



## **Preliminary results from 1979 and 2017 ICDP SUSTAIN drilling: the sub-terrestrial biosphere of the neo-island Surtsey**

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During the ICDP SUSTAIN drilling operation at Surtsey, from 10 August to 4 September 2017, a total of 56 core samples were collected for microbial detection and analysis from three drill holes. These are two vertical holes to 152 m (SE-02A) and 192 m depth (SE-02B) and one inclined hole 354 m long (SE-03). Downhole water samples were also collected in 2016 and 2017 at successive depths from a hole drilled in 1979 (SE-01), and in 2017 from the three new drill holes (SE-02A, SE-02B and SE-03). In total, 17 core samples were collected from SE-02A, the first vertical hole, cored using filtered, doubly sterilized seawater drilling fluid; 17 from SE-02B, the second vertical hole, cored using filtered, doubly sterilized seawater drilling fluid and occasional attapulgitic mud; and 22 from SE-03, the inclined drill core, cored with untreated seawater and occasional attapulgitic mud. A 30 cm core section was cut from the lower end of the upper one meter of every third core run and divided into three sub-sections at the drill site: 10 cm for molecular analyses (MA), 8 cm for cultivation (Cu), and 2 cm for microscopic investigation (M). MA sections were kept in the plastic core liner, taped at both ends, wrapped in a plastic bag and kept in liquid nitrogen (-196°C) until delivery into long term laboratory storage at -80°C. Cu sections were immediately removed from the liner, put into a sterile plastic bag, oxygen removed by gas-pack; and stored at 4°C. M sections were incubated in a PBS 1X and 2% formaldehyde solution, washed twice with PBS 1X, transferred into a solution of PBS 1X and ethanol 96%, and stored at -80°C.

Initial results of 16S rDNA tag sequencing analysis of DNA isolated from the underwater volcanic biosphere in the 1979 drill hole (SE-01), identified unique sequences of bacteria and archaea (e.g. methanomicrobia-related sequences and archaeoglobus-like sequences) [1]. Some viruses with icosahedral symmetry were detected as well. Further investigation of the diversity, metabolism and function of cultivated and non-cultivated microbes in water samples from drill hole SE-01 is ongoing. Core samples collected at successive depths from 2017 drilling are currently being enriched in different media for autotrophic (e.g. methane, iron, sulfur metabolisms) and heterotrophic microorganisms at various temperatures, 22°C, 60°C, and 80°C. Some enrichments show microbial growth. For example, 8 strains have been isolated and identified from a 15 m deep SE-02A sample. At least one strain has been isolated at 80°C from a 180 m deep SE-02B sample. Enrichments, isolations and identification of new strains are ongoing. In addition to this cultivation-dependent approach, the analysis of 2017 SUSTAIN core samples using high-throughput amplicon sequencing, functional metagenomics and metatranscriptomic analysis will be performed and the interactions between minerals present in the rock and microorganisms will be studied by Fluorescent In-Situ Hybridization.

[1] Marteinsson et al. 2015, Microbial colonization in diverse surface soil types in Surtsey and diversity analysis of its subsurface microbiota, *Biogeosciences*, 12, 1191–1203.