Bacterial communities of soils under different forest ecosystems as revealed by molecular approaches based on 16S rDNA clone library

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Abstract A 16S rDNA clone library was established following soil community DNA extraction, polymerase chain reaction (PCR) and cloning. A total of 324 clones, including 27 from each sample collected from the natural forest (YNF), the first rotation (Y1R) and the second rotation (Y2R) of hoop pine plantations (each treatments with four replicates) at Yarraman, in subtropical Australia, were randomly selected and sequenced to represent the bacterial composition and diversity of the clone library. Phylogenetic analyses indicated that Yarraman soil had a putative bacterial community composition of Unclassified bacteria (34.4%), Proteobacteria (22.0%), Chloroflexi/ Verrucomicrobia (15.7%), Fibrobacteres/Acidobacteria (10.2%), Chloroflexi (6.9%), Gemmatimonadetes (5.6%), and Actinobacteria (5.2%). There was a significant difference among different treatments in the taxonomic group distribution. The soil bacterial diversity tended to decrease from the natural forest to the first and then to the second rotation of hoop pine plantations.

Introduction

Microorganisms are vital to the forest ecosystem through their roles in nutrient cycling and their associations with other organisms, including plants. Forest clearance, tree harvesting and replanting to re-establish a forest represent a severe disturbance to a forest ecosystem, and these activities are likely to disturb soil microbial communities. DNA-based approaches have been used increasingly to study the genetic structure of the total bacterial community and the impact of environmental disturbance on the soil bacterial community. Smit et al. (2001) studied the soil microbial community of a wheat field in the Netherlands and found significant seasonal changes in DGGE patterns. Soil type and properties can influence microbial community composition. Johnson et al. (2003) compared the composition of microbial communities in 47 agricultural soil samples in San Joaquin Valley, California, USA and found that soil bacterial DNA fingerprints were significantly correlated with soil electrical conductivity, soil texture, inorganic carbon and nitrogen content. He et al. (2006) investigated the soil bacterial composition and diversity under forest residue management practices but no significant difference was detected among the residue management regimes at 6.4 years of forest growth. The objectives of this study were to compare the soil bacterial community composition and diversity under different forest ecosystems, i.e., the natural forest and the first and the second rotation of hoop pine plantations by using 16S rDNA-based cloning and sequencing approaches.

Materials and Methods

Soil samples were collected from three contrasting forest ecosystems located at a long-term experimental site (2403 YMN, 26°52'S, 151°51'E) at Yarraman, Queensland in subtropical Australia. The natural forest (YNF) first rotation (Y1R) and second rotation (Y2R) hoop pine plantation sites are adjacent to each other on the same position of the slope at approximately 2-3°.

The YNF site is classified as a rainforest/bastard scrub and is dominated by bunya pine (Araucaria bidwilli), yellowwood (Terminalia oblongata), crows ash (Pentaceras australis) and lignum-vitae (Premana lignum-vitae). The Y1R hoop pine plantation was established in 1949 and the Y2R hoop pine plantation was planted in November 2000 after the harvest part of the IR hoop pine plantation. Under each forest ecosystem, 4 areas of 10x10 m² were selected and 5 soil cores were taken from each area to form a composite sample. Each sample was placed in a sterile plastic bag, transported to the laboratory in ice and stored at 4°C. The soil was classified as a Smirf Mesotrophic Red Ferrosol with a medium, non-gravel, clay-loamy surface horizon. Organic matter contents were 10-11%, and pH values were 5.79, 5.47 and 6.26 for YNF, Y1R and Y2R samples, respectively.

Soil community DNA from each sample was extracted and purified as described by He et al. (2005). 16S rDNA PCR amplification was carried out using the universal bacterial primer pair of P4-P5, which produces 433 bp products (Kirsie & Wellington, 1999). The PCR products were purified by agarose gel electrophoresis, ligated into the pGEM-T Easy Vector and transformed into competent cells (E. Coli JM109). The white colonies were counted and 27 were randomly selected for sequencing. The clone sequences were checked using the Chimera Check program, analysed with the BLAST program, and the phylogenetic trees were constructed using the neighbour-joining (NJ) method. The taxonomic groups of clusters in the NJ tree were sorted by comparing with the Genbank database.

For the convenience of description and data statistics, a sequence or a cluster of sequences with less than 5% and 10% base substitutions to the adjacent sequence(s) was defined as a 5% or 10% operational taxonomic unit (OTU), respectively. OTUs distribution in the NJ tree was described and OTUs of each sample and treatment were counted. All the
nucleotide sequences were submitted to Genbank and assigned accession no. AY930113 to AY930430.

Results and Discussion
A 16S rDNA clone library of Yarraman soil samples was constructed, containing 100 clones from each of 12 samples. 27 clones were sequenced for each sample and 318 effective clone sequences in total were obtained. These 16S rDNA clone sequences had most similar Genbank species or sequences with identities ranging from 85% to 99%. Most sequences had their identity value ≥ 95% (accounted for 76.7% of the total sequences) and only 2.2% had a value < 90%. The taxonomic classification of the NCBI database. These putative bacterial groups were Unclassified, Fibrobacteres/Acidobacteria (mostly Acidobacteria class), Chlamydiae/Verrucomicrobia (mostly Verrucomicrobia class), Proteobacteria, Gemmatimonadetes, Actinobacteria, and Chloroflexi groups. On average, the Unclassified group was the largest group and accounted for 34.4%, followed by Proteobacteria (22.0%), Chlamydiae/Verrucomicrobia (15.7%), Fibrobacteres/Acidobacteria (10.2%), Chloroflexi (6.9%), Gemmatimonadetes (5.6%), and Actinobacteria (5.2%).

The putative taxonomic groups were not distributed randomly among the treatments of YNF, YIR and Y2R as confirmed by the Chi-Square test (p=0.0001). In other words, there were significant differences in the proportions made up by different soil bacterial groups among the three treatments. Comparing YNF with YIR and Y2R, Fibrobacteres/Acidobacteria, Chlamydiae/Verrucomicrobia and Chloroflexi made up a greater proportion in YNF than in YIR and Y2R. YNF clones belonging to Unclassified group accounted for 18.0%, but YIR and Y2R accounted for 38.5% and 46.5%, respectively, double the percentage of the YNF clones in the group. On the other hand, YNF had 18.0%, 23.0% and 9.0% clones of Fibrobacteres/Acidobacteria, Chlamydiae/Verrucomicrobia and Chloroflexi groups, respectively, higher than the corresponding YIR and Y2R. The differences in the proportions of the three treatments and replications were some differences in OTU numbers among different treatments and replications. There were 82, 73 and 59% OTUs and 59, 53 and 43% OTUs for YNF, YIR and Y2R, respectively, tending to decrease from YNF to YIR and Y2R. The Shannon index H' of different treatments were calculated for 5% OTUs. The Shannon index H' of different treatments were calculated for 5% OTUs. YNF, YIR and Y2R had the H' values of 5.41, 5.19 and 4.79, respectively. The Shannon index H' of different treatments were calculated for 5% OTUs. YNF, YIR and Y2R had the H' values of 4.90, 4.66 and 4.35, respectively. The H' values tended to decrease from YNF to YIR and to Y2R. These results implied that, with the conversion from natural forest to hoop pine plantations, i.e., from YNF to YIR and Y2R, soil bacterial diversity tended to decrease.

References
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