



## Supporting Online Material for

### **Bacterial Diversity in Tree Canopies of the Atlantic Forest**

M. R. Lambais,\* D. E. Crowley,\* J. C. Cury, R. C. Büll, R. R. Rodrigues

\*To whom correspondence should be addressed. E-mail: [mlambais@esalq.usp.br](mailto:mlambais@esalq.usp.br)  
(M.R.L.); [crowley@ucr.edu](mailto:crowley@ucr.edu) (D.E.C.)

Published 30 June 2006, *Science* **312**, 1917 (2006)

DOI: 10.1126/science.1124696

#### **This PDF file includes:**

Materials and Methods  
SOM Text  
Figs. S1 and S2  
Tables S1 to S4  
References

#### **Other Supporting Online Material for this manuscript includes the following:**

available at [www.sciencemag.org/cgi/content/full/312/5782/1917/DC1](http://www.sciencemag.org/cgi/content/full/312/5782/1917/DC1)

Database 1 as zipped archive: 1124696s.zip

# Bacterial Diversity in Tree Canopies of the Atlantic Forest

## Supporting Online Material

### Materials and Methods

**Sampling location.** Samples were collected from mature trees within a semideciduous forest stand within the Atlantic Forest at the Estação Ecológica dos Caetetus (between 22°41' and 22°46'S and 49°10' and 49°16'W), Gália, São Paulo State, Brazil. The experimental site is in a semideciduous mesophytic forest ecotone, which contains 118 different tree species in 85 genera and 45 families, and has been described in relation to its climate and biogeography (<http://www.lerf.esalq.usp.br/parcelas/caetetus.html>). The sampled trees were located in a preserved experimental parcel (10 hectare) that was established as part of the Biota Program (FAPESP, Brazil).

**Leaf sampling.** Samples of mature leaves were collected from three individual trees of 9 different species at different locations in the forest (Table S1). To collect the leaves, a 10 m extended pole clipper was used to cut small branches that were thereafter handled only by the branch stems to avoid contamination of the leaves. Leaf samples were collected from the lower shaded, interior part of the canopy from mature trees which ranged in height from 10 to 30 meters tall. Detailed descriptions of the tree species are provided online at <http://www.lerf.esalq.usp.br/matrizes/banco.html>. The leaves were removed from the felled branches using hand shears to clip individual leaves directly into 4 L plastic bags. Samples were maintained on ice during transportation and immediately processed to extract the bacteria from the leaf surface.

**Bacteria isolation.** Using axenic procedures, 12 g of leaves (fresh weight) from individual trees were placed into 50 mL polypropylene tubes containing washing solution (0.1 M potassium phosphate buffer, pH 7.0) and sonicated for 10 min at 22.5 kHz in an ultrasonic cell disrupter (Misonix Inc., Atlantic Beach, NY, USA ) (6). The resulting bacterial suspension was centrifuged at 30,000 g for 15 min at 4°C. The pellet was resuspended in 1.5 mL of washing solution and further concentrated by centrifugation at 30,000 g for 15 min at 4°C. Bacteria were resuspended in 200 µL of washing solution and frozen at -20°C until processing for DNA extraction.

**DNA extraction.** Metagenomic DNA was extracted from 100 µL of the bacterial washings using the Fast DNA kit (Qbiogene, Irvine, CA, USA), according to the manufacturer's instructions. DNA integrity was determined by electrophoresis in 0.5X TBE-0.8% agarose gels, after staining with Vistra Green (Amersham Biociences, São Paulo, Brazil). DNA concentration was determined by densitometry, using the Low DNA Mass Ladder (Invitrogen, São Paulo, Brazil), as standard, and the program Fragment Analyses (Amersham Biosciences, São Paulo, Brazil).

**PCR-DGGE analyses.** Bacterial community structures on different trees were initially surveyed using a low resolution method that separates out 16S rRNA gene sequences based on differences in their GC content. The V3 regions of the 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the metagenomic DNA extracted as template

and primers BA338fGC and UN518r (*S1*). Amplification was performed in 1X Taq Platinum DNA polymerase buffer containing 0.2 mM dNTPs, 3 mM MgCl<sub>2</sub>, 1 U Taq Platinum DNA polymerase (Invitrogen, São Paulo, Brazil); 5 pmol of each primer and 10 ng of metagenomic DNA. PCR amplification conditions were 5 min at 95°C; 30 cycles 1 min at 95°C, 1 min at 55°C and 1 min at 72°C, and final extension for 10 min at 72°C. The concentration of PCR products (amplicons) was determined by densitometry, after electrophoresis in 0.5X TBE-0.8% agarose gels and staining with Vistra Green (Amersham Biosciences, São Paulo, Brazil), using the Low DNA Mass Ladder (Invitrogen, São Paulo, Brazil), as standard, a FluorImager laser densitometer and the program Fragment Analyses (Amersham Biosciences, São Paulo, Brazil).

Amplicons (300 ng) were analyzed by denaturing gradient gel electrophoresis (DGGE) using 8% (w/v) acrylamide:bisacrylamide (37.5:1, m:m) gels containing a 15 to 55% linear gradient of formamide and urea (100% denaturing solution contained 40% formamide and 7 M urea) (*S1*). Electrophoreses were performed at 200V constant and 60°C, using a DCode System (BioRad, Hercules, CA, USA), in 1X TAE buffer. Gels were stained with Vistra Green (Amersham Biosciences, São Paulo, Brazil) and analyzed by densitometry, using a FluorImager laser densitometer and the program Diversity Database (BioRad, Hercules, CA, USA). Two gels were cast and run simultaneously to compare the communities under uniform electrophoresis conditions. Each gel contained 3 marker lanes that were run with a standard mixture of DNA to allow for alignments between gels.

Statistical analyses of the 16S rRNA gene profiles were conducted to compare the similarities in the community compositions within and between tree species (5). The DNA band patterns were analyzed to identify specific bands according to their R<sub>f</sub> (relative front) values and pixel intensities. The resulting band profiles were analyzed using linear discriminant analysis and cluster analysis with the software MINITAB version 14 (Minitab Inc., State College, PA, USA). For discriminant analysis, the DNA band data were pruned to discard bands that comprised less than 2% of the total pixel intensity, or that appeared only once across the sample set. The excel data sheet containing the raw data and summary output of the statistical analyses is provided as a supplementary file.

**16S rRNA gene cloning and sequencing.** The V1-V3 regions of the 16S rRNA genes were amplified by PCR using the metagenomic DNA extracted as template and primers UN518R (*S1*) and BA63F (*S2*). Amplification was performed in 1X Taq Platinum DNA polymerase buffer containing 0.2 mM dNTPs, 3 mM MgCl<sub>2</sub>, 1 U Taq Platinum DNA polymerase (Invitrogen, São Paulo, Brazil); 5 pmol of each primer and 10 ng of metagenomic DNA. PCR amplification conditions were 5 min at 95°C; 30 cycles 1 min at 95°C, 1 min at 55°C and 1 min at 72°C, and final extension for 10 min at 72°C.

Amplicons were purified using GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences, São Paulo, Brazil) and ligated to pGEM-T Easy Vector (Promega, Madison, WI, USA) at 4°C overnight, according to the manufacturer's instructions. The ligation products were transformed into *E. coli* DH5α competent cells by heat shock and transformed cells plated on LB-agar, containing ampicilin (100µg mL<sup>-1</sup>), X-Gal (5-bromo-4-chloro-3-indolil-β-D-galactoside). Colonies containing recombinant plasmids were selected and cells were grown in liquid LB medium containing 100 µg ampicilin mL<sup>-1</sup> at

37°C overnight.

Plasmids were extracted through alkaline lysis. Nucleic acids solutions were incubated with 15 µg RNase A for 20 min at 37°C. DNA was precipitated with equal volume of cold isopropanol for 5 min on ice and centrifugation at 4,000 g for 40 min at 4°C. DNA pellet was washed with 70% cold ethanol, dried and suspended in ddH<sub>2</sub>O. DNA concentration was determined spectrophotometrically at 260 nm.

Sequencing was performed using 200-500 ng of plasmid DNA, 10 pmol of T7 or SP6 primers, 2 µL of DYEmanic ET Terminator (Amersham Biosciences, São Paulo, Brazil), 2 µL of buffer (200 mM Tris-HCl pH 9.0 and 5 mM MgCl<sub>2</sub>.6H<sub>2</sub>O) and ultrapure H<sub>2</sub>O to a final volume of 10 µL, in 25 cycles of 20 sec at 95°C, 15 sec at 50°C and 1 min at 60°C. PCR products were precipitated with ethanol, dried and resuspended in deionized formamide. Electrophoresis was performed using an ABI 3100 automatic sequencer, according to the manufacturer's instructions (Applied Biosystems, São Paulo, Brazil).

**Sequence analyses and OTU definition.** A total of 109, 153 and 166 clones of the V1-V3 region of the 16S rDNA from bacterial communities of the phyllosphere of *Trichillia catigua*, *Trichillia clausenii* and *Campomanesia xanthocarpa* were analyzed. Nucleotide sequences (reads) were trimmed for the removal of low quality bases (quality parameter > 20, i.e. less than one error in 100 nucleotides) and vector sequences using Phred program (S3). Valid sequences were then clustered into operational taxonomic units (OTUs) using the program DOTUR and cut-off evolutionary distances of 0.01 or 0.03 (S4). Jukes-Cantor evolutionary distances were calculated using DNADIST of the PHYLIP 3.63 Package (J. Felsenstein; <http://evolution.genetics.washington.edu/phylip.html>), after aligning the sequences using ClustalX 1.83 (S5) under the default parameters, except gap-opening penalty of 10.0 and gap-extension penalty of 0.1 for pairwise and multiple alignments. The number of sequences for each OTU was computed and used for diversity estimations. Valid sequences were compared to sequences in the Ribosomal Database Project II for taxonomic affiliation, using the program RDPquery (S6).

**Diversity indices.** The estimated minimum number of OTUs in the samples was determined through ACE and Chao1 non-parametric estimators, using SPADE (Chao & Chen; <http://chao.stat.nthu.edu.tw>). SPADE was also used for the determination of Shannon's and the reciprocal of Simpson's indices (maximum likelihood estimators) and estimated sample coverage.

**Coverage comparisons.** Homologous and heterologous coverage comparisons were performed using the program LIBSHUFF 1.22 (S7) after aligning the sequences using ClustalX 1.83 (S5) under the default parameters, except gap-opening penalty of 10.0 and gap-extension penalty of 0.1 for pairwise and multiple alignments, and calculating Jukes-Cantor evolutionary distances using DNADIST of the PHYLIP 3.63 Package.

## Supplementary Text

Bacterial cells are found at an average density of  $10^6$ - $10^7$  cells cm<sup>-2</sup> on plants from

temperate regions (2), and may be even higher on tropical plants where dense canopies are conducive for bacterial growth. Considering that the estimated leaf area of terrestrial plants is  $6.4 \times 10^8 \text{ km}^2$  (58), the number of bacterial cells on leaf surfaces globally is as high as  $10^{26}$  cells. For some time it has been presumed that this harsh environment supports a relatively low bacterial diversity and that those bacteria that colonize plant leaves are transported in wind-borne dust and are broadly distributed. However, there are many conditions that may lead to ecological selection for different microbial communities on leaves from different plant species (8). Prior surveys have mainly described bacteria that are associated with agricultural plants and have used culture based methods that typically reveal only a few dozen species of bacteria on any one plant. More recent studies using molecular methods have shown microbial diversity on the leaves of agricultural plants is actually much greater and suggest that different plants harbor distinct bacterial communities (6). The discovery of high bacterial diversity for agricultural plants has prompted many questions about the true extent of microbial diversity that is associated with plant canopies in natural ecosystems.

Sequence data obtained from molecular analysis of the microbial communities were used to compare the genetic diversity in the phyllospheres and to identify the predominant taxonomic groups that were present on leaf surfaces. Two lines of evidence were used to ascertain between tree species and within species variation in microbial community composition. The 16S rRNA gene profiles generated by the low resolution DNA fingerprinting method, PCR-DGGE, suggest that there was considerable variation in bacterial community species composition both within and among the 9 tree species studied (Fig. S1). Discriminant analysis of the DNA band profiles for replicates of bacterial communities yielded consistent groups corresponding to tree species (Table S2). Such groupings support the hypothesis that within tree species variation is lower than between tree species, but such data must be viewed cautiously as DNA bands located at the same gel migration distance may not necessarily represent the same bacterial taxa.

Evidence for high variation between tree species is derived from statistical analysis of the clone libraries that were generated for the three tree species using the program LIBSHUFF (Fig. S2). LIBSHUFF analyses use the approximation form of the Cramér-von Mises statistics to compare homologous and heterologous coverage curves. Results of the analyses indicated that the clone sample sizes were sufficient to obtain good coverage of the bacterial species composition for each tree (67-81%), and that the clone libraries from the three bacterial phyllosphere communities were significantly different ( $P = 0.001$ ). Using RDPquery (56) to assign a phylogenetic affiliation at different taxonomic levels to the 16S rRNA gene sequences, we observed that a majority of the bacteria were from the Proteobacteria (86, 73 and 48% in *T. catigua*, *T. clausenii* and *C. xanthocarpa*, respectively; Table S4). Within this phylum, bacteria of the gamma-Proteobacteria class (*Enterobacteriales* and *Pseudomonadales*) were predominant in the phyllospheres of *T. catigua* and *T. clausenii*, whereas alpha-Proteobacteria (*Sphingomonadales*) were predominant in the phyllosphere of *C. xanthocarpa*. The taxons identified with the different tree species are described in detail in Table S4.

## References

- S1. L. Øvreås, L. Forney, F. L. Daae, V. Torsvik, *Appl. Environ. Microbiol.* **63**, 3367 (1997).
- S2. J. R. Marchesi *et al.*, *Appl. Environ. Microbiol.* **64**, 795 (1998).
- S3. B. Ewing, P. Green, *Genome Res.* **8**, 186 (1998).
- S4. P. D. Schloss, J. Handelsman, *Appl. Environ. Microbiol.* **71**, 1501 (2005).
- S5. J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, D. G. Higgins, *Nuc. Acids Res.* **24**, 4876 (1997).
- S6. G. Dyszynski, W. M. Sheldon, [http://simo.marsci.uga.edu/public\\_db/rdp\\_query.htm](http://simo.marsci.uga.edu/public_db/rdp_query.htm) (December 13, 2005).
- S7. D. R. Singleton, M. A. Furlong, L. R. Stephen, W. B. Whitman, *Appl. Environ. Microbiol.* **67**, 4374 (2001).
- S8. C. E. Morris, L. L. Kinkel, in *Phyllosphere microbiology*, S. E. Lindow, E. I. Hecht-Poinar, V. Elliott, Eds. (APS Press, St. Paul, MN, 2002), pp. 365-375.

Table S1. Tree species at Estação Ecológica dos Caetetus (Gália, São Paulo, Brazil) selected for analysis of phyllosphere bacterial community structures.

| <b>Species</b>                            | <b>Family</b>  | <b>Order</b>   |
|---|----------------|----------------|
| <i>Aspidosperma polyneuron</i> Müll. Arg. | Apocynaceae    | Gentianales    |
| <i>Campomanesia xanthocarpa</i> O. Berg   | Myrtaceae      | Myrtales       |
| <i>Holocalyx balansae</i> Micheli         | Fabaceae       | Fabales        |
| <i>Ocotea indecora</i> Schott ex Meisn.   | Lauraceae      | Lurales        |
| <i>Seguiera floribunda</i> Benth.         | Phytolaccaceae | Caryophyllales |
| <i>Trichilia catigua</i> A.Juss.          | Meliaceae      | Sapindales     |
| <i>Trichilia clausenii</i> C.DC.          | Meliaceae      | Sapindales     |
| <i>Trichilia pallida</i> Sw.              | Meliaceae      | Sapindales     |
| <i>Urera baccifera</i> (L.) Gaudich.      | Urticaceae     | Rosales        |

Table S2. Discriminant analysis of bacterial communities associated with different tree species.

|                            | <i>Control</i> | <i>C. xanthocarpa</i> | <i>H. balansae</i> | <i>O. indecora</i> | <i>S. floribunda</i> | <i>A. polyneuron</i> | <i>T. pallida</i> | <i>T. catigua</i> | <i>T. clauseni</i> | <i>U. baccifera</i> |
|----------------------------|----------------|-----------------------|--------------------|--------------------|----------------------|----------------------|-------------------|-------------------|--------------------|---------------------|
| <i>Control</i>             | 6              | 0                     | 0                  | 0                  | 0                    | 0                    | 0                 | 0                 | 0                  | 0                   |
| <i>C. xanthocarpa</i>      | 0              | 3                     | 0                  | 0                  | 0                    | 0                    | 0                 | 0                 | 0                  | 0                   |
| <i>Holocalyx balansae</i>  | 0              | 0                     | 3                  | 0                  | 0                    | 0                    | 0                 | 0                 | 0                  | 0                   |
| <i>Ocotea indecora</i>     | 0              | 0                     | 0                  | 3                  | 0                    | 0                    | 0                 | 0                 | 0                  | 0                   |
| <i>Sequiera floribunda</i> | 0              | 0                     | 0                  | 0                  | 3                    | 0                    | 0                 | 0                 | 0                  | 0                   |
| <i>A. polyneuron</i>       | 0              | 0                     | 0                  | 0                  | 0                    | 3                    | 0                 | 0                 | 0                  | 0                   |
| <i>Trichillia pallida</i>  | 0              | 0                     | 0                  | 0                  | 0                    | 0                    | 3                 | 0                 | 0                  | 0                   |
| <i>Trichillia catigua</i>  | 0              | 0                     | 0                  | 0                  | 0                    | 0                    | 0                 | 2                 | 0                  | 0                   |
| <i>Trichillia clauseni</i> | 0              | 0                     | 0                  | 0                  | 0                    | 0                    | 0                 | 0                 | 2                  | 0                   |
| <i>Urera baccifera</i>     | 0              | 0                     | 0                  | 0                  | 0                    | 0                    | 0                 | 0                 | 0                  | 2                   |
| Total N                    | 6              | 3                     | 3                  | 3                  | 3                    | 3                    | 3                 | 2                 | 2                  | 2                   |
| N correct                  | 6              | 3                     | 3                  | 3                  | 3                    | 3                    | 3                 | 2                 | 2                  | 2                   |



Table S3. Estimated diversity indices for bacterial communities in the phyllosphere of *T. catigua*, *T. clausenii* and *C. xanthocarpa*.

| Community             | NDO | Estimated OTUs |                | Shannon <sup>a</sup> | 1/D <sup>b</sup>  | ESC   |
|-----------------------|-----|----------------|----------------|----------------------|-------------------|-------|
|                       |     | ACE            | Chao1          |                      |                   |       |
| <b>D = 0.01</b>       |     |                |                |                      |                   |       |
| <i>T. catigua</i>     | 58  | 172 (109, 312) | 152 (95, 292)  | 3.74 (3.58, 3.89)    | 31.0 (25.1, 40.5) | 0.642 |
| <i>T. clausenii</i>   | 89  | 253 (178, 390) | 221 (154, 357) | 4.20 (4.06, 4.33)    | 46.2 (36.5, 62.8) | 0.588 |
| <i>C. xanthocarpa</i> | 101 | 417 (265, 708) | 378 (235, 671) | 4.28 (4.14, 4.42)    | 44.1 (31.8, 72.1) | 0.527 |
| <b>D = 0.03</b>       |     |                |                |                      |                   |       |
| <i>T. catigua</i>     | 40  | 95 (60, 190)   | 84 (54, 180)   | 3.36 (3.21, 3.50)    | 22.5 (18.0, 29.8) | 0.807 |
| <i>T. clausenii</i>   | 68  | 167 (117, 266) | 145 (102, 242) | 3.79 (3.64, 3.95)    | 29.2 (23.5, 38.7) | 0.719 |
| <i>C. xanthocarpa</i> | 83  | 200 (147, 296) | 172 (127, 265) | 3.99 (3.83, 4.15)    | 32.1 (25.0, 45.1) | 0.667 |

<sup>a</sup>Maximum likelihood estimator. <sup>b</sup>Reciprocal of Simpson's index, maximum likelihood estimator. NDO, Number of distinct OTUs; ESC, Estimated sample coverage. Values in parentheses represent the 95% confidence intervals.

Table S4. Frequencies (%) of different bacterial taxons in the phyllosphere of *T. catigua* (Tca), *T. clausenii* (Tcl) and *C. xanthocarpa* (Cxa).

| PHYLUM         | CLASS               | ORDER              | FAMILY              | GENUS            | Tca | Tcl | Cxa  |
|----------------|---------------------|--------------------|---------------------|------------------|-----|-----|------|
| Actinobacteria | Actinobacteria      | Actinomycetales    | Mycobacteriaceae    | Mycobacterium    | 0.0 | 0.0 | 0.6  |
|                |                     |                    | Unknown             | Unknown          | 0.0 | 0.0 | 0.6  |
| Bacteroidetes  | Bacteroidetes       | Unknown            | Unknown             | Unknown          | 0.0 | 1.3 | 0.0  |
| Bacteroidetes  | Flavobacteria       | Flavobacteriales   | Flavobacteriaceae   | Chryseobacterium | 3.7 | 0.0 | 1.2  |
|                |                     |                    |                     | Flavobacterium   | 0.9 | 1.3 | 0.0  |
|                |                     |                    |                     | Unknown          | 3.7 | 0.7 | 0.6  |
| Bacteroidetes  | Sphingobacteria     | Sphingobacteriales | Flexibacteraceae    | Unknown          | 0.0 | 0.7 | 0.0  |
| Bacteroidetes  | Sphingobacteria     | Sphingobacteriales | Sphingobacteriaceae | Pedobacter       | 0.0 | 3.3 | 1.2  |
|                |                     |                    |                     | Sphingobacterium | 0.0 | 0.0 | 0.6  |
|                |                     |                    |                     | Unknown          | 0.0 | 9.2 | 9.7  |
|                |                     |                    |                     | Unknown          | 0.9 | 1.3 | 0.6  |
| Bacteroidetes  | Sphingobacteria     | Sphingobacteriales | Unknown             | Unknown          | 0.9 | 1.3 | 0.6  |
| Bacteroidetes  | Sphingobacteria     | Unknown            | Unknown             | Unknown          | 2.8 | 5.2 | 6.1  |
| Bacteroidetes  | Unknown             | Unknown            | Unknown             | Unknown          | 0.9 | 0.7 | 0.6  |
| Cyanobacteria  | Cyanobacteria       | Deferribacterales  | Unknown             | Unknown          | 0.0 | 0.0 | 10.9 |
| Cyanobacteria  | Cyanobacteria       | Unknown            | Unknown             | Unknown          | 0.0 | 0.0 | 3.0  |
| Cyanobacteria  | Unknown             | Unknown            | Unknown             | Unknown          | 0.0 | 0.0 | 0.6  |
| Firmicutes     | Bacilli             | Lactobacillales    | Lactobacillaceae    | Lactobacillus    | 0.0 | 0.0 | 8.5  |
| Firmicutes     | Bacilli             | Bacillales         | Staphylococcaceae   | Staphylococcus   | 0.0 | 0.0 | 3.6  |
| Firmicutes     | Bacilli             | Unknown            | Unknown             | Unknown          | 0.0 | 0.0 | 1.8  |
| Proteobacteria | Alphaproteobacteria | Rhizobiales        | Beijerinckiaceae    | Beijerinckia     | 0.0 | 0.0 | 0.6  |
|                |                     |                    |                     | Unknown          | 0.9 | 0.0 | 0.0  |
| Proteobacteria | Alphaproteobacteria | Rhizobiales        | Brucellaceae        | Ochrobactrum     | 0.9 | 0.0 | 0.0  |
| Proteobacteria | Alphaproteobacteria | Rhizobiales        | Methylobacteriaceae | Methylobacterium | 1.8 | 0.0 | 1.8  |
|                |                     |                    |                     | Unknown          | 0.0 | 1.2 | 0.0  |
| Proteobacteria | Alphaproteobacteria | Rhizobiales        | Rhizobiaceae        | Unknown          | 0.9 | 0.0 | 0.0  |
|                |                     |                    |                     | Rhizobium        | 0.0 | 0.7 | 0.6  |
| Proteobacteria | Alphaproteobacteria | Rhizobiales        | Unknown             | Unknown          | 0.0 | 0.0 | 0.6  |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales   | Sphingomonadaceae   | Sphingomonas     | 3.7 | 4.6 | 20.6 |
|                |                     |                    |                     | Sphingopyxis     | 0.9 | 0.0 | 0.0  |
|                |                     |                    |                     | Novosphingobium  | 0.0 | 0.0 | 0.6  |
|                |                     |                    |                     | Sphingobium      | 0.0 | 0.0 | 0.6  |
|                |                     |                    |                     | Unknown          | 0.0 | 0.0 | 1.8  |
| Proteobacteria | Alphaproteobacteria | Unknown            | Unknown             | Unknown          | 0.9 | 1.3 | 4.8  |
| Proteobacteria | Betaproteobacteria  | Burkholderiales    | Comamonadaceae      | Acidovorax       | 0.9 | 0.0 | 0.0  |
|                |                     |                    |                     | Variovorax       | 0.0 | 0.7 | 0.0  |

|                |                     |                   |                    |                  |      |      |     |
|----------------|---------------------|-------------------|--------------------|------------------|------|------|-----|
|                |                     |                   |                    | Delftia          | 0.0  | 0.0  | 0.6 |
| Proteobacteria | Betaproteobacteria  | Burkholderiales   | Incertae sedis     | Xylophilus       | 0.0  | 0.0  | 0.6 |
| Proteobacter   | Betaproteobacteria  | Burkholderiales   | Unknown            | Unknown          | 0.0  | 0.7  | 0.0 |
| Proteobacter   | Betaproteobacteria  | Unknown           | Unknown            | Unknown          | 0.0  | 0.0  | 1.2 |
| Proteobacteria | Deltaproteobacteria | Myxococcales      | Cystobacteraceae   | Cystobacter      | 0.0  | 0.0  | 0.6 |
| Proteobacteria | Gammaproteobacteria | Enterobacteriales | Enterobacteriaceae | Pantoea          | 5.5  | 0.7  | 0.0 |
|                |                     |                   |                    | Enterobacter     | 12.8 | 9.2  | 0.0 |
|                |                     |                   |                    | Raoultella       | 0.0  | 3.9  | 0.0 |
|                |                     |                   |                    | Erwinia          | 8.3  | 10.5 | 0.0 |
|                |                     |                   |                    | Salmonella       | 4.6  | 0.0  | 0.0 |
|                |                     |                   |                    | Citrobacter      | 1.8  | 0.7  | 0.0 |
|                |                     |                   |                    | Klebsiella       | 0.0  | 1.3  | 0.0 |
|                |                     |                   |                    | Kluyvera         | 0.0  | 1.3  | 0.0 |
|                |                     |                   |                    | Yersinia         | 0.0  | 0.7  | 0.0 |
|                |                     |                   |                    | Unknown          | 2.8  | 2.6  | 0.6 |
| Proteobacteria | Gammaproteobacteria | Enterobacteriales | Unknown            | Unknown          | 0.0  | 0.7  | 0.6 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales   | Moraxellaceae      | Acinetobacter    | 30.3 | 1.3  | 0.0 |
|                |                     |                   |                    | Unknown          | 0.0  | 0.0  | 0.6 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales   | Pseudomonadaceae   | Pseudomonas      | 7.3  | 19.6 | 2.4 |
|                |                     |                   |                    | Unknown          | 0.0  | 3.9  | 0.0 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales   | Unknown            | Unknown          | 0.0  | 0.7  | 0.0 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales   | Xanthomonadaceae   | Stenotrophomonas | 1.8  | 2.6  | 0.0 |
|                |                     |                   |                    | Unknown          | 0.0  | 0.7  | 3.6 |
| Proteobacteria | Gammaproteobacteria | Unknown           | Unknown            | Unknown          | 0.0  | 3.3  | 3.6 |
| Proteobacteria | Unknown             | Unknown           | Unknown            | Unknown          | 0.0  | 1.3  | 0.0 |
| Unknown        | Unknown             | Unknown           | Unknown            | Unknown          | 0.9  | 3.3  | 1.8 |

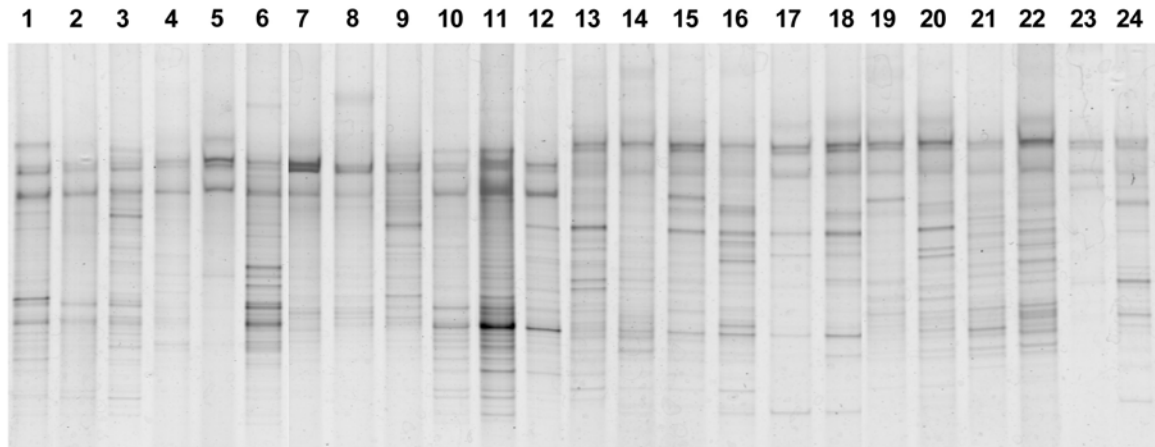


Fig. S1. Bacterial communities associated with the leaves of nine forest tree species from Estação Ecológica dos Caetetus (SP, Brazil) as determined by PCR-DGGE of 16S rRNA genes. Lane headings denote samples as follows: 1-3, *Campomanesia xanthocarpa*; 4-6, *Holocalix balansae*; 7-9, *Ocotea indecora*; 10-12, *Seuieria floribunda*; 13-15, *Aspidosperma polyneuron*; 16-18, *Trichilia pallida*; 19-20, *Trichilia catigua*, 21-22, *Trichilia clausenii*; 23-24, *Urera baccifera*.

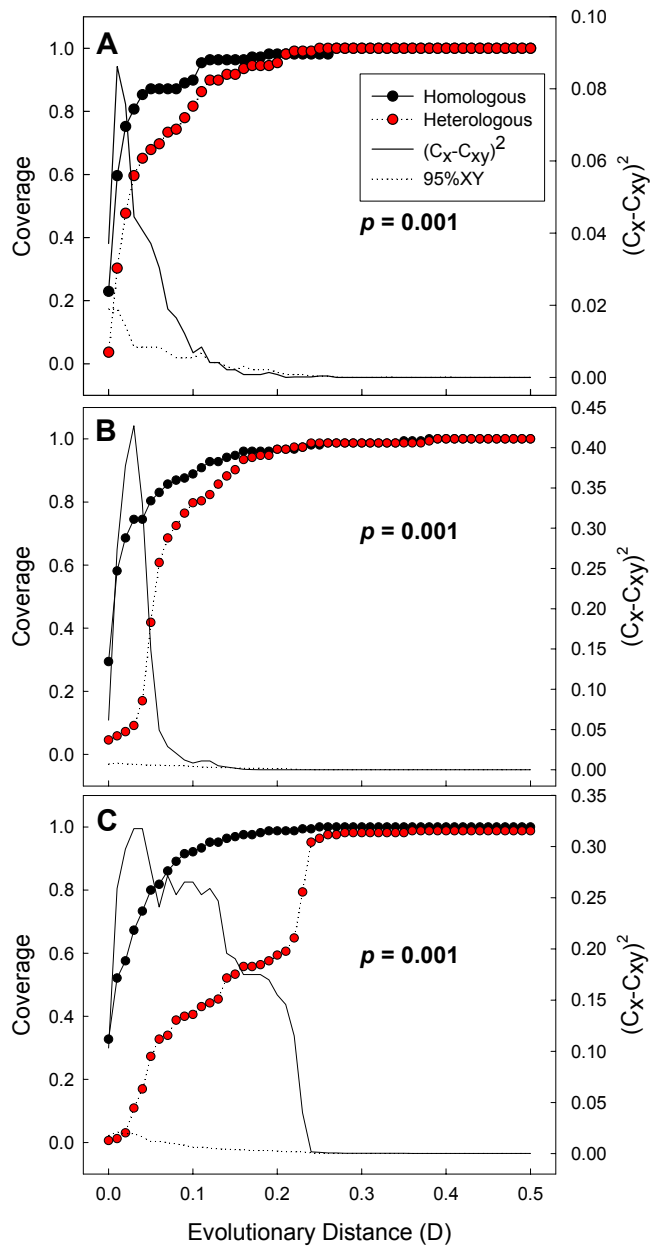


Fig. S2. LIBSHUFF analysis of the bacterial communities of in the phyllosphere of *T. catigua*, *T. clausenii* and *C. xanthocarpa*. **A**, *T. catigua* (homologous) x *T. clausenii*; **B**, *T. clausenii* (homologous) x *C. xanthocarpa*; **C**, *C. xanthocarpa* (homologous) x *T. catigua*. Communities are significantly different at  $P = 0.001$ . The distribution of  $(C_x - C_{xy})^2$  as a function of D indicates that the bacterial communities of the phyllosphere of *T. catigua* and *T. clausenii*, *T. clausenii* and *C. xanthocarpa* and *C. xanthocarpa* and *T. catigua* differ mostly at  $D < 0.12$ ,  $D < 0.15$  and  $D < 0.36$ , respectively.