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Tufa microbial diversity revealed by 16S rRNA cloning in Taroko National Park, Taiwan

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Abstract

Tufa is a carbonate sediment contains inorganic and organic substances such as algae, microorganism and invertebrate. Microbial diversity of tufa found in Taroko National Park was investigated using 16S rRNA cloning and fluorescent in situ hybridization (FISH). Eleven 16S rRNA phylotypes and 37 genus and group of bacteria were identified. Of total 381 clones isolated, proteobacteria occupied 25–30% whereas cyanobacteria dominated 16–28% in total microbial population in the three sites. Acidobacteria, agricultural soil bacterium, verrucomicrobia and firmicutes were, generally, distributed in the three sampling sites. Among the three sampling sites, Baiyang walkway is found to be the most diverse site in its tufa microbial composition, indicated by species richness plot and FISH.
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Keywords: 16S rRNA; Cyanobacteria; Microbial diversity; Proteobacteria; Tufa

1. Introduction

Taroko National Park covers more than 92,000 ha in Taiwan, is feature of its high mountain, steep gorges, waterfalls, rivers and diverse fauna and flora ecologies. The action of rainwater and streams to these marbles has formulated the valleys and geographical landforms nowadays. The formation of this world distinguish geographical Park is caused by the collision of Philippine and Eurasian Continental Plate, thick layer of calcareous rock had been raised from marine. For over million years of immense tectonics interaction, the original limestone rocks have turned into tufa, a product of calcium carbonate precipitation under a cool water regime and typically contain the remains of microorganism and invertebrate (Ford and Pedley, 1996).

The complexity of tufa is determined by interactions among prokaryotes, plants and physico-chemical reactions (Violante et al., 1994; Ford and Pedley, 1996). This fact is important in the field of sedimentology and geomorphology. In some extreme environments, such as volcanic regions, sulfur bacteria were found in the tufa deposit. They oxidizes H_2S converting it to sulfur and soluble gypsum (Hubbard et al., 1990). For the tufa in low-temperature and alkaline environment, alkaliphilic cyclobacterium were the dominant microbial flora (Peter et al., 2002). Algea such as *Oocardium* and *Lyngbya* and cyanobacteria *Phormodium* and *Schizothrix*, as reported by Golubic et al. (1993), are commonly found in such environments and are important in carbonate accumulation in organic and inorganic systems. Heterotrophic bacteria and fungi are closely associated with biofilms and organic matter formed on tufa. Previous study also elucidated its role in the formation of tufa, and as a fundamental agent in the growth of bacterioherm fabrics (Folk, 1994). The understanding of the microbial communities in this natural environment is interesting to ecologists and microbiologists because of their usefulness in a diverse range of medical and agricultural contexts. They have been successfully isolated from an enormous genetic pool. Plant–soil events, soil–microorganism interactions, spatial heterogeneity, temporal variability, horizontal gene transfer and other factors contribute to the high diversity and occurrence

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of new microbial species in soil environment (Trevors, 1998; Wall and Virginia, 1999; Franklin and Mills, 2003; Kirk et al., 2004).

In order to understand microbial community of tufa in Taroko National Park, microorganisms from tufa samples were described using molecular approaches. FISH was also employed to elucidate the microbes found in Tufa, and demonstrated the diverse microbial flora in this environment. The establishment of this fundamental information concerning this unique ecosystem can be extended further in coming future for species discovery, exploration and conservation of useful microbes. Microbial in tufa may also serve as an alternative indicator, which we can use for the evaluation on microbial community changes resulted by human or natural disturbances.

2. Materials and methods

2.1. Sampling

Tufa samples, weighing around 100 g each, were collected from three sampling sites—Eternal Spring Shrine (N24°09′40.1″; E 121°36′13.2″), Baiyang walkway (N 24°11′29.2″; E 121°29′21.9″) and Cross-Island Swallow valley (N24°10′23″; E 121°32′21″), respectively. Three samples were collected at each sampling site. A core sampler was used to bore the tufa specimens to depths of 5 cm below the surface. The water sample was obtained from the sampling site using aseptic plastic dropper. The environmental temperature and pH were measured in the field using a Cardy Twin pH meter (Cole Palmer, USA). The electrical conductivity of the water sample was measured using a microprocessor conductivity meter (WTW LF96, Germany). The specimens were transported at 0 °C and processed immediately upon arrival at laboratory.

2.2. Amplification and cloning of the 16S rRNA gene

Ten grams of three samples collected at each sampling site were mixed using autoclaved mortar, and the total DNA of tufa specimens were extracted using FastDNA® Spin Kit (BIO101, USA) as instructed by user manual. Further, purified by Wizard® DNA purification system (Promega, USA). Primers BSF8 (5′-AGAGTTTGATCCTGGC TCAG-3′) and BSF1507 (5′-TACCTTGTTACGACTT-3′) (Ribosomal Database Project II) were used for the amplification of 16S rRNA gene. The 25 μl PCR reaction mixture contained DNA template 5 μl, 5 μl of 1 mM dNTP, 0.5 μM of each primer, 0.5 μl *Taq* DNA polymerase (5 U/μl, AmpliTaq; Perkin–Elmer, USA), 10X PCR buffer 2.5 μl. The amplification was performed with Gene Amp® PCR system 9700 (Applied Biosystems, USA), using condition: initial heating 95 °C for 2 min, 30 cycles consisting of 95 °C for 5 s, 60 °C for 30 s, 72 °C for 1. 5 min, and final extension 72 °C for 10 min. The amplificates were purified with Gel Extraction kit (Viogene, Taiwan) and subsequently ligated into pGEM®-T Vector Systems (Promega, USA). The ligation product was transformed into ECOS® competent cell (Yeastern Biotech, Taiwan) screened by blue–white screening with IPTG and X-Gal. White colonies were selected and plated on LB agar plate contains 50 μg/ml Ampicilin. 16S rRNA inserts were then amplified from the colonies using primers TAF (5′-CAAGGC GATTAAGTTGGGTA-3′) and TAR (5-GGAATTGTGAGCGATAACA-3′) provide by the pGEM®-T Vector Systems. The amplified fragments were sequenced (Mission Biotech. Taiwan) and submitted to Genbank.

2.3. Species richness

Species richness was plotted using BioDiversity Pro ver 2 as instructed by software manual. The theory basis of this plot provides a measurement of species diversity which is robust to sample size, from this plot we can compare composition of different community (Simberloff, 1972). Steeper curves indicate more diverse communities found in the sample.

2.4. Phylogenetic analysis

Sequence alignment and phylogenetic analysis was performed with the multiple sequence alignment software CLUSTAL W ver. 1.82 (Higgins et al., 1994). Phylogenetic tree was constructed by the neighbour-joining with robustness of 100 bootstrapping using PHYLIP package ver. 3.6b.

2.5. FISH samples fixation

Samples for FISH were fixed in 3.7% (wt/vol) formamide overnight at 4 °C. Cells from tufa region were extracted based on the method of Christensen et al. (1999), cells from water region were filtered onto a 25 mm diameter, 0.2-μm-pore-size polycarbonate white GTTP filter (Millipore, USA) under vacuum 5 mmHg. The vacuum was released and 1 ml of 2% (wt/vol) NaCl-50% (vol/vol) ethanol was incubated on the filter for 1 min and

Table 1
Properties and locations of sampling sites

	Location	Elevation (meter)	Tufa pH	Water pH	Tufa temp. (°C)
1	Eternal Spring Shrine	68	9.0	7.8	18
2	Baiyang walkway	546	8.0	8.2	17
3	Cross-Island Swallow valley	450	10.1	8.4	17

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