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Verification of the Safety from the Diversity of *Haloarchaea* in Commercial Salts Made in Japan

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Summary

In Japan, we have become possible to easily obtain various solar salt and rock salt since 2002. In 2009, the kind of salt that circulates in Japan has been more than 1,500. We have isolated a lot of halophilic microorganisms from commercial salt to date. However, the ecology of halophilic microorganisms in commercial salt has not been still clear. Salt is an ingredient extensively used in food preparations. Miso, soy sauce and pickles as unique Japanese fermented food contain this salt in a large amount. It gives a specific flavor to the product. Most importantly, salt acts as a food preservative by reducing water activity. In this study, we isolated many halophiles capable of growth in 30 kinds of JCM medium from 205 commercially available salts in Japan.

Commercial salt samples (0.3 g each) were dissolved in 1.5 ml sterile water, and one drop each was spread on agar plates of each JCM medium. After incubation for 2 weeks at 37 ˚C, colonies were picked up and transferred to new plates. The 16S rRNA gene was amplified by PCR with haloarchaea specific primer sets.

An extremely halophilic microorganisms were observed, mostly on JCM No. 298, 457 and 574 medium. These media have pyruvate as carbon source. Additionally, these meida have included sodium chloride, magnesium sulfate, potassium chloride, ammonium salt and phosphate as a common component. We separated 1096 strains from commercial salts. And we analyzed 16S rRNA gene sequences about 384 strains of them.

The diversity of viable archaea from the family *Halobacteriaceae* in commerical salts in Japan was important with 16 genera identified out of the 48 genera validly published to date. The most represented genus was the *Halobacterium*. The second most important genera were *Haloplanus* and *Haloarcula*. In contrast, many genera were identified in various salts: *Halarchaeum*, *Halobaculum*, *Halobellus*, *Halococcus*, *Halolamina*, *Halomicrobium*, *Halopenitus*, *Halorubrum*, *Halosimplex*, *Halostagnicola*, *Haloterrigena*, *Natronomonas* and *Salarchaeum*. On the other hand, new species or genera have been included 81 strains.