

2. Isolation and use metabolite analysis of actinomycetes collected from the deep-sea water of Toyama Bay -2nd report-

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1. Introduction

Our laboratory has analyzed actinomycetes in the deep-sea water (DSW) and their bioactive compounds for over 20 years using the membrane filter method. However, it was unable to find strains characteristic to the DSW of Toyama Bay. It is possible that the amount of DSW filtered was insufficient to obtain the rare actinomycetes in DSW. In the last meeting, we reported the result of isolation of actinomycetes from the filter by which 600,000L of DSW was passed through at Nyuzen DSW facility.¹⁾ Most of the isolates were *Streptomyces* and the secondary metabolites were clearly different from previous results. In this study, we investigated the taxonomy and secondary metabolites from Nyuzen DSW.

2. Material and Methods

138 actinomycetes strains were isolated from the DSW filter of the Nyuzen DSW intake station (from 384 m depth). Genus level identification of the isolated strains was performed based on the 16S rRNA gene sequence. Metabolite analysis was performed as follows. : The strains cultured on an agar plate was inoculated into seed medium and cultured for 4 days. The seed culture was transferred into production medium and cultured for 6 days. The culture broth was extracted with an equal volume of 1-BuOH under stirring for 1 hour and the organic layer was allowed to evaporate. BuOH extracts were

dissolved in DMSO for the high-performance liquid chromatography (HPLC) analysis. Then, observed peaks of HPLC were checked with our in-house UV spectrum database to eliminate known compounds. Also, antimicrobial and anticancer assays were carried out using BuOH extracts of 83 strains.

3. Results and Discussions

A Total of 138 actinomycetes strains were isolated from the filter which was obtained in November, 2016. To date, a total of 62 strains were identified to belong to *Streptomyces* (43 strains), *Micromonospora* (18 strains), and *Actinomadura* (1 strain). 39 of 52 strains produced some metabolites and 22 strains of them produced unknown compounds which were not present in the database. Twenty-nine strains showed strong activity against cancer cell lines and 6 strains exhibited significant antimicrobial activity against MRSA. Our currently ongoing study suggests that four isolates produce structurally new compounds. Here we report the results of phylogenetic and secondary metabolites analysis, also screening of novel anti-MRSA compounds.

¹⁾Ogino · Igarashi et al., Proceeding of The 21st DOWAS National Convention in Hokkaido 2017, p16