

Bacterial Diversity in Tree Canopies of the Atlantic Forest

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The leaf surface, also known as the phyllosphere, is one of the most common habitats for terrestrial microorganisms (1), but almost nothing is known about the diversity of microorganisms that inhabit this environment (2). Here, we report a survey of bacterial diversity in the leaf canopy of a tropical Atlantic forest. The Atlantic Forest of Brazil is a biodiversity hotspot that has been reduced to less than 8% of its original size over the past 4 centuries and is considered to be the oldest forest on the planet, containing about 20,000 vascular plant species, of which about one-half are endemic (3, 4). Initially we compared the bacterial communities on the leaf surfaces of nine tree species (table S1) by using a molecular method that generates a DNA fingerprint of the predominant bacteria from their 16S ribosomal RNA (rRNA) gene sequences (5) (fig. S1). Our results showed that bacterial communities from the same tree species varied but could be consistently grouped by discriminant analyses (table S2). These data are consistent with previous research showing that different plants select for distinct microbial communities (6).

To identify the bacteria in the phyllospheres of *Trichilia catigua*, *T. clausenii*, and *Campomanesia xanthocarpa*, we analyzed 418 partial DNA sequences encoding 16S rRNA genes (5). Comparison of homologous and heterologous

coverage curves indicated that all three phyllosphere communities were significantly different in their bacterial species compositions (fig. S2). For all clone libraries, the sample size was sufficient to recover the most abundant deep phylogenetic groups. At evolutionary distances (*D*) higher than 0.20 (the cutoff value for group sequences at the phylum level), the homologous coverages were greater than 96% (fig. S2). At a *D* of 0.03, corresponding to bacterial species, coverages varied from 67 to 81% (fig. S2). Species richness was estimated by using Chao1 nonparametric estimator (table S3). Each phyllosphere community harbored from at least 95 to 671 bacterial species (Fig. 1 and table S3), of which only 0.5% were common to all of the trees studied. Almost all of the bacteria (97%) were from undescribed species, suggesting they may be unique to the phyllosphere habitat (table S4).

Although this initial survey was limited in scope, extrapolation of our results for the 20,000 vascular plant species in the Atlantic Forest would yield about 2 to 13 million new bacterial species. The absolute diversity of bacteria in nature is unknown, but by comparison the Earth's oceans have been estimated to contain up to 2 million species, whereas a ton of soil may have 4 million species (7). The estimates for phyllosphere diversity could be decreased considerably should future surveys reveal higher amounts of overlap

in bacterial community composition between tree species. On the other hand, the bacterial species richness for the individual trees surveyed represent minimum estimates of that which may occur on individual trees. Variations in community structures within tree species may possibly correspond to different leaf ages, location in the canopy, light incidence, and microclimate conditions that influence the leaf environment (8). The current study provides a glimpse into the microbial diversity in tree canopies of tropical forests, and there are many questions that arise from this research. Do the same tree species in completely different locations or continents harbor similar communities? To what degree do various environmental factors affect the composition and structure of phyllosphere communities? What is the diversity of fungi and archaea on the plant leaf surfaces, and what role does phyllosphere microbial community play in protection against herbivory or infection by pathogens? As we begin to survey the bacterial species through systematic surveys of different plants, there will be exciting opportunities for studies of the metabolic capabilities and the ecological functions of phyllosphere microorganisms in terrestrial ecosystems.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5782/1917/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S4
Data and Analyses

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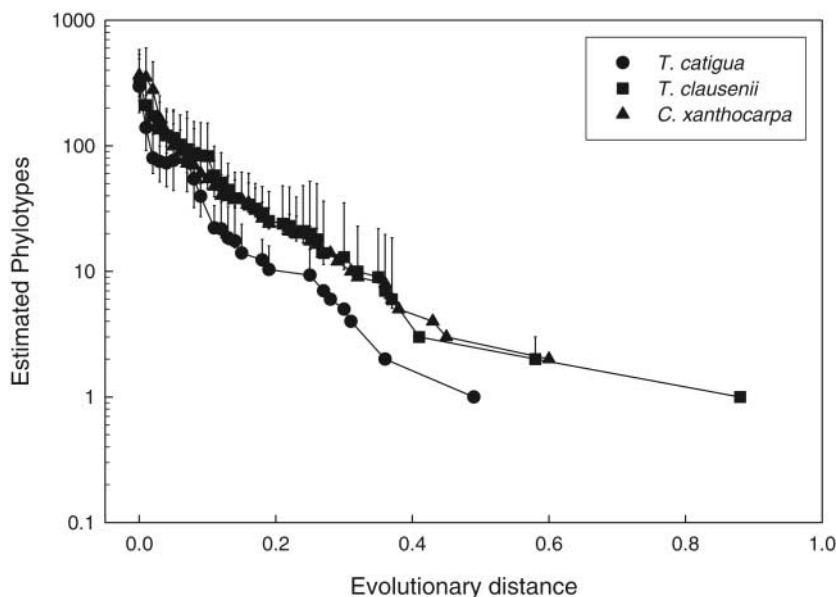


Fig. 1. Estimated number of phylotypes at different evolutionary distances, using Chao1 nonparametric estimator, on the leaf surface of different tree species. Bars represent 95% confidence intervals.

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