

Morphological changes during growth of microorganisms newly isolated from Suiyo Seamount

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We have developed an in situ cultivation device (ISCD) that allows mixing of hydrothermal fluids and seawater for growth of microorganisms, and have collected a cell-fusing Thermococcus species from the southern Mariana Trough. It is presently unknown whether our device is especially suited for collection of cell-fusing microorganisms. It will be clarified through accumulation of data on microorganisms collected using this device. In the present study, I isolated a microorganism strain MN14 using ISCD and characterized morphological changes during growth, which were suggestive of possible cell fusion.

We deployed ISCD containing apatite and dacite pumice as carriers for 2 days at a hydrothermal vent of Suiyo Seamount (depth, 1,384 m; temperature, 281C) by ROV Hyper Dolphin (JAMSTEC) during NT05-16 cruise (from Sep. 22 to Oct. 7, 2005) of R/V Natsushima (JAMSTEC). After deployment, the carriers were anaerobically pouched on the mother ship and kept at 4C during transportation to the laboratory. Carriers were crashed and the resulting fragments were inoculated into TF medium for heterotrophs. Enrichment was performed at 60C for 20 h. After successive cultures, microorganisms were isolated by the dilution-to-extinction method and then by single colony isolation using 0.8% Gelrite-containing plate.

From dacite pumice, a microorganism strain MN14 was isolated. Strain MN14 was a rod and formed a filamentous body with a width of 1 micro m and a length of 4-15 micro m. Examination by phase-contrast and epifluorescence microscopy in various growth phases suggested that the morphology of strain MN14 changed from a rod to a large coccus during growth. Characteristic 4 morphologies were: 1, a rod whole body of which epifluoresces; 2, a long filament in which epifluorescent bodies are fragmented; 3, similar to morphology 2, but with a part of filamentous body transformed to a balloon; and 4, a coccus as large as 10 micro m in diameter possessing some strongly epifluorescing large nucleoids, in the order of growth phases. These morphological characteristics suggest that strain MN14 belongs to the genus Thermosipho in the order Thermotogales. The filament may contain some microbial cells that are confined in an outer membrane. Balloon might be formed from toga. Cells were discernible in morphology 1, but how many cells were in a filament was not discernible in morphologies 2 and 3. In morphology 4, strongly epifluorescing large nucleoids suggest that the DNA might be extraordinarily replicated or the nucleoids might be fused. If the latter is the case, it means that the cells in a filament fuse in morphology 2 or later. Gram staining suggested that the filamentous bodies were Gram-negative, but the balloons were Gram-positive, suggesting that they had different surface structures. When a culture was stood for 1 month at room temperature, it became rich in microorganisms having morphology 4. Inoculation of this culture into a fresh medium did not regenerate microorganisms, suggesting that the cells were living but could not make a descendent or were dead end microorganisms. The 16S rRNA gene sequence is now being determined to fix the phylogenetic position of strain MN14.