The Enigmatic Planctomycetes May Hold a Key to the Origins of Methanogenesis and Methylotrophy

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Methanogenesis and methane oxidation are the major biological processes affecting the global cycling of the powerful greenhouse gas methane. To carry out the two alternative bioconversions, Nature has cleverly recycled key reactions for the C_1 transfers between the oxidation levels of formaldehyde and formate, and these involve analogous enzyme systems and common specialized cofactors, methanopterin and methanofuran. Until recently, the distribution of these functions has been limited to methanogenic archaea and methylotrophic proteobacteria, and their evolutionary history remained obscure. Single interdomain lateral transfer of the respective genes has been suggested to play a role. Here we show that genes for C_1 transfer reactions linked to methanopterin and methanofuran are also present in diverse representatives of the enigmatic bacterial clade, the Planctomycetes. Phylogenetic analysis places the planctomycete sequences as distantly from their archaeal counterparts as from their proteobacterial counterparts, suggesting novel scenarios for the evolution of the C_1 transfer functions in both methanogens and methylotrophs. This finding suggests a possible role for Planctomycetes in the evolution of the methane cycle on Earth.

Introduction

Most if not all of the methane in the atmosphere of Earth is of biogenic origin, and although its concentration has been steadily increasing over the past 300 years, it is maintained at a low level considering the amounts of methane produced as a result of human activities, as well as from natural environments. The two major players in maintaining methane balance on this planet are the methanogens (methane producers) and methanotrophs (methane consumers). The known methanogens belong to a number of phylogenetically diverse groups within the domain of Archaea (archaebacteria), kingdom Euryarchaeota, most species of which are strict anaerobes (http://link.springer.de/link/service/books/10125/). Methanotrophy is carried out via two different modes, aerobic and anaerobic, by two distinct groups of microorganisms, methanotrophic representatives of Bacteria (eubacteria) and methanotrophic representatives of Archaea. Aerobic methanotrophs fall within two groups of Proteobacteria, alpha and gamma (http://link.springer.de/link/service/books/10125/), whereas the recently discovered anaerobic methanotrophic archaea appear to be phylogenetically close to methanogens of the Methanosarcinales group (Hinrichs et al. 1999; Boetius et al. 2000; Michaelis et al. 2002). Methanogenesis (e.g., from carbon dioxide, $CO_2 \rightarrow CH_4$) and methanotrophy ($CH_4 \rightarrow CO_2$) may be viewed chemically as reversed reactions. However, in biology, both processes involve energy metabolism; therefore they are not mirror images of each other, due to the specific enzymes involved (fig. 1). Methyl-CoM reductase catalyzes the final step of methanogenesis and is unique to methanogens, whereas methane monooxygenase initiates the aerobic oxidation of methane and is unique to methanotrophic bacteria (http://link.springer.de/link/service/books/10125/). The pathway responsible for anaerobic oxidation of methane remains unknown, but the phylogenetic position of the archaea that carry out this metabolism suggests the core C_1 transfers may resemble those in methanogenesis (Hinrichs et al. 1999; Boetius et al. 2000; Michaelis et al. 2002). The similarity between methanogenesis and methylotrophy lies in the core reactions, that is, C_1 transfers between the oxidation levels of formaldehyde and formate that are carried out in both by homologous enzymes that involve similar coenzymes (fig. 1, gray box), pointing to the common evolutionary root of the two bioconversions (Chistoserdova et al. 1998; Vorholt et al. 1999; Chistoserdova et al. 2003). Until now, these reactions have been found in only two microbial groups, Euryarchaea and Proteobacteria, and their origin and evolutionary histories have remained elusive. The possibility of lateral gene transfer between Euryarchaea and Proteobacteria has been widely discussed in this context, and the most probable direction of the transfer has been assumed to be from Archaea into Bacteria (Chistoserdova et al. 1998; Vorholt et al. 1999; DeLong 2000; Gogarten, Doolitle, and Lawrence 2002; Boucher et al. 2003; Martin and Russell 2003). The genes in question have even been viewed as part of the "archaeal genomic signature," that is, a set of genes uniquely present in Archaea and missing from most members of the Bacteria and Eucarya (Graham et al. 2000), based on an assertion of the ancestral role of the methanopterin/methanofuran-linked C_1 transfer pathway in the reductive direction in deeply branching methanogenic archaea. However, no fossil record exists dating methanogenesis as a more ancient process compared to methylotrophy (Grassineau et al. 2001; Brocks et al. 2003). In addition, an archaeal prototype for such a transfer is missing. In all known methanogenic archaea the genes in question are scattered on the chromosomes, and lateral transfer of small numbers of genes in each event would not result in a metabolic innovation providing a selective advantage to the recipient. A scenario of lateral transfer of

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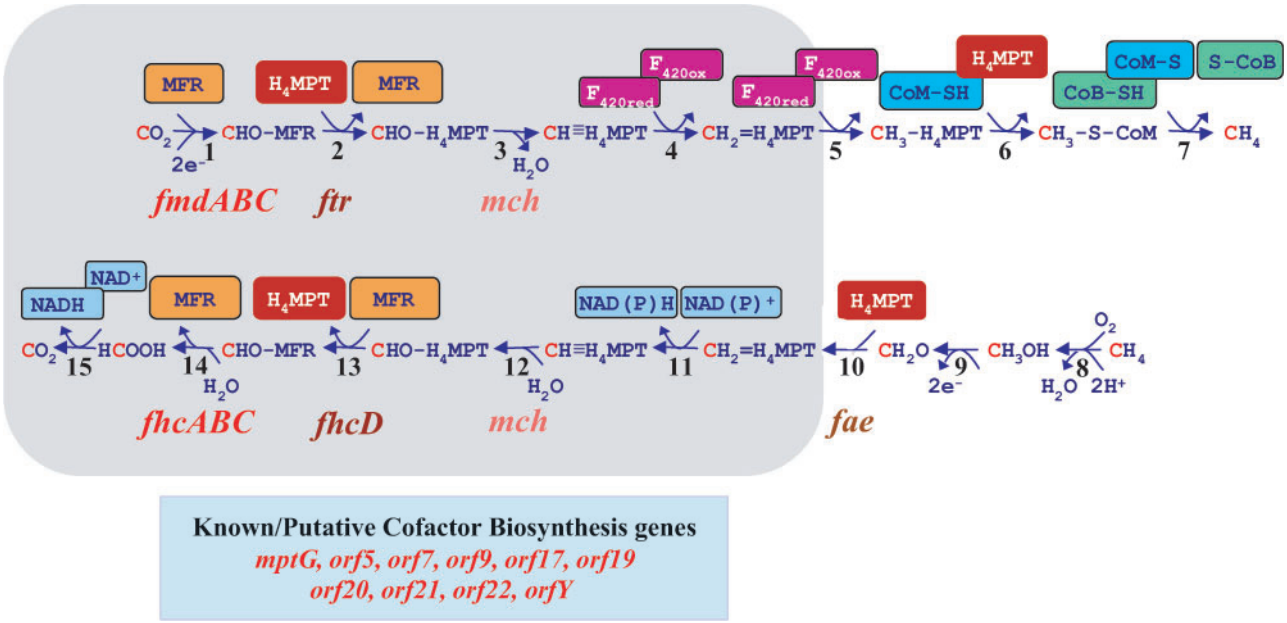

FIG. 1.—The commonality of methanogenesis and methane oxidation. Reactions 1–4 of methanogenesis are analogous to reactions 11–15 of methane oxidation (boxed in gray). MFR, methanofuran; H_4MPT, tetrahydromethanopterin; F_{420}, coenzyme F_{420}; CoM, Coenzyme M; CoB, Coenzyme B. 1, formyl-MFR dehydrogenase; 2, formyl-MFR:H_4MPT-formyltransferase; 3, 12, methenyl-H_4MPT cyclohydrolase; 4, 11, methylene-H_4MPT dehydrogenase (these enzymes are not conserved between methanogens and methylotrophs and apparently have independent evolutionary histories); 5, methylene-H_4MPT reductase; 6, methyl-H_4MPT:CoM methyltransferase; 7, methyl-CoM reductase; 8, methane monooxygenase; 9, methanol dehydrogenase; 10, formaldehyde activating enzyme; 13, 14, formyltransferase/hydrolase complex; 15, formate dehydrogenase. Genes conserved in archaeal methanogens and bacterial methylotrophs are in italics, same color indicates sequence identity at protein level. For more details on cofactors, enzymes, and reactions see Thauer (1998) and Vorholt (2002).

these genes from a (aerobic) proteobacterial methylotroph into a euryarchaeon has also been suggested (Cavalier-Smith 2002), but this scenario would necessitate aerobic methylotrophy preceding anaerobic methanogenesis, which contradicts the current understanding of the history of Earth's atmosphere (Kasting and Siefert 2002). The distinct separation of these two groups of genes (Vorholt et al. 1999) suggested that the understanding of their evolutionary history was incomplete. Recently, the presence of the genes in question has been reported in *Pirellula* sp. strain 1, a representative of Plancomycetales (Glöckner et al. 2003). In this work, we demonstrate that these genes are also present in other, distantly related representatives of Planctomycetes, thus making this enigmatic division of bacteria the third major microbial group possessing genes for C_1 transfers mediated by methanopterin and methanofuran. Phylogenetic analysis of these new sequences suggests novel scenarios for the evolution of C_1 transfer functions.

Materials and Methods

Genome Analysis

The complete genome sequence of *Pirellula* sp. strain 1 (Glöckner et al. 2003) was assessed at http://blast.mpi-bremen.de/m_status.php. Preliminary sequenced data for *Gemmata obscuriglobus* were assessed at http://www.tigr.org/. A draft (approximately 1.5X) sequence of *Gemmata* sp. Wa1-1 was generated by Integrated Genomics, Inc. (Chicago, Ill.) and is proprietary at this time. The genome sequences of *Methanothermobacter thermoautotrophicus*, *Methanococcus jannaschii*, *Methanopyrus kandleri*, *Methanosarcina mazei*, *Methanosarcina acetivorans*, and *Archaeoglobus fulgidus* were assessed at http://www.ncbi.nlm.nih.gov/. Preliminary sequence data for *Methylococcus capsulatus* were assessed at http://www.tigr.org/, the draft sequence of *Burkholderia fungorum* was assessed at http://www.jgi.doe.gov/, and the draft sequence of *Methylobacterium extorquens* was assessed at http://www.intergatedgenomics.com/genomereleases.html#list6. Sequences pertaining to this work were initially identified in the genomes mentioned above via BLAST analyses using the sequences of the 16 ("archaeal-like") polypeptides from *M. extorquens* (Chistoserdova et al. 2003) as queries. Then all the candidate sequences were cross-checked in further BLAST analyses against all the genome sequences mentioned earlier, to ensure that the sequences from various genomes homologous to the *M. extorquens* queries were homologous to each other. Resulting from these BLAST analyses, the highest E-value obtained for the two homologs was 0.096 (Orf22 homologs from *M. extorquens* and *Pirellula* sp. strain 1) and the lowest was e-127 (FhcA homologs from *G. obscuriglobus* and *B. fungorum*). In the case of *Gemmata* sp. Wa1-1, DNA fragments containing complete or partial genes of interest were PCR amplified, cloned into the pCR2.1 vector (Invitrogen, Carlsbad, Calif.), and resequenced. These are deposited with the GenBank accession numbers AY515679 to AY515684. BLAST searches were also performed with the nonredundant database (http://www.ncbi.nlm.nih.gov/BLAST/) in order

to assess the distribution of the respective genes outside the divisions of methanogenic Euryarchaea, Proteobacteria, and Planctomycetes.

Sequencing

To expand the databases of bacterial counterparts of the archaeal-like genes, pWEB (Epicentre, Madison, Wisc.)–based cosmid genomic libraries were constructed for the following bacteria: *Methylobacillus flagellatus* (beta-proteobacterial methylotroph), *Hyphomicrobium zavarzinii*, *Xanthobacter autotrophicus* (alpha-proteobacterial methylotrophs), *Methylosinus* sp. LW2 (alpha-proteobacterial methanotroph), *Methylomonas* sp. LW13, and *Methylomicrobium* sp. AMO1 (gamma-proteobacterial methanotrophs). The *mch* genes have been previously identified for the former three organisms (Vorholt et al. 1999). Partial *mch* genes from *Methylosynus* sp. LW2, *Methylomonas* sp. LW13, and *Methylomicrobium* sp. AMO1 were polymerase chain reaction (PCR) amplified using a pair of degenerate primers previously described (Vorholt et al. 1999). ^{32}P-labeled *mch* genes were hybridized with the respective cosmid libraries, and inserts positive for *mch* were (partially) sequenced, to identify neighboring archaeal-like genes. The sequences of these gene clusters have been deposited with GenBank under accession numbers AF139592, AY506552, AF139593, and AY515685-AY515687, respectively. The complete sequence of the *fae* gene from an environmental clone of unknown phylogenetic affiliation was obtained by probing a CopyControl pCC1Fos (Epicentre)–based metagenomic library constructed from DNA isolated from Lake Washington sediment (unpublished data) with ^{32}P-labeled PCR probes specific for the novel *fae* group identified in the Lake Washington environmental *fae* library (Kalyuzhnaya, Lidstrom, and Chistoserdova 2004), followed by sequencing the insert of a fosmid specific for the probes. This *fae* sequence has been deposited with GenBank under accession number AY532483.

Mutant Complementation

The homologs of *fae, mptG, orf7*, and *orf19* were PCR amplified from the chromosome of *Gemmata* sp. Wa1-1 and cloned into an expression vector pCM80 (Marx and Lidstrom 2001). The resulting plasmids were conjugated into the respective (methanol-negative) mutants of *M. extorquens* and the transconjugants tested for the ability to grow on methanol. The transconjugants revealed wild-type growth characteristics on this substrate.

Phylogenetic Analysis

The Phylip package (Felsenstein 2003) was used for this analysis. Distance, parsimony and maximum likelihood (ML) analyses were performed. One thousand bootstrap analyses were run for distance and parsimony methods, and 100 bootstrap analyses for the ML method. The ML analyses were run using the gamma distribution option with the coefficients of variation optimized for each tree, to reduce the long-branch attraction effect (Swofford et al. 1996).

Results and Discussion

C_1 Transfer Genes in Planctomycetes

A total of 16 genes involved in methanopterin and methanofuran-mediated C_1 transfers are shared between archaeal methanogens and bacterial methylotrophs (Chistoserdova et al. 2003) (fig. 1). We used the 16 polypeptides translated from these genes as queries to search for homologous genes in the newly generated genomic databases of representatives of Planctomycetes, the *Gemmata* and the *Pirellula* strains (see *Materials and Methods*). We identified all genes in the genomic sequence of *G. obscuriglobus* (http://www.tigr.org/). Fifteen of these genes were identified in the genome of *Pirellula* sp. strain 1 (Glöckner et al. 2003) (*fhcB* is either missing from this genome or is not recognizable). We also identified most of these genes in a partial genomic database of another *Gemmata* sp. Wa1-1. No genomic sequences for representatives of two other deeply branching genera within Planctomycetales, *Isosphaera* and *Planctomyces*, are available at this time. The genome of a representative of another deeply branching group of Planctomycetes, the one involved in anaerobic ammonium oxidation, of Candidatus "*Kuenenia stuttgartiensis*," is being sequenced by Genoscope, France, University of Vienna, Austria, and the University of Nijmegen, the Netherlands (http://www.anammox.com/research.html#kuenenia), but the sequence is not available for analysis at this time. It would be of great interest to analyze the presence of the C_1 transfer genes in these groups of Planctomycetes. So far, we were able to amplify *fhcD* and *fae* sequences from representatives of *Isosphaera* and *Planctomyces*, demonstrating that at least some of the genes of the pathway are present beyond the *Pirellula* and *Gemmmata* genera (unpublished results). Thus, Planctomycetales, a deeply branching division of bacteria (Brochier and Philippe 2002; Di Giulio 2003), represent the third major microbial group to possess the functions in question. However, no homologs for the known genes for the final steps of methanogenesis or the primary oxidation or assimilation of C_1 compounds were found in the genomes of the Planctomycetes. Based on these predictions from the genomes, Planctomycetes are not expected to be capable of methylotrophy or methanogenesis. Indeed, our attempts to cultivate Planctomycete strains on methanol or formaldehyde, or observe stimulation of growth on heterotrophic media by these C_1 substrates, produced negative results (unpublished data). Most likely, the archaeal-like C_1 transfer module in Planctomycetes fulfills a formaldehyde detoxification function, as has been demonstrated for both methylotrophic (Chistoserdova et al. 2000; Marx, Chistoserdova, and Lidstrom 2003) and nonmethylotrophic (Marx et al. 2004) proteobacteria. Testing this proposed function by mutant analysis is not possible at this time, as no genetic systems exist for Planctomycetes.

Functional Expression of Planctomycete Genes

We tested the functionality of the C_1 transfer pathway in Planctomycetes by expressing four of the genes, *fae*

(encoding the formaldehyde activating enzyme, Vorholt et al. 2000), *mptG* (encoding β-ribofuranosylaminobensene phosphate synthase, a H_4MPT biosynthesis enzyme, Scott and Rasche 2002), *orf7* and *orf19* (putative H_4MPT biosynthesis enzymes, Chistoserdova et al. 2003) from *Gemmata* sp. Wa1-1 in a heterologous host, *M. extorquens*, whose mutants in the archaeal-like genes fail to grow on C_1 compounds (Chistoserdova et al. 2003). Wild-type phenotype restoration was obtained in the presence of the respective gene homologs from *Gemmata* sp. Wa1-1. This indicates that the planctomycete genes in question do potentially encode functional proteins that carry out the predicted reactions. Additional evidence of the expression of genes in question in Planctomycetes was obtained via fluorescent in situ hybridization with sediment samples from Lake Washington, using two differentially labeled probes, one specific to Planctomycete 16S rRNA (Neef et al. 1998), and another specific to *fae* mRNA (unpublished data). We have also demonstrated, by environmental library construction and sequence analysis, that this site harbors a variety of Planctomycete strains possessing *fae* genes (Kalyuzhnaya, Lidstrom, and Chistoserdova 2004).

Phylogenetic Analysis

One of the major outcomes of discovering the third major deeply branching microbial group possessing methanopterin/methanofuran-linked functions is the potential to obtain new insights into the possible scenarios for the evolution of these functions and to test the currently prevalent hypothesis of lateral transfer of these genes from Euryarchaeota to Proteobacteria (Gogarten, Doolitle, and Lawrence 2002; Boucher et al. 2003; Martin and Russell 2003). We performed phylogenetic analysis of all 16 polypeptides shared by archaeal methanogens (as well as *A. fulgidus*), Proteobacteria (including a nonmethylotroph, *B. fungorum* LB400), and Planctomycetes (*fhcB* is missing or not recognizable in the *Pirellula* sp. strain 1 genome, so analysis of FhcB homologs only included the *Gemmata* sequences). This analysis shows that in general, the polypeptide counterparts from Planctomycetes are as distant from their archaeal homologs as they are from their proteobacterial homologs, and in most cases they form a distinct third group on the phylogenetic trees, with significant bootstrap confidence for the node defining this group's monophyly (six representative trees are shown in fig. 2).

However, some of the trees built in this study showed deviations from this common pattern. For example, topologies of trees built for Orf5, Orf17, and Fae were complicated by the presence of multiple homologs that must reflect a more complex evolution of these genes including early duplications, and early and recent gene transfers. One example of such a complex tree topology, for Fae polypeptides and their homologs is shown in figure 3. In this tree, as in those shown in figure 2, the Planctomycete sequences form a monophyletic group separated from both the proteobacterial branch and the branch containing archaeal counterparts that represent domains of longer polypeptides (function unknown). However, representatives of Methanosarcinales contain additional homologs of Fae (function unknown), and these branch between proteobacterial and planctomycete sequences. These latter genes might have resulted from early lateral transfers from bacteria. At least some bacteria also contain multiple *fae* homologs. For example, *M. extorquens* contains three apparent *fae* homologs. Although the function of the protein encoded by "true" *fae* (Fae1 in fig. 3) has been established (Vorholt et al. 2000), the potential functions of Fae2 and Fae3 remain unknown. Remarkably, Fae3 from *M. extorquens* shows 82% identity to Fae2 from *Pirellula* sp. strain 1, possibly representing an example of a recent lateral transfer between Proteobacteria and Planctomycetes. The two Fae homologs from *Methylosinus* sp. LW2 (83% identity) might be a result of a lateral transfer, or a result of a recent duplication (these are adjacent on the chromosome, not shown).

Trees built for FhcA, FhcC, and FhcD polypeptides represent another deviation from the pattern shown in figure 2. In these trees, the sequences of *Pirellula* sp. strain 1 tended to cluster within the proteobacterial branch, while the *Gemmata* sequences followed the pattern similar to the one shown in figure 2 (not shown). However, it has been argued previously that genes encoding formylmethanofuran dehydrogenases (Fmd's; encoded by homologs of the *fhc* genes) might not represent the best targets for phylogenetic analysis, due to potential complications inherent in isoenzymes and functionally equivalent enzymes (Reeve et al. 1997). Moreover, although clearly homologous to the α, β, and γ-subunits of tungsten- or molybdenum-dependent Fmd's, the *fhcABC* genes in proteobacteria encode subunits of an enzyme catalyzing the hydrolysis rather than the dehydrogenation of the formyl-group of formyl-methanofuran (Pomper et al. 2002). Possibly, the tendency of Fhc sequences from *Pirellula* sp. strain 1 to cluster with proteobacterial sequences reflects a selective pressure on these genes, or these genes may be a result of lateral transfers from Proteobacteria.

In contrast to *fch* genes, *mch* has been suggested before as one of the most reliable genes for following the evolutionary history of methanogenesis (and likely of C_1 transfers in bacteria), based on a few criteria, such as its essential function, the lack of duplication in any known organism, and the absence of substitution by functionally equivalent enzymes (Reeve et al. 1997). Mch phylogeny also seems to agree with the 16S rRNA phylogeny in both Euryarchaeota (Reeve et al. 1997) and in Proteobacteria (Kalyuzhnaya, Lidstrom, and Chistoserdova 2004). Thus the analysis that follows is mostly based on the topology of the Mch tree and similar trees depicted in figure 2. At least five alternative scenarios for the evolution of genes in question in the three prokaryotic divisions, Euryarchaeota, Planctomycetales, and Proteobacteriales may be suggested (fig. 4). (*A*) The genes in question arose in Euryarchaeota and were subsequently transferred into Bacteria, via lateral gene transfer (LGT), before the separation of Planctomycetes and Proteobacteria. If this scenario were true, the sequences from Planctomycetes and from Proteobacteria would be expected to branch together. This would also be the case for a transfer from Euryarchaea to Planctomycetes, followed by another transfer from Planctomycetes to

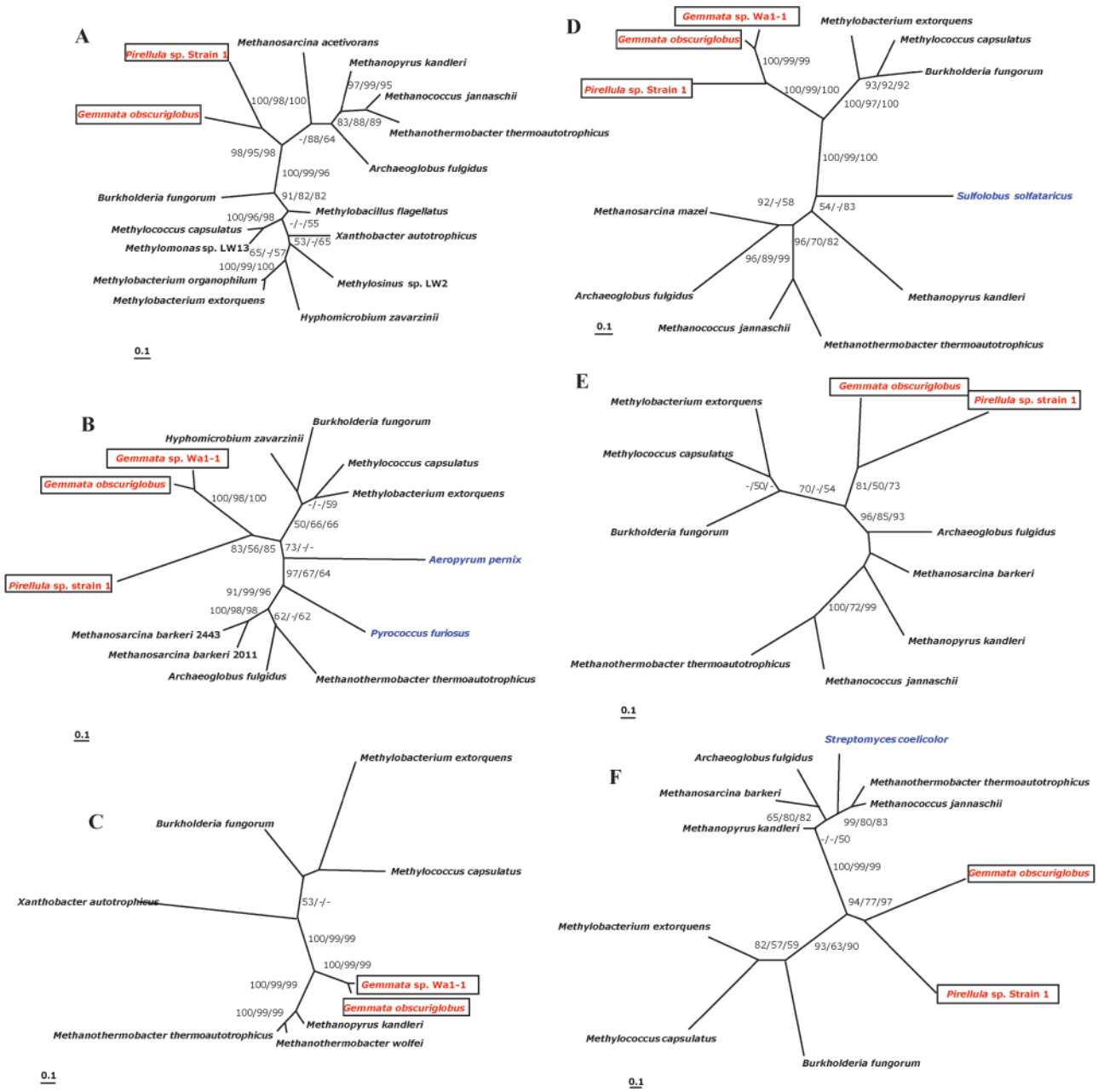

FIG. 2.—Phylogenetic analysis of polypeptides involved in C_1 transfers conserved in archaeal methanogens, proteobacterial methylotrophs, and Planctomycetes. (*A*) Mch, (*B*) MptG, (*C*) FhcB, (*D*) Orf20, (*E*) Orf21, (*F*) Orf22. The Phylip package (Felsenstein 2003) was used for this analysis. The bootstrap values (%) are shown for distance, parsimony, and ML analyses, respectively. One thousand bootstrap analyses were run for distance and parsimony methods, and 100 bootstrap analyses for the ML method. The Planctomycete strains are boxed and shown in red. The strains not belonging to Planctomycetes, Proteobacteria or methanogenic or sulphate-reducing archaea are shown in blue.

Proteobacteria (not shown). However, the planctomycete and the proteobacterial sequences do separate in the phylogenetic trees. (*B*) The genes in question arose in Euryarchaeota, and were transferred into Planctomycetes and into Proteobacteria after the groups separated, as a result of independent LGT events. If such independent transfers did take place, one would expect each group of bacterial sequences to branch within the group of archaeal sequences from which transfer occurred, but instead they separate. In addition, a certain degree of gene order conservation in Planctomycetes and in Proteobacteria argues against independent transfer events. For example, *mch* is always linked to *orf5* in bacteria, but they are separated from each other in all known archaeal genomes (data not shown). (*C*) The genes in question arose in Proteobacteria, and were transferred separately into Euryarchaea and into Planctomycetes. We argued earlier against a scenario in which these genes arise in Proteobacteria and are transferred into Archaea (see *Introduction*), which would suggest late emergence of methanogenesis relative to aerobic methylotrophy. The same reasoning would argue against transfer from Pro-

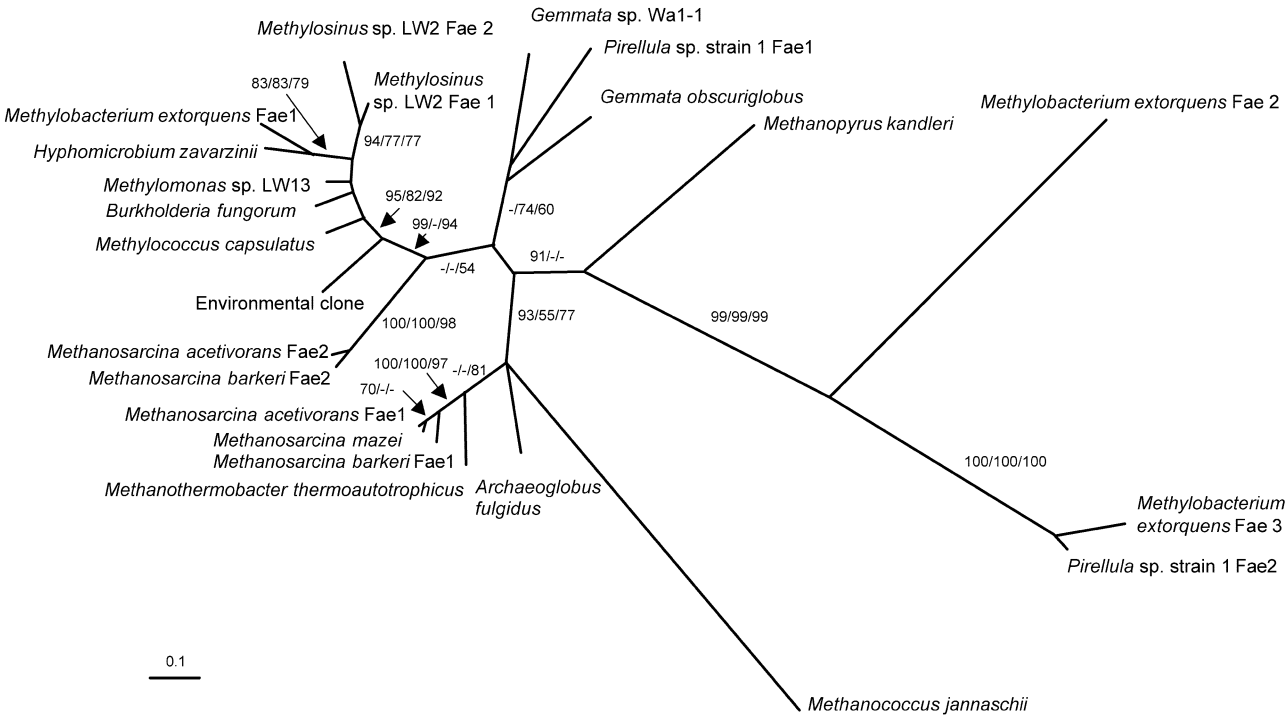

FIG. 3.—Phylogenetic analysis of Fae and Fae homolog polypeptides. See legend for figure 2 for analyses description.

teobacteria into Planctomycetes and then to Euryarchaea (not shown). (*D*) The genes in question emerged in Planctomycetes and were transferred, early in life history, to an early euryarchaeon and an early proteobacterium. The clear separation of archaeal and proteobacterial sequences on phylogenetic trees is in agreement with this scenario. (*E*) The genes in question were present in the last universal common ancestor (LUCA), before the separation of Archaea and Bacteria, followed by the loss of the genes in most known lineages of Bacteria, and the loss in Crenarchaeota after their separation from Euryarchaeota. This scenario also agrees with the topology of the trees. Thus we argue that scenarios (*D*) and (*E*) must be the most probable scenarios for the evolution of methanopterin/methanofuran-linked C_1 transfers in Archaea and Bacteria, of the multiple alternative scenarios previously presented.

Antiquity of the C_1 Transfer Pathway

In both scenarios *D* and *E*, a selective pressure would be required to prevent the loss of the whole complement of the genes. Such a fitness advantage in early life on Earth is in fact predicted. Formaldehyde is thought to have been abundant on early Earth (Arrhenius, Arrhenius, and Paplawsky 1994). Therefore, it could be argued that early cells would have benefited from a system to reduce the toxic effect of formaldehyde, the role proposed here for the methanopterin/methanofuran-linked C_1 transfer pathway in the Planctomycetes. At later stages, an additional fitness would be derived from the ability to draw energy from these reactions. At this time, both hypotheses outlined by scenarios (*D*) and (*E*) seem plausible. The existence of the C_1 transfer gene prototypes in LUCA is supported by the presence of the homologs of some of them (but not the complete set) in representatives of nonmethanogenic Euryarchaeota, some Crenarchaeota, and some bacteria (e.g., shown in figure 2 *B, D*, and *F*, MptG, Orf20, and Orf22). The scenario in which the genes for C_1 transfer arose in Planctomycetes is supported by the suggested antiquity of this group of bacteria (Brochier and Philippe 2002; Di Giulio 2003). This enigmatic group, although classified within the Bacteria, reveals unique features not common in the bacterial domain, such as a cell wall lacking peptidoglycan that is reminiscent of Archaea, intricate cell compartmentalization reminiscent of eukaryotes, division by budding, similarity to yeasts, and unique ladderane lipids in some autotrophic ammonium-oxidizing (anammox) representatives (Fuerst 1995; Lindsay et al. 2001; Sinninghe Damste et al. 2002). The recently discovered ubiquitous nature of the Planctomycetes, including thermophilic, halotolerant, and anaerobic species (Strous et al. 1999; Dalsgaard et al. 2003; Kuypers et al. 2003), the large size of their genomes, and the variety of metabolic capabilities encoded (Glöckner et al. 2003) make them prospective candidates for physiological similarity to the LUCA. In some models the LUCA has been proposed to be not a single organism but a community possessing a variety of biochemical capabilities, with few restrictions for their exchange (Woese 1998). Although no physical record of the antiquity of Planctomycetes exists at this time to support their position near the root of the universal phylogenetic tree, to the best of our knowledge, our results suggest that the origin of the methanopterin/methanofuran-linked C_1 transfer pathway in the Planctomycetes predated the divergence of the Proteobacteria, pointing to the antiquity of this pathway. Currently, no

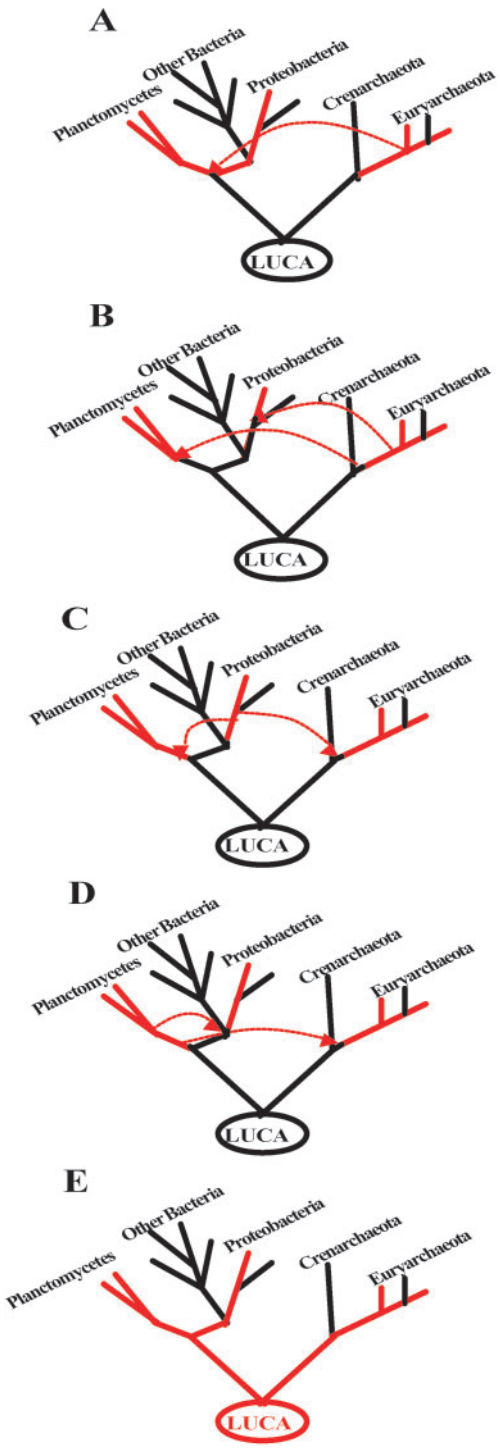

FIG. 4.—Alternative scenarios for the evolution of methanofuran/methanopterin-linked C_1 transfers in methanogens and methylotrophs. The branches containing genes in question are shown in red. (*A*) The genes emerge in Euryarchaea and are laterally transferred into bacteria before the separation of Proteobacteria and Planctomycetes. (*B*) The genes emerge in Euryarchaea and are transferred separately into Planctomycetes and into Proteobacteria. (*C*) The genes emerge in Proteobacteria and are transferred separately into Planctomycetes and into Euryarchaea. (*D*) The genes emerge in Planctomycetes and are transferred into Proteobacteria and Euryarchaea. (*E*) The genes are present in the LUCA; remain in Planctomycetes, Proteobacteria, and Euryarchaea; and are lost in most of the known lineages. For simplicity, Eucarya are not considered.

fossil record or biomarkers exist to indicate whether microbial methanogenesis preceded methylotrophy or vice versa, but evidence exists for both being present as long ago as 2.78 Gyr ago (Grassineau et al. 2001; Brocks et al. 2003). The new results presented here suggest the possibility that the methanopterin/methanofuran-linked C_1 transfer pathway between the oxidation levels of formaldehyde and formate may have been an early, important function for life, and then became the first building block in the formation of both methanogenesis and methanotrophy. The functions specific to either methanogenesis or methanotrophy, such as methyl-CoM reductase, methane monooxygenase, and the specific accessory functions would have emerged later in prokaryotic history.

In conclusion, the data we present here provide new insight into the history of two environmentally significant bioconversions, methanogenesis and methylotrophy, pointing to Planctomycetes, an enigmatic division of Bacteria, as potential ancestors of the key C_1 transfer functions and thus as potential players in the evolution of the global methane cycle.

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Literature Cited

Arrhenius, T., G. Arrhenius, and W. Paplawsky. 1994. Archean geochemistry of formaldehyde and cyanide and the oligomerization of cyanohydrin. Orig. Life Evol. Biosph. **24**:1–17.

Boetius, A., K. Ravenschlag, C. J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann., B. B. Jorgensen, U. Witte, and O. Pfannkuche. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature **407**:623–626.

Boucher, Y., C. J. Douady, R. T. Papke, D. A. Walsh, M. E. R. Boudreau, C. Nesbø, R. J. Case, and W. F. Doolitle. 2003. Lateral gene transfer and the origins of prokaryotic groups. Annu. Rev. Genet. **37**:283–328.

Brochier, C., and H. Philippe. 2002. Phylogeny: a non-hyperthermophilic ancestor for bacteria. Nature **417**:244.

Brocks, J. J., R. Buick, R. E. Summons, and G. A. Logan. 2003. A reconstruction of Archaean biological diversity based on molecular fossils from the 2.78 to 2.45 billion year-old Mount Bruce Supergroup, Hamersley Basin, Western Australia. Geochim. Cosmochim. Acta **67**:4321–4335.

Cavalier-Smith, T. 2002. The neomuran origin of archaebacteria, the negibacterial root of the universal tree and bacterial megaclassification. Int. J. Syst. Evol. Microbiol. **52**:7–76.

Chistoserdova, L., S.-W. Chen, A. Lapidus, and M. E. Lidstrom. 2003. Methylotrophy in *Methylobacterium extorquens* AM1 from a genomic point of view. J. Bacteriol. **185**:2980–2987.

Chistoserdova, L., L. Gomelsky, J. A. Vorholt, M. Gomelsky, Y. D. Tsygankov, and M. E. Lidstrom. 2000. Analysis of two formaldehyde oxidation pathways in *Methylobacillus flagellatus* KT, a ribulose monophosphate cycle methylotroph. Microbiol. **146**:233–238.

Chistoserdova, L., J. A. Vorholt, R. K. Thauer, and M. E. Lidstrom. 1998. C_1 transfer enzymes and coenzymes linking methylotrophic bacteria and methanogenic Archaea. Science **281**:99–102.

Dalsgaard, T., D. E. Canfield, J. Petersen, B. Thamdrup, and J. Acuna-Gonzalez. 2003. N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature **422**:606–608.

DeLong, E. F. 2000. Resolving a methane mystery. Nature **407**:577.

Di Giulio, M. 2003. The ancestor of the Bacteria domain was a hyperthermophile. J. Theor. Biol. **224**:277–283.

Felsenstein, J. 2003. Inferring phylogenies. Sinauer Associates, Inc., Sunderland, Mass.

Fuerst, J. A. 1995. The planctomycetes: emerging models for microbial ecology, evolution and cell biology. Microbiology **141**:1493–1506.

Glöckner, F. O., M. Kube, M. Bauer et al. (14 coauthors). 2003. Complete genome sequence of the marine planctomycete *Pirellula* sp. strain 1. PNAS USA **100**:8298–8303.

Gogarten, J. P., W. F. Doolittle, and J. G. Lawrence. 2002. Prokaryotic evolution in light of gene transfer. Mol. Biol. Evol. **19**:2226–2238.

Graham, D. E., R. Overbeek, G. J. Olsen, and C. R. Woese. 2000. An archaeal genomic signature. PNAS USA **97**: 3304–3308.

Grassineau, N. V, E. G. Nisbet, M. J. Bickle, C. M. Fowler, D. Lowry, D. P. Mattey, P. Abell, and A. Martin. 2001. Antiquity of the biological sulphur cycle: evidence from sulphur and carbon isotopes in 2700 million-year-old rocks of the Belingwe Belt, Zimbabwe. Proc. R. Soc. Lond. B Biol. Sci. **268**:113–119.

Hinrichs, K.-U., J. M. Hayes, S. P. Sylva, P. G. Brewer, and E. F. DeLong. 1999. Methane-consuming archaebacteria in marine sediments. Nature **398**:802–805.

Kalyuzhnaya, M. G., M. E. Lidstrom, and L. Chistoserdova. 2004. Utility of environmental primers targeting ancient enzymes: methylotroph detection in Lake Washington. Microb. Ecol. (in press).

Kasting, J., and J. L. Siefert. 2002. Life and the evolution of Earth's atmosphere. Science **296**:1066–1068.

Kuypers, M. M., A. O. Sliekers, G. Lavik, M. Schmid, B. B. Jorgensen, J. G. Kuenen, J. S. Sinninghe Damste, M. Strous, and M. S. Jetten. 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature **422**:608–611.

Lindsay, M. R., R. I. Webb, M. Strous, M. S. Jetten, M. K. Butler, R. J. Forde, and J. A. Fuerst. 2001. Cell compartmentalisation in planctomycetes: novel types of structural organisation for the bacterial cell. Arch. Microbiol. **175**: 413–29.

Martin, W., and M. J. Russell. 2003. On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Phil. Trans. R. Soc. Lond. **358**:59–83.

Marx, C. J., L. Chistoserdova, and M. E. Lidstrom. 2003. Formaldehyde-detoxifying role of the tetrahydromethanopterin-linked pathway in *Methylobacterium extorquens* AM1. J. Bacteriol. **185**:7160–7168.

Marx, C. J., and M. E. Lidstrom. 2001. Development of improved versatile broad-host-range vectors for use in methylotrophs and other Gram-negative bacteria. Microbiology **147**:2065–2075.

Marx, C. J., J. A. Miller, L. Chistoserdova, and M. E. Lidstrom. 2004. Multiple formaldehyde oxidation/detoxification pathways in *Burkholderia fungorum* LB400. J. Bacteriol. **186**:2173–2178.

Michaelis, W., R. Seifert, K. Nauhaus et al. (17 coauthors). 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science **297**:1013–1015.

Neef, A., R. Amann, H. Schlesner, and K. H. Schleifer. 1998. Monitoring a widespread bacterial group: *in situ* detection of planctomycetes with 16S rRNA-targeted probes. Microbiology **144**:3257–3266.

Pomper, B. K., O. Saurel, A. Milon, and J. A. Vorholt. 2002. Generation of formate by the formyltransferase/hydrolase complex (Fhc) from *Methylobacterium extorquens* AM1. FEBS Lett. **523**:133–137.

Reeve, J. N., J. Nölling, R. M. Morgan, and D. R. Smith. 1997. Methanogenesis: genes, genomes, and who's on first? J. Bacteriol. **179**:5975–5986.

Scott, J. W., and M. E. Rasche. 2002. Purification, overproduction, and partial characterization of beta-RFAP synthase, a key enzyme in the methanopterin biosynthesis pathway. J. Bacteriol. **184**:4442–4448.

Sinninghe Damste, J. S., M. Strous, W. I. Rijpstra, E. C. Hopmans, J. A. Geenevasen, A. C. van Duin, L. A. van Niftrik, and M. S. Jetten, M.S.2002. Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature **419**:708–712.

Strous, M., J. A. Fuerst, E. H. Kramer, S. Logemann, G. Muyzer, K. T. van de Pas-Schoonen, R. Webb, J. G. Kuenen, and M. S. Jetten. 1999. Missing lithotroph identified as new planctomycete. Nature **29**:446–449.

Swofford, D., G. Olson, P. Waddell, and D. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *in* D. Hillis, C. Moritz, and B. Mable, eds. Molecular systematics, 2nd edition. Sinauer Associates, Sunderland, Mass.

Thauer, R. K. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Microbiology **144**:2377–2406.

Vorholt, J. A. 2002. Cofactor-dependent pathways of formaldehyde oxidation in methylotrophic bacteria. Arch. Microbiol. **178**:239–249.

Vorholt, J. A., L. Chistoserdova, S. M. Stolyar, R. K. Thauer, and M. E. Lidstrom. 1999. Distribution of tetrahydromethanopterin-dependent enzymes in methylotrophic bacteria and phylogeny of methenyl tetrahydromethanopterin cyclohydrolases. J. Bacteriol. **181**:5750–5757.

Vorholt, J. A., C. J. Marx, M. E. Lidstrom, and R. K. Thauer. 2000. Novel formaldehyde-activating enzyme in *Methylobacterium extorquens* AM1 required for growth on methanol. J Bacteriol. **182**:6645–6650.

Woese, C. R. 1998. The universal ancestor. PNAS USA **95**: 6854–6859.

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