

7. Microbial community structure analysis of deep-sea water and isolation of useful microorganisms

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Introduction

The discovery of novel and useful bioactive compounds from terrestrial sources is decreasing year by year. For this reason, much attention¹⁾ has been paid to marine microorganisms, and especially microorganisms living in deep-sea water (DSW). It is well-known that populations of microorganisms in DSW are much lower than those in surface seawater (SSW)²⁾. Microorganisms in DSW have been adapted to the deep-sea environmental conditions such as low temperature, high hydrostatic pressure and low concentrations of organic matter. As a result, microorganisms might acquire the ability to produce new products. In the present study, microbial population analysis of DSW and SSW was performed using a pyrosequencer in order to isolate novel or unknown microorganisms. Among the various kinds of microorganisms, actinomycetes were well-known to produce bioactive substances, and therefore were selected for further study. The characterization of actinomycetes which produce anticancer substances was performed.

Methods

About 21 samples of DSW and SSW were obtained from 7 pumping stations across Japan; Hokkaido-Rausu, Hokkaido-Iwanai, Izu-Akazawa, Kochi-Muroto, Kagoshima-Koshikishima, and Okinawa-Kumejima. Those samples were used for the microbial population analysis. Each sample was prefiltered using a nucleopore filter (pore size 3 µm) and the filtrate was filtered using a pore size 0.2 µm filter in order to trap suspended solids on the filter. The microbial DNA on the filter was extracted and the 16S rDNA was amplified by PCR using bacterial universal primers. The amplicons were subject to nucleotide sequence analysis by pyrosequencer (GSFLX Titanium, Roche) and the microbial community structure was determined. The bag type filter which was used for the removal of the suspended solid in

DSW was obtained from the pumping station in Izu-Akazawa and used for the isolation of actinomycetes. The colonies developed on ISP-No. 4 medium after incubation at 27°C for 4 weeks were isolated and stored. All the isolates were incubated in a liquid medium and the culture supernatant was obtained. The cytotoxicity against B16 mouse melanoma cells (cancer cell) was tested and the strain having the highest cytotoxicity was selected. The cytotoxicity against normal human dermal fibroblasts was also tested and identification of the strain was performed by 16 S rDNA analysis.

Results and discussion

In the present study, the microbial community structure analysis was performed on water samples collected from 7 DSW pumping stations in Japan. Among them, the population of unknown or new species of microorganisms was the highest in Izu-Akazawa and Kumejima DSW. Actinomycete strains were isolated from these stations and 215 strains were incubated in a liquid medium. Among them, the 15 strains showed cytotoxicity against B16 mouse melanoma cells whereas 12 strains showed no cytotoxicity against fibroblasts. One strain (named as strain AKA32) from Izu-Akazawa had high cytotoxicity against B16 cells and no cytotoxicity against fibroblasts. The strain was a new species and was selected for further studies on the characterization of anticancer substance from the strain.

¹⁾ Terahara et al., *Gene*, 576, 696-700 (2016).

²⁾ Imada, *Deep Ocean Water Research*, 13, 33-40 (2012) (in Japanese).