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Bacterial 16S rDNA Sequences in Immature Volcanic Ash Soil on Volcanoes Mt. Sakurajima and Mt. Fugen in Japan Determined by PCR Amplification

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Volcanogenous soils are widely distributed in Japan. Andosols, a group of volcanogenous soil, are known to show several physicochemical characteristics such as high porosity, presence of allophane, and high content of organic carbon (FitzPatrick 1980). The formation of Andosols is a very rapid process resulting from the large surface area of the volcanic ash-derived parent materials.

Also volcanogenous soils exhibit several microbiological characteristics. Ishizawa and Toyoda (1964) reported that the bacterial counts obtained by the culture method were smaller in volcanogenous than in non-volcanogenous soils while the counts of actinomycetes and anaerobes were higher. We were interested to determine what kinds of microorganisms are involved in soil formation. In this study, we aimed at analyzing the bacterial community in immature volcanic ash soils. A culture-independent, 16S rDNA analysis method was adopted because it is well known that only a part of the bacteria in soil can be cultured and because we anticipated that the population of culturable bacteria was small due to the low content of organic matter in the soil samples used in this study. Rhizobacteria of the pioneer plants in the low-nutrient immature volcanic ash soils were also investigated.

Samples from soils or plant roots were taken on Mt. Fugen in June 1996 and on Mt. Sakurajima in October 1996, both located in the Kyushu district of Japan. At Mt. Fugen, strong eruptions occurred in 1990-1991, when pyroclastic flows destroyed the pre-eruption vegetation. In 1996, the sampling area was covered with patches of a few plant species such as *Polygonum cuspidatum, Eragrostis curvula*, and *Paspalum* spp. Mount Sakurajima has been erupting intermittently and the sampling area was covered with patches of *Miscanthus sinensis*. On those sites, immature volcanic ash soils (1-10 cm depth; Mt. Fugen: pH(H_2O) 5.0, organic C 0.15%, aerobic bacteria on YG agar (yeast extract 1.0 g, glucose 1.0 g, K_2HPO_4 0.3 g, KH_2PO_4 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, agar 15.0 g, distilled water 1,000 mL, pH 6.8) 1.3×10^6 cfu per g; Mt. Sakurajima: pH(H_2O) 4.5, organic C 0.08%, aerobic bacteria on YG agar 6.0×10^4 cfu per g) and plant roots (*P. cuspidatum* on Mt. Fugen and *M. sinensis* on Mt. Sakurajima) were sampled with a sterilized scoop. The samples were transported to the laboratory in sterilized boxes within one day after sampling, frozen with liquid N_2, and stored at $-30°C$ until analysis. For the root samples, the adhering soil particles were removed by gentle shaking in sterile water and with tweezers, then the roots from four plants

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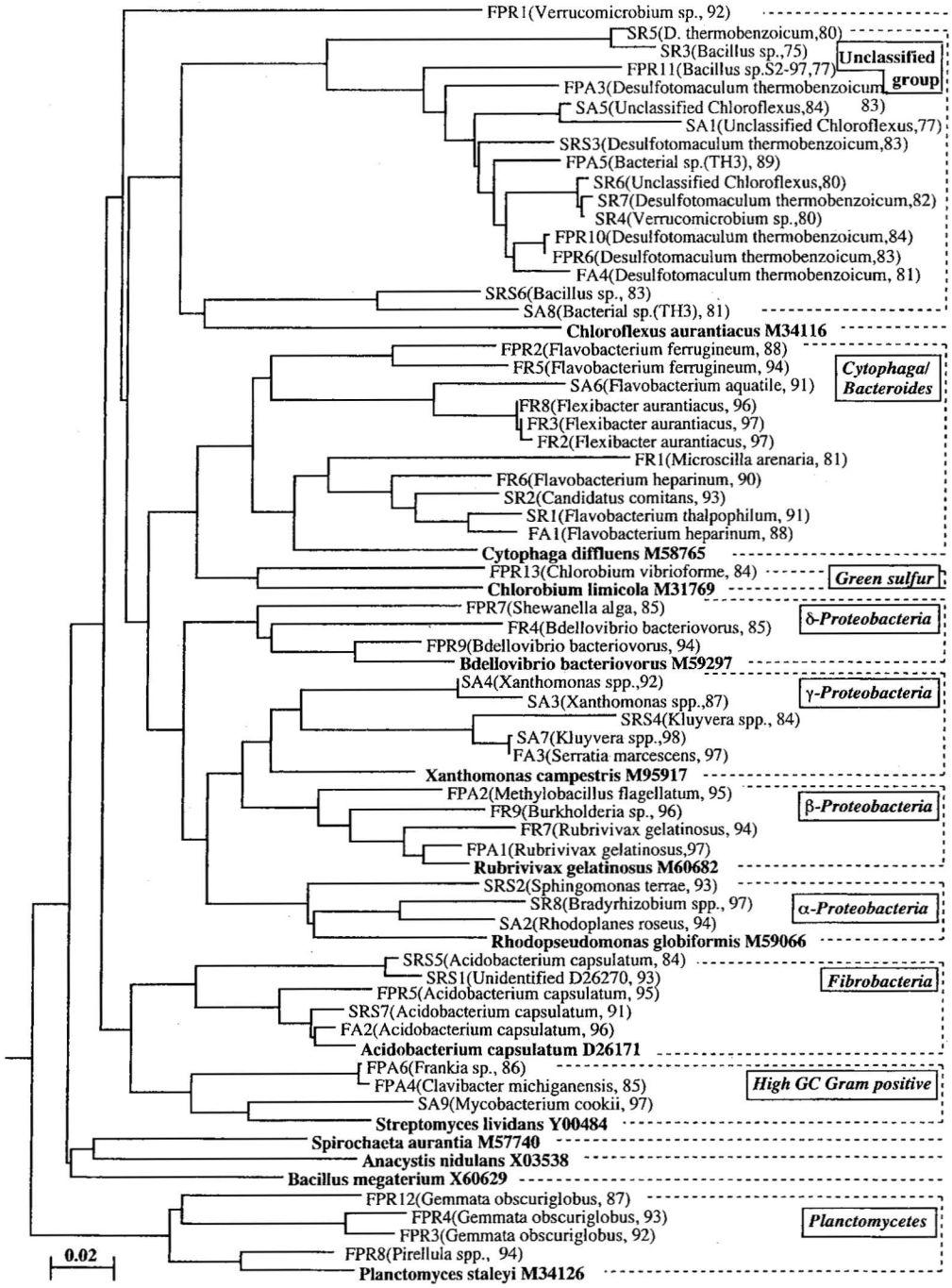
were cut into pieces before freezing. A portion of soil samples of Mt. Fugen was used for a pot experiment. The soil (800 g) was placed in a pot (11.5 cm ϕ, 16 cm height), and five seeds of *Polygonum* sp. were sown and cultivated in a greenhouse with 60 mL of water a day, an amount nearly equivalent to the average daily precipitation at Mt. Fugen. At 75 d after



cultivation, the non-rhizosphere soil, rhizosphere soil obtained by gentle shaking of the roots in the air, and roots were sampled.

DNA was directly extracted from 2 g portions of soil (dry wt.) or of root pieces (fresh wt.) by the method of Zhou et al. (1996). Extracted DNA was purified through low-melting agarose gel (Porteous and Armstrong 1993). A part of 16S rDNA (positions 530–1494 in *Escherichia coli* numbering) in purified DNA corresponding to 50 mg of soil or root was amplified by PCR (*TaKaRa Ex Taq* polymerase 2 U, primers 0.5 μM each, dNTP 0.2 mM each, 10×*Ex Taq* buffer containing 20 mM Mg^{2+} 5 μL, total 50 μL). The primers were 5′-GTGCCAGCMGCCGCGG-3′ as forward primer and 5′-GGYTACCTTGTTACGACTT-3′ as reverse primer, both of which being universal primers (Borneman et al. 1996). The reaction conditions were: 2 min at 90°C, 40 cycles of 1 min at 94°C, 0.5 min at 55°C, and 2.5 min at 70°C, and 10 min at 70°C. The amplified fragments were cloned into the pT7 Blue-T vector (Novagen). The ligated plasmids were transformed into competent *E. coli* JM109. Nineteen clones from immature volcanic ash soils, 30 clones from roots, and 7 clones from rhizosphere soil were analyzed with an automated DNA sequencer (ABI PRISM 377, Perkin-Elmer) using the PCR reverse primer as sequencing primer. The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank database (AB005820–AB005876). Phylogenetic relationships among the sequences of the clones (positions 1167–1420 in *E. coli* numbering) and the 14 bacterial strains in the DNA databases were analyzed using the CLUSTAL W program (Thompson et al. 1994) and the neighbor-joining method

Table 1. Distribution of partial 16S rDNA clones from immature volcanic ash soil area within the domain *Bacteria*.

Sample[a]	*Proteobacteria*				*Cyto-phaga/ Bacteroides*	High GC Gram positive	*Plancto-mycetes*	*Fibro-bacteria*	Un-classified	Green sulfur (*Chlorobium*)	*Verruco-micro-bium*	Total
	α	*β*	*γ*	*δ*								
FA	0	0	1	0	1	0	0	1	1	0	0	4
FR	0	2	0	1	6	0	0	0	0	0	0	9
FPA	0	2	0	0	0	2	0	0	2	0	0	6
FPR	0	0	0	2	1	0	4	1	3	1	1	13
SA	1	0	3	0	1	1	0	0	3	0	0	9
SR	1	0	0	0	2	0	0	0	5	0	0	8
SRS	1	0	1	0	0	0	0	3	2	0	0	7
Total	3	4	5	3	11	3	4	5	16	1	1	56

Data are expressed by the number of clones obtained. [a] Abbreviations for samples; FA, Mt. Fugen immature volcanic ash soil; FR, Mt. Fugen *Polygonum cuspidatum* roots; FPA, Mt. Fugen immature volcanic ash soil in pot experiment; FPR, *Paspalum* sp. roots in pot experiment; SA, Mt. Sakurajima immature volcanic ash soil; SR, Mt. Sakurajima *Miscanthus sinensis* roots; SRS, Mt. Sakurajima soil from rhizosphere of *M. sinensis*.

Fig. 1. Phylogenetic relationships of partial 16S rDNA sequences (positions 1167–1420, *E. coli* numbering) from 56 clones in immature volcanic ash soil area and 13 bacteria in DNA databases (bold, with their accession number). FA, Mt. Fugen immature volcanic ash soil; FR, plant roots in Mt. Fugen; FPA, immature volcanic ash soil in pot experiment; FPR, roots in pot experiment; SA, Mt. Sakurajima immature volcanic ash soil; SR, roots in Mt. Sakurajima; SRS, rhizosphere soil in Mt. Sakurajima. As for the environmental clones, the name of known organism closest to each clone and the percentage of homology were shown in parenthesis. Names of the known major groups in the domain *Bacteria*, with the exception of an unclassified group, were shown in boxes, and the range of each group was indicated by a dashed line. Bar indicates the number of changes per sequence.

(Saitou and Nei 1987). *Methanococcus thermolithotrophicus* (M59128) was used as root in the tree. The FASTA program (Pearson and Lipman 1988) was run for the homology search. According to the tree and the homology value, each clone was assigned to one of the major groups of the domain *Bacteria* (Olsen et al. 1994).

Distribution of the clones from immature volcanic ash soil area among the major groups of *Bacteria* is shown in Table 1, and their relationships and results of homology search are indicated in Fig. 1. Similarity of the clones to the most related strains in the database was 75–97%. Sixteen out of 56 sequences did not correspond to those of the known major groups of *Bacteria*, as reported in previous studies in the field of molecular, soil microbial diversity (Stackebrandt et al. 1993; Ueda et al. 1995; Borneman et al. 1996).

Bacterial 16S rDNA sequences from a variety of groups were recovered in the immature volcanic ash soil area (Table 1). Although the bacterial community structure could not be determined precisely by the methods used in this study due to the bias during DNA extraction and PCR amplification (Smit et al. 1997) as well as the limited number of the clones analyzed, the results obtained at least indicate the existence of bacteria with the sequences. Therefore, the results suggest that various bacteria occurred in the immature volcanic ash soil area.

The results obtained showed that *Acidobacterium*- and *Planctomycetes*-like bacteria were present in the immature volcanic ash soil area (Fig. 1). So far, *Acidobacterium capsulatum* has been isolated only from an acidic mine (Kishimoto et al. 1991). It has been recently suggested that *Planctomycetes* occurred profusely in soils in an uncultured form (Lee et al. 1996; Zarda et al. 1997). The results obtained in this report may raise questions regarding their role in the immature volcanic ash soil area.

Our primary concern is to determine whether the bacterial community is ecologically important in terms of the genesis and development of volcanic ash soils. Yoshida (1989) reported that initial colonizers of culturable bacteria in a new tephra of Usu Volcano in Japan consisted of *Arthrobacter, Corynebacterium*, and *Streptomyces*. Actinomycetes were reported to be relatively abundant in volcanogenous soils (Ishizawa and Toyoda 1964). In the present study, one *Clavibacter* (formerly *Corynebacterium*)-like clone was recovered while other easily culturable actinomycetes or high GC Gram positive bacteria were not detected. It is suggested that the bacterial community may change toward a community rich in high GC Gram positive bacteria along with soil formation.

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