

ISOLATION OF EXTREMOPHILIC MICROORGANISMS FROM THE ACIDIC, HIGH-TEMPERATURE SYSTEM OF THE GOSHOGAKE MUD VOLCANO FIELD, TOHOKU, NORTHERN JAPAN. N. Miyake¹, R. Ishimaru¹, G. Komatsu², K. Kawai³, M. Kobayashi⁴, H. Sakuma⁵, and T. Matsui¹
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Introduction: Over the last decades, microorganisms inhabit in various extreme environments, known as extremophiles, have been vigorously studied by scientists. They have been found at more than 10,000 m deep in the ocean – at pressures of up to 110 MPa; from extreme acid (pH 0); and from hydrothermal vents at 122 °C to frozen sea water, at -20 °C. For every extreme environmental conditions investigated, many microorganisms have shown that they not only can tolerate these conditions, but that they also often require those conditions for survival.

Recent studies show that a variety of extremophiles, such as mesophilic sulfate-reducers and chemolithotrophic Fe(III) reducers, are found in the active on-land mud volcanoes which are related to sedimentary processes at relatively low temperatures [e.g., 1, 2]. Mud volcanoes can be found in a numerous geological settings all around the world whereby fluid-rich, fine-grained sediments ascend within a lithologic succession mainly due to their buoyancy [3], simultaneously bringing any extremophiles that are thriving in the path.

The Goshogake mud volcano field in Tohoku, Japan is associated with magmatic volcanism hence characterized as a high-temperature geothermal field (Fig. 1). The detailed description of this field is introduced elsewhere [4, 5, 6]. Oyunuma (Grand hot pond) has the conditions of highly extreme (52 °C, pH 2.5). In Yellowstone national park, US, similar mud pots with high-temperature geothermal acidic hot spring (~88 °C, pH ~3) can be found, and from there one of the well-known archaeal thermoacidophile, *Sulfolobus*, was isolated [7]. However, the study of microbial communities within mud volcanoes in magmatic settings is still scarce. Since the understanding of microbiology associated with mud volcanoes has important implications for astrobiology of Mars [8, 9] and possibly of Enceladus, we carried a molecular analysis of the Goshogake mud volcano field for the first time.

Material and Method:

Sampling. Mud samples were collected from Oyunuma, (steamy salse) as shown in Fig. 1. By wearing heat-resistant gloves, 50 ml glass serum bottles were plunged into the salse using sterile tongue, and



Fig. 1. A) Over view of the Goshogake mud volcano field in Tohoku, Japan. Arrow shows Oyunuma (Grand hot pond) area (52 °C, pH 2.5). B) View of Oyunuma which is a 70 x 60 m wide steamy salse. A gryphon field is visible next to Oyunuma and a small group of mud pots are located beyond Oyunuma. C) A picture of author collecting the sample from the edge of salse.

the mud samples were collected at approximately 30 cm in depth. Glass serum bottles were hard-sealed immediately after the collection, by trying not to leave any air inside. The surrounding mud sample was also collected for a control. Those samples were kept frozen until molecular analysis.

Molecular Analysis. From those samples collected, DNA was directly extracted using ISOIL for beads Beating kit (Nippon gene), followed by 16S rDNA PCR amplification using GC341f-534r primer (Bacterial) and GC344f-519r primer (archaeal) with the conditions shown elsewhere [10, 11]. Denaturing gradient gel electrophoresis (DGGE) was then carried out using Dcode DGGE complete system (Bio Rad). Target bands were sequenced using DGGE band sequencing kit (TecknoSuruga).

Results: From the bacterial PCR-DGGE profiles, two clearly dominant bands were detected and their BLAST search for the nucleotide sequence of 16S rRNA gene to the Apollon database DB-BA 9.0 indicated that they were a member of the genus *Hydrogenobaculum* and *Desulfuella*. (Table 1) The first band

Table 1. Comparison of the closest phylotype and described relatives of DGGE bands from the GenBank database using BLAST.

DGGE Band Number	Closest Phylotype				Closest Described Relative					
	Accession Number	Percent Similarity	Organism	Reference	Accession Number	Percent Similarity	Organism	Reference	Phylogenetic Group	Characteristics of Described Organisms
B05c	EF156502	98.1	Uncultured <i>Hydrogenobaculum</i> sp. clone BY137	Kord, S.E. et al. (2006) Unpublished	NR_025844	96	<i>Hydrogenobaculum acidophilum</i> strain 3H-1	Shima, S. et al. (1994) FEMS Microbiol. Lett. 119(1-2), 119-122	Aquificales	Thermosacidophilic, chemolithotrophic, H ₂ -oxidizer
B05d	HF677548	98.1	Uncultured bacterium clone AM2-15	Brito, E.M. et al. (2014) Extremophiles 18(2), 385-398	NR_026109	96	<i>Desulfurella acetivorans</i> strain A63	Rainey, F.A. et al. (1993) Syst. Appl. Microbiol. 16, 373-379	Desulfurellales	Thermophilic obligately aerobic, S-reducer
A01a	JF305833	99.1	Uncultured <i>Thaumarchaeote</i> Kam37	Eme, L. et al. (2013) Res. Microbiol. 164(5), 425-438	NR_040779	82	<i>Thermocladium modestius</i> strain IC-125	Itoh, T. et al. (1998) Int. J. Syst. Bacteriol. 48(PT3), 879-887	Crenarchaeota	Thermophilic anaerobe, obligately heterotrophic, S-reducer

has shown the closest described relatives as *Hydrogenobaculum acidophilum* strain 3H-1 (96% identity; accession number NR_025844) isolated from a solfataric field, Tsumagoi, Japan [12]. *Hydrogenobaculum* is a genus in the order of Aquificales, which are widespread in both deep-sea and terrestrial hydrothermal systems [e.g., 13, 14]. *H. acidophilum* is a thermoacidophilic, hydrogen-oxidizing bacterium, which grows chemolithoautotrophically. It requires elemental sulfur as the electron acceptor, hydrogen as electron donors and carbon dioxide as a carbon source. Some other *Hydrogenobaculum* sp. use reduced Fe(II) or perhaps As(III) [12, 15]. The second band has shown the closest described relatives as *Desulfurella acetivorans* strain A63 (96% identity; accession number NR_026109) isolated from a hot water pool in the eastern solfataric field of the Uzon volcano, the Kamchatka peninsula [16, 17]. A genus, *D. acetivorans*, is an obligately anaerobic, thermophilic, sulfur-reducing bacterium, capable of chemolithotrophic growth with molecular hydrogen and elemental sulfur. It also uses elemental sulfur as the electron acceptor and acetate as the sole source of energy and carbon [18].

From the archaeal PCR-DGGE profiles, a single dominant band was detected and the BLAST search indicated that it belongs to the phylum Crenarchaeota. Its closest described relative was *Thermocladium modestius* strain IC-125 (82% identity; accession number NR_040779) isolated from the solfataric mud in Noji-osen, Fukushima, Japan [19]. *T. modestius* is an anaerobic/microaerobic, thermophilic and obligately heterotrophic archaeon, utilizes wide range of carbon sources for growth. Sulfur or sulfate with molecular hydrogen can be used as electron acceptor. Growth stimulated by CO₂ in the gas phase [20].

Discussion: In the recent years, characterization of microbial communities associated with the active on-land mud volcanoes are rapidly investigated [e.g., 1, 2] since they can be the terrestrial analog to those features interpreted to be mud volcanoes found widely in the northern plains of Mars [e.g., 8]. In this paper, we investigated a unique terrestrial mud volcano field, located at Goshogake, Tohoku, Japan, which is associated with magmatic volcanism hence characterized as a high-temperature system of a geothermal field (52 °C, pH 2.5). From here, three types of extremophiles were

isolated, namely genus *Hydrogenobaculum* bacterium, genus *Desulfurella* bacterium and phylum Crenarchaeota archaeon. They are all thermophilic capable of thriving in extremely high temperature. *Hydrogenobaculum* is also acidophilic so can tolerate extreme low pH as well. These species have never been found from the conventional sedimentary mud volcanoes with relatively low temperature, which implies the uniqueness of this microbial community associated with magmatic volcanism.

In October 2015, the Cassini spacecraft flew directly through the plume of Enceladus, and found that the plume contains molecular hydrogen, which suggest the water in Enceladus' ocean is reacting with rocks through hydrothermal systems [21]. Since the isolates from the Goshogake mud volcano can utilize molecular hydrogen for their growth, Enceladus' ocean floor bed can be their ideal niche.

We are currently in a process of Illumina MiSeq 16S amplicon sequence analysis on the Goshogake mud volcano samples. The further results on microbial community are expected to come in a near future.

Reference: [1] Green-Saxena A. et al. (2012) *Environ. Microbiol.*, 14(12), 3271–3286. [2] Miyake N. et al. (2015) *LPS 46*, Abstract #1944. [3] Kopf A. J. (2002) *Rev. Geophys.*, 40(2), 2-52. [4] Komatsu G. et al. (2018) *LPS 49*, this volume. [5] Ishimaru R. et al. (2018) *LPS 49*, this volume. [6] Kobayashi M. et al. (2018) *LPS 49*, this volume. [7] Brock T. D. et al. (1972) *Arch. Microbiol.*, 84, 54-68. [8] Komatsu G. et al. (2016) *Icarus*, 268, 56–75. [9] Hosein R. et al. (2014) *Life*, ISSN 2075-1729, doi:10.3390/life4040566. [10] Muyzer G. et al. (1993) *Appl. Environ. Microbiol.*, 59, 695-700. [11] Nishijima M. et al. (2010) *Mar. Biotechnol.*, 12, 253-60. [12] Shima S. et al. (1994) *FEMS Microbiol. Lett.*, 119(1-2), 119-122. [13] Takai K. et al. (2001) *Int. J. Syst. Evol. Microbiol.*, 51, 823-827. [14] Yamamoto H. et al. (1998) *Appl. Environ. Microbiol.*, 64, 1680-1687. [15] Stohr R. (2001) *Int. J. Syst. Evol. Microbiol.*, 51, 1853-1862. [16] Rainey F. A. et al. (1993) *Syst. Appl. Microbiol.*, 16, 373-379. [17] Bonch-Osmolovskaya E. A. (1990) *Arch. Microbiol.*, 153, 151-155. [18] Miroshnichenko M. L. (1998) *Int. J. Syst. Bacteriol.*, 48, 475-479. [19] Itoh T. et al. (1998) *Int. J. Syst. Bacteriol.*, 48(PT3), 879-887. [20] Reysenbach A. (2015) *Bergey's Manual Syst. Arch. Bac.*, doi:10.1002/9781118960608.gbm00407. [21] Waite J. H. (2017) *Science*, 356(6334), 155-159.