Supplemental Figures

PYRUVATE FORMATE-LYASE AND A NOVEL ROUTE OF EUKARYOTIC ATP-SYNTHESIS IN *CHLAMYDOMONAS* MITOCHONDRIA

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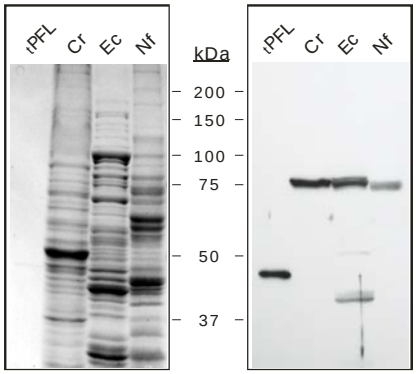
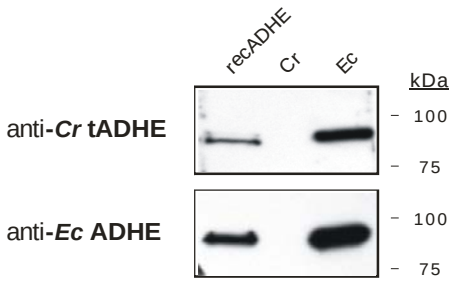


Supplemental Figure I- Proteins were separated on a 10% (w/v) acrylamide SDS-PAGE, and either stained with Coomassie brilliant blue (CBB) or transferred to nitrocellulose for further immunodetection (anti-PFL). tPFL, purified truncated PFL, used for antibody production (1 ng). Cr, exponentially grown *C. reinhardtii* cells, transferred to darkness for one day (40 μg); Ec, anaerobically grown *E. coli* cells (40 μg); Nf, anaerobically-grown *N. frontalis* cells (40 μg).

Truncated *C. reinhardtii* PFL protein (tPFL; Leu236-Val677 of the precursor protein) was expressed in *E. coli* and used for antibody production. The specificity of polyclonal anti-PFL antiserum was tested by protein-blot analysis using protein extracts from *C. reinhardtii*, *E. coli* and *N. frontalis*. Anti-PFL antiserum recognized the 45 kDa tPFL used for immunization (tPFL). In anaerobically-grown *E. coli* cells, the antiserum detected two protein bands of related apparent mass (*Ec*), which correspond to the mature PFL protein and to its C-terminal cleaved product - a consequence of oxygenolytic cleavage of activated enzyme. In *C. reinhardtii* cells growing on TAP medium and transferred to darkness for one day, anti-PFL antiserum recognized a protein of ~78 kDa (*Cr*). The antiserum also recognized a protein of ~76 kDa in cell extract from the amitochondriate protist *N. frontalis* (*Nf*). These data indicate a broad specificity of the antibody.



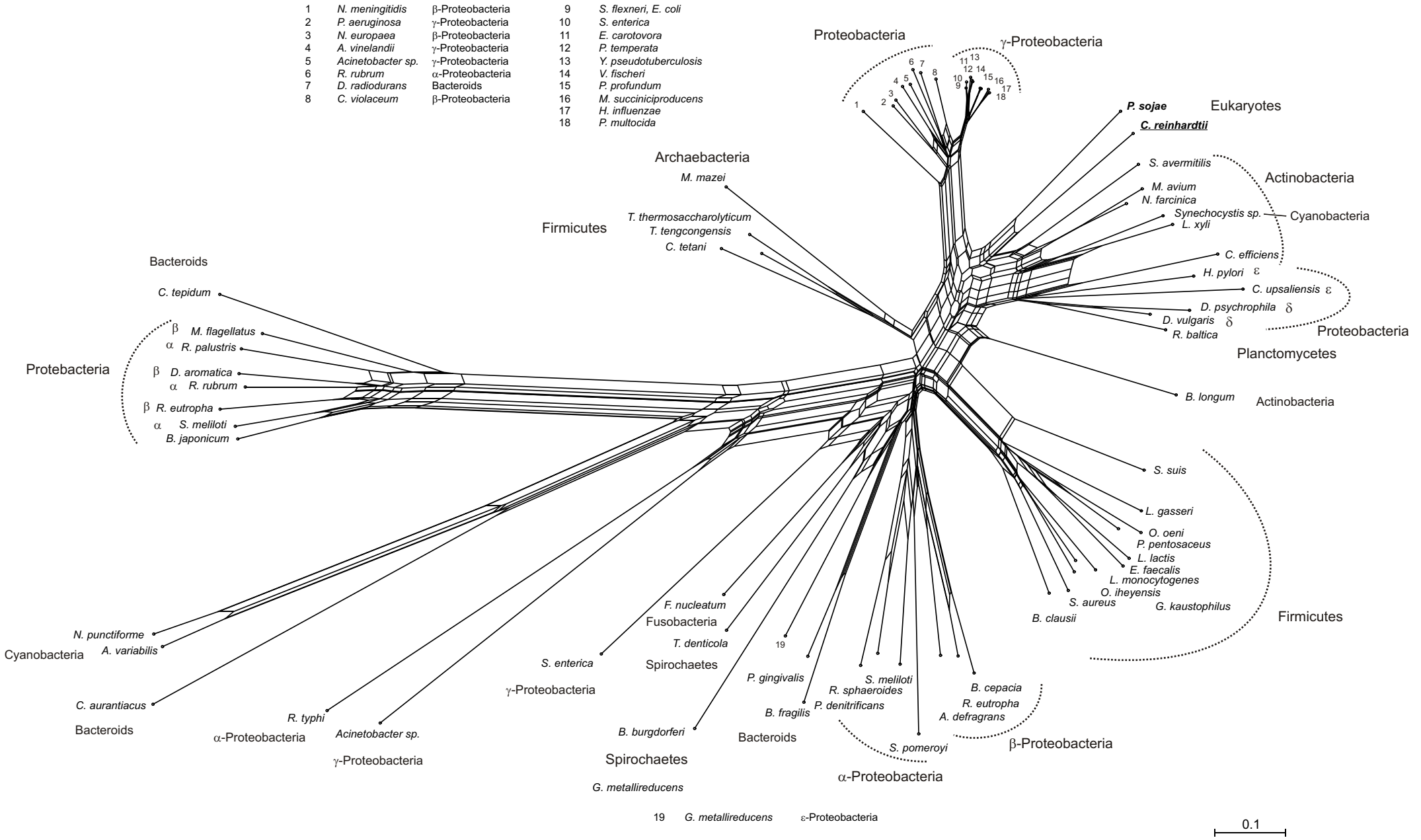
Supplemental Figure II. Proteins were separated on a 10% (w/v) acrylamide SDS-PAGE and transferred to nitrocellulose for further immunodetection. Immunoblot analysis with antisera against *C. reinhardtii* ADHE (anti-*Cr* tADHE) (dilution of 1:1000) and *E. coli* ADHE (anti-*Ec* ADHE) (Courtesy of Dr. J. Ros, Lleida, Spain; dilution of 1:1000). recADHE, recombinant predicted mature ADHE purified on Ni-NTA column (1 ng); *Cr*, exponentially grown *C. reinhardtii* cells, transferred to darkness for one day (40 μg); *Ec*, anaerobically grown *E. coli* cells (40 μg).

Our anti-ADHE antiserum recognized the truncated 45-kDa ADHE (tADHE; Val354-Pro703 in the precursor protein) used for immunization (not shown) and overexpressed *C. reinhardtii* ADHE (recADHE; Ala62-Asn953). Although the antiserum recognized *E. coli* ADHE, no signal was detected in *C. reinhardtii* cells maintained in the dark, in aerated conditions for one day. Similarly, an antiserum against *E. coli* ADHE recognized recADHE but did not recognize any protein in *C. reinhardtii* cell extract.

Attempts to detect ADHE in the two sets of cells analyzed for PFL steady-levels (see Figure 4 of the manuscript) using either anti-*C. reinhardtii* ADHE or anti-*E. coli* ADHE antisera were unsuccessful (not shown), indicating very low steady-state levels of ADHE under these conditions.

ADHE expression and antibody production

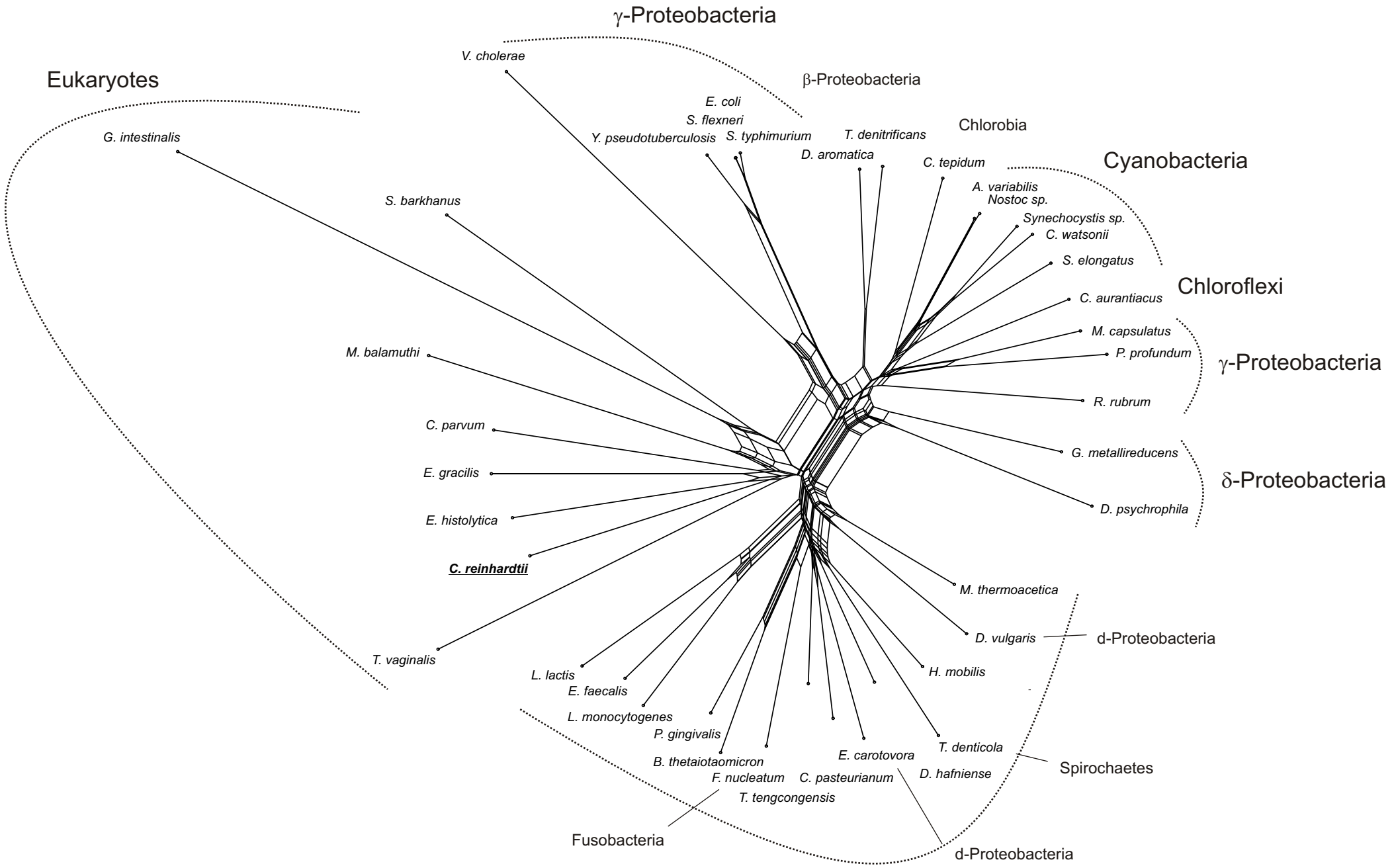
The DNA sequence encoding putative *C. reinhardtii* ADHE mature protein (recADHE) (Ala62-Asn953 in the precursor protein) was amplified by PCR with primers containing the *Bam*HI and *Hind*III restriction sites at their 5' and 3' terminus, respectively. The primers used were: 5'-GACGGATCCGCCACCCCCCATGCTGAG GTG-3', and 5'-GTCAAGCTTGTTGATCTTGGAGAAGAACTC-3'. A part of *C. reinhardtii* ADHE cDNA encoding residues Val254-Pro703 (tADHE) was amplified by PCR using the following primers: 5'-GACGGATCCGTGTCCCAGGCGCTGATGCAG-3', and 5'-GTCAAGCTTGGGGGTCAGGGCGTAATCG GC-3'. PCR products were cloned into pGEM-T Easy (Promega) and recloned into the *Bam*HI/*Hind*III sites of the overexpression vector pQE30 (Qiagen). The constructs were introduced into *E. coli* XL1 Blue MRF' to produce the recombinant proteins. His-tagged proteins were purified under denaturing conditions by affinity column chromatography using Ni-NTA matrix (Qiagen), as recommended by the supplier. Antibodies against tADHE were produced at Eurogentec (Leuven, Belgium).



Supplemental Figure III

Supplemental Figure III. Neighbor-Net planar graph of phosphotransacetylase (PTA) sequences

Sources of sequences are as given. *Phytophthora sojae* (Contig 4 in Scaffold 8 reverse complement of nucleotides 288672-291088), *Acinetobacter sp.* (YP_045288), *Alcaligenes defragrans* (AAN08490), *Anabaena variabilis* (ZP_00158828), *Azotobacter vinelandii* (ZP_00091995), *Bacillus clausii* (YP_177402), *Bacteroides fragilis* (YP_097761), *Bifidobacterium longum* (NP_696142), *Borrelia burgdorferi* (NP_212723), *Bradyrhizobium japonicum* (NP_770097), *Burkholderia cepacia* (ZP_00213733), *Campylobacter upsaliensis* (EAL53303), *Chlorobium tepidum* (NP_661976), *Chloroflexus aurantiacus* (ZP_00357389), *Chromobacterium violaceum* (AAQ59205), *Clostridium tetani* (NP_781870), *Corynebacterium efficiens* (NP_739201), *Dechloromonas aromatica* (ZP_00150564), *Deinococcus radiodurans* (AAF09663), *Desulfotalea psychrophila* (YP_064294), *Desulfovibrio vulgaris* (YP_012240), *Enterococcus faecalis* (NP_814687), *Erwinia carotovora* (YP_051130), *Escherichia coli* (S50130), *Fusobacterium nucleatum* (ZP_00143396), *Geobacillus kaustophilus* (YP_149268), *Geobacter metallireducens* (ZP_00299463), *Haemophilus influenzae* (NP_439359), *Helicobacter pylori* (NP_223559), *Acinetobacter sp.* (YP_046896), *Lactobacillus gasseri* (ZP_00046191), *Lactococcus lactis* (ZP_00383172), *Leifsonia xyli* (YP_061462), *Listeria monocytogenes* (NP_465627), *Mannheimia succiniciproducens* (YP_088190), *Methanosarcina mazei* (NP_632520), *Methylobacillus flagellatus* (ZP_00350388), *Mycobacterium avium* (AAR92165), *Neisseria meningitidis* (CAB84122), *Nitrosomonas europaea* (NP_840385), *Nocardia farcinica* (YP_121562), *Nostoc punctiforme* (ZP_00107450), *Oceanobacillus iheyensis (*NP_693944), *Oenococcus oeni (*ZP_00319267), *Paracoccus denitrificans* (AAS78789), *Pasteurella multocida* (NP_245642), *Pediococcus pentosaceus* (ZP_00323036), *Photobacterium profundum* (YP_130973), *Photorhabdus temperata* (AAN08360), *Porphyromonas gingivalis* (AAQ66196), *Pseudomonas aeruginosa* (NP_249526), *Ralstonia eutropha* (ZP_00166541), *Ralstonia eutropha* (ZP_00165579), *Rhodobacter sphaeroides* (ZP_00006686), *Rhodopirellula baltica* (NP_869002), *Rhodopseudomonas palustris* (CAE30007), *Rhodospirillum rubrum* (AAN75024), *Rhodospirillum rubrum* (ZP_00269282), *Rickettsia typhi* (YP_067322), *Salmonella enterica* (YP_149840), *Salmonella enterica* (YP_217449), *Shigella flexneri* (NP_708179), *Silicibacter pomeroyi* (AAV96785), *Sinorhizobium meliloti* (NP_437512), *Sinorhizobium meliloti* (Q9X448), *Staphylococcus aureus* (YP_040042), *Streptococcus suis* (ZP_00333252), *Streptomyces avermitilis* (BAC70534), *Synechocystis sp.* (NP_441027), *Thermoanaerobacter tengcongensis* (NP_623097), *Thermoanaerobacterium thermosaccharolyticum* (CAA06174), *Treponema denticola* (NP_970659), *Vibrio fischeri* (YP_204219), *Yersinia pseudotuberculosis* (YP_071108).



Supplemental Figure IV

Supplemental *Figure IV. Neighbor-Net planar graph of pyruvate: ferredoxin oxidoreductase (PFO) sequences*

Sources of sequences are as given. *Anabaena variabilis* (ZP_00161270) *Bacteroides thetaiotaomicron* (NP_810660) *Chlorobium tepidum* (NP_662511) *Chloroflexus aurantiacus* (ZP_00356572) *Clostridium pasteurianum* (CAB43935) *Crocosphaera watsonii* (ZP_00175454) *Cryptosporidium parvum* (EAK87662) *Dechloromonas aromatica* (ZP_00348794) *Desulfitobacterium hafniense* (ZP_00098862) *Desulfotalea psychrophila* (YP_066622) *Desulfovibrio vulgaris* (YP_012236) *Entamoeba histolytica* (EAL51636) *Enterococcus faecalis* (NP_816200) *Erwinia carotovora* (YP_051048) *Escherichia coli* (AAG56382) *Euglena gracilis* (CAC37628) *Fusobacterium nucleatum* (ZP_00143398) *Geobacter metallireducens* (ZP_00301732) *Giardia intestinalis* (AAA74894) *Heliobacillus mobilis* (AAN87538) *Lactococcus lactis* (NP_266578) *Listeria monocytogenes* (ZP_00232449) *Mastigamoeba balamuthi* (AAM53401) *Methylococcus capsulatus* (AAU92952) *Moorella thermoacetica* (ZP_00329821) *Nostoc sp.* (BAB74502) *Novosphingobium aromaticivorans* (ZP_00303726) *Photobacterium profundum* (YP_130193) *Porphyromonas gingivalis* (AAQ65740) *Rhodopseudomonas palustris* (NP_950055) *Rhodospirillum rubrum* (ZP_00270012) *Salmonella typhimurium* (AAL20569) *Shigella flexneri* (NP_707680) *Spironucleus barkhanus* (AAD55754) *Synechococcus elongatus* (ZP_00165363) *Synechocystis sp.* (NP_442703) *Thermoanaerobacter tengcongensis* (NP_622125) *Thiobacillus denitrificans* (ZP_00333731) *Treponema denticola* (NP_972799) *Trichomonas vaginalis* (AAA85494) *Vibrio cholerae* (AAF96433) *Yersinia pseudotuberculosis* (YP_070768).