

Supplementary Material
A major clade of prokaryotes with ancient adaptations to life on land
Fabia U. Battistuzzi and S. Blair Hedges

Data assembly and phylogenetic analyses

Protein data set: Amino acid sequences of 25 protein-coding genes (“proteins”) were concatenated in an alignment of 18,586 amino acid sites and 283 species. These proteins included: 15 ribosomal proteins (RPL1, 2, 3, 5, 6, 11, 13, 16; RPS2, 3, 4, 5, 7, 9, 11), four genes (RNA polymerase alpha, beta, and gamma subunits, Transcription antitermination factor NusG) from the functional category of Transcription, three proteins (Elongation factor G, Elongation factor Tu, Translation initiation factor IF2) of the Translation, Ribosomal Structure and Biogenesis functional category, one protein (DNA polymerase III, beta subunit) of the DNA Replication, Recombination and repair category, one protein (Preprotein translocase SecY) of the Cell Motility and Secretion category, and one protein (O-sialoglycoprotein endopeptidase) of the Posttranslational Modification, Protein Turnover, Chaperones category, as annotated in the Cluster of Orthologous Groups (COG) (Tatusov et al. 2001).

After removal of multiple strains of the same species, GBlocks 0.91b (Castresana 2000) was applied to each protein in the concatenation to delete poorly aligned sites (i.e., sites with gaps in more than 50% of the species and conserved in less than 50% of the species) with the following parameters: minimum number of sequences for a conserved position: 110, minimum number of sequences for a flank position: 110, maximum number of contiguous non-conserved positions: 32000, allowed gap positions: with half. The signal-to-noise ratio was determined by altering the “minimum length of a block” parameter. This was increased, starting from a minimum of two to a maximum of 80, in order to obtain different data sets retaining approximately 40% (the longest alignment obtainable with the parameters chosen), 30%, 20%, 10%, 5%, and 2% of the original alignment. A phylogeny was built with MEGA4 (NJ, JTT+gamma, with the alpha parameter estimated by the program RAxML (Stamatakis 2006) and the number of monophyletic classes, their bootstrap support, and the monophyly of the phyla Proteobacteria (excluding the position of Solibacteres) and Firmicutes were compared. Solibacteres (Phylum Acidobacteria) was not considered in assessing Proteobacteria monophyly because its taxonomic position as an independent phylum has been questioned in light of recent phylogenetic results (Ciccarelli et al. 2006). In the evaluation of Firmicutes monophyly the position of *Symbiobacterium thermophilum* was not considered (see below). An increase in stringency levels caused a decrease in bootstrap support for the monophyly of classes (used as an approximation of the strength of the phylogenetic signal) because fewer sites were available, yet there was no apparent effect on the recovery of monophyletic classes. For this reason, we selected the 40% stringency level because it maximized the length of the alignment and the number of monophyletic eubacterial classes (Fig. S1).

Preliminary phylogenetic analyses showed a potential bias caused by the presence in the data set of the thermophile *Thermus thermophilus* (Phylum *Deinococcus-Thermus*), most likely caused by its thermophilic adaptations (Omelchenko et al. 2005). In the final data set, we decided to remove this species so that the final composition included 218 species and 6,884 sites (37% of the original alignment). This data set was analyzed with ML (RAxML v. 2.2.1, PROT MIXJTT+gamma) and bayesian methods (MrBayes3, partitioned data set, 2 independent runs of 20 million generations each, sample frequency=1000, model=jones, rates=gamma)

(Ronquist and Huelsenbeck 2003). One representative per class and one for the Phylum Bacteroidetes were chosen in the Bayesian analysis for a total of 31 species. Support for the use of a concatenation of genes came from a consensus analysis of the 25 ML protein trees. This was built using the program Consense of the Phylip package (Felsenstein 1989). This tree showed a generally poor phylogenetic signal in single phylogenies for relationships among classes and phyla and supported the use of a concatenation of these genes to increase the signal to noise ratio (Fig. S2).

Additional analyses were carried out on a data set created by applying the Slow-Fast (SF) method (Brinkmann and Philippe 1999; Philippe et al. 2000) to the original concatenation and building the phylogeny as described above (Fig. S3). This method progressively eliminates from the data set variable sites (i.e., sites with a number of changes above a threshold) leaving only slow evolving positions to estimate the phylogeny. PAUP* v.4 beta10 (Swofford 1998) was used to calculate the number of changes per site in each class represented by multiple species (a maximum of six species representing different genera was used when available). Archaeobacteria were analyzed at the domain level because only one class was represented by more than three species. The threshold between slow and fast evolving sites was based on the sum of changes across all phylogenetic categories for a given site: any site showing fewer changes than the selected threshold was considered slow evolving and retained in the alignment. Distance trees (NJ, JTT+gamma, with the alpha parameter estimated by the program RAxML) were built for each data set with threshold of 45, 30, 15, ten, five, and two changes per site. Increase threshold stringency resulted in paraphyly of classes and phyla, and loss of phylogenetic signal. We selected a threshold of 45 changes because it maximized the number of monophyletic classes and phyla (Fig. S1).

Rooting of phylogenetic trees: For the primary phylogenetic analyses, Eubacteria were rooted with Archaeobacteria, as has been the consensus in the field based on analyses of duplicated genes (Zhaxybayeva, Lapierre, and Gogarten 2005). However, this is an active area of research and other positions for the root have been suggested.

Symbiobacterium thermophilum: This species is a thermophilic bacterium dependent on microbial commensalism for growth (Ohno et al. 2000). It was classified as an actinobacterium based on its high GC content (Ueda et al. 2001) but recent studies have shown its affiliation with Firmicutes based on genome characteristics, indels, and the absence of proteins uniquely shared with Actinobacteria (Ueda et al. 2004; Gao and Gupta 2005; Gao, Paramanathan, and Gupta 2006). A recent supertree analysis also showed *S. thermophilum* clustering with Clostridia (Pisani, Cotton, and McInerney 2007) as in our phylogeny (both ML and NJ, BP 68% and 58% respectively). Given the amount of evidence, we consider this species as a misclassified actinobacterium and the first high GC member of the Class Clostridia.

Ribosomal RNA (rRNA) data set: small subunit (SSU) and large subunit (LSU) sequences available at the European Ribosomal RNA Database (Van de Peer et al. 2000; Wuyts, Perriere, and de Peer 2004) were used in their aligned form. The alignment was based on the secondary structure of rRNA using *Methanococcus jannachii* and *Sulfolobus acidocaldarius* as models (Van de Peer et al. 2000). A few classes present in the protein data set were absent from the rRNA data set (Bacteroidetes, Chloroflexi, Fusobacteria, and Solibacteres in the eubacteria, and Methanopyri and Nanoarchaeota in the archaeobacteria). Two sequences for archaeobacteria, *Methanopyrus kandleri* and *Nanoarchaeum equitans*, were added and manually aligned. The

missing eubacterial classes were not added because of the ambiguities in manually aligning a few species of uncertain phylogenetic position with hundreds of highly divergent sequences. The sequences for the two subunits were concatenated. As for the protein data set, GBLOCKS was applied to remove non-conserved sites and the stringency level was chosen using a criterion based on monophyly of eubacterial classes. The parameters used were: minimum number of sequences for a conserved position: 95, minimum number of sequences for a flank position: 95, maximum number of contiguous non-conserved positions: 32000, allowed gap positions: with half. The “minimum length of a block” parameter was progressively increased to obtain different data sets retaining approximately 60%, 50%, 40%, 30%, 20%, and 10% of the original alignment (columns with only gaps are deleted at the beginning of the analysis). A phylogeny was built with MEGA4 (NJ, TamuraNei+gamma, with the alpha parameter estimated by the program RAxML) and the number of monophyletic classes, their bootstrap support and the monophyly of Proteobacteria and Firmicutes were calculated. In the evaluation of Proteobacteria monophyly the position of *Zoogloea ramigera* was not considered (see below). Higher stringency levels caused a decrease in number of monophyletic classes (paraphyly of Gamma and Deltaproteobacteria, Spirochaetes, and Bacilli) as well as a decrease in bootstrap support of the remaining monophyletic ones. Monophyly of the two phyla is unaffected. We selected a stringency of 60% to maximize the number of sites (Fig. S1). The final data set was composed of 189 species for 3,786 sites (approximately 60% of the original alignment) (Table S2). ML and Bayesian trees were built with RAxML and MrBayes3 using GTRMIX+gamma and GTR+gamma, respectively, and partitioning the two subunits. One representative per class was chosen in the Bayesian analysis run with the following parameters: 2 independent runs of 20 million generations each, sample frequency=1000, model=GTR, rates=gamma.

An additional data set was created using the SF method and analyzed as explained above (Fig. S5). The number of changes per site in each eubacterial class represented by multiple species was calculated using the program PAUP* v.4 beta10. Archaeobacteria were treated at the domain level because only two classes were represented by more than three species. A maximum of six species was used in each class, spanning different genera when available. As for the protein data set, the number of changes within each class was summed across the two domains to obtain an estimate of variability of each site. Based on this, four threshold levels were tested: 15, 10, 5, and 3 changes per site. Distance trees (NJ, JTT+gamma, with the alpha parameter estimated by the program RAxML) were built for each one of these levels and monophyly of classes and phyla, and bootstrap supports were calculated. Increasing stringency (i.e., lower threshold) resulted in paraphyly of many classes and phyla, and lower bootstrap supports. We selected a threshold of 15 changes because it maximized the number of monophyletic classes, phyla, and their bootstrap values. This new data set includes approximately 60% of the variable sites present in the original data set (Fig. S1).

Zoogloea ramigera: The original classification of this species had placed it within the Gammaproteobacteria (Shin, Hiraishi, and Sugiyama 1993). A more detailed analysis of various strains revealed that this was a misclassification and placed the type strain within the Betaproteobacteria. Nonetheless, some strains did not cluster with the type strain in an SSU phylogenetic tree and were also found missing a particular rhodoquinone-8 (RQ-8) synthesized by the type strain. The putatively misclassified strains were shown to cluster within the Alphaproteobacteria close to *Agrobacterium tumefaciens* (Shin, Hiraishi, and Sugiyama 1993). This position is the same found in our phylogenetic tree of rRNA subunits (BP 100%) and

suggests that the sequence named *Z. ramigera* X88894 in the European Ribosomal Database belongs to one of the misclassified strains. We thus consider it an alphaproteobacterium.

Time estimation

Protein data set: One representative per class in Eubacteria and Archaeobacteria was chosen for a total of 21 ingroup eubacterial species and ten ingroup archaeobacterial species. Five additional data sets were created using randomly chosen eubacterial species to test for sampling bias. Divergence times were estimated with a Bayesian method, Multidivtime T3 (Thorne and Kishino 2002), both with partitioned (T3p) and non partitioned (T3np) genes, and rate smoothing methods: nonparametric rate smoothing (NPRS) and penalized likelihood (PL) (Sanderson 1997). The Bayesian method and NPRS performed as expected but PL showed inconsistent results. The monotonic decrease in square-errors with increasing smoothing factor obtained under this method suggests either a constant rate throughout the tree or rate variations that do not follow a specific pattern (Sanderson 2002). When this case occurs, use of the constant rate molecular clock (LF) is favored, although the reliability of these time estimates remains unclear under the circumstances of uncorrelated rate variations. However, in the absence of other evidence, neither of the methods can be excluded.

Multiple calibration points were used in both the eubacterial and archaeobacterial data sets. We used three calibrations within Eubacteria. The first was a maximum boundary for the ingroup root node at 4.2 Ga, which is the mid-point of the time range estimated for the last ocean-vaporizing event (Sleep et al. 1989), while acknowledging a late heavy bombardment at 3.9 Ga (Zahnle et al. 2007) may have included an ocean-boiling impact, and that life may have survived such an event (Wells, Armstrong, and Gonzalez 2003; Zahnle et al. 2007). The second is a minimum time for the divergence of Chlorobia and Bacteroidetes at 1.64 Ga, based on biomarker evidence for chlorobactane in the Barney Creek Formation of the MacArthur Group, Northern Australia (Brocks et al. 2005). The third is a minimum time for the divergence of Gamma and Betaproteobacteria at 1.64 Ga, which comes from biomarker evidence of okenane in the Barney Creek Formation of the MacArthur Group, Northern Australia (Brocks et al. 2005).

For the primary time estimation analyses, we avoided additional calibrations that included Cyanobacteria or involved oxygen metabolism so that we could draw inferences about those organisms and metabolisms. However, two additional calibrations were used to test the robustness of the time estimates. One was a minimum at 2.3 Ga for the divergence of Cyanobacteria and Dehalococcoidetes (Phylum Chloroflexi), corresponding to the presence of oxygen in the atmosphere (Holland 2002). The other was a maximum of 4.0 Ga for the earliest land-dwelling taxa (Group-I), corresponding to the presence of continents (Rosing et al. 2006). The small number of calibration points available for Archaeobacteria is a reflection of the poor geologic record of these organisms. Fluid inclusions in dykes of the Dresser Formation (North Pole area, Pilbara craton, Western Australia) have a content of methane highly depleted in the heavy carbon isotope ^{13}C . This depletion is comparable to that produced by methanogenic prokaryotes, offering a calibration point for the origin of these organisms at a minimum of 3.46 Ga (Baptiste, Brochier, and Boucher 2005; Ueno et al. 2006). A second calibration point is determined by the time of the last ocean-vaporizing event, inferred to have happened at 4.2 (maximum boundary) Ga (Sleep et al. 1989) on the ingroup root node.

Ribosomal RNA (rRNA) data set: The same methods used in the analysis of the protein data set were applied to the ML phylogeny of the combined SSU and LSU rRNA data set.

Habitat

We categorized the different lineages of Terrabacteria (Group-I) based on the ecological habitat of terminal taxa to infer the habitat of the common ancestor of this group (Table S4). Information for families, when available, or single genera was retrieved from the literature (Jackson, Ramaley, and Meinsch 1973; Holt 1984; Mohagheghi et al. 1986; Rao and Kumar 1989; Jensen, Dwight, and Fenical 1991; Takizawa, Colwell, and Hill 1993; Fletchner, Johansen, and Clarck 1998; Silva and Pienaar 1999; Wade et al. 1999; Löffler et al. 2000; Gich, Garcia-Gil, and Overmann 2001; Webster et al. 2001; Fletchner et al. 2002; Hanada et al. 2002; Hentschel et al. 2002; Nakamura et al. 2003; Hugenholtz and Stackebrandt 2004; Leiva et al. 2004; Albuquerque et al. 2005; Cox and Battista 2005; Jimenez, Magos, and Collado-Vides 2005; Montalvo et al. 2005; Pires et al. 2005; Thomas 2005; Beleneva and Zhukova 2006; Costello and Schmidt 2006; Hunter, Mills, and Kostka 2006; Miller et al. 2006; Miroshnichenko and Bonch-Osmolovskaya 2006; Rivera-Aguilar et al. 2006; Taddei et al. 2006; Yamada et al. 2006; Anderson and Haygood 2007; Fermani, Mataloni, and Van de Vijver 2007; Garrity et al. 2007; Gorbushina 2007; Jiang et al. 2007; Jumas-Bilak et al. 2007; Li and Brand 2007; Liang et al. 2007; Moore et al. 2007; Rusch et al. 2007; Zhou et al. 2007; Zvyagintsev et al. 2007). A ML family-level phylogeny for each of the classes Actinobacteria, Cyanobacteria, and *Deinococcus-Thermus* was estimated from an SSU alignment (secondary structure) using one representative per family, when available. One member of each of the other classes in Terrabacteria was used as outgroup. The phylogeny of Chloroflexi used was after Costello and Schmidt (Costello and Schmidt 2006), while Firmicutes were considered at the class level. The habitat assignments of the lineages and of the common ancestor was estimated using MacClade (Maddison and Maddison 1989) (maximum parsimony reconstruction of an unordered character) and Mesquite (Maddison and Maddison 2008) (ML reconstruction, Mk1 model) (Figs. S6 and S7). The ancestral states reconstruction shown by the ML method reflects the uncertainty in reconstructing characters for deep phylogenetic nodes. However, the high probability of a terrestrial ancestry for the last common ancestor of the clade (73% terrestrial, 3% marine) is in agreement with the maximum parsimony analysis.

Environmental distribution of eubacterial species was obtained from culture-independent studies, which were considered to avoid biases introduced by culturing methods. However, these studies present biases as well. In deep sea studies, for example, because it is not possible to identify those species that are metabolically active, it is possible that a fraction of the sampled species is, in reality, surface derived (Lauro and Bartlett 2008). Ranges shown in Table 1 in the main text are the lowest and highest fractions for each group found among all studies and sites for each habitat; only Group-I and Group-II taxa are considered.

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Table S1 List of species of Eubacteria and Archaeobacteria used in the protein data set and their classification (genome accession numbers can be found at <http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi>). Species in bold are the ones used in the final ML data set (218 species). Asterisks denote species used in the Bayesian phylogenetic analysis.

Species name	Classification
EUBACTERIA	
Acinetobacter sp. ADP1 *	Gamma proteobacteria
Agrobacterium tumefaciens str. C58 *	Alpha proteobacteria
Anabaena variabilis ATCC 29413 *	Cyanobacteria
Anaeromyxobacter dehalogenans 2CP-C *	Delta proteobacteria
Anaplasma marginale str. St. Maries	Alpha proteobacteria
Anaplasma phagocytophilum HZ	Alpha proteobacteria
Aquifex aeolicus VF5 *	Aquificae
Aster yellows witches'-broom phytoplasma AYWB *	Firmicutes/Mollicutes
Azoarcus sp. EbN1 *	Beta proteobacteria
Bacillus anthracis str. 'Ames Ancestor' *	Firmicutes/Bacilli
Bacillus anthracis str. Ames	Firmicutes/Bacilli
Bacillus anthracis str. Sterne	Firmicutes/Bacilli
Bacillus cereus ATCC 10987	Firmicutes/Bacilli
Bacillus cereus ATCC 14579	Firmicutes/Bacilli
Bacillus cereus E33L	Firmicutes/Bacilli
Bacillus clausii KSM-K16	Firmicutes/Bacilli
Bacillus halodurans C-125	Firmicutes/Bacilli
Bacillus licheniformis ATCC 14580	Firmicutes/Bacilli
Bacillus subtilis subsp. subtilis str. 168	Firmicutes/Bacilli

Bacillus thuringiensis serovar konkukian str. 97-27	Firmicutes/Bacilli
Bacteroides fragilis NCTC 9343 *	Bacteroidetes
Bacteroides fragilis YCH46	Bacteroidetes
Bacteroides thetaiotaomicron VPI-5482	Bacteroidetes
Bartonella henselae str Houston-1	Alphaproteobacteria
Bartonella quintana str. Toulouse	Alphaproteobacteria
Bdellovibrio bacteriovorus HD100	Deltaproteobacteria
Bifidobacterium longum NCC2705 *	Actinobacteria
Bordetella bronchiseptica RB50	Betaproteobacteria
Bordetella parapertussis 12822	Betaproteobacteria
Bordetella pertussis Tomaha I	Betaproteobacteria
Borrelia burgdorferi B31 *	Spirochaetes
Borrelia garinii Pbi	Spirochaetes
Bradyrhizobium japonicum USDA 110	Alphaproteobacteria
Brucella abortus biovar 1 str. 9-941	Alphaproteobacteria
Brucella melitensis 16M	Alphaproteobacteria
Brucella melitensis biovar Abortus 2308	Alphaproteobacteria
Brucella suis 1330	Alphaproteobacteria
Buchnera aphidicola str. APS	Gammaproteobacteria
Buchnera aphidicola str. Bp	Gammaproteobacteria
Buchnera aphidicola str. Sg	Gammaproteobacteria
Burkholderia mallei ATCC 23344	Betaproteobacteria
Burkholderia pseudomallei 1710b	Betaproteobacteria
Burkholderia pseudomallei K96243	Betaproteobacteria
Burkholderia sp. 383	Betaproteobacteria
Burkholderia thailandensis E264	Betaproteobacteria

Campylobacter jejuni RM1221 *	Epsilonproteobacteria
Campylobacter jejuni subsp. Jejuni NCTC 11168	Epsilonproteobacteria
Candidatus Blochmannia floridanus	Gammaproteobacteria
Candidatus Blochmannia pennsylvanicus str. BPEN	Gammaproteobacteria
Candidatus Pelagibacter ubique HTCC1062	Alphaproteobacteria
Candidatus Protochlamydia amoebophila UWE25	Chlamydiae
Carboxydothemus hydrogenoformans Z-2901	Firmicutes/Clostridia
Caulobacter crescentus CB15	Alphaproteobacteria
Chlamydia muridarum Nigg *	Chlamydiae
Chlamydia trachomatis A/HAR-13	Chlamydiae
Chlamydia trachomatis D/UW-3/CX	Chlamydiae
Chlamydophila abortus S26/3	Chlamydiae
Chlamydophila caviae GPIC	Chlamydiae
Chlamydophila felis Fe/C-56	Chlamydiae
Chlamydophila pneumoniae AR39	Chlamydiae
Chlamydophila pneumoniae CWL029	Chlamydiae
Chlamydophila pneumoniae J138	Chlamydiae
Chlamydophila pneumoniae TW-183	Chlamydiae
Chlorobium chlorochromatii CaD3 *	Chlorobia
Chlorobium tepidum TLS	Chlorobia
Chromobacterium violaceum ATCC 12472	Betaproteobacteria
Clostridium acetobutylicum ATCC 824 *	Firmicutes/Clostridia
Clostridium perfringens str. 13	Firmicutes/Clostridia
Clostridium tetani E88	Firmicutes/Clostridia
Colwellia psychrerythraea 34H	Gammaproteobacteria
Corynebacterium diphtheriae NCTC 13129	Actinobacteria

Corynebacterium efficiens YS-314	Actinobacteria
Corynebacterium glutamicum ATCC 13032	Actinobacteria
Corynebacterium jeikeium K411	Actinobacteria
Coxiella burnetii RSA 493	Gammaproteobacteria
Dechloromonas aromatica RCB	Betaproteobacteria
Dehalococcoides ethenogenes 195 *	Chloroflexi/Dehalococcoidetes
Dehalococcoides sp. CBDB1	Chloroflexi/Dehalococcoidetes
Deinococcus radiodurans R1 *	Deinococci
Desulfitobacterium hafniense Y51	Firmicutes/Clostridia
Desulfotalea psychrophila LSv54	Deltaproteobacteria
Desulfovibrio desulfuricans G20	Deltaproteobacteria
Desulfovibrio vulgaris subsp.vulgaris str. Hildenborough	Deltaproteobacteria
Ehrlichia canis str. Jake	Alphaproteobacteria
Ehrlichia chaffeensis str. Arkansas	Alphaproteobacteria
Ehrlichia ruminantium str. Gardel	Alphaproteobacteria
Ehrlichia ruminantium str. Welgevonden	Alphaproteobacteria
Enterococcus faecalis V583	Firmicutes/Bacilli
Erwinia carotovora subsp. atroseptica SCRI1043	Gammaproteobacteria
Erythrobacter litoralis HTCC2594	Alphaproteobacteria
Escherichia coli CFT073	Gammaproteobacteria
Escherichia coli K12	Gammaproteobacteria
Escherichia coli O157:H7	Gammaproteobacteria
Escherichia coli O157:H7 EDL933	Gammaproteobacteria
Escherichia coli W3110	Gammaproteobacteria
Francisella tularensis subsp. holarctica	Gammaproteobacteria
Francisella tularensis subsp. tularensis SCHU S4	Gammaproteobacteria

Frankia sp. CcI3	Actinobacteria
Fusobacterium nucleatum subsp. nucleatum ATCC 25586 *	Fusobacteria
Geobacillus kaustophilus HTA426	Firmicutes/Bacilli
Geobacter metallireducens GS-15	Deltaproteobacteria
Geobacter sulfurreducens PCA	Deltaproteobacteria
Gloeobacter violaceus PCC 7421	Cyanobacteria
Gluconobacter oxydans 621H	Alphaproteobacteria
Haemophilus ducreyi 35000HP	Gammaproteobacteria
Haemophilus influenzae 86-028NP	Gammaproteobacteria
Haemophilus influenzae Rd KW20	Gammaproteobacteria
Hahella chejuensis KCTC 2396	Gammaproteobacteria
Helicobacter hepaticus ATCC 51449	Epsilonproteobacteria
Helicobacter pylori 26695	Epsilonproteobacteria
Helicobacter pylori J99	Epsilonproteobacteria
Idiomarina loihiensis L2TR	Gammaproteobacteria
Jannaschia sp. CCS1	Alphaproteobacteria
Lactobacillus acidophilus NCFM	Firmicutes/Bacilli
Lactobacillus johnsonii NCC 533	Firmicutes/Bacilli
Lactobacillus plantarum WCFS1	Firmicutes/Bacilli
Lactobacillus sakei subsp. sakei 23K	Firmicutes/Bacilli
Lactococcus lactis subsp. Lactis II1403	Firmicutes/Bacilli
Legionella pneumophila str.Lens	Gammaproteobacteria
Legionella pneumophila str.Paris	Gammaproteobacteria
Legionella pneumophila subsp. pneumophila str. Philadelphia 1	Gammaproteobacteria
Leifsonia xyli subsp. xyli str. CTCB07	Actinobacteria
Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130	Spirochaetes

Leptospira interrogans serovar Lai str. 56601	Spirochaetes
Listeria innocua Clip11262	Firmicutes/Bacilli
Listeria monocytogenes EGD-e	Firmicutes/Bacilli
Listeria monocytogenes str. 4b F2365	Firmicutes/Bacilli
Magnetospirillum magneticum AMB-1	Alphaproteobacteria
Mannheimia succiniciproducens MBEL55E	Gammaproteobacteria
Mesoplasma florum L1	Firmicutes/Mollicutes
Mesorhizobium loti MAFF303099	Alphaproteobacteria
Methylococcus capsulatus str. Bath	Gammaproteobacteria
Moorella thermoacetica ATCC 39073	Firmicutes/Clostridia
Mycobacterium avium subsp. paratuberculosis K-10	Actinobacteria
Mycobacterium bovis AF2122/97	Actinobacteria
Mycobacterium leprae TN	Actinobacteria
Mycobacterium tuberculosis CDC1551	Actinobacteria
Mycobacterium tuberculosis H37Rv	Actinobacteria
Mycoplasma capricolum subsp. capricolum ATCC 27343	Firmicutes/Mollicutes
Mycoplasma gallisepticum R	Firmicutes/Mollicutes
Mycoplasma genitalium G37	Firmicutes/Mollicutes
Mycoplasma hyopneumoniae 232	Firmicutes/Mollicutes
Mycoplasma hyopneumoniae 7448	Firmicutes/Mollicutes
Mycoplasma hyopneumoniae J	Firmicutes/Mollicutes
Mycoplasma mobile 163K	Firmicutes/Mollicutes
Mycoplasma mycoides subsp. Mycoides SC str. PG1	Firmicutes/Mollicutes
Mycoplasma penetrans HF-2	Firmicutes/Mollicutes
Mycoplasma pneumoniae M129	Firmicutes/Mollicutes
Mycoplasma pulmonis UAB CTIP	Firmicutes/Mollicutes

<i>Mycoplasma synoviae</i> 53	Firmicutes/Mollicutes
<i>Neisseria gonorrhoeae</i> FA 1090	Betaproteobacteria
<i>Neisseria meningitidis</i> MC58	Betaproteobacteria
<i>Neisseria meningitidis</i> Z2491	Betaproteobacteria
<i>Neorickettsia sennetsu</i> str. Miyayama	Alphaproteobacteria
<i>Nitrobacter winogradskyi</i> Nb-255	Alphaproteobacteria
<i>Nitrosococcus oceani</i> ATCC 19707	Gammaproteobacteria
<i>Nitrosomonas europaea</i> ATCC 19718	Betaproteobacteria
<i>Nitrospira multiformis</i> ATCC 25196	Betaproteobacteria
<i>Nocardia farcinica</i> IFM 10152	Actinobacteria
<i>Nostoc</i> sp. PCC 7120	Cyanobacteria
<i>Novosphingobium aromaticivorans</i> DSM 12444	Alphaproteobacteria
<i>Oceanobacillus iheyensis</i> HTE831	Firmicutes/Bacilli
Onion yellows phytoplasma OY-M	Firmicutes/Mollicutes
<i>Pasteurella multocida</i> subsp. <i>multocida</i> str. Pm70	Gammaproteobacteria
<i>Pelobacter carbinolicus</i> DSM 2380	Deltaproteobacteria
<i>Pelodictyon luteolum</i> DSM 273	Chlorobia
<i>Photobacterium profundum</i> SS9	Gammaproteobacteria
<i>Photorhabdus luminescens</i> subsp. <i>laumondii</i> TTO1	Gammaproteobacteria
<i>Porphyromonas gingivalis</i> W83	Bacteroidetes
<i>Prochlorococcus marinus</i> str. MIT 9312	Cyanobacteria
<i>Prochlorococcus marinus</i> str. MIT 9313	Cyanobacteria
<i>Prochlorococcus marinus</i> str. NATL2A	Cyanobacteria
<i>Prochlorococcus marinus</i> subsp. <i>marinus</i> str CCMP1375	Cyanobacteria
<i>Prochlorococcus marinus</i> subsp. <i>pastoris</i> str. CCMP1986	Cyanobacteria
<i>Propionibacterium acnes</i> KPA171202	Actinobacteria

Pseudoalteromonas haloplanktis TAC125	Gammaproteobacteria
Pseudomonas aeruginosa PAO1	Gammaproteobacteria
Pseudomonas fluorescens Pf-5	Gammaproteobacteria
Pseudomonas fluorescens PfO-1	Gammaproteobacteria
Pseudomonas putida KT2440	Gammaproteobacteria
Pseudomonas syringae pv. phaseolicola 1448A	Gammaproteobacteria
Pseudomonas syringae pv. syringae B728a	Gammaproteobacteria
Pseudomonas syringae pv. tomato str. DC3000	Gammaproteobacteria
Psychrobacter arcticus 273-4	Gammaproteobacteria
Ralstonia eutropha JMP134	Betaproteobacteria
Ralstonia solanacearum GMI1000	Betaproteobacteria
Rhizobium etli CFN 42	Alphaproteobacteria
Rhodobacter sphaeroides 2.4.1	Alphaproteobacteria
Rhodoferax ferrireducens DSM 15236	Betaproteobacteria
Rhodopirellula baltica SH1 *	Planctomycetacia
Rhodopseudomonas palustris CGA009	Alphaproteobacteria
Rhodopseudomonas palustris HaA2	Alphaproteobacteria
Rhodospirillum rubrum ATCC 11170	Alphaproteobacteria
Rickettsia conorii str. Malish 7	Alphaproteobacteria
Rickettsia felis URRWXCa12	Alphaproteobacteria
Rickettsia prowazekii str. Madrid E	Alphaproteobacteria
Rickettsia typhi str. Wilmington	Alphaproteobacteria
Salinibacter ruber DSM 13855	Bacteroidetes
Salmonella enterica subsp. enterica serovar Choleraesuis str. SC-B67	Gammaproteobacteria
Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150	Gammaproteobacteria
Salmonella enterica subsp. enterica serovar Typhi Ty2	Gammaproteobacteria

Salmonella enterica subsp. enterica serovar Typhi str. CT18	Gammaproteobacteria
Salmonella typhimurium LT2	Gammaproteobacteria
Shewanella oneidensis MR-1	Gammaproteobacteria
Shigella boydii Sb227	Gammaproteobacteria
Shigella dysenteriae Sd197	Gammaproteobacteria
Shigella flexneri 2a str. 2457T	Gammaproteobacteria
Shigella flexneri 2a str. 301	Gammaproteobacteria
Shigella sonnei Ss046	Gammaproteobacteria
Silicibacter pomeroyi DSS-3	Alphaproteobacteria
Sinorhizobium meliloti 1021	Alphaproteobacteria
Sodalis glossinidius str. 'morsitans'	Gammaproteobacteria
Solibacter usitatus Ellin6076 *	Acidobacteria/Solibacteres
Staphylococcus aureus RF122	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus COL	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus MRSA252	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus MSSA476	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus MW2	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus Mu50	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus N315	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus NCTC 8325	Firmicutes/Bacilli
Staphylococcus aureus subsp. aureus USA300	Firmicutes/Bacilli
Staphylococcus epidermidis ATCC 12228	Firmicutes/Bacilli
Staphylococcus epidermidis RP62A	Firmicutes/Bacilli
Staphylococcus haemolyticus JCSC1435	Firmicutes/Bacilli
Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305	Firmicutes/Bacilli
Streptococcus agalactiae 2603V/R	Firmicutes/Bacilli

Streptococcus agalactiae A909	Firmicutes/Bacilli
Streptococcus agalactiae NEM316	Firmicutes/Bacilli
Streptococcus mutans UA159	Firmicutes/Bacilli
Streptococcus pneumoniae R6	Firmicutes/Bacilli
Streptococcus pneumoniae TIGR4	Firmicutes/Bacilli
Streptococcus pyogenes M1 GAS	Firmicutes/Bacilli
Streptococcus pyogenes MGAS10394	Firmicutes/Bacilli
Streptococcus pyogenes MGAS315	Firmicutes/Bacilli
Streptococcus pyogenes MGAS5005	Firmicutes/Bacilli
Streptococcus pyogenes MGAS6180	Firmicutes/Bacilli
Streptococcus pyogenes MGAS8232	Firmicutes/Bacilli
Streptococcus pyogenes SSI-1	Firmicutes/Bacilli
Streptococcus thermophilus CNRZ1066	Firmicutes/Bacilli
Streptococcus thermophilus LMG 18311	Firmicutes/Bacilli
Streptomyces avermitilis MA-4680	Actinobacteria
Streptomyces coelicolor A3 (2)	Actinobacteria
Symbiobacterium thermophilum IAM 14863	Actinobacteria
Synechococcus elongatus PCC 6301	Cyanobacteria
Synechococcus elongatus PCC 7942	Cyanobacteria
Synechococcus sp. CC9605	Cyanobacteria
Synechococcus sp. CC9902	Cyanobacteria
Synechococcus sp. JA-2-3B'a (2-13)	Cyanobacteria
Synechococcus sp. JA-3-3Ab	Cyanobacteria
Synechococcus sp. WH 8102	Cyanobacteria
Synechocystis sp. PCC 6803	Cyanobacteria
Thermoanaerobacter tengcongensis MB4	Firmicutes/Clostridia

Thermobifida fusca YX	Actinobacteria
Thermosynechococcus elongatus BP-1	Cyanobacteria
Thermotoga maritima MSB8 *	Thermotogae
Thermus thermophilus HB27	Deinococci
Thermus thermophilus HB8	Deinococci
Thiobacillus denitrificans ATCC 25259	Betaproteobacteria
Thiomicrospira crunogena XCL-2	Gammaproteobacteria
Thiomicrospira denitrificans ATCC 33889	Epsilonproteobacteria
Treponema denticola ATCC 35405	Spirochaetes
Treponema pallidum subsp. pallidum str. Nichols	Spirochaetes
Tropheryma whipplei TW08/27	Actinobacteria
Ureaplasma parvum serovar 3 str. ATCC 700970	Firmicutes/Mollicutes
Vibrio cholerae O1 biovar eltor str. N16961	Gammaproteobacteria
Vibrio fischeri ES114	Gammaproteobacteria
Vibrio parahaemolyticus RIMD 2210633	Gammaproteobacteria
Vibrio vulnificus CMCP6	Gammaproteobacteria
Vibrio vulnificus YJ016	Gammaproteobacteria
Wigglesworthia glossinidia endosymbiont of Glossina brevipalpis	Gammaproteobacteria
Wolbachia	Alphaproteobacteria
Wolinella succinogenes DSM 1740	Epsilonproteobacteria
Xanthomonas axonopodis pv. citri str. 306	Gammaproteobacteria
Xanthomonas campestris pv. campestris str. 8004	Gammaproteobacteria
Xanthomonas campestris pv. campestris str. ATCC 33913	Gammaproteobacteria
Xanthomonas campestris pv. vesicatoria str. 85-10	Gammaproteobacteria
Xanthomonas oryzae pv. oryzae KACC10331	Gammaproteobacteria
Xylella fastidiosa 9a5c	Gammaproteobacteria

Xylella fastidiosa Temecula1	Gammaproteobacteria
Yersinia pestis CO92	Gammaproteobacteria
Yersinia pestis KIM	Gammaproteobacteria
Yersinia pestis biovar Medievalis str. 91001	Gammaproteobacteria
Yersinia pseudotuberculosis IP 32953	Gammaproteobacteria
Zymomonas mobilis subsp. Mobilis ZM4	Alphaproteobacteria
ARCHAEBACTERIA	
Aeropyrum pernix K1	Crenarchaeota/Thermoprotei
Archaeoglobus fulgidus DSM 4304 *	Euryarchaeota/Archaeoglobi
Haloarcula marismortui ATCC 43049 *	Euryarchaeota/Halobacteria
Halobacterium sp. NRC-1	Euryarchaeota/Halobacteria
Methanocaldococcus jannaschii DSM 2661 *	Euryarchaeota/Methanococci
Methanococcus maripaludis S2	Euryarchaeota/Methanococci
Methanopyrus kandleri AV19 *	Euryarchaeota/Methanopyri
Methanosarcina acetivorans C2A	Euryarchaeota/Methanomicrobia
Methanosarcina barkeri str. Fusaro	Euryarchaeota/Methanomicrobia
Methanosarcina mazei Go1 *	Euryarchaeota/Methanomicrobia
Methanosphaera stadmanae DSM 3091 *	Euryarchaeota/Methanobacteria
Methanospirillum hungatei JF-1	Euryarchaeota/Methanomicrobia
Methanothermobacter thermoautotrophicus str. Delta H	Euryarchaeota/Methanobacteria
Nanoarchaeum equitans Kin4-M *	Nanoarchaeota
Natronomonas pharaonis DSM 2160	Euryarchaeota/Halobacteria
Picrophilus torridus DSM 9790 *	Euryarchaeota/Thermoplasmata
Pyrobaculum aerophilum str. IM2	Crenarchaeota/Thermococci
Pyrococcus abyssi GE5 *	Euryarchaeota/Thermococci

Pyrococcus furiosus DSM 3638	Euryarchaeota/Thermococci
Pyrococcus horikoshii OT3	Euryarchaeota/Thermococci
Sulfolobus acidocaldarius DSM 639	Crenarchaeota/Thermoprotei
Sulfolobus solfataricus P2 *	Crenarchaeota/Thermoprotei
Sulfolobus tokodaii str. 7	Crenarchaeota/Thermoprotei
Thermococcus kodakarensis KOD1	Euryarchaeota/Thermococci
Thermoplasma acidophilum DSM 1728	Euryarchaeota/Thermoplasmata
Thermoplasma volcanium GSS1	Euryarchaeota/Thermoplasmata

Table S2 List of Eubacteria and Archaeobacteria species used in the ribosomal RNA data set (shared by SSU and LSU) and their classification. Species used in the Bayesian analysis are marked with an asterisk.

Species	Classification
EUBACTERIA	
Acetobacter europaeus AJ012698 *	Alphaproteobacteria
Acetobacter intermedius AJ012697	Alphaproteobacteria
Acetobacter xylinum X75619	Alphaproteobacteria
Acinetobacter calcoaceticus M34139 *	Gammaproteobacteria
Aeromonas hydrophila AF099021	Gammaproteobacteria
Agrobacterium radiobacter AJ130719	Alphaproteobacteria
Agrobacterium rubi D12787	Alphaproteobacteria
Agrobacterium tumefaciens D12784	Alphaproteobacteria
Agrobacterium vitis D12795	Alphaproteobacteria
Alcaligenes faecalis AF155147 *	Betaproteobacteria
Aquifex aeolicus AE000751 *	Aquificae
Bacillus alcalophilus AF078812 *	Firmicutes/Bacilli
Bacillus anthracis AF155951	Firmicutes/Bacilli
Bacillus cereus AF155952	Firmicutes/Bacilli
Bacillus globisporus X68415	Firmicutes/Bacilli
Bacillus halodurans D AP001507	Firmicutes/Bacilli
Bacillus licheniformis AF234844	Firmicutes/Bacilli
Bacillus stearothermophilus AJ005760	Firmicutes/Bacilli
Bacillus subtilis B K00637	Firmicutes/Bacilli
Bacillus thuringiensis AF155954	Firmicutes/Bacilli

Bartonella bacilliformis M65249	Alphaproteobacteria
Bordetella avium AF177666	Betaproteobacteria
Bordetella bronchiseptica U04948	Betaproteobacteria
Bordetella parapertussis U04949	Betaproteobacteria
Bordetella pertussis AF142326	Betaproteobacteria
Borrelia burgdorferi X85202 *	Spirochaetes
Bradyrhizobium japonicum Z35330	Alphaproteobacteria
Bradyrhizobium lupini U69636	Alphaproteobacteria
Brevundimonas diminuta AB021415	Alphaproteobacteria
Brucella melitensis AF220148	Alphaproteobacteria
Buchnera aphidicola L18927	Gammaproteobacteria
Burkholderia gladioli AB012916	Betaproteobacteria
Burkholderia mallei AF110187	Betaproteobacteria
Burkholderia pseudomallei	Betaproteobacteria
Campylobacter coli L04312 *	Epsilonproteobacteria
Campylobacter hyoilei L19738	Epsilonproteobacteria
Campylobacter jejuni AL139074	Epsilonproteobacteria
Campylobacter lari L04316	Epsilonproteobacteria
Carsonella ruddii AF211123	Gammaproteobacteria
Chlamydia muridarum aA16S AE002280 *	Chlamydiae
Chlamydia trachomatis AE001347	Chlamydiae
Chlamydophila abortus U76710	Chlamydiae
Chlamydophila felis U68457	Chlamydiae
Chlamydophila pecorum U68434	Chlamydiae
Chlamydophila pneumoniae aA16S AE002256	Chlamydiae
Chlamydophila psittaci U68447	Chlamydiae
Chlorobium limicola Y10640 *	Chlorobia

Citrobacter freundii AJ233408	Gammaproteobacteria
Clostridium botulinum A L37586 *	Firmicutes/Clostridia
Clostridium histolyticum M59094	Firmicutes/Clostridia
Clostridium tyrobutyricum L08062	Firmicutes/Clostridia
Coxiella burnetii D89791	Gammaproteobacteria
Enterococcus faecalis AB012212	Firmicutes/Bacilli
Erysipelothrix rhusiopathiae AB034200 *	Firmicutes/Mollicutes
Erysipelothrix tonsillarum AB034201	Firmicutes/Mollicutes
Escherichia coli B AE000471	Gammaproteobacteria
Fibrobacter succinogenes M62683 *	Fibrobacteres
Flavobacterium odoratum D14019 *	Bacteroidetes/Flavobacteria
Flexibacter flexilis M62794 *	Bacteroidetes/Sphingobacteria
Frankia sp. M55343 *	Actinobacteria
Haemophilus influenzae D U32847	Gammaproteobacteria
Helicobacter pylori A AE000620	Epsilonproteobacteria
Klebsiella pneumoniae AB004753	Gammaproteobacteria
Lactobacillus amylolyticus Y17361	Firmicutes/Bacilli
Lactobacillus confusus M23036	Firmicutes/Bacilli
Lactobacillus delbrueckii AB007908	Firmicutes/Bacilli
Lactococcus lactis X64887	Firmicutes/Bacilli
Leptospira interrogans M71241	Spirochaetes
Leuconostoc carnosum AB022925	Firmicutes/Bacilli
Leuconostoc lactis M23031	Firmicutes/Bacilli
Leuconostoc mesenteroides AB023243	Firmicutes/Bacilli
Leuconostoc oenos M35820	Firmicutes/Bacilli
Leuconostoc paramesenteroides M23033	Firmicutes/Bacilli
Leucothrix mucor X87277	Gammaproteobacteria

Listeria grayi X56150	Firmicutes/Bacilli
Listeria innocua S55473	Firmicutes/Bacilli
Listeria ivanovii X98529	Firmicutes/Bacilli
Listeria monocytogenes U84150	Firmicutes/Bacilli
Listeria murrayi X56154	Firmicutes/Bacilli
Listeria seeligeri X56148	Firmicutes/Bacilli
Listeria welshimeri X56149	Firmicutes/Bacilli
Microbispora bispora U58524	Actinobacteria
Micrococcus luteus AF234843	Actinobacteria
Mycobacterium avium M29573	Actinobacteria
Mycobacterium kansasii M29575	Actinobacteria
Mycobacterium leprae X55022	Actinobacteria
Mycobacterium paratuberculosis M61680	Actinobacteria
Mycobacterium phlei M29566	Actinobacteria
Mycobacterium smegmatis AJ131761	Actinobacteria
Mycobacterium tuberculosis X55588	Actinobacteria
Mycoplasma flocculare X63377	Firmicutes/Mollicutes
Mycoplasma gallisepticum L08897	Firmicutes/Mollicutes
Mycoplasma genitalium A16S U39694	Firmicutes/Mollicutes
Mycoplasma hyopneumoniae Y00149	Firmicutes/Mollicutes
Nannocystis exedens AJ233946*	Deltaproteobacteria
Neisseria gonorrhoeae AF146369	Betaproteobacteria
Neisseria meningitidis AF059671	Betaproteobacteria
Paracoccus denitrificans AJ288159	Alphaproteobacteria
Peptococcus niger X55797	Firmicutes/Clostridia
Pirellula marina X62912 *	Planctomycetacia
Plesiomonas shigelloides M59159	Gammaproteobacteria

Propionibacterium freudenreichi AJ009989	Actinobacteria
Pseudomonas aeruginosa AF023658	Gammaproteobacteria
Pseudomonas fluorescens AF068010	Gammaproteobacteria
Pseudomonas stutzeri AF038653	Gammaproteobacteria
Ralstonia pickettii AB004790	Betaproteobacteria
Ralstonia solanacearum AB024604	Betaproteobacteria
Renibacterium salmoninarum AB017538	Actinobacteria
Rhizobium galegae AF025853	Alphaproteobacteria
Rhizobium leguminosarum D12782	Alphaproteobacteria
Rhizobium tropici D11344	Alphaproteobacteria
Rhodobacter capsulatus D13474	Alphaproteobacteria
Rhodobacter sphaeroides B X53854	Alphaproteobacteria
Rhodococcus erythropolis AJ237967	Actinobacteria
Rhodococcus fascians X81932	Actinobacteria
Rhodopseudomonas palustris AB017261	Alphaproteobacteria
Rhodospirillum rubrum D30778	Alphaproteobacteria
Rickettsia akari L36099	Alphaproteobacteria
Rickettsia australis L36101	Alphaproteobacteria
Rickettsia bellii L36103	Alphaproteobacteria
Rickettsia canada L36104	Alphaproteobacteria
Rickettsia conorii L36105	Alphaproteobacteria
Rickettsia parkeri L36673	Alphaproteobacteria
Rickettsia prowazekii AJ235272	Alphaproteobacteria
Rickettsia rhipicephali L36216	Alphaproteobacteria
Rickettsia rickettsii U11021	Alphaproteobacteria
Rickettsia sibirica D38628	Alphaproteobacteria
Rickettsia typhi L36221	Alphaproteobacteria

Ruminobacter amylophilus AB004908	Gammaproteobacteria
Salmonella typhi U88545	Gammaproteobacteria
Serpulina hyodysenteriae U14931	Spirochaetes
Serpulina innocens U14924	Spirochaetes
Simkania negevensis U68460	Chlamydiae
Staphylococcus aureus AF076030	Firmicutes/Bacilli
Staphylococcus carnosus AB009934	Firmicutes/Bacilli
Staphylococcus condimenti Y15750	Firmicutes/Bacilli
Staphylococcus piscifermentans Y15754	Firmicutes/Bacilli
Stigmatella aurantiaca AJ233935	Deltaproteobacteria
Streptococcus macedonicus Z94012	Firmicutes/Bacilli
Streptococcus oralis S70359	Firmicutes/Bacilli
Streptococcus parauberis X89967	Firmicutes/Bacilli
Streptococcus thermophilus X59028	Firmicutes/Bacilli
Streptococcus uberis AB002527	Firmicutes/Bacilli
Streptomyces ambofaciens M27245	Actinobacteria
Streptomyces coelicolor A AL356612	Actinobacteria
Streptomyces griseus B AB030568	Actinobacteria
Streptomyces lividans AB037565	Actinobacteria
Streptomyces rimosus F X62884	Actinobacteria
Synechocystis sp. D64000 *	Cyanobacteria
Thermomonospora chromogena AF002261	Actinobacteria
Thermotoga maritima aA16S AE001703 *	Thermotogae
Thermus thermophilus L09659 *	Deinococcus-Thermus
Treponema pallidum AE001208	Spirochaetes
Tropheryma whippelii AF190688	Actinobacteria
Ureaplasma urealyticum AE002127	Firmicutes/Mollicutes

<i>Vibrio cholerae</i> AE004096	Gammaproteobacteria
<i>Vibrio vulnificus</i> X56582	Gammaproteobacteria
<i>Waddlia chondrophila</i> AF042496	Chlamydiae
<i>Wolbachia pipientis</i> AF179630	Alphaproteobacteria
<i>Wolinella succinogenes</i> M26636	Epsilonproteobacteria
<i>Xylella fastidiosa</i> aA16S AE003870	Gammaproteobacteria
<i>Yersinia enterocolitica</i> M59292	Gammaproteobacteria
<i>Zoogloea ramigera</i> D14254	Betaproteobacteria
<i>Zymobacter palmae</i> AF211871	Gammaproteobacteria
<i>Zymomonas mobilis</i> C AF117351	Alphaproteobacteria
ARCHAEBACTERIA	
<i>Aeropyrum pernix</i> AB019552 *	Crenarchaeota/Thermoprotei
<i>Archaeoglobus fulgidus</i> AE000965 *	Euryarchaeota/Archaeoglobi
<i>Desulfurococcus mobilis</i> M36474	Crenarchaeota/Thermoprotei
<i>Haloarcula marismortui</i> AF034620 *	Euryarchaeota/Halobacteria
<i>Halobacterium halobium</i> AJ002949	Euryarchaeota/Halobacteria
<i>Halobacterium marismortui</i> X61689	Euryarchaeota/Halobacteria
<i>Halococcus morrhuae</i> D11106	Euryarchaeota/Halobacteria
<i>Haloferax mediterranei</i> D11107	Euryarchaeota/Halobacteria
<i>Methanobacterium thermoautotrop</i> AE000940 *	Euryarchaeota/Methanobacteria
<i>Methanococcus jannaschii</i> B U67517 *	Euryarchaeota/Methanococci
<i>Methanococcus vanniellii</i> M36507	Euryarchaeota/Methanococci
<i>Methanopyrus kandleri</i> *	Euryarchaeota/Methanopyri
<i>Methanospirillum hungatei</i> M60880 *	Euryarchaeota/Methanomicrobia
<i>Nanoarchaeum equitans</i> *	Nanoarchaeota
<i>Natronobacterium magadii</i> X72495	Euryarchaeota/Halobacteria

Pyrobaculum islandicum L07511	Crenarchaeota/Thermoprotei
Pyrococcus abyssi AJ248283 *	Euryarchaeota/Thermococci
Pyrococcus horikoshii AP000001	Euryarchaeota/Thermococci
Sulfolobus acidocaldarius U05018	Crenarchaeota/Thermoprotei
Sulfolobus shibatae M32504	Crenarchaeota/Thermoprotei
Sulfolobus solfataricus X90483	Crenarchaeota/Thermoprotei
Thermococcus celer M21529	Euryarchaeota/Thermococci
Thermofilum pendens X14835	Crenarchaeota/Thermoprotei
Thermoplasma acidophilum M38637 *	Euryarchaeota/Thermoplasmata

Table S3 Total number of species per group (source: DSMZ, NCBI, Algaebase). P: phylum; C. Class.

EUBACTERIA	Total number of species
Acidobacteria (p)	4
Actinobacteria (p, c)	1784
Alphaproteobacteria (c)	711
Aquificae (p, c)	22
Bacilli (c)	845
Bacteroidetes (p)	493
Betaproteobacteria (c)	373
Chlamydiae (p, c)	13
Chlorobia (p, c)	17
Chloroflexi (p)	45
Clostridia (c)	578
Cyanobacteria (p)	2654
Deinococci (c)	45
Deltaproteobacteria (c)	226
Epsilonproteobacteria (c)	77
Fibrobacteres (p, c)	2
Fusobacteria (p, c)	37
Gammaproteobacteria (c)	1177
Mollicutes (c)	204
Planctomycetes (p)	12
Spirochaetes (p, c)	98
Thermolithobacteria (c)	2
Thermotogae (p, c)	30

ARCHAEBACTERIA

Archaeoglobi (c)	5
Halobacteria (c)	82
Methanobacteria (c)	37
Methanococci (c)	13
Methanomicrobia (c)	61
Methanopyri (c)	1
Nanoarchaeota (p)	1
Thermococci (c)	33
Thermoplasmata (c)	5
Thermoprotei (c)	53

Table S4 Habitat preference of families in Group-I phyla. Symbols: t, terrestrial; m, marine; m/t, marine and terrestrial. Bacilli, Clostridia, and Mollicutes are treated at the class level and have been conservatively coded as m/t (most classes within Clostridia and Mollicutes are strictly terrestrial while Bacilli colonize both habitats).

Phylum	Family	Habitat
Actinobacteria	Acidimicrobiaceae	m/t
	Acidothermaceae	t
	Actinomycetaceae	t
	Actinospicaceae	t
	Actinosynnemataceae	t
	Beutenbergiaceae	t
	Bogoriellaceae	t
	Brevibacteriaceae	t
	Catenulisporaceae	t
	Corynebacteriaceae	t
	Dermabacteraceae	t
	Dermacoccaceae	m/t
	Dermatophilaceae	t
	Dietziaceae	t
	Frankiaceae	t
	Geodermatophilaceae	t
	Glycomycetaceae	t
	Gordoniaceae	t
	Intrasporangiaceae	m/t
	Jonesiaceae	t
Kineosporiaceae	m/t	
Microbacteriaceae	m/t	

	Micrococcaceae	m/t
	Micromonosporaceae	m/t
	Mycobacteriaceae	t
	Nakamurellaceae	t
	Nocardiaceae	m/t
	Nocardiodiaceae	m/t
	Promicromonosporaceae	m/t
	Propionibacteriaceae	m/t
	Pseudonocardiaceae	t
	Rarobacteraceae	t
	Sanguibacteraceae	t
	Segniliparaceae	t
	Sporichthyaceae	t
	Streptomycetaceae	m/t
	Streptosporangiaceae	t
	Thermomonosporaceae	t
	Tsukamurellaceae	m/t
	Williamsiaceae	m/t
	Yaniaceae	t
	Bifidobacteriaceae	t
	Coriobacteriaceae	t
	Conexibacteraceae	t
Actinobacteria	Patulibacteraceae	t
	Rubrobacteraceae	t
	Solirubrobacteraceae	t
	Thermoleophilaceae	t
Bacilli		m/t

	Chloroflexaceae	m/t
	Herpetosiphonaceae	t
	Thermomicrobiaceae	t
Chloroflexi	Sphaerobacteraceae	t
	<i>Dehalococcoides</i>	t
	Anaerolinaceae	t
	Caldilineaceae	t
Clostridia		m/t
	Chroococcaceae	m/t
	Cyanobacteriaceae	m/t
	Dermocarpellaceae	m
	Entophysalidaceae	m/t
	Gloeobacteraceae	t
	Hydrococcaceae	m
	Microcystaceae	m/t
	Prochloraceae	m
Cyanobacteria	Xenococcaceae	m/t
	Chlorogloeopsidaceae	t
	Hapalosiphonaceae	t
	Microchaetaceae	m/t
	Nostocaceae	m/t
	Rivulariaceae	m/t
	Scytonemataceae	m/t
	Stigonemataceae	t
	Symphyonemataceae	m/t
	Oscillatoriaceae	m/t
	Phormidiaceae	m/t

	Schizotrichaceae	t
	Pseudanabenaceae	m/t
	Mastigocladaceae	t
	Chamaesiphonaceae	m/t
	Merismopediaceae	m/t
	Synechococcaceae	m
	Deinococcaceae	t
<i>Deinococcus-Thermus</i>	Trueperaceae	t
	Thermaceae	m/t
Mollicutes		m/t

Fig. S1 Effects of increasing GBLOCKS (panels A) and SF (panels B) stringencies on the phylogeny of the protein and rRNA data set. Diamonds: number of monophyletic eubacterial classes; Squares: number of significantly supported monophyletic classes; Triangles: number of monophyletic eubacterial phyla. Black rectangles show the selected stringency level.

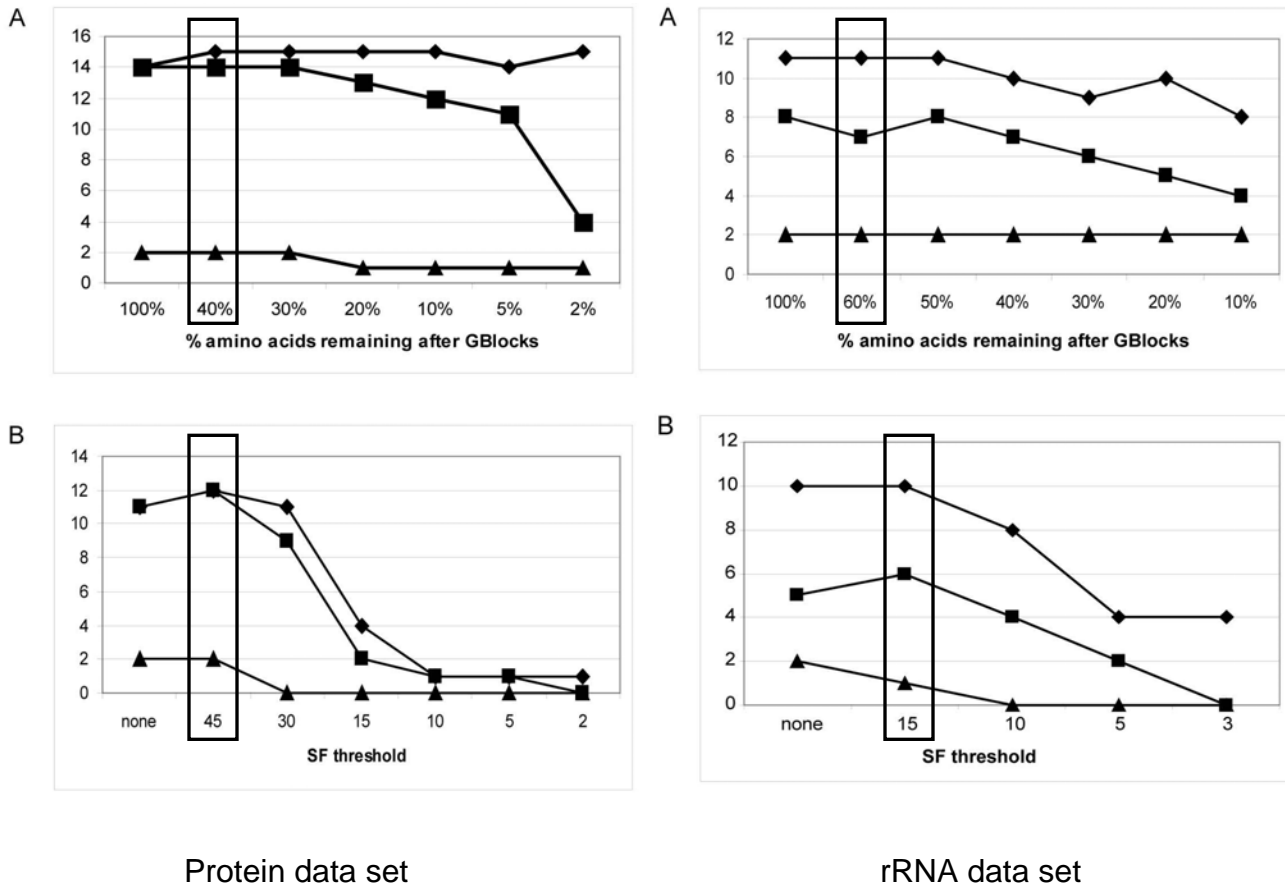


Fig. S2 Consensus of 25 single ML gene trees from the protein data set. Triangles are proportional to the number of sequences analyzed in each class. Numbers represent the percentage of genes supporting the cluster.

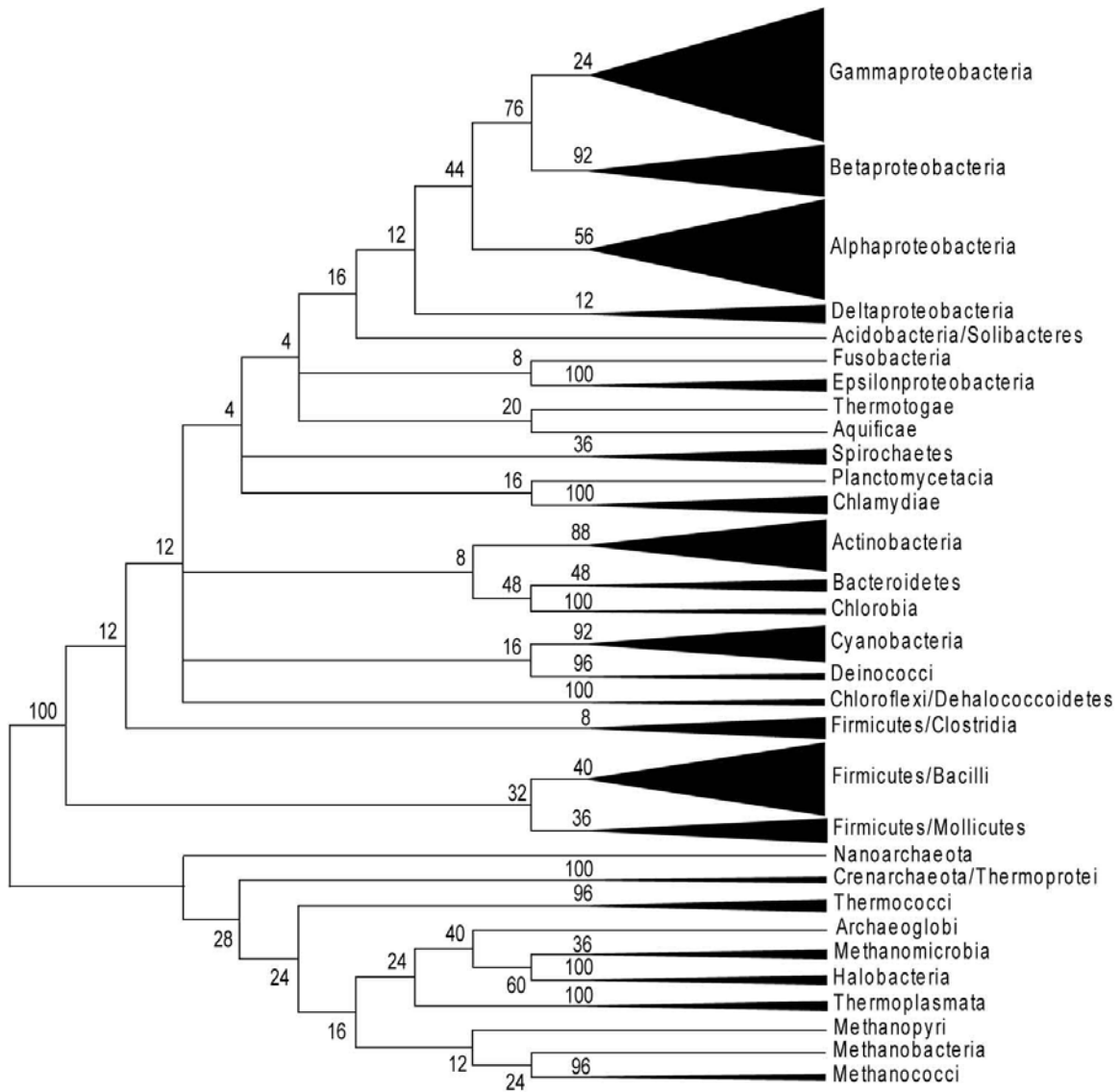


Fig. S3 Maximum likelihood phylogeny of slow evolving sites in the protein data set (Eubacteria and Archaeobacteria). Asterisks: bootstrap values equal to or higher than 95%. Triangles are proportional to the number of sequences analyzed in each lineage. Values at each node are for 100 bootstrap replicates.

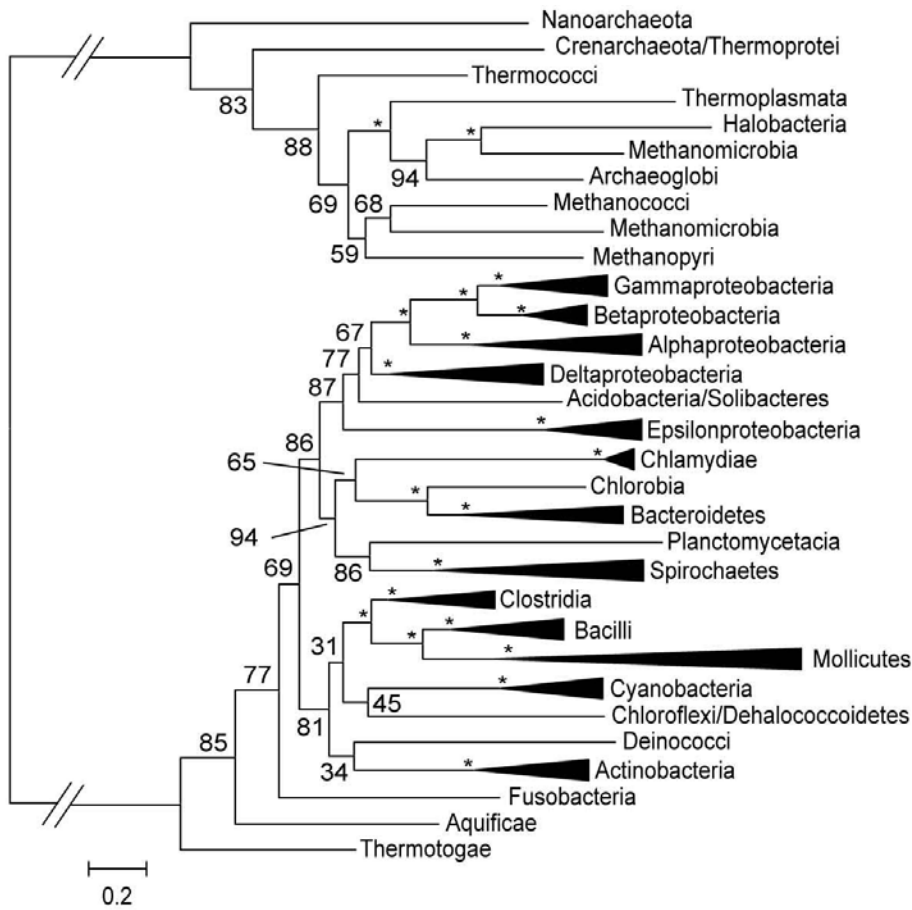


Fig. S4 LogDet phylogeny of rRNA (SSU+LSU) data set. Triangles are proportional to the number of sequences analyzed in each lineage. Values at each node are percentage support for 100 bootstrap replicates.

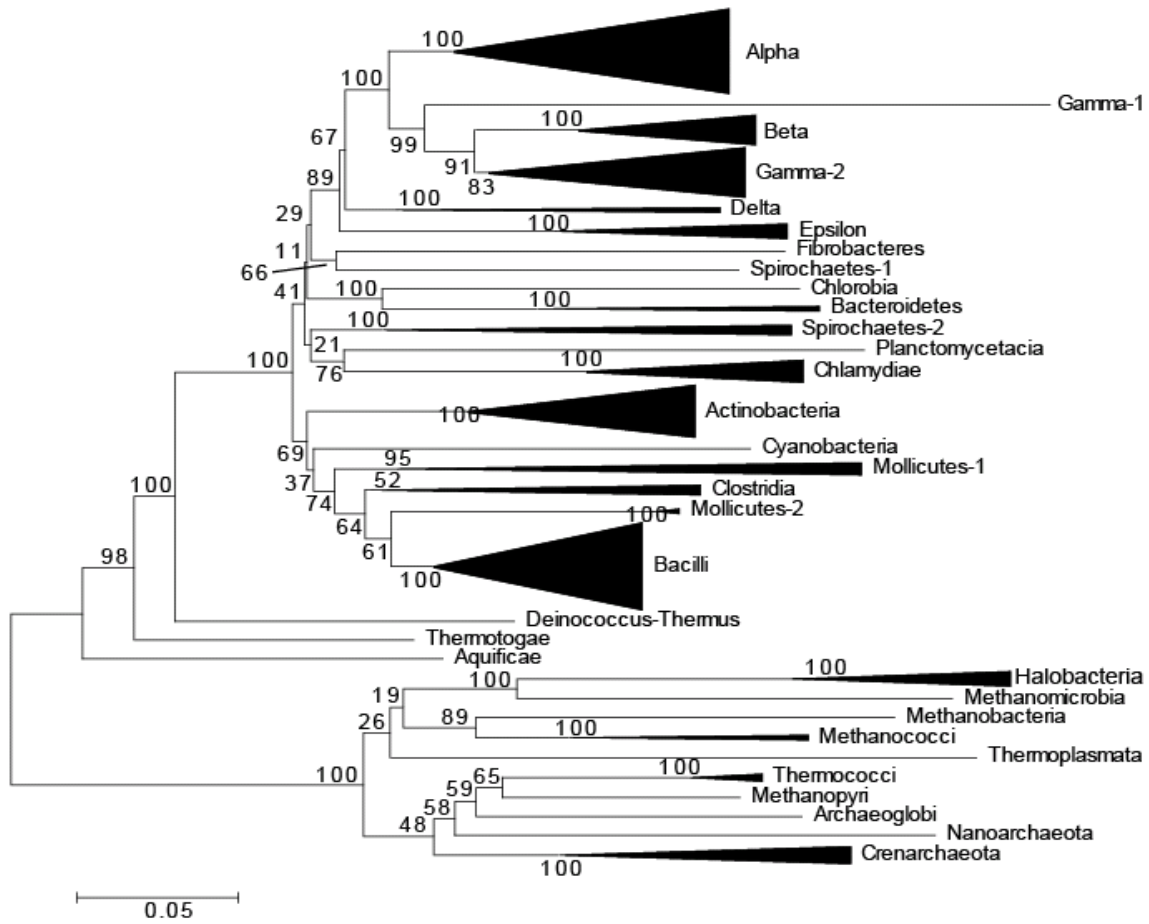


Fig. S5 Maximum likelihood phylogeny of slow evolving sites in the rRNA (SSU+LSU) data set (Eubacteria and Archaeobacteria). Asterisks: bootstrap values equal to or higher than 95%. Triangles are proportional to the number of sequences analyzed in each lineage. Values at each node are for 100 bootstrap replicates.

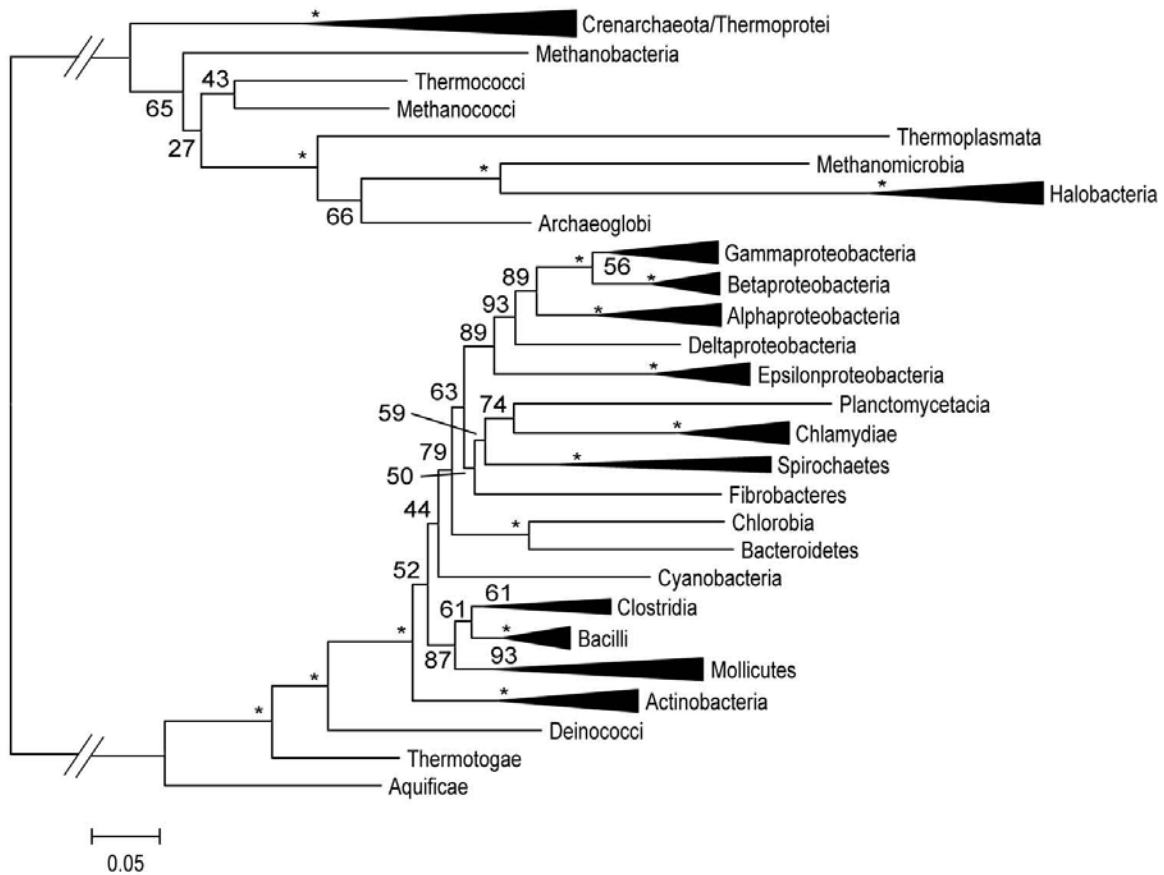


Fig. S6 Maximum parsimony ancestral states reconstruction in major lineages of Terrabacteria (Group-1). Terrestrial states (species) are shown in tan and marine states in blue; dashed lines indicate lineages in which there is at least one terrestrial and one marine species. The phylum-level topology of the tree and relationships within Firmicutes are from the ML protein analysis whereas the topology within other phyla (Actinobacteria, *Deinococcus-Thermus*, and Cyanobacteria) is from the ML SSU rRNA analysis. The phylogeny within Chloroflexi is from elsewhere (Costello and Schmidt 2006). The branch leading to Firmicutes is either terrestrial or mixed (assigned here conservatively as mixed). Each phylum is represented at the lowest determinable monophyletic taxonomic level beginning with family. Therefore, within a phylum if orders were not monophyletic then families were used; orders were used if they were monophyletic. Firmicutes are represented at the class level as in the protein data set.

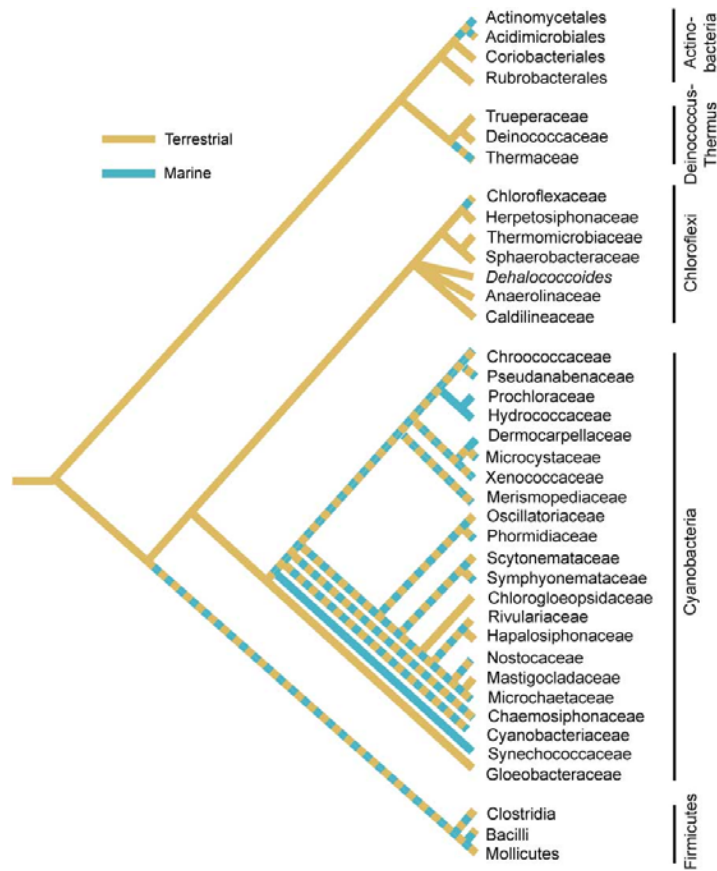


Fig. S7 Maximum likelihood ancestral states reconstruction of Terrabacteria (Group-I) lineages. Phylogenetic details are as in Fig. S6. Terrestrial state is shown in tan, marine state in blue, mixed state in gray. Probabilities of each state in the last common ancestor of the group are: 73% terrestrial, 24% mixed, and 3% marine.

