5. Characterization of antitumor substance from strain AKA32 derived from deep-sea water in Izu-Akazawa, Japan

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1.Introduction

Bioactive substances such as antibiotics and anticancer drugs derived from microorganisms have contributed to improving human life. These bioactive substances have been mainly produced by microorganisms derived from terrestrial soils. However in recent years, the discovery of novel bioactive substances has drastically decreased. Instead of the terrestrial source, the marine environment will be a promising source for finding novel microorganisms. To date, most microorganisms have been isolated from coastal seawater and marine organisms; however, deep-sea water (DSW) has not been paid attention as a a source for finding novel microorganisms. Previously, we analyzed the bacterial community structure of DSW from seven pumping stations in Japan using the pyrosequencing method. The result showed that DSW had a unique bacterial community structure differing from that of SSW[1)]. In addition, the relative abundance of unknown geni of bacteria including new species was highest in Izu-Akazawa among the seven DSW stations, suggesting that Izu-Akazawa DSW would be a promising source for finding new bioactive compounds. In this study, we isolated and characterized antitumor substances from DSW-derived actinomycetes in Izu-Akazawa.

2.Materials and Methods

Actinomycetes were isolated from Izu-Akazawa DSW. Antitumor activity of culture supernatant was examined using B16 murine melanoma cell line (B16). Those actinomycete strains, which showed high cytotoxicity against B16 cell, were identified using 16S rRNA gene analysis and physiological characters of the strain were compared with its closest type strains. From the candidate strain, antitumor substances were purified from the culture supernatant and the chemical structure was determined by ^{1}H, ^{13}C NMR, and mass spectral analysis. Cytotoxicity against B16, Human hepatocellular carcinoma (Hep G2), Human epithelial colorectal adenocarcinoma (Caco-2) cells of each compound was examined.

3.Result and Discussion

In total 131 actinomycetes were isolated from Izu-Akazawa DSW and 12 strains had cytotoxicity against B16 cell. One strain named as “AKA32” was identified with *Nonomuraea indica* DRQ-2^{T} (98.5% similarity), *Nonomuraea asiatica* A299 (97.8%) and *Nonomuraea muscovyensis* FMN03^{T} (97.8 %) based on 16S rRNA gene analysis. However, the colony color of the strain and the requirement of amino acids were different from N. indica and its closest type strains. Therefore, strain AKA32 can be considered a novel species of actinomycete. Further purification and chemical structure determination of compounds reveal that strain AKA32 produced two known compounds (*N*-formylanthranilic acid, actinofuranone C) and one novel compound named as “Akazamicin”. These coumpounds showed selective cytotoxicity against each tumor cells.

1) Terahara et al. Gene **576**, 696-700, (2017).