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Acquisition of Resistance to Osmotic Pressure by Co-existing with Marine Microbes

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Summary

The objective of this study is to reveal the mechanisms by which a non-marine microorganism acquires salt tolerance through coexistence with microorganisms. A salt manufacturing process in Japan use an ion-exchange membrane to obtain brine. When microorganisms in seawater attach to the membrane, biofilm is formed leading to increase electrical resistance. For investigating the survival strategies of a non-marine microorganisms in seawater, a single species isolate is needed to evaluate its growth activity under various cultivation conditions. Previously, our research group enriched non-marine nitrite-oxidizing bacterium "*Nitrotoga*". The sample source was a costal sediment, but the isolated *Nitrotoga* cells did not grow in artificial seawater medium (ASM). This indicates that coexistence with other microorganisms allow non-marine *Nitrotoga* to acquires salt tolerance for survival in the sea environment.

We expected that coexisting microorganisms were in close proximity to *Nitrotoga* cells. The enriched *Nitrotoga* culture was applied to a cell sorting system to collect coexisting microorganisms with *Nitrotoga*. The dot plot area with forward scatter (FSC) and side scatter (SSC), reflecting the target particle size and complexity, respectively. Microscopic observation of the sorted aggregates showed that *Acidovorax* was attached to the *Nitrotoga* cells. *Acidovorax* was isolated using solid medium. *Nitrotoga* and *Acidovorax* were cultured together in ASM, however, no nitrite oxidation by *Nitrotoga* was observed. *Nitrotoga* did not form aggregates with *Acidovorax* during the cultivation. *Nitrotoga* may consume nitrite after *Nitrotoga–Acidovorax* aggregates pre-culture in ASM as "adaptation period". A previous study reported that *Nitrotoga* was predominant nitrite-oxidizing bacteria in a bioreactor supplied with ASM over 60 days.

Then we tried to obtain other salt tolerant microorganisms from a salt plant. Seawater, sand filters, and ion-exchange membranes were collected. DNA extracted seawater and sand filters was amplified by universal bacterial primers targeting the 16S rRNA genes. The PCR products were sequenced using an Ion PGM system, and the microbial community compositions were analyzed. *Nitrosomonadaceae*, known as a non-marine ammonia-oxidizing bacterium, was detected from sand filters. The amplicon sequencing suggests that non-marine bacteria can acquire salt tolerance and grow in sand filters. In future, we will isolate non-marine bacteria with salt tolerance and reveal the mechanisms how to acquire salt tolerance.