

1. Isolation of Microorganisms from Deep-sea Water using the Membrane Filter Method and Screening of Bioactive Compounds

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1. Introduction

Various drugs such as antibiotics have been commercialized by fermentation of actinomycetes and fungi. However, as a result of intensive screening efforts, the discovery of new compounds from terrestrial microorganisms has become difficult, and thus we turned our attention to marine microorganisms. Microbes collected from invertebrates such as sponges in particular are the main target of drug discovery, but the microorganisms in deep-sea water (DSW) had not been studied at all when we initiated this research in 1996. Our research on the exploration of microbes for drug production from DSW was a totally new challenge. In order to collect the microorganisms from 'clean' DSW, we selected the membrane filter method by which the microbial cells in DSW can be trapped on the filter.

2. Method

DSW samples were collected at the Namerikawa DSW facility from the depth of 333 m and on a vessel equipped with DSW collection tool from the depth of 700 m in Toyama Bay. The collected DSW was immediately filtered through a membrane filter (Advantec, 0.20 μ m pore) in a clean room. Then, the filter paper was placed on a nutrient agar medium and incubated. After two weeks, the filter was removed from the agar plate and the plate was incubated for additional two weeks. Bacterial cells and fungi are not able to pass through the filter, while only the mycelium of actinomycetes of which diameter is less than 0.20 μ m can penetrate into the

filter and reach the agar medium. Therefore, only actinomycete colonies can be obtained by this method. Isolates were cultured in three kinds of liquid medium and the culture broth was extracted by organic solvent. After evaporation, the solvent extracts were analyzed by HPLC for metabolite analysis and were subjected to antimicrobial and antitumor assays.

3. Results and Discussion

From the DSW sample (10 L) collected from -333 m depth in 1995, 38 actinomycete strains comprising 14 *Streptomyces* and 24 *Micromonospora* strains were isolated. Meanwhile, from the DSW sample (10 L) collected from -700 m in 2004, only 8 actinomycete strains comprising 6 *Streptomyces* and 2 *Micromonospora* strains were isolated. Afterward, continuous isolation was carried out from the DSW sample of -700 m depth and obtained 5 to 7 strains from 10 L on average. From these DSW-derived actinomycetes we discovered new bioactive compounds with antitumor and antimicrobial activity.

In this presentation, we will provide an outline of our research activity on the screening of bioactive compounds from DSW-derived actinomycetes carried out from 1996 to 2018.¹⁾

¹⁾ Y. Igarashi, Antitumor compounds from actinomycetes in deep-sea water of Toyama Bay, Handbook of Anticancer Drugs from Marine Origin, pp. 367-382, Springer (2016)