Analyses of the influencing factors of soil microbial functional gene diversity in tropical rainforest based on GeoChip 5.0

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Abstract

To examine soil microbial functional gene diversity and causal factors in tropical rainforests, we used a microarray-based metagenomic tool named GeoChip 5.0 to profile it. We found that high microbial functional gene diversity and different soil microbial metabolic potential for biogeochemical processes were considered to exist in tropical rainforest. Soil available nitrogen was the most associated with soil microbial functional gene structure. Here, we mainly describe the experiment design, the data processing, and soil biogeochemical analyses attached to the study in details, which could be published on BMC microbiology Journal in 2015, whose raw data have been deposited in NCBI’s Gene Expression Omnibus (accession number GSE69171).

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2.3. GeoChip 5.0 experiment

GeoChip 5.0 was manufactured by Agilent (Agilent Technologies Inc., Santa Clara, CA) in the 8 x 60 K format. 600 ng of purified soil DNA of each sample was labeled with the fluorescent dye Cy-3 (GE Healthcare, CA, USA) using a random priming method as described previously [3] purified using a QIAquick Purification kit (Qiagen, CA, USA), and dried in a SpeedVac (Thermo Savant, NY, USA) into a powder. Subsequently, the labeled DNA was resuspended into 27.5 μl of DNase/RNase-free distilled water, and mixed completely with 42 μl of hybridization solution, containing 1 x Acgh blocking, 1 x HI-RPM hybridization buffer, 10 pM universal standard DNA, 0.05 μg/μl Cot-1 DNA, and 10% formamide (final concentrations). After these, the solution was denatured at 95 °C for 3 min, and then incubated at 37 °C for 30 min, then hybridized with GeoChip 5.0 arrays (60 K). GeoChip hybridization was preceded at 67 °C in Agilent hybridization oven for 24 h. After hybridization, the slides were washed using Agilent Wash Buffers at room temperature. Then the arrays were scanned at 633 nm by a laser power of 100% and a photomultiplier tube gain of 75% with a NimbleGen MS200 Microarray Scanner (Roche NimbleGen, Inc., Madison, WI, USA). The images data were extracted by following Agilent Feature Extraction program.

2.4. Raw data processing

The microarray data were preprocessed by the microarray analysis pipeline on IEG website (http://ieg.ou.edu/microarray/) as previously described [3]. The main steps were in the following steps: (i) removing the spots of poor quality, which was a signal to noise ratio of less than 2.0; (ii) the relative abundance of each soil sample was calculated by dividing the total intensity of the detected probes, then multiplying a constant and taking the natural logarithm transformation; (iii) the detected probes in spots of poor quality, which was a signal to noise ratio of less than 2.0; (iv) the detected probes in the total intensity of the detected probes, then multiplying a constant and taking the natural logarithm transformation; (v) the detected probes in spots of poor quality, which was a signal to noise ratio of less than 2.0; (vii) the detected probes in spots of poor quality, which was a signal to noise ratio of less than 2.0; (viii) the detected probes in spots of poor quality, which was a signal to noise ratio of less than 2.0;

2.5. Statistical analysis

A one-way analysis of variance was used to analyze the statistical differences at a significance level of P < 0.05. The Shannon index and Simpson index were used to characterize the soil microbial community diversity. The normalized signal intensity of each gene category was used to represent the gene relative abundance [4]. Detrended correspondence analysis was used to evaluate the differences of sampling sites. Canonical correspondence analysis was used to examine the linkages between soil microbial communities and environmental factors. The multivariate regression tree was used to determine the important factors in influencing the soil microbial functional gene diversity. All the data analyses were performed in the Vegan package (v. 1.15-1) in R (v. 2.9.1).

3. Discussion

In this study, we described the functional gene diversity and metabolic potential of soil microbial community in the tropical rainforests of JFL based on GeoChip 5.0 technology. GeoChip 5.0 is comprised of more than 57,000 oligonucleotide probes, covering over 144,000 gene sequences from 393 functional gene families involved in carbon, nitrogen, sulfur, phosphorus cycling and others.[5] We found that soil available nitrogen and plant diversity were significantly correlated with soil microbial functional gene structure. Our results indicated that the metabolic potential for soil microbial community could acclimatize to acid tropical rainforest soils.

Conflict of interest

We declare that there is no conflict of interest on our work published in this paper.

Acknowledgements

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