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The Archaea Community Associated with Lava-Formed Gotjawal Forest Soil in Jeju, Korea

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Abstract

The abundance and diversity of archaeal assemblages were analyzed in soils collected from Gyorae Gotjawal forest, Jeju, Korea. Gotjawal soil refers to soil derived from a lava-formed forest, characterized by high organic matter content, fertility, and poor rocky soil. Using domain-specific primers, archaeal 16S rRNA gene sequences were PCR amplified for clone library construction, and a total of 185 archaeal clones were examined. The archaeal clones were affiliated with the phyla Thaumarchaeota (96.2%) and Euryarchaeota (3.8%). The most abundant thaumarchaeal group (90.3% of the clones) was the group I.1b clade, which includes soil ammonia-oxidizing archaea. The unique characteristics of Gotjawal soil, including basalt morphology, vegetation, and groundwater aquifer penetration, may be reflected in the archaeal community composition. Further study is necessary to understand the unique factors of Gotjawal soils that influence archaeal abundance, composition, and diversity.

Keywords

Archaea, Gotjawal Soil, 16S rRNA Gene, Phylogeny

1. Introduction

In Jeju, Korea, the word “Gotjawal” refers to any natural forest with trees that grows from basalt-flow rock in

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the poorest soil, and forms a virtually impassable mixture of trees and undergrowth [1]. These forests are considered to represent sites of international importance because of their unique forest ecosystems and the support provided for ecological communities [2]. The Gotjawal are referred to as the "lungs of Jeju" and provide clean air in exchange for carbon dioxide, which is necessary within the ecosystem. It also functions as a roof over pristine aquifers, as well as porous rocks that purify and recharge rainwater. The Gotjawal represents a biosphere; it has an enormous biodiversity, which includes plant species of both the northern and southern hemispheres. In addition, the Gotjawal is a microclimate regulated by openings into the earth that act as breathing holes and provide water to the aquifers below [3] [4]. However, lava forests have been gradually disappearing in recent decades, with ~50% having been destroyed, leaving 6% of the landmass remaining as a result of unregulated construction and urbanization [5].

The forest is crucial for replenishing the only source of water for Jeju's population of half a million, and for nursing its unique flora and fauna, which include some endangered species and other species endemic to the island. Until recently, no studies had characterized the microorganisms in the soil environments of a Gotjawal forest. Little is known about the soil microbial community or the characteristics that direct its formation [6] [7].

Here, we analyzed the abundance, composition, and diversity of archaea in Gotjawal soils using a polymerase chain reaction (PCR)-based approach. Our study will expand the understanding of soil microbial communities to include those of lava forest soils.

2. Materials and Methods

2.1. Collection of Gotjawal Soil Samples

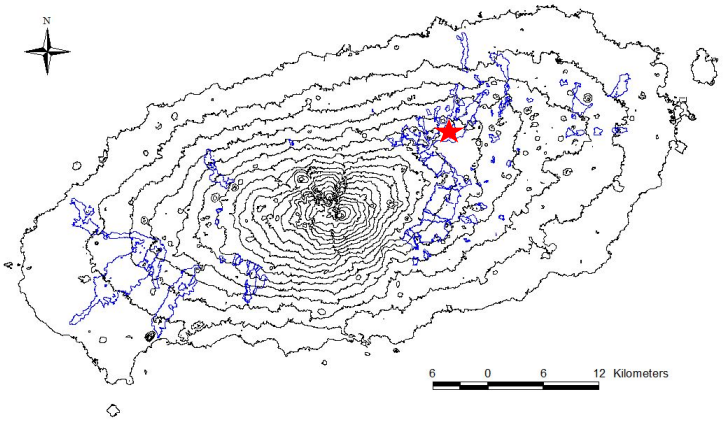
The geographic coordinates of the sample collection site were 33˚26.023'N, 26˚39.46'W (Gyorae Natural Recreation Forest, **Figure 1(a)**). Samples of soil located below the lava and trees were aseptically collected using ethanol-disinfected spatulas and placed into sterile, sealable plastic bags (**Figure 1(b)**). Soil samples were stored in a cooler during transfer to our laboratory and then stored at 5˚C until further processing. Prior to total DNA extraction, soil subsamples were stored at −70˚C. Soil analysis and DNA extraction were conducted within a week of collection. Soil pH and electrical conductivity (EC) were measured in soil extracts using a 1:5 soil: water ratio. Organic matter was analyzed using the Walkey-Black method [8] and nitrate was quantified with a Kieltec auto 2400 system (Tecato AB, Sweden) at the National Instrumentation Center for Environmental Management (NICEM) in Seoul National University.

2.2. DNA Extraction, PCR, and Cloning

DNA was directly extracted from several soil subsamples using the FastDNA® SPIN Kit for Soil (QBiogene Inc., Vista, CA, USA) according to the manufacturer's instructions. The extracted DNA was purified using a FastPure DNA™ Kit (Takara Bio Inc., Japan) and concentrated using a Zymoclean™ Gel DNA Recovery Kit (Zymo Research Corp., Orange, CA, USA). Purified DNA from multiple subsamples was combined and used to construct the clone library. The forward and reverse primers used to amplify archaeal 16S rRNA genes were Ar4F and Ar958R [9], respectively. For clone library construction, 1 µL of DNA template was placed in a 20-µL reaction mixture and PCR-amplified according to conditions described previously [10]. The thermocycler conditions were 95˚C for 5 min; 30 cycles of 95˚C for 45 s, 55˚C for 45 s, and 72˚C for 90 s; and a final extension step of 72˚C for 5 min. The amplification products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). The purified PCR products were ligated into pUC118 Hinc II/BAP (Takara Bio Inc., Japan), transformed into competent *Escherichia coli* DH4*α* cells (Invitrogen Corp., Carlsbad, CA, USA), and then plated on Luria-Bertani (LB) agar plates for selection of transformants. White recombinant transformants were selected and grown overnight at 37˚C in LB medium containing 0.1 $g \cdot L^{-1}$ ampicillin. Plasmids from *E. coli* DH4*α* transformants were isolated using the PureLink® Quick Plasmid Miniprep Kit (Invitrogen Corp., Carlsbad, CA, USA).

2.3. Sequencing and Phylogenetic Analysis

The 16S rRNA genes from the archaeal clones were sequenced using an Applied BioSystems model 3730xl automated DNA sequencing system (Foster City, CA, USA). Putative chimeric sequences were identified using Bellerophon [11] and removed from analysis. Multiple sequence alignment of 16S rRNA gene sequences was performed using the online aligner SINA using the SILVA database (http://www.arb-silva.de/aligner [12]),



(a)

(b)

Figure 1. (a) A geologic map of Jeju Island showing the Gotjawal distribution (blue line) and sampling sites; Gotjawal forest (red star symbol); (b) Photograph of an undisturbed Gyorae Gotjawal forest (33˚26.023'N, 26˚39.46'W) in the Gyorae Natural Recreation Forest, Jeju, Korea.

which takes into consideration the secondary structure of the rRNA gene. Phylogenetic trees were constructed using the neighbor-joining method [13] with MEGA version 5.0 for Windows [14]. Evolutionary distances were calculated using the Kimura 2-parameter method [15]. Bootstrap analyses of the neighbor-joining data were conducted based on 1000 samples to assess the support for inferred phylogenetic relationships.

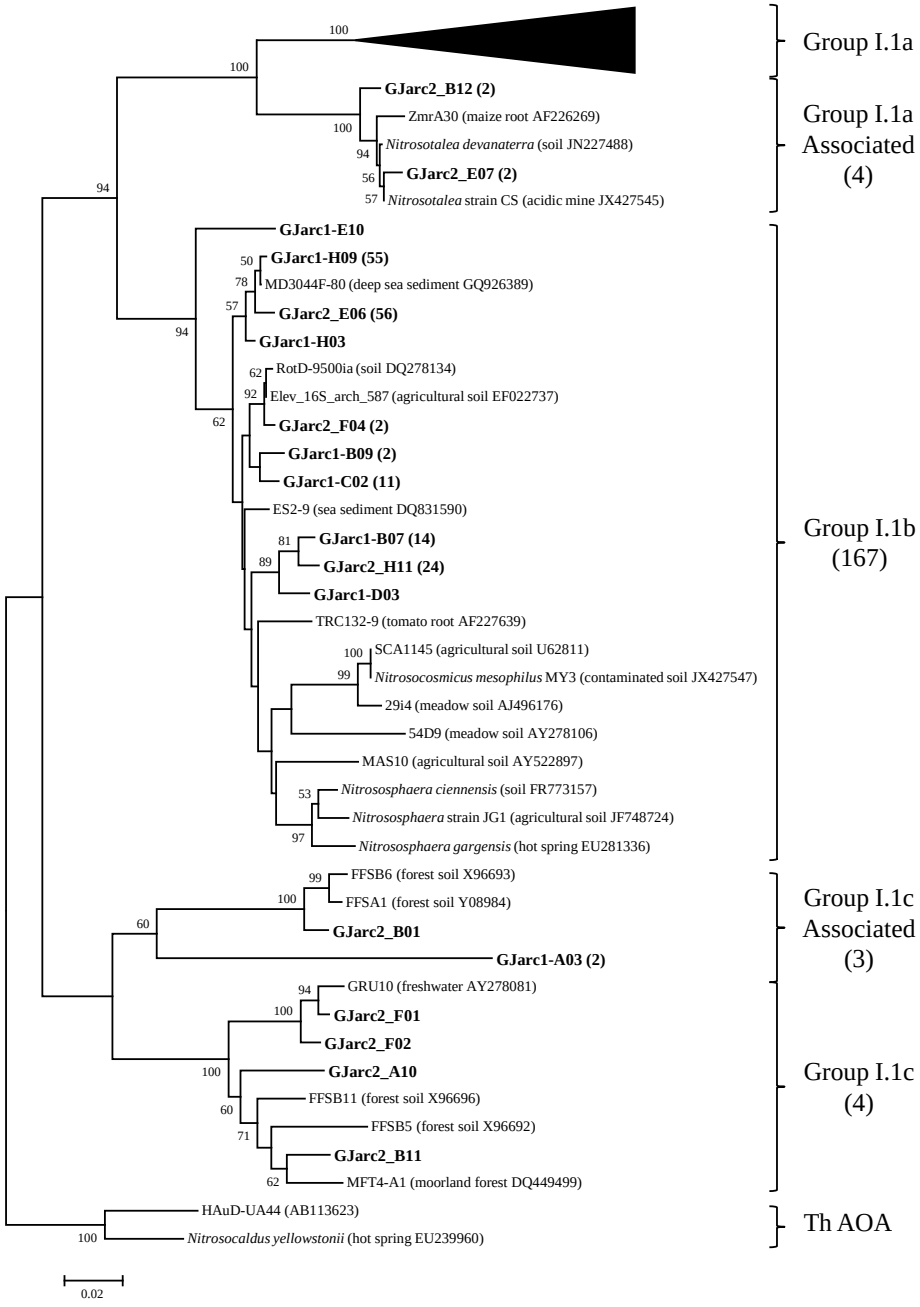
2.4. Nucleotide Sequence Accession Numbers

All sequences were deposited in DDBJ/GenBank/EMBL under the following accession numbers: AB848738-AB848922 (185 archaeal clones).

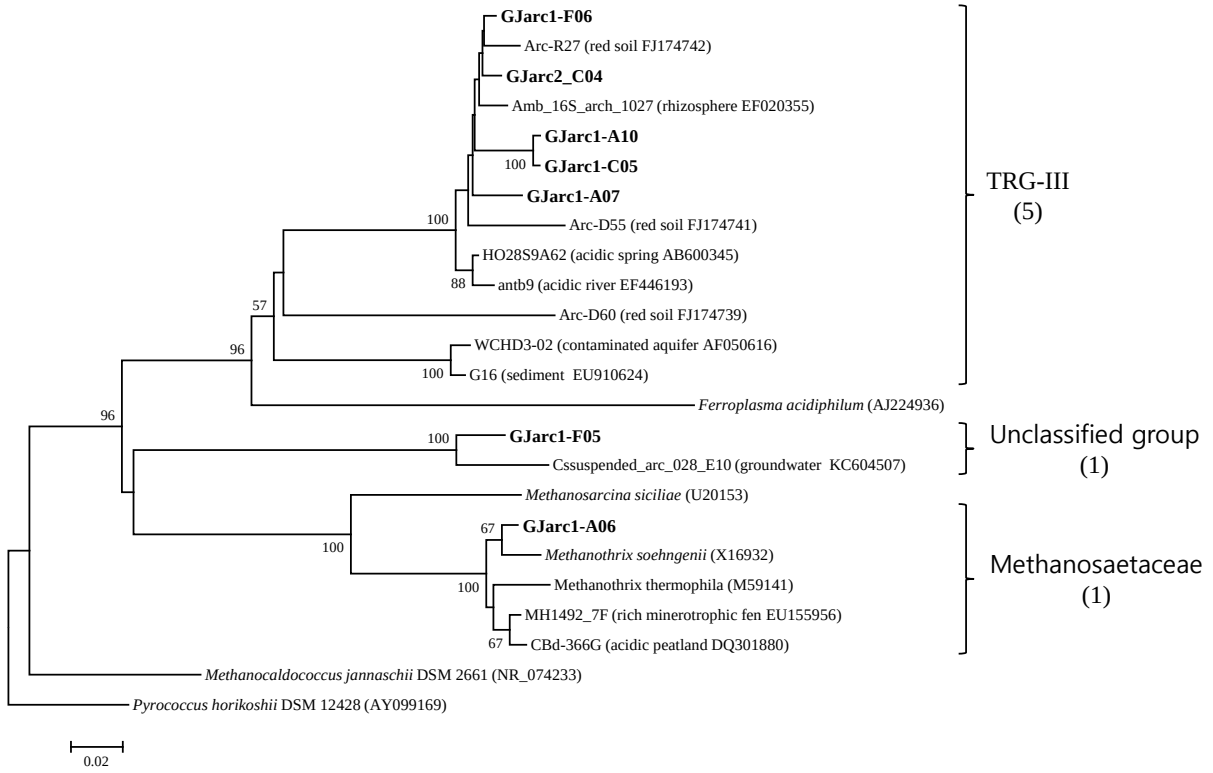
3. Results and Discussion

The soil samples collected from Gyorae Gotjawal had a pH of 4.5, an electrical conductivity of 3.44 $dS \cdot m^{-1}$, 34% organic matter content, and an NO_3^- concentration of 300.48 $mg \cdot kg^{-1}$ dry soil.

Analysis of archaeal 16S rRNA gene sequences was performed with a total of 185 clones. Of them, 178 clones (96.2%) belonged to the phylum Thaumarchaeota and 7 (3.8%) to the phylum Euryarchaeota (**Figure 2**).



(a)



(b)

Figure 2. Phylogenetic tree of 185 archaeal 16S rRNA gene sequences from Gotjawal soil: (a) Thaumarchaeota; (b) Euryarchaeota. Bootstrap values > 50% (1000 iterations, neighbor-joining method) are shown for supported branches. The scale bar represents 2% estimated sequence divergence. The numbers in parentheses following clone names indicate the number of additional clones represented by the sequence (≥99.5% identity).

Most Thaumarchaeota clones belonged to group I.1b, which has been reported in various soils [16] and is known to include ammonia-oxidizing archaea (AOA) [4] [17]. Despite the low pH (4.5) of the lava soils, only four Thaumarchaeota clones were detected that were affiliated with the group I.1a-associated acidophilic AOA group, which includes acidophilic ammonia-oxidizing archaea [18]. Seven group I.1c, and group I.1c-associated clones were identified (**Figure 2(a)**). Archaeal communities in many acidic forest soil systems, including grassland pasture, moorland, and alpine soils, are dominated by the group I.1c thaumarchaeal lineage [19], although the ecological functions of this group remain unknown. Notably, thaumarchaeal group I.1b, which is frequently detected in various soil environments, was prevalent despite the low soil pH. The group I.1a thaumarchaeal lineage, which is the most abundant in the marine environment, was not identified in the clone library (**Figure 2(a)**).

A total of five euryarchaeal clones belonged to *Thermoplasma*-related group III (TRG-III) [19]. The phylotypes in TRG-III were related to environmental clones recovered from acidic environments [20]-[22]. One euryarchaeal clone was related to methanogens of the Methanosaetaceae family (**Figure 2(b)**).

Most notably, clones related to AOA dominated (92.4%) the archaeal clone library. The absence of clones of ammonia-oxidizing bacteria (AOB) and the relative abundance of archaeal *amoA* genes compared to bacterial *amoA* genes tempted us to speculate that AOA might be important players in lava forest soils. It has been proposed that pristine environments may be preferred by AOA more than by AOB, based on ecological studies of the numerical abundance of ammonia oxidizing microorganisms [23] and studies of soil AOA affinity for ammonia and oxygen [24]. There remains controversy regarding the relationship between the numerical abundance of AOA and their ecological activity [23] [25], and a mixotrophic metabolism has been proposed specifically for the soil AOA of thaumarchaeal group I.1b.

4. Conclusion

In conclusion, most of the archaeal clones were affiliated with thaumarchaeal group I.1B, which are related to

nitrifying archaea. Further studies are required to fully understand their abundance, diversity and functions in soil.

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