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An in-situ culturing system for hydrothermal vent microorganisms

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Hydrothermal vents are suggested to be the site of the birth of the life. For microbial growth, there are many differences between hydrothermal vent and laboratory conditions with respect to living space, temperatures, pressure, light, nutrition, pH, oxygen and poisons, etc. In the present study, an in-situ culturing system was developed, thinking much of living space, temperature and nutrition among the conditions listed above. The in-situ culturing system consisted of the incubator, the neck and the cone. It had the characteristics of allowing mixing of hydrothermal fluid and sea water in a semi-open systgem. Temperature gradient was self-formed by making three layers inside the incubator, and living space and nutrition were supplied from solid supports. We used the following solid supports; apatite containing phosphorus, pumice with porosity and dacite as control. This device was installed at the vent of APSK07 and culture was performed for 3 days. Upon the installation, the temperature inside the cone was 278C and that just above the incubator was 44C. Therefore, the temperature of the center of the incubator must be in between, and thus, suited for the growth of hydrothermal vent microorganisms. By the in-situ culturing, solid supports turned black by binding of metal sulfide, indicative of the presence of microorganisms. Laboratory culturing was carried out using the black parts of the solid supports as inoculum with the heterotrophic mediums for Archaeoglobus, Pyrococcus, Thermococcus, and Thermotoga for 16h at 60C under an anaerobic condition (N2:H2:CO2=80:10:10). Cocci were obtained from apatite and the rods from the pumice. Thus, different microorganisms were grown from the different solid supports. This fact indicates that geology influences microorganisms. Two rods were able to be subcultured with Pyrococcus medium, one furiously rotating adhering an end of the cell to cover glass upon observation by microscope and the other moderately motile. The phylogenetic analysis was performed on the two rods according to the 16S rDNA sequences. The rotating one belonged to Deferribacter, near to Deferribacter desulfuricans. The genus of Deferribacter was newly established in 1997 for Deferribacter thermophilus, and only two species have been reported. The other one belonged to Thermosipho, near to Thermosipho japonicus. Unexpectedly and fortunately, precipitates were formed during the in-situ culturing at the neck, which was made of metal sulfides and clay-like substances. Then, the black powder bound to the precipitates was inoculated into Thermococcus and Thermotoga mediums, and they were incubated at 90C. Two different cocci were cultured, one with Thermococcus medium and the other with Thermotoga medium. They were different because each one cannot grow in the other medium. It was necessary to add sterilized precipitates for the growth of coccus with Thermotoga medium. Phylogenetic analysis suggested that the coccus obtained with Thermotoga medium belonged to Thermococcus. We named this TS3. TS3 positioned near to Thermococcus kodakaraensis. The in-situ culturing system developed in this study was effective for the culture of microorganisms of hydrothermal vents. The precipitate made during the in-situ culturing at the neck was considered to be a pseudochimney and a site of microbial growth.