CHARACTERIZATION OF MICROBIAL COMMUNITIES IN THE LAND-BASED MUD VOLCANOES OF TOKAMACHI, NIIGATA, NORTHERN JAPAN. N. Miyake[1], R. Ishimaru[1], G. Komatsu[2], and T. Matsui[1], [1]Planetary Exploration Research Center, Chiba Institute of Technology, Japan (n.miyake@perc.it-chiba.ac.jp), [2]International Research School of Planetary Sciences, Università d'Annunzio, Italy.

Introduction: Mud volcanism (MV) is one of the most intriguing phenomena observed in various geological settings in the world, which is frequently used as an important "window" into the unknown biosphere of underlying strata. In recent years, MVs on seafloor-beds were preferentially studied to reveal a complex configuration of microbial communities thriving in the methane-rich submarine sedimentary biosphere. Many of those areas were dominated mostly by methane consumers, known as anaerobic methanotrophs (ANMEs), which can catalyze anaerobic methane oxidation with their symbiont sulfate-reducing bacteria in the sulfate-methane transition zone (SMTZ) [e.g., 1, 2]. SMTZ is usually located at a shallow subsurface layer, where diffused sulfate from the ocean above and seeped methane from beneath MVs can coexist. On the contrary, active MVs located across on-land sites have been less investigated. Since the origins and the compositions of erupted muddy fluids are very different between land-based and submarine MVs, their impact on the microbial community structure and potential activity in each location must be variable. Hereby, we report the results of the biological investigation on two of the most active methane-seeping on-land MVs in Japan.

Geological setting: Investigated MVs are situated at Murono (37°7'15.16''N, 138°33'27.76''E) and Kamou (37°7'58.45''N, 138°34'30.24''E) districts in the Tokamachi city, Niigata prefecture, Northern Japan (**Fig. 1**). The Late Miocene Sugawa Formation consists primarily of dark-color massive mudstones and accompanying alternating mudstones-sandstones, and it is highly folded and widely distributed in the Tokamachi area (**Fig. 1a**). The MVs occur in the areas of this exposed Sugawa Formation. The erupted mud contains slightly alkaline (pH 7.5) groundwater with very high salinity and electrical conductivity (14 mS/cm in Murono, 13 mS/cm in Kamou) [3]. The oxygen and hydrogen isotopic ratio of groundwater and vitrinite reflectance of coal fragments show the estimated origin of those mud to be at the depth range of 3400 m to 4000 m [4]. The temperature inside the sampled vents (~40 cm in "depth") were 15°C in Murono site and 17°C in Kamou site during the investigation (October 2018, outside temp. of 23°C). The temperature at the origin (3400~4000 m) is estimated to be about 120°C [5], therefore the mud must have cooled down during the slow accession.

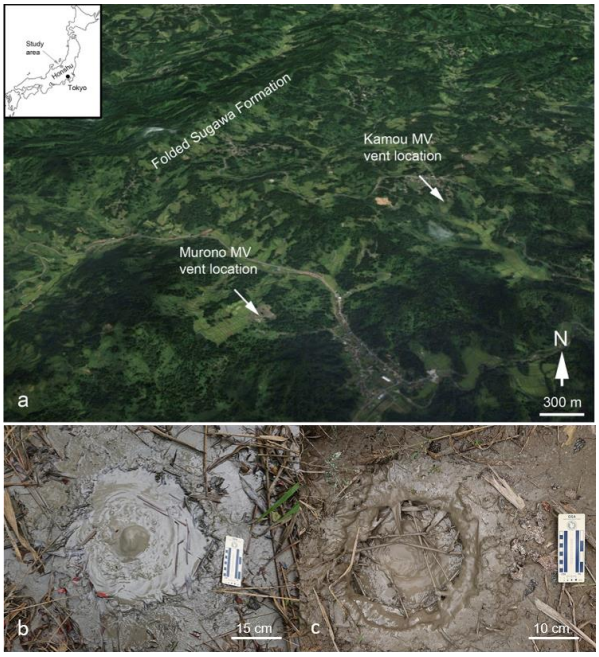

Fig. 1. Google Earth views and ground photos of the investigated MVs in Tokamachi, Niigata prefecture, Japan. (a) Location of Murono and Kamou MVs which are distanced approx. 2 km. (b) The sampled vent at the Murono site, which has a diameter of ~30 cm. (c) The sampled vent at the Kamou site, which has a diameter of ~20 cm.

Geochemical characterization: Murono and Kamou MVs have spouting of mud together with seepage of natural gas (e.g., methane), groundwater, and oil [6]. We measured the amount of methane seepage which was 2483.68 ppm*m in Murono and 827.05 ppm*m in Kamou. Their column densities are 2~3 orders of magnitude higher than those in the backgrounds. Previous studies carried out carbon isotopic analysis of the gas at the Murono site, which suggested a possible biodegradation of hydrocarbons taking place in a deep subsurface [6, 7].

Molecular characterization: Mud samples were sterilely collected from approximately 40 cm in "depth" and "surface" of vents of Murono (**Fig. 1b**) and Kamou (**Fig. 1c**) sites, then stored frozen (-80°C) until molecular analysis. The DNA extracted from the mud samples (1 ml) were measured and by using the mean value of 2.5 fg of DNA-content cell^{-1} [e.g., 8, 9] and the operational taxonomic units (OTUs) provided from 16S rRNA gene (V4) amplicon sequencing, the total

number of microbial cells expected in each sample were calculated (**Table 1**). The results show a high number of archaeal cells in "depth" of MVs compared to "surface" (about 14-fold at Murono and 9-fold at Kamou), which suggests the archaeal communities might have some kinds of significant role in depth of on-land MVs.

Sampled Site		Extracted DNA from the Sample (per 1 ml)	OTUs from 16S rRNA (V4) Amplicon Sequencing		Expected Number of Cells in Tokamachi Mud Volcanoes (per 1 ml)		
			Archaeal	Bacterial	Total	Archaeal	Bacterial
Murono	"depth"	13.56 ng	16.88 %	83.02 %	0.054 E+8	0.916 E+6	0.450 E+7
	"surface"	936.87 ng	1.17 %	98.65 %	3.747 E+8	4.385 E+6	36.969 E+7
Kamou	"depth"	252.15 ng	14.41 %	85.59 %	1.009 E+8	14.534 E+6	8.633 E+7
	"surface"	525.42 ng	1.62 %	97.96 %	2.102 E+8	3.405 E+6	20.588 E+7

Table 1. The table is showing the expected total number of cells, and the ratio of archaeal and bacterial cells in 1 ml of mud samples from the Murono and Kamou MV sites.

Archaeal and bacterial 16S rRNA gene (ARC V4 and Bac V3V4) amplicon sequencings were also carried out. Their OTUs were summarized in pie-charts (**Fig. 2**).

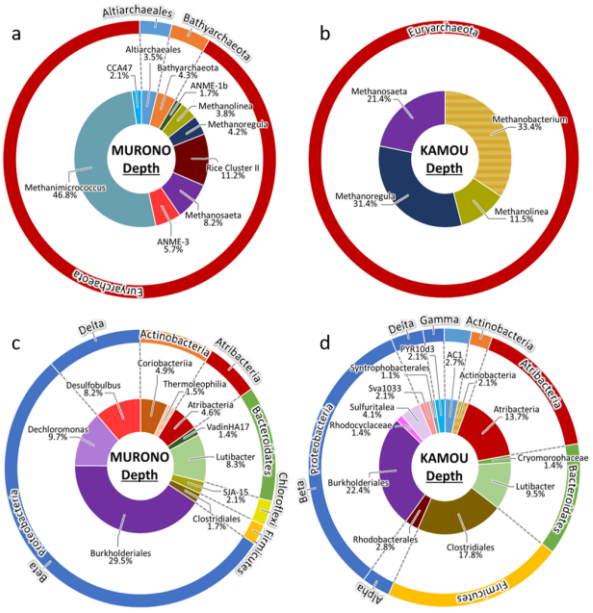

Fig. 2. The Pie-charts represent the OTUs (%) obtained from 16S rRNA amplicon sequencing. (a)(b) Archaea in Murono and Kamou sites. (c)(d) Bacteria in Murono and Kamou sites.

The archaeal results show a high abundance of methanogens, such as *Methanolinea*, *Methanoregula*, *Methanosaeta*, *Rice Cluster II group*, *Methanimicrococcus*, and *Methanobacterium* in both Murono and Kamou sites (**Fig. 2a, 2b**). On other hand, only the Murono site had anaerobic methanotrophs of *ANME-1b* (1.7%) and *ANME-3* (5.7%). The bacterial results show a high abundance of uncultured *Comamonadaceae*, which belongs to *Burkholderiales* of Beta-proteobacteria, in both sites (**Fig. 2c, 2d**). It is noteworthy that a high number of Delta-proteobacteria were found, which include sulfate-reducing bacteria (SRB), *Desulfobulbus* at the Murono site and Fe(III)-reducing bacteria, *Desulfuromonadales Sva1033 group* at the Kamou site. Moreover, a high number of *Atribacteria* were found from both sites, which are recently suggested to mediate anaerobic oxidation of methane (AOM) possibly using humic acids as electron shuttles in some cold-seep environments [10].

Discussion: The Murono site is affiliated with ANME, which form a syntrophic consortium with SRBs, such as *Desulfobulbus*, to mediate anaerobic oxidation of methane (AOM) coupled with sulfate reduction. The ANME-SRB consortium is usually found at the SMTZ of a shallow layer of submarine MVs [e.g., 1, 2]. In the case of on-land MVs, sulfate is expected from the microbial oxidation of sulfur-bearing minerals, such as pyrite and greigite from the ascended mud [e.g., 11, 12]. However, an XRD analysis of the mud from the Murono site has revealed the presence of halite, quartz, mica, and smectite but no sulfur-related minerals (personal communication with M. Kobayashi). The erupted materials are originated from the depth range of 3400 m to 4000 m where the Miocene marine strata exist [4], indicating the possibility of the old sea-related juvenile water being the source of additional sulfur-related components for the SRB in Murono MV. Furthermore, this can hypothesize the origin of ANME at our study area to be deeper than other on-land MV investigations have suggested.

There are many features interpreted to be land based MVs on Mars [e.g., 13], and searching for extant life or biomarkers in the sedimentary deposits [14] associated with MVs should consider terrestrial on-land MVs for their analogs.

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