

Supplementary Material
A major clade of prokaryotes with ancient adaptations to life on land
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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 MIX JTT+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 Phylib 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). Archaeabacteria 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 Archaeabacteria, 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 archaeabacteria). Two sequences for archaeabacteria, *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. Archaeabacteria 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 Archaeabacteria was chosen for a total of 21 ingroup eubacterial species and ten ingroup archaeabacterial 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 archaeabacterial 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 Archaeabacteria 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; Loffler 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 Archaeabacteria 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 * | Gammaproteobacteria |
| Agrobacterium tumefaciens str. C58 * | Alphaproteobacteria |
| Anabaena variabilis ATCC 29413 * | Cyanobacteria |
| Anaeromyxobacter dehalogenans 2CP-C * | Deltaproteobacteria |
| Anaplasma marginale str. St. Maries | Alphaproteobacteria |
| Anaplasma phagocytophilum HZ | Alphaproteobacteria |
| Aquifex aeolicus VF5 * | Aquificae |
| Aster yellows witches'-broom phytoplasma AYWB * | Firmicutes/Mollicutes |
| Azoarcus sp. EbN1 * | Betaproteobacteria |
| 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 |

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| 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 |

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| 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 |
| Carboxydothermus 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 |

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| 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. holaretica | Gammaproteobacteria |
| Francisella tularensis subsp. tularensis SCHU S4 | Gammaproteobacteria |

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|---|-----------------------|
| Frankia sp. CcI3 | Actinobacteria |
| Fusobacterium nucleatum subsp. nucleatum ATCC 25586 * | Fusobacteria |
| Geobacillus kaustophilus HTA426 | Firmicutes/Bacilli |
| Geobacter metallireducens GS-15 | Delta proteobacteria |
| Geobacter sulfurreducens PCA | Delta proteobacteria |
| 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 |

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

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

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| 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 |

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| <i>Salmonella enterica</i> subsp. <i>enterica</i> 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 |

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

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|---|-----------------------|
| 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 |

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

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

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

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

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

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

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

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|---|-------------------------------|
| <i>Vibrio cholerae</i> AE004096 | Gammaproteobacteria |
| <i>Vibrio vulnificus</i> X56582 | Gammaproteobacteria |
| <i>Waddlia chondrophila</i> AF042496 | Chlamydiae |
| <i>Wolbachia pipiensis</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 |
| ARCHAEABACTERIA | |
| <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 vannielii</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 |

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|--|------------------------------|
| <i>Pyrobaculum islandicum</i> L07511 | Crenarchaeota/Thermoprotei |
| <i>Pyrococcus abyssi</i> AJ248283 * | Euryarchaeota/Thermococci |
| <i>Pyrococcus horikoshii</i> AP000001 | Euryarchaeota/Thermococci |
| <i>Sulfolobus acidocaldarius</i> U05018 | Crenarchaeota/Thermoprotei |
| <i>Sulfolobus shibatae</i> M32504 | Crenarchaeota/Thermoprotei |
| <i>Sulfolobus solfataricus</i> X90483 | Crenarchaeota/Thermoprotei |
| <i>Thermococcus celer</i> M21529 | Euryarchaeota/Thermococci |
| <i>Thermofilum pendens</i> X14835 | Crenarchaeota/Thermoprotei |
| <i>Thermoplasma acidophilum</i> 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 |
| | Nocardioidaceae | 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 |
| | | m/t |
| | | m/t |
| Clostridia | Chroococcaceae | m/t |
| | Cyanobacteriaceae | m/t |
| | Dermocarpellaceae | m |
| | Entophysalidaceae | m/t |
| | Gloeobacteraceae | t |
| | Hydrococcaceae | m |
| | Microcystaceae | m/t |
| | Prochloraceae | m |
| | Xenococcaceae | m/t |
| | Chlorogloeopsidaceae | t |
| | Hapalosiphonaceae | t |
| | Microchaetaceae | m/t |
| | Nostocaceae | m/t |
| Cyanobacteria | Rivulariaceae | m/t |
| | Scytonemataceae | m/t |
| | Stigonemataceae | t |
| | Sympytonemataceae | m/t |
| | Oscillatoriaceae | m/t |
| | Phormidiaceae | m/t |
| | | 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.

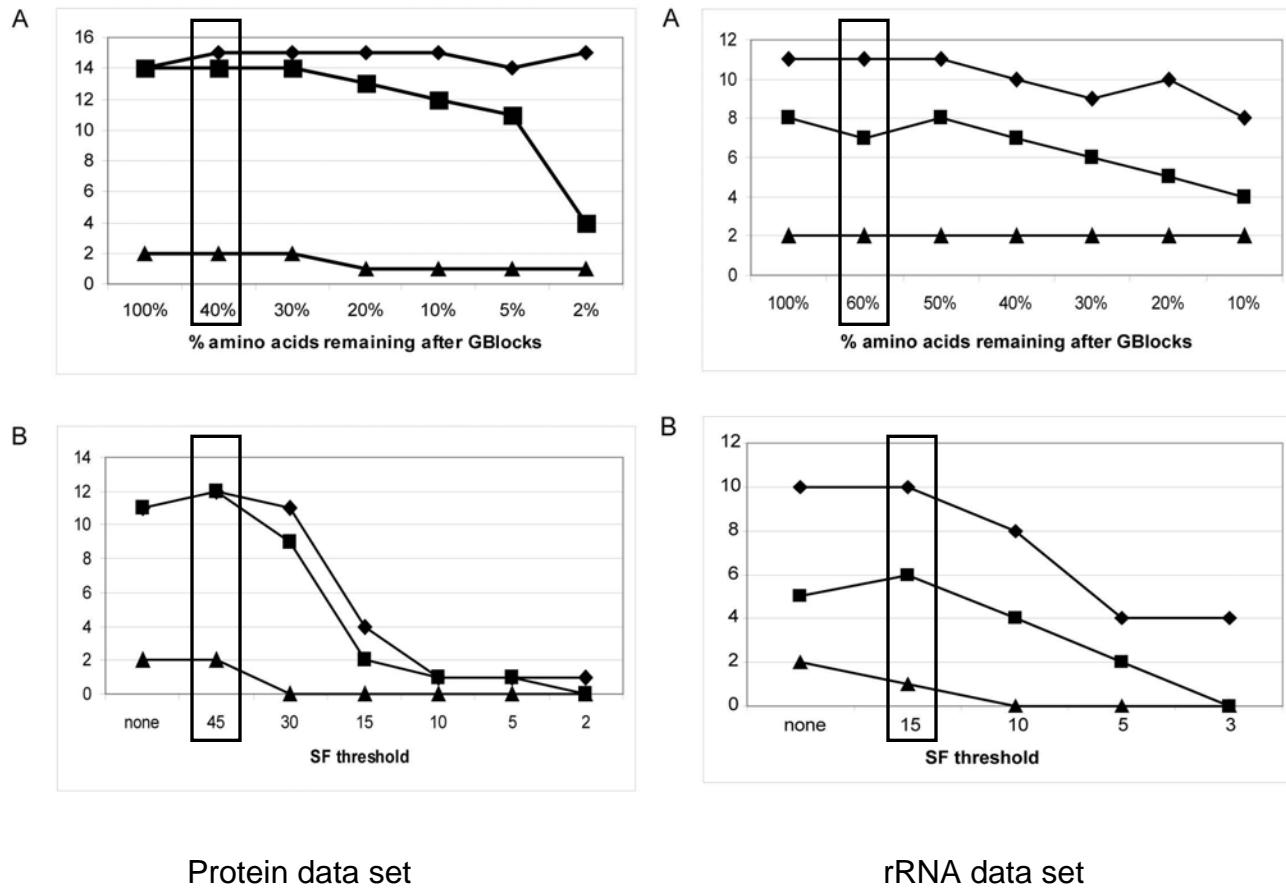


Fig. S2 Consensus of 25 single ML gene trees from the protein data set. Triangles are proportional to the number of sequences analyzed in each class. Numbers represent the percentage of genes supporting the cluster.

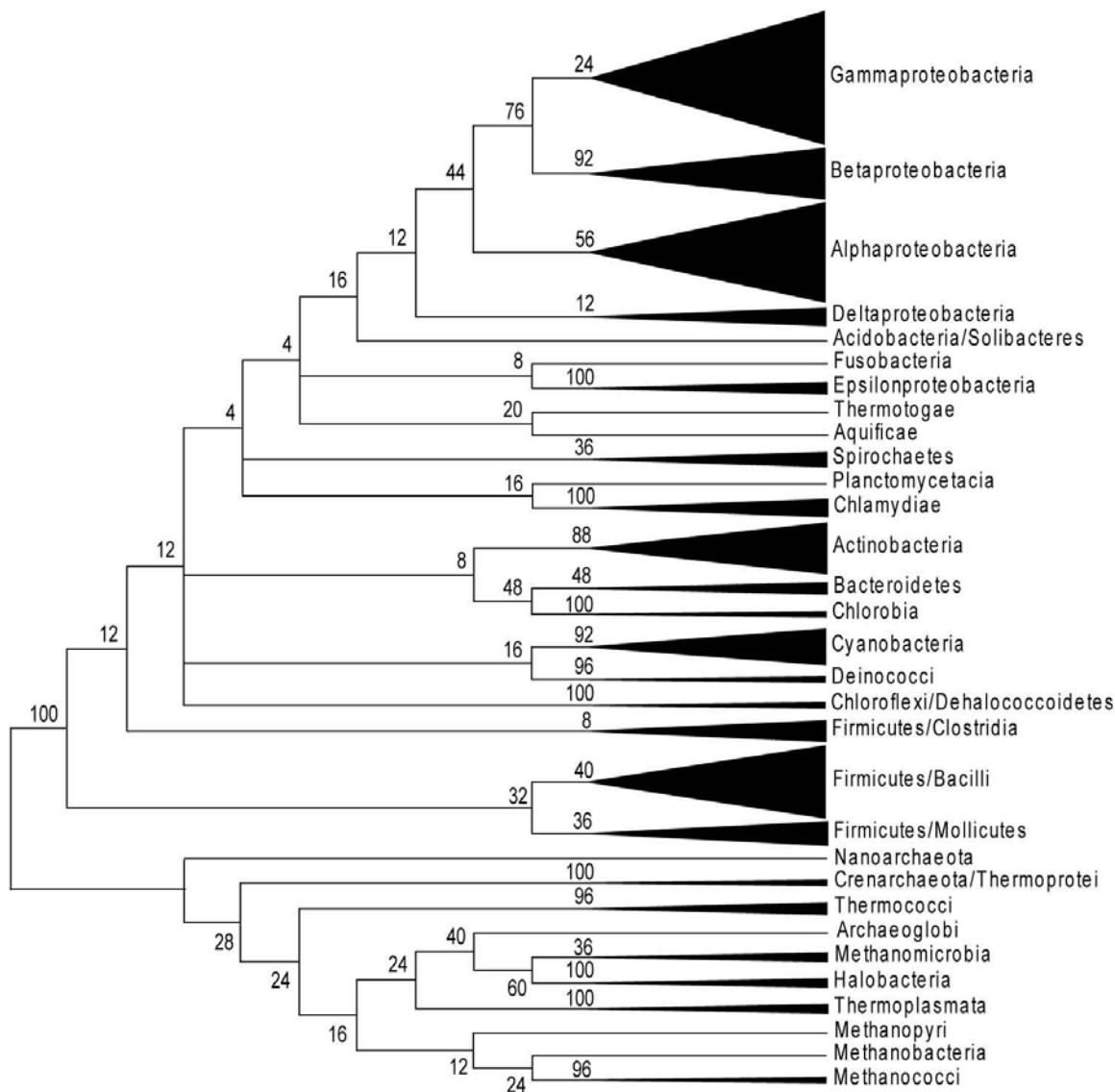


Fig. S3 Maximum likelihood phylogeny of slow evolving sites in the protein data set (Eubacteria and Archaeabacteria). Asterisks: bootstrap values equal to or higher than 95%. Triangles are proportional to the number of sequences analyzed in each lineage. Values at each node are for 100 bootstrap replicates.

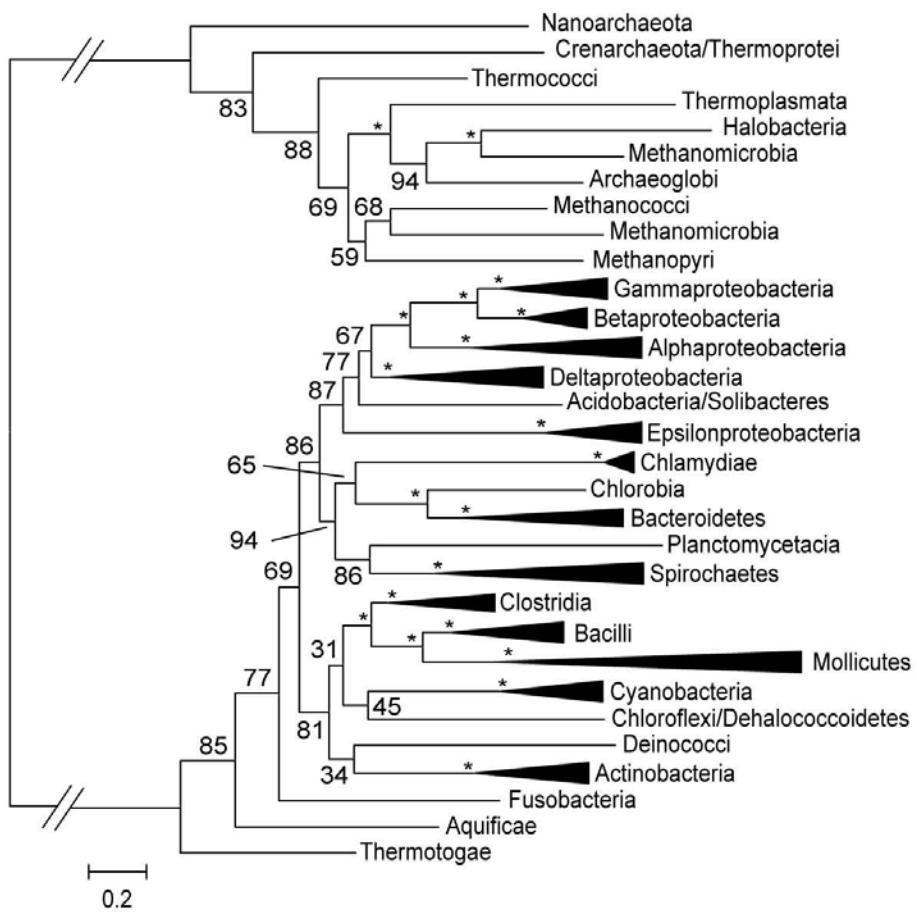


Fig. S4 LogDet phylogeny of rRNA (SSU+LSU) data set. Triangles are proportional to the number of sequences analyzed in each lineage. Values at each node are percentage support for 100 bootstrap replicates.

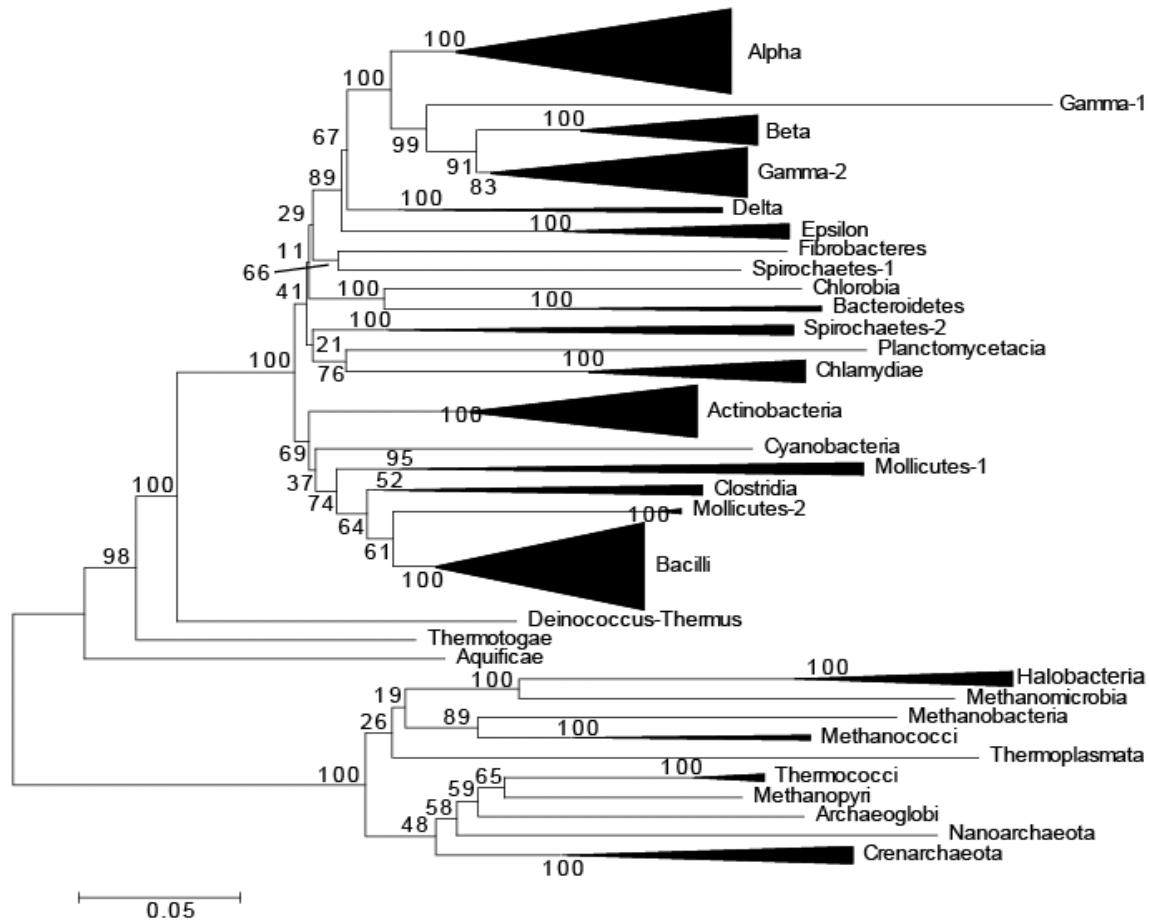


Fig. S5 Maximum likelihood phylogeny of slow evolving sites in the rRNA (SSU+LSU) data set (Eubacteria and Archaeabacteria). Asterisks: bootstrap values equal to or higher than 95%. Triangles are proportional to the number of sequences analyzed in each lineage. Values at each node are for 100 bootstrap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

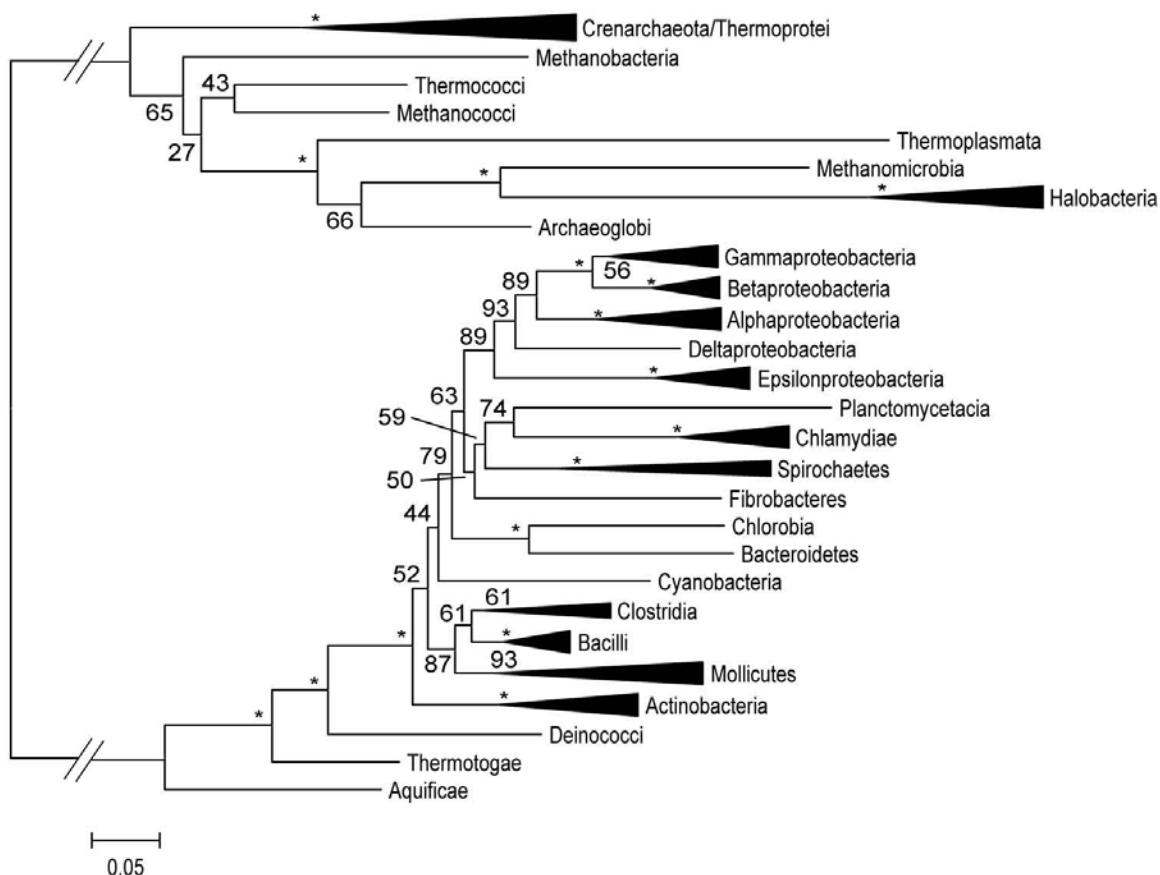


Fig. S6 Maximum parsimony ancestral states reconstruction in major lineages of Terrabacteria (Group-1). Terrestrial states (species) are shown in tan and marine states in blue; dashed lines indicate lineages in which there is at least one terrestrial and one marine species. The phylum-level topology 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.

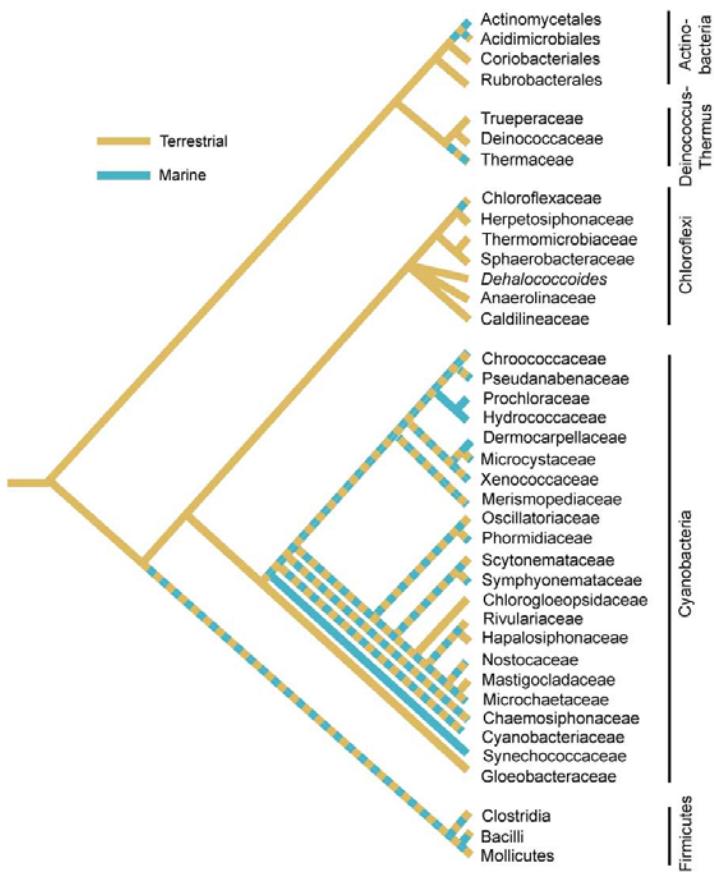


Fig. S7 Maximum likelihood ancestral states reconstruction of Terrabacteria (Group-I) lineages. Phylogenetic details are as in Fig. S6. Terrestrial state is shown in tan, marine state in blue, mixed state in gray. Probabilities of each state in the last common ancestor of the group are: 73% terrestrial,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

