (ORFs; 4283 total) in biological roles compared with α-Proteobacteria *Caulobacter crescentus* (3767 ORFs), *Mesorhizobium loti* (7281 ORFs) and *Rhodobacter capsulatus* (3616 ORFs) and with a composite of all genes in the TIGR Comprehensive Microbial Database (CMR).

Supplementary Figure S2.
Demonstration of CO oxidation by *S. pomeroyi* was carried out in triplicate cultures with an initial headspace concentration of approximately 10 ppm CO. CO decreased in an exponential fashion to 0.13 ± 0.05 ppm (equivalent to 0.11 nM equilibrium dissolved CO concentration) after 50 h of incubation.

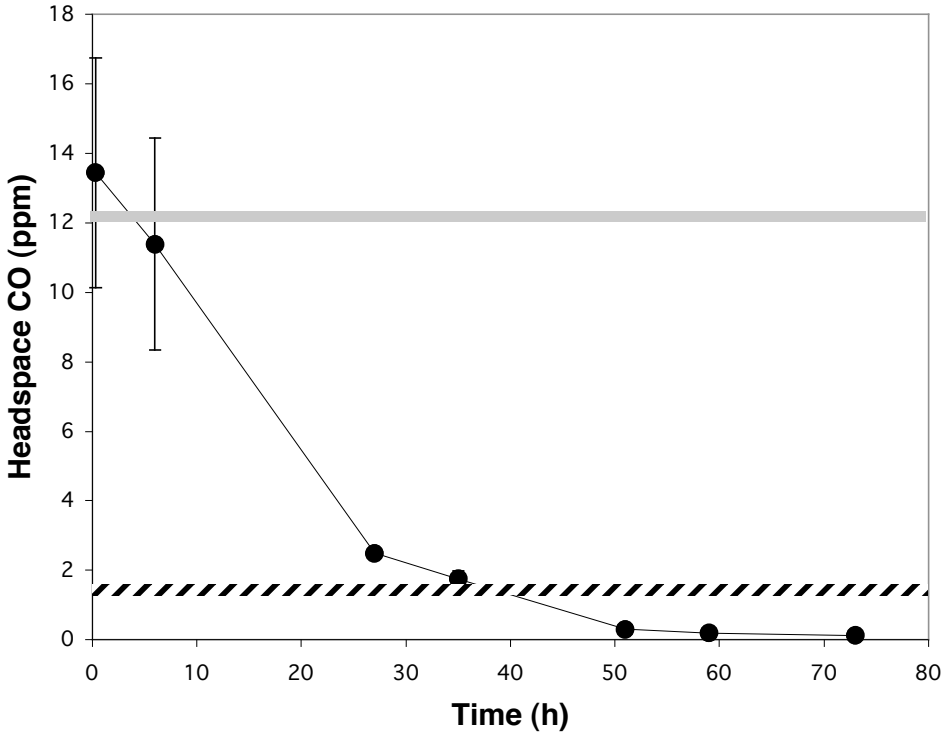

Figure S2. Consumption of CO in the headspace of triplicate cultures of *S. pomeroyi* (mean, ± 1 S.E.). Headspace CO concentrations equivalent to equilibrium dissolved CO concentrations typical of sunlit coastal ocean surface waters are indicated by the gray line (Jones and Amador 1993) and of open ocean surface waters (area-weighted for the Atlantic and Pacific[9]) by the diagonally striped line.

The ecological relevance of CO-based lithoheterotrophy is two-fold: as a mechanism for controlling atmospheric CO concentrations, and as an energy source for the marine microbial food web. Marine bacterioplankton are the dominant sink for CO in the surface ocean, consuming >75% of the CO that is produced photochemically from DOM[9]. Little is known about the taxa that consume CO, but information from the *S. pomeroyi* genome suggests that lithoheterotrophs (including some in the Roseobacter clade) may be responsible. CO lithoheterotrophy could account for only a small fraction of total bacterial respiration in the open ocean [<0.5% assuming net primary productivity = 50 x 10^{15} g C y^{-1} (Hedges 1992), bacterial respiration = 60% of primary productivity (Cole et al. 1988), blue water CO formation = 50 x 10^{12} g C y^{-1} (ref 9), and bacterial uptake of 80% of the CO]. However, CO lithoheterotophy could play more important roles in some environments, such as coastal surface waters fully exposed to sunlight, where CO lithotrophy could account for ~8% of bacterial respiration [assuming full sunlight production of 0.8 μM CO-C d^{-1} (ref 21) and coastal bacterial respiration = 10 μM C d^{-1} (Pomeroy et al. 1994)].

Supplementary Figure S 3.
S. pomeroyi cultures were inoculated into medium containing three different concentrations of acetate (2, 5, and 10 mM initial concentration), with and without the addition of 10 mM thiosulfate. After 50 h of growth, cultures with supplements of 10 mM thiosulfate had reached optical densities that were 43% (2 mM), 52% (5 mM), and 52% (10 mM) higher than those with equivalent acetate but without a thiosulfate supplement (Figure S3).

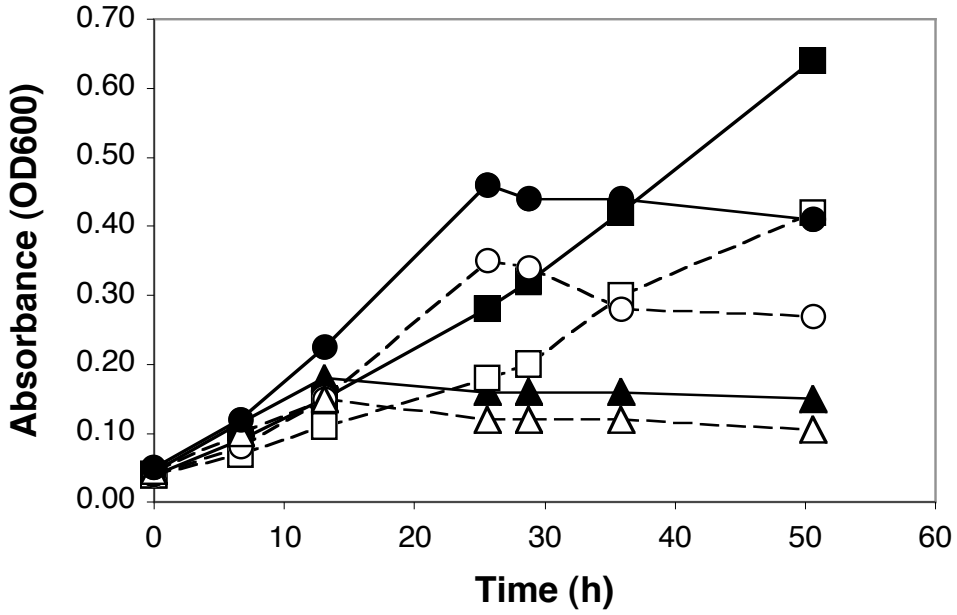

Figure S3. Growth of *S. pomeroyi* cultures in acetate medium with (closed symbols) or without (open symbols) 10 mM thiosulfate. Acetate was added at 2 mM (▲, △), 5 mM (●, ○), or 10 mM (■ , □) final concentration.

Supplementary Figure S4.
The two *luxI*-type homologs from *S. pomeroyi* (SilI1 and SilI2) were individually expressed in *Escherichia coli*. Each ORF produced a distinct set of AHL-type activities (Figure S4).

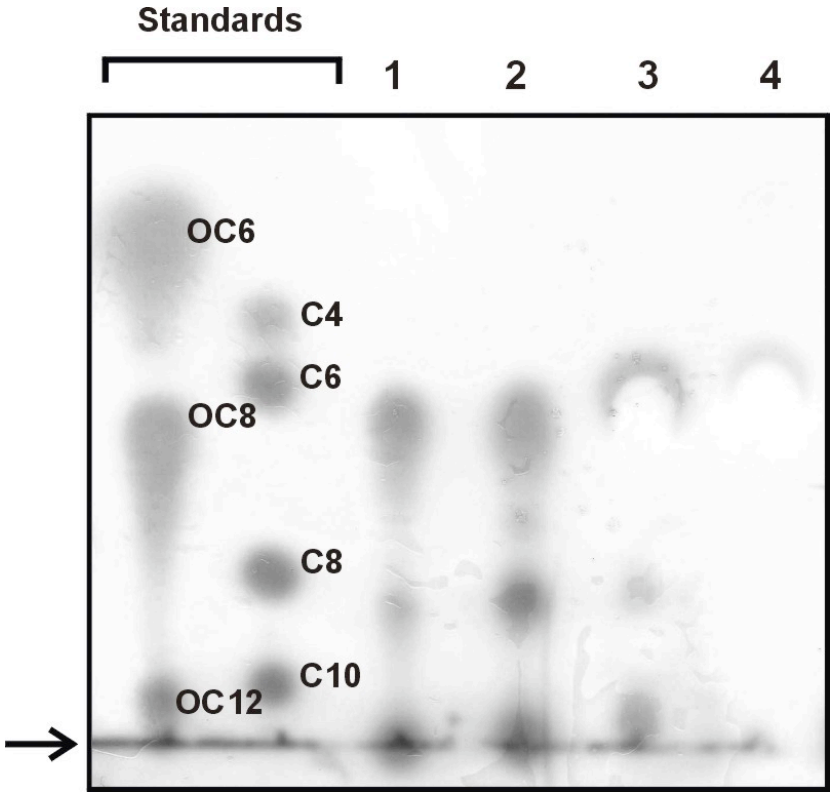

Figure S4. Production of AHL-type activities directed by *S. pomeroyi* DSS-3 LuxI-type gene expression. C18 reverse phase TLC plate fractionation (60% methanol mobile phase) of dichloromoethane extracts of *S. pomeroyi* DSS-3 (lane 1), *Escherichia coli* TOP10 (P_{Lac}::*silI1*) (lane 2), *E. coli* XL1-Blue (P_{Lac}::*silI2*) (lane 3) and *E. coli* XL1-Blue (pCR2.1-TOPO) (lane 4). Post-fractionation TLC plate overlaid with 0.6% ATGN minimal salts agar with X-Gal (40 μg/ml) and the ultrasensitive AHL reporter strain *A. tumefaciens* KYC55 (pJZ372)(pJZ384)(pJZ410). Standards marked OC6, OC8, and OC12 are 3-oxo-AHLs, and those marked C4, C6, C8, and C10 are fully reduced AHLs. Arrow marks point of application. Weak crescent-shaped activity in *E. coli* (pCR2.1-TOPO) vector control is likely to be a cyclic dipeptide(s) derived from LB broth.

Supplementary Figure S5.
Cultures of acetate-grown *S. pomeroyi* with various nitrogen sources indicated that the bacterium utilizes ammonium and urea for growth, but not nitrate or nitrite (Figure S5).

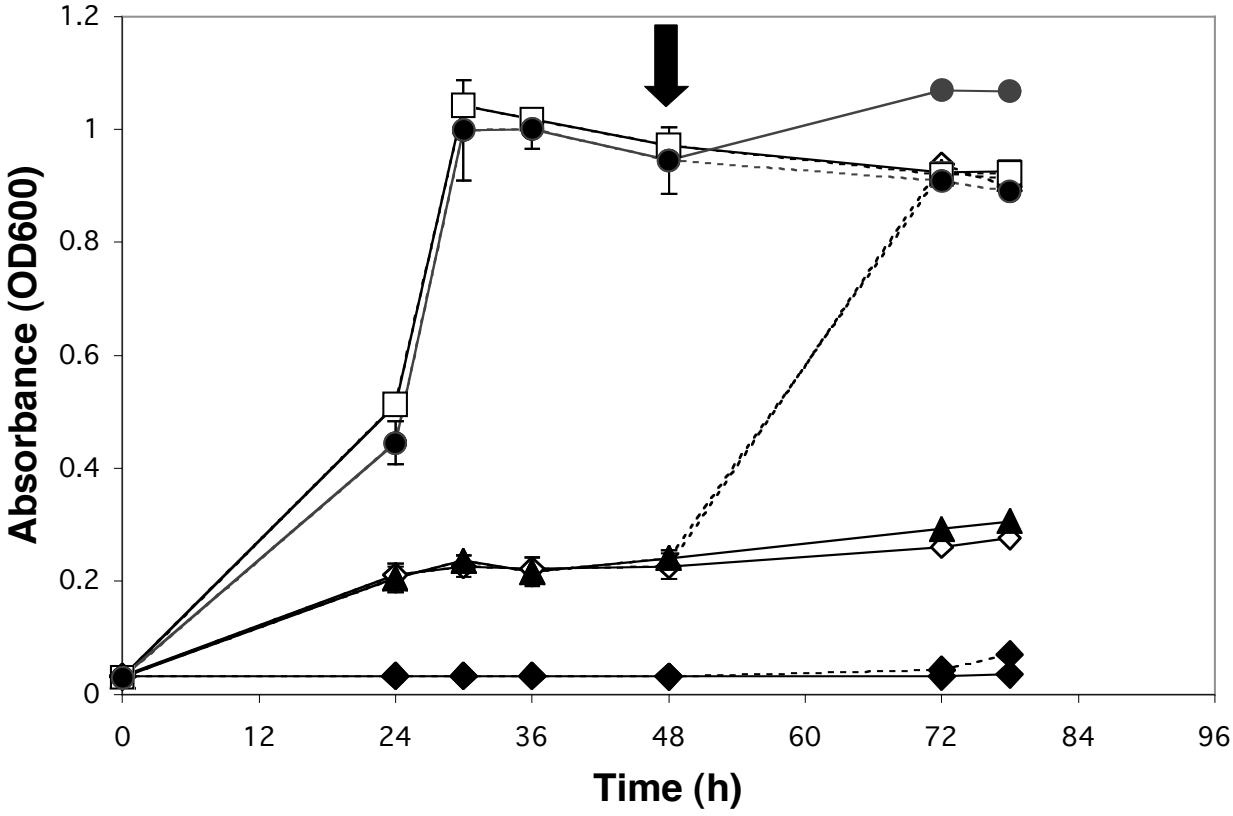

Figure S5. Growth of triplicate cultures of *S. pomeroyi* (mean ± 1 S.D.) in acetate medium with ammonium (□), urea (●), nitrate (▲), or nitrite (◆) supplied at 10 mM concentration or with no nitrogen addition (◇). Growth observed in the no nitrogen control is due to carryover from the rich medium used for cell preparation. After 48 h (black arrow), 10 mM ammonium was added to one replicate of each treatment (dotted line).

Supplementary Figure S6.
Identification of putative CO oxidation genes in the Sargasso Sea environmental shotgun library was carried out by local BLAST analysis of trace files downloaded from NCBI (http://www.ncbi.nlm.nih.gov/Traces/trace.fcgi?) using the *coxL* sequences from *S. pomeroyi* as the query sequences and a cut-off for E of 10^{-95}. Putative CoxL proteins were aligned with authentic CoxL from CO-oxidizing isolates, and those clustering with the authentic proteins were used for calculations in Table 2. Two trees spanning different regions of the CoxL protein were constructed to accommodate the partial sequences retrieved from the Sargasso Sea library (Figure S6A and S6B). Of the 91 sequences with BLAST scores $<10^{-95}$, 49 clustered with authentic CoxL of the "BMS" type, 28 clustered with CoxL of the "OMP" type[11], and 14 were considered non-CoxL proteins.

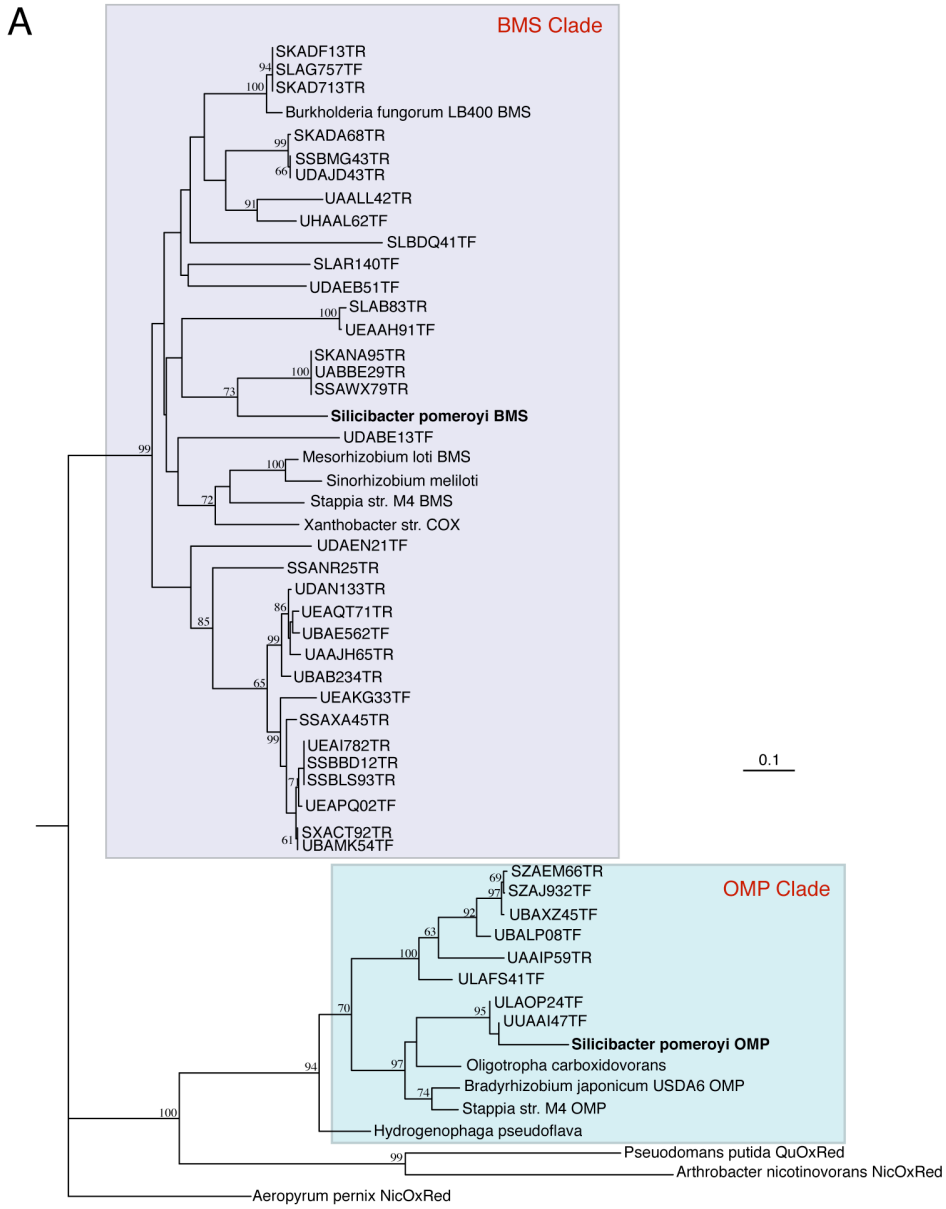

Figure S6A. Phylogenetic tree of CoxL sequences based on 125 positions aligning to residues 268 through 388 of *Sinorhizobium meliloti* (NP387097). Sequences from selected CO-oxidizing isolates and putative CoxL sequences from the Sargasso Sea environmental shotgun library are included, along with non-CoxL molybdenum hydroxylases from *Aeropyrum pernix* (nicotine dehydrogenase, NP148464), *Arthrobacter nicotinovorans* (nicotine dehydrogenase, AAK;6423) and *Pseudomonas putida* (quinoline 2-oxidoreductasem CAA66830). Alignments were carried out using the PILEUP program of Wisconsin Package v 10.2 (Accelrys, San Diego, CA). Trees were constructed with the PHYLIP package using evolutionary distances (Kimura) and the Neighbor-Joining Method. Bar represents Kimura distance and bootstrap values >60 (out of 100 runs) are provided at branch nodes. *Aeropyrum pernix* nicotine dehydrogenase served as the outgroup. Two clades of CoxL sequences have been previously identified[11], designated BMS and OMP.

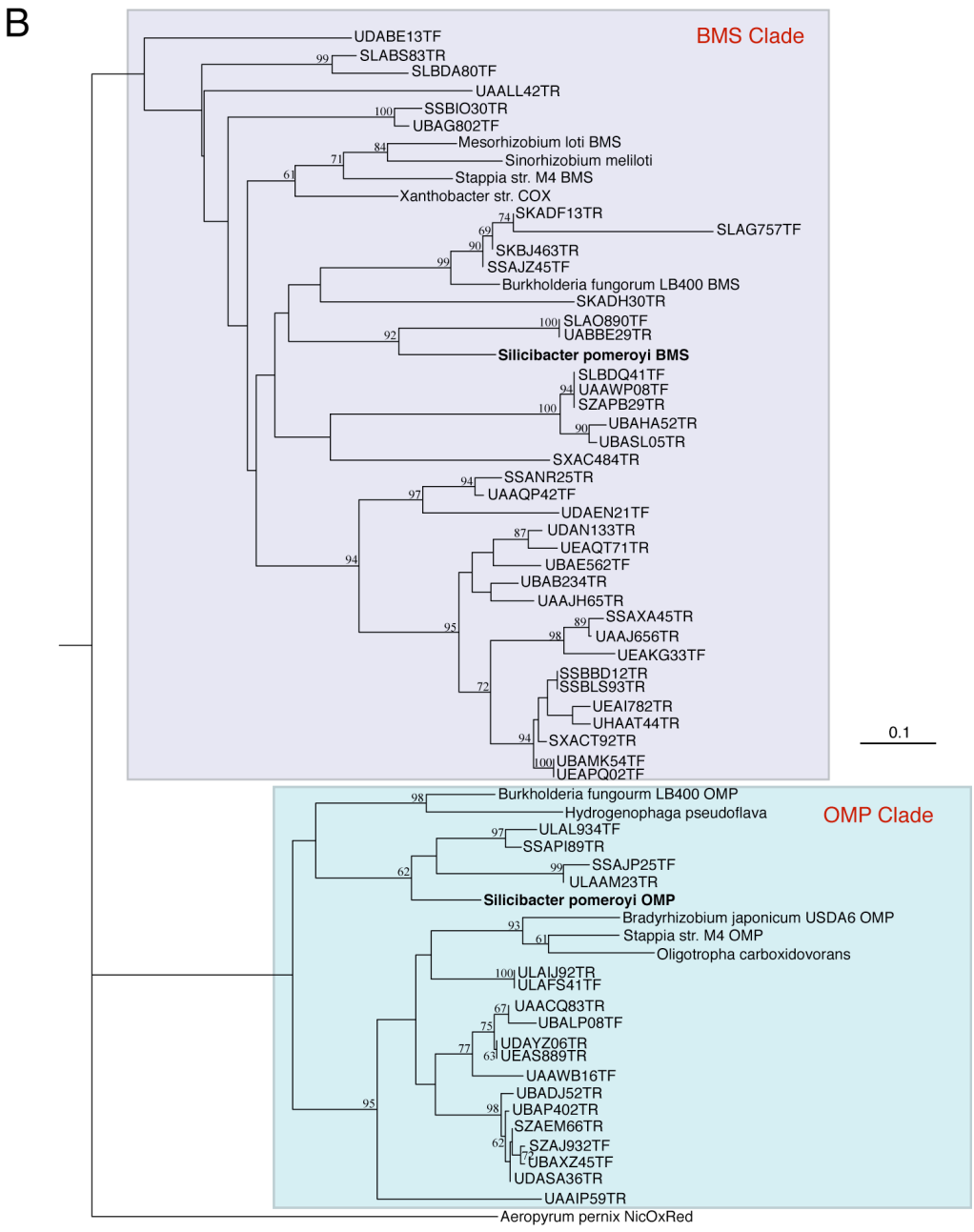

Figure S6B. Phylogenetic tree of CoxL sequences based on 100 positions aligning to residues 385 through 472 of *Sinorhizobium meliloti* (NP387097). Sequences from selected CO-oxidizing isolates and putative CoxL sequences from the Sargasso Sea environmental shotgun library are included, along with non-CoxL molybdenum hydroxylase from *Aeropyrum pernix* (nicotine dehydrogenase, NP148464). Alignments were carried out using the PILEUP program of Wisconsin Package v 10.2 (Accelrys, San Diego, CA). Trees were constructed with the PHYLIP package using evolutionary distances (Kimura) and the Neighbor-Joining Method. Bar represents Kimura distance and bootstrap values >60 (out of 100 runs) are provided at branch nodes. *Aeropyrum pernix* nicotine dehydrogenase served as the outgroup. Two clades of CoxL sequences have been previously identified[11], designated BMS and OMP.

Additional References:

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Hedges, J. I. Global biogeochemical cycles: Progress and problems. Mar. Chem. **39**, 67-93 (1992).

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