

Isolation and Characterization of Marine Chemolithoautotrophic Bacteria from a Sand Filter for Pretreatment of a Salt Production Process

Satoshi Tsuneda¹, Hirotsugu Fujitani², Kento Ishii¹, Yu Takahashi¹

¹ Department of Life Science and Medical Bioscience, Waseda University

² Department of Biological Sciences, Faculty of Science and Engineering, Chuo University

Summary

The sand filtration systems have been used in salt manufacturing processes for decades in Japan. Those systems are treated by back-washing to prevent contaminants adhering to the sand filters. However, back-washing cannot remove most of the microorganisms attaching to the sands. Furthermore, the phylogenies and properties of microorganisms have not been sufficiently studied. Here, we focused on the detailed characteristics of microorganisms inhabiting sand filtration systems. Since the ocean is an oligotrophic environment with low concentration of organic carbon source, autotrophic bacteria are thought to contribute to biofilm formation on the sand filters. However, their physiological properties are almost unknown because of the difficulty of cultivation. Therefore, we aimed to cultivate autotrophic bacteria inhabiting sand filtration system in a salt manufacturing process and propose a methodology to improve the performance of the sand filtration system.

As a representative of autotrophic bacteria, we focused on the genus *Nitrospira*. Although this genus oxidizes nitrite and play an important role in biofilm formation, little is known about this group derived from oceans. Therefore, we tried to isolate and cultivate *Nitrospira* inhabiting sand filters. First, the original sand was collected from the sand filtration system of Naikai Salt Industries. The biomass was cultivated in an inorganic medium in a fed-batch system with a supply of nitrite. The bacterial community analysis based on 16S amplicon sequencing revealed the presence of phylogenetically novel *Nitrospira* in the filter. Furthermore, *Nitrospira* was found to increase when the original sand was transferred directly into the fed-batch cultures. On the other hand, the supernatants of sonicated sands hardly contained *Nitrospira*. This result supports the high adhesive ability of *Nitrospira*. Thus, this genus might tolerate the pressure of backwashing and remain in the sand filtration system. In this study, *Nitrospira* from the sand filtration system was successfully cultivated, and further study to obtain their isolated strains is needed to reveal the detailed properties.