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Short communication

Bacterial diversity of terra preta and pristine forest soil from the Western Amazon

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Abstract

The survey presented here describes the bacterial diversity and community structures of a pristine forest soil and an anthropogenic terra preta from the Western Amazon forest using molecular methods to identify the predominant phylogenetic groups. Bacterial community similarities and species diversity in the two soils were compared using oligonucleotide fingerprint grouping of 16S rRNA gene sequences for 1500 clones (OFRG) and by DNA sequencing. The results showed that both soils had similar bacterial community compositions over a range of phylogenetic distances, among which Acidobacteria were predominant, but that terra preta supported approximately 25% greater species richness. The survey provides the first detailed analysis of the composition and structure of bacterial communities from terra preta anthrosols using noncultured-based molecular methods.

Keywords: Bacterial diversity; Forest soils; Microbial ecology; Terra preta

1. Introduction

Terra preta anthrosols in the Amazon basin are nutrient rich soils that contain thick, dark colored, surface horizons with a high organic matter content (Lima et al., 2002) and are noted for their exceptional fertility and ability to accumulate stable organic carbon. Prior studies suggest these soils were formed by pre-Columbian, Amerindians, who practiced a "slash and char" agriculture (Mann, 2002). The ability of terra preta to accumulate stable organic matter has attracted attention as a possible means for increasing fertility and carbon storage in tropical soils (Sombroek, 1966; Lehmann et al., 2003), and has provoked questions regarding the role of microorganisms in the formation and plant growth promotion characteristics of these soils (Thies and Suzuki, 2003). The objective of this study was to carry out a survey of the bacterial diversity in terra preta and pristine forest soil as the basis for studies on the ecology of these forest soils.

To characterize bacterial diversity in the terra preta and pristine forest soil, samples were collected from two adjacent locations in the Jamari National Forest, of Rondonia, Brazil (latitude: 8° 45′ 0 S, longitude: 63° 27′ 0 W). The soil from the pristine forest was a clay loam ultisol having a 1 cm A horizon under a layer of moist duff. The B horizon consisted of a highly oxidized ultisol with an acid pH of 3.1 and had negligible organic matter. The terra preta samples were collected at a site two hundred meters distant where the soil contained a deep, organic matter rich, sandy loam in the A horizon that was approximately 1 m thick before transitioning into the underlying clay ultisol. Intact soil cores measuring 10 cm in depth and 8 cm in width were collected from the surface horizon below the organic litter layer using sterile, stainless steel soil coring sleeves. The cores were shipped immediately to the University of California, Riverside, USA, where they were frozen at −80 °C. At the time of analysis, three cores from

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each site were combined into one composite sample and sieved to pass a 1.0 mm screen. The composite samples were analyzed for their chemical and physical characteristics and metal contents (Supplemental Table 1, online supplementary material) and were subjected to molecular analyses to identify the predominant bacteria and to describe the bacterial community structures at different phylogenetic distances based on comparisons of 16S rRNA gene sequences recovered from the samples.

The analysis of bacterial diversity employed a two step procedure in which the first step was to sort 16S rRNA clone libraries with a method referred to as oligonucleotide fingerprinting of ribosomal genes (OFRG) (Valinsky et al., 2002). This was followed by sequencing of random clones from the taxonomic clusters generated by OFRG to identify the predominant bacteria. The gene sequences were also used to construct phylogenetic trees based on actual sequence data. To produce the 16S rRNA gene clone libraries, near full-length (1465 bp) small-subunit rRNA gene sequences were PCR amplified from soil DNA. The purified DNA was ligated into pGEM-T (Promega) and transformed into competent *Escherichia coli* JM109 (Promega). Libraries of the rRNA genes were represented by 768 clones for each sample, which were arrayed on replicate nylon membranes for hybridization with ^{33}P DNA end-labeled oligonucleotide probes as described by Valinsky et al., (2002). Signal intensities with background correction were obtained using ImaGene 4.0 software (Biodiscovery) and were transformed for every probe into three values: 0, 1, and N, where 0 and 1 indicate negative and positive hybridization events, respectively, and N indicates an uncertain assignment. This process creates a hybridization fingerprint for each clone, which is a vector of values resulting from hybridizations with all probes. Estimates of diversity using 16S rRNA gene sequences were examined based at 95%, and 90% similarities using the PHYLIP-formatted distance matrices generated by the computer program PAUP 4.0 (Sinauer Associates, Inc.).

To identify the taxonomic groups separated by OFRG, nucleotide sequences were determined for 76 and 49 rRNA gene clones that were randomly selected from within each of the clusters generated by OFRG. The 16S rRNA gene sequences were submitted to the BLAST server (Basic Local Alignment Search Tool), National Center for Biotechnology Information (NCBI) to determine the closest matching sequences in the GenBank and to infer phylogenetic affiliations. Novel sequences were deposited at GenBank and were assigned accession numbers AY326512–AY326636.

Phylogenetic trees based on the sequenced clones were constructed using the 16S rRNA gene sequence data. Nucleotide sequences were aligned using Clustal X, after which an evolutionary distance matrix was generated using the program MEGA3 (Kumar et al., 2004) using the N-J method. Bootstrap analyses of the neighbor-joining data were conducted based on 1000 samplings to assess the stability of the phylogenetic relationships. Rarefaction curves and estimates of species diversity at different phylogenetic distances were determined using the computer program DOTUR (Schloss et al., 2004). Comparisons of the species coverage and overall community similarities at different phylogenetic distances were determined using the program LIBSHUFF (Singleton et al., 2001).

2. Results and discussion

The Amazon contains diverse soils of which only a few have been characterized with respect to their microbiology. To date there has been only one prior survey of Amazon soils that used molecular methods to analyze soil microbial diversity in which 100 clones were analyzed (Borneman and Triplett, 1997). The survey data presented here thus provide a more comprehensive survey and the first analysis of terra preta using molecular methods. Inspection of the taxonomic trees generated by OFRG showed that the two soils differed with respect to the number of operational taxonomic units (OTUs), and the number of branches that represent different taxonomic groups, with terra preta having overall greater diversity (Fig. 1). As shown using rarefaction, there was significant separation of OTU richness (95% similarity level) as sample size increased above 50 for each sample set (Fig. 2). Enumeration of the unique 16S rRNA gene sequences yielded 396 OTUs from terra preta as compared to 291 OTUs in the forest soil (Table 1). Calculation of Shannon index values using DOTUR yielded significantly different ($P > 0.05$) values for terra preta and forest soil (5.2 and 4.37, respectively). Other commonly used measures of diversity including Simpsons Index of Diversity and Chao I, which employ independent mathematical approaches to measuring diversity revealed the same phenomenon. Altogether, these analyses suggest that bacterial species richness was approximately 25% greater for terra preta than that in the forest soil.

Using the program LIBSHUFF to compare the similarities of the communities from each soil at different phylogenetic distances, it was determined that overall coverage of sequence diversity was high, with values of 60% and 68% for the forest soil and terra preta samples sets, respectively (Fig. 3). Comparison of the forest soil sample set against terra preta revealed that all of the OTU diversity in the forest soil was represented in terra preta and that the forest sample was not significantly different from the terra preta ($P = 0.64$; data not shown). In contrast, comparison of the terra preta versus the forest soil showed that terra preta was significantly different ($P = 0.05$), in that it contained additional sequences that did not occur in the forest soil. The greatest difference in 16S rRNA gene sequences between terra preta and the forest soil occurred for OTUs having up to 5% evolutionary distance (95% 16S rRNA similarity),

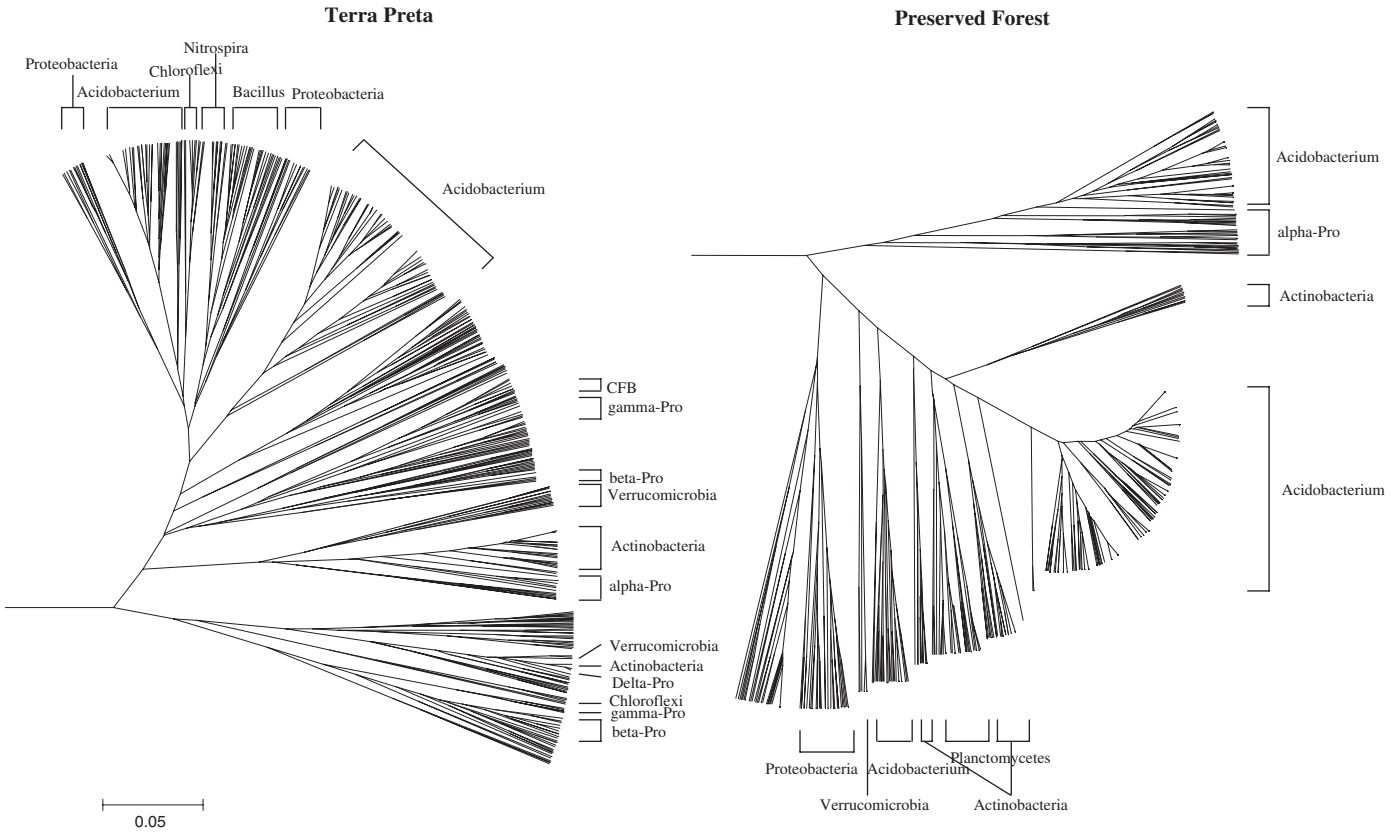

Fig. 1. Taxonomic cluster analysis of 16S rRNA gene sequences from terra preta and adjacent pristine forest soil based on oligonucleotide fingerprinting of 16S rRNA gene sequences. The 16S rRNA clone libraries were generated for single samples consisting of three composited replicate soil cores (litter layer removed) taken from the top 10 cm of the soil profile at each location.

corresponding to species and genera. The coverage curves converged at 95% evolutionary distance and then separated again at distances from 96% to 90%, indicating that there were also significant differences between the communities at deeper phylogenetic levels. Complete coverage of the two communities was obtained at greater than 90% similarity, suggesting that the large data set that was analyzed here, with ca. 700 clones per sample, represented all of the major bacterial phyla that were present in the soils.

The differences in community composition revealed by the LIBSHUFF analysis were confirmed by the phylogenetic analyses based on DNA sequencing. Identities of the major bacterial groups by direct sequencing of clones representing different clusters revealed 14 phylogenetic groups in terra preta, as compared to 9 from the forest soil (Fig. 4). Among the major groups represented, *Acidobacterium* was predominant, comprising approximately 30% of the bacteria in terra preta and 50% of the forest soil. The phylogenetic trees included two possible new clades of *Acidobacterium* that were distinct from the previously described subgroups for this phylum. Other bacterial groups that were common to both two soils included the *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Verrucomicrobia*. Representatives of the α, β, and γ *Proteobacteria* were similarly abundant in terra preta; whereas mostly α *Proteobacteria* were found in the forest soil.

Acidobacterium sp are common in many forest soils around the world, comprising from 12% of the non-cultured species surveyed in Austrian forest soils under pine to 35% in spruce-fir-beech soils in Europe (Hackl et al., 2004). At least four subgroups of these bacteria have been recognized previously using specific 16S rRNA gene primers in a survey of 43 soils and sediments representing a range of environments (Barns et al., 1999). This earlier survey suggested that the A and G subgroups are ubiquitous, whereas the Y and O subgroups are largely absent from acid soils. The A subgroup is thought to be the most phylogenetically diverse, with deep branches in the phylogenetic tree that may represent distinct lineages. Our results are largely in agreement with these suppositions. Here, most of the cloned bacterial sequences were in the A and G subgroups. Terra preta contained sequences from the Y subgroup, which purportedly favors non-acid soils. Only the A subgroup was found in the acid soil from the pristine forest. In the phylogenetic tree generated by DNA sequencing, the BLAST analysis revealed three possible

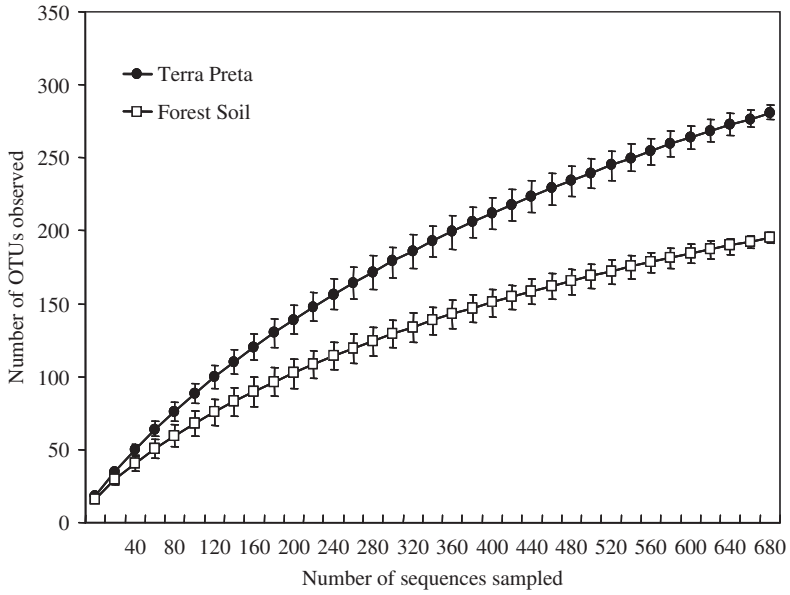

Fig. 2. Rarefaction analysis of 16S rRNA gene sequences from terra preta and forest soil. Gene sequences were differentiated by oligonucleotide fingerprint grouping at 0.05 dissimilarity level. Vertical bars indicate 95% confidence intervals.

Table 1
Measures of bacterial species diversity based on OFRG analysis of microbial communities from terra preta and Amazon forest soil

Index	Terra preta	Forest soil
OTU richness		
Unique OFRG groups / clones	396/742	291/768
0.05 evolutionary distance	287	196
0.10 evolutionary distance	210	142
Shannon index	5.2	4.37
Simpsons index	0.015	0.34
Estimated richness Chao1 (0.05 evolutionary distance)	413	277

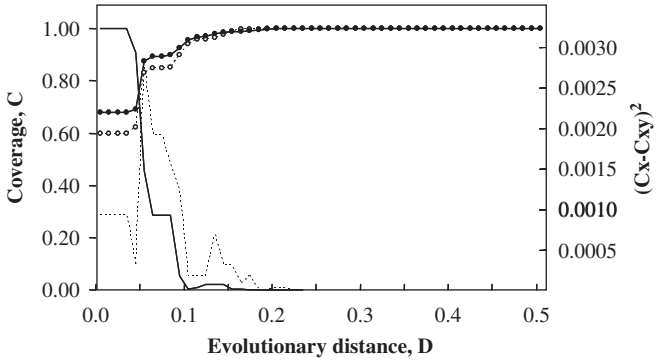

Fig. 3. LIBSHUFF analysis of 16S rRNA gene sequences from terra preta (closed circles) compared to adjacent forest soil (open circles) showing differences in composition of the bacterial communities (y-axis) over a range of evolutionary distances (D) (x-axis). Solid lines indicate the value of (CX-CXY)2 for samples at each value of D. Broken lines indicate the $P = 0.05$ value of (CX-CXY)2 for the randomized samples.

new subgroups of *Acidobacterium*. Two new clades of *Acidobacterium* were found in the terra preta, one that was most closely related to the G subgroup, and a second well-defined cluster of five clones that were most closely related to the Y-subgroup. The new clade of *Acidobacterium* in the forest soil was comprised by four clones that occurred within the A-subgroup and were most similar to previous accessions designated as *Holophaga*.

Based on prior knowledge, soil pH is likely to be one of the most important selection factors affecting the microbial species composition in different soils (Fierer and Jackson, 2006). Here, both soils supported abundant plant growth and carried similar above ground vegetation consisting of a diverse community of Amazon forest tree species and understory plants. The relationship between organic matter inputs from different overstory trees, rhizosphere effects, and the bacterial community composition of forest soils is still not understood, but likely contributes to differences in diversity and community composition along the forest floor. There was also increased earthworm activity and soil aggregation in terra preta that may provide a wide range of niches and thereby contribute to increased bacterial species diversity. Additional surveys and comparisons at different locations will be needed to characterize other locations with terra preta. It may then be possible to unravel the ecology of these bacterial communities and study the role of specific bacterial groups that contribute to the many interesting properties of these soils.

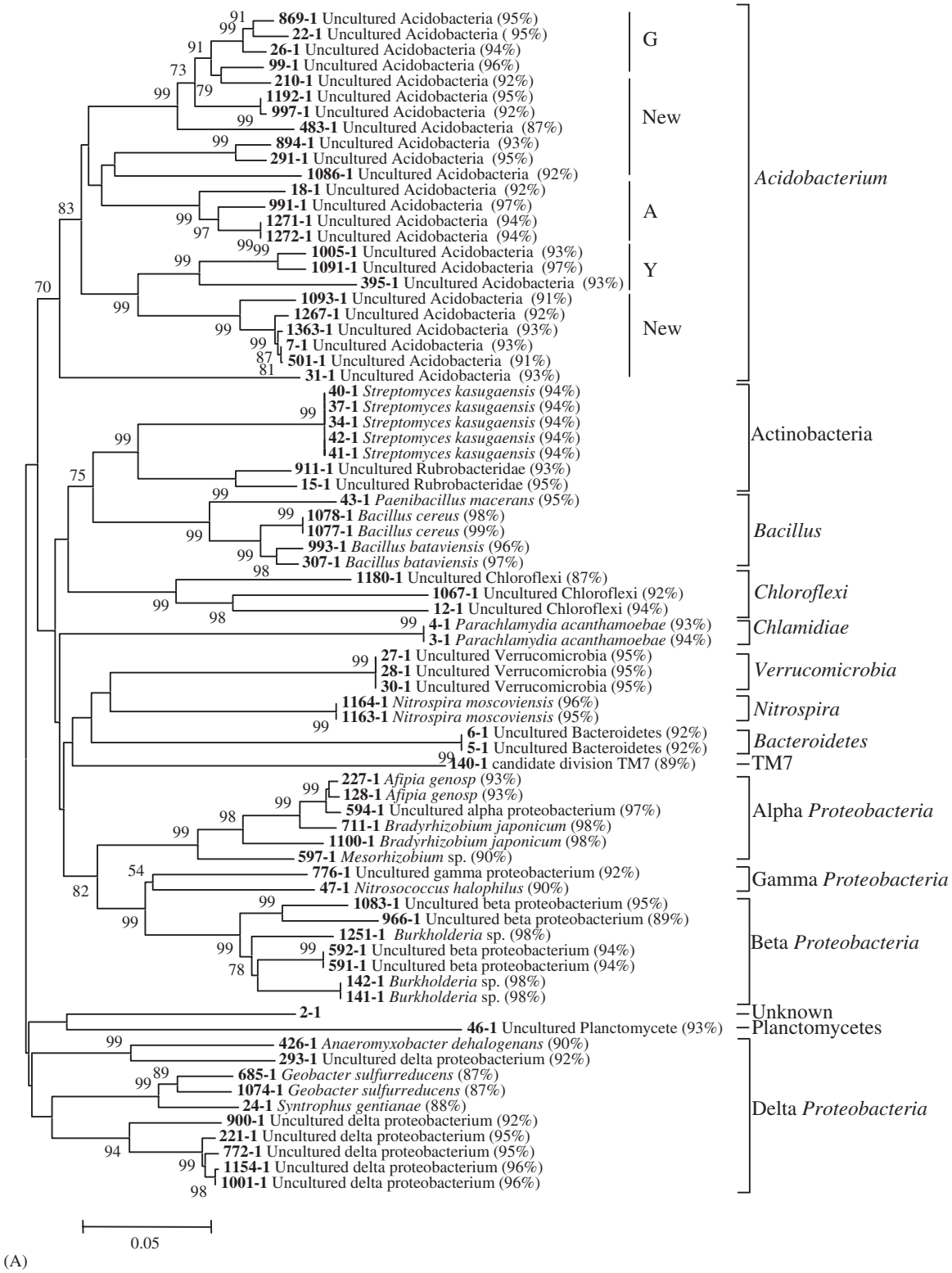

Fig. 4. Phylogenetic trees for unique 16S rRNA gene sequences based on rRNA gene sequencing. (A) Terra preta soil (B) Forest soil.

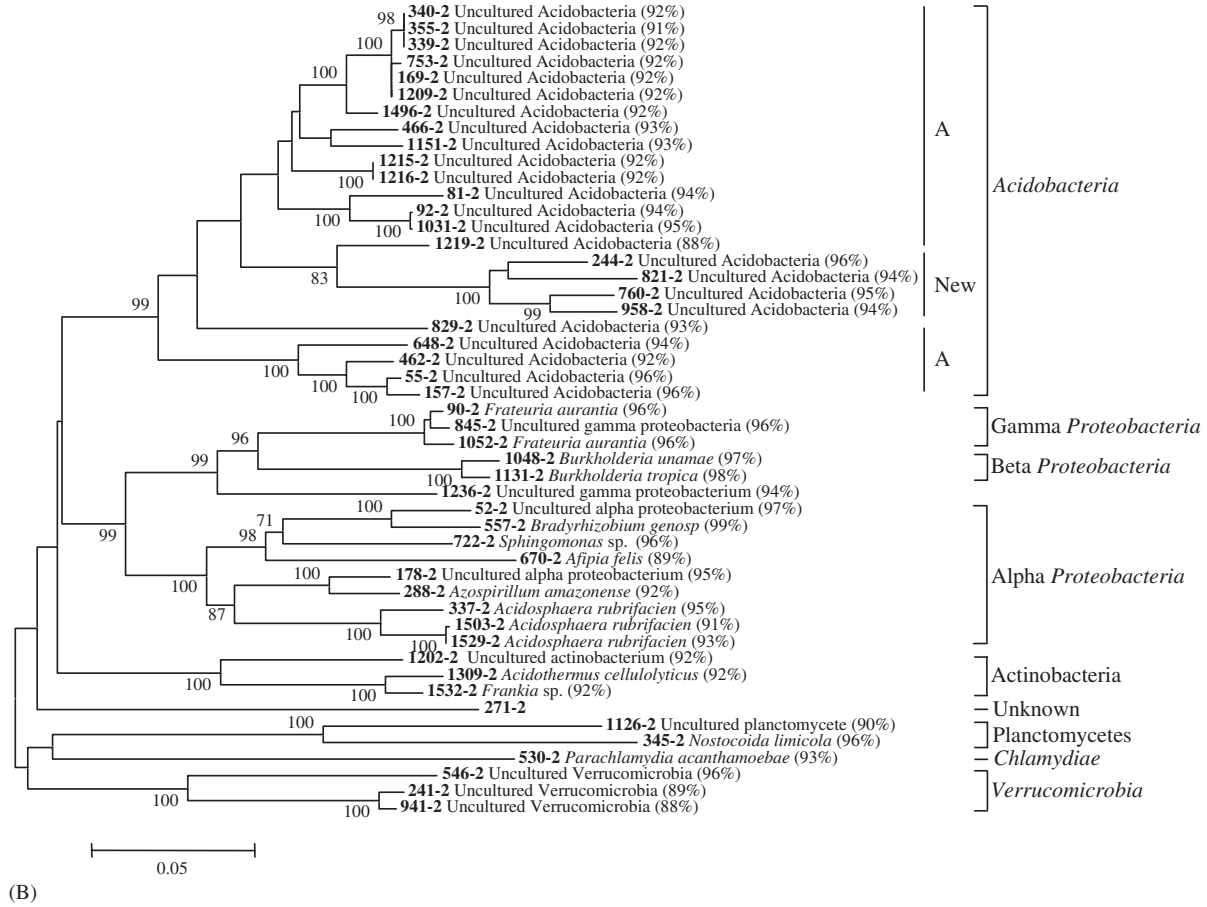

Fig. 4. (*Continued*)

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilbio.2006.08.010

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