

## 論文の内容の要旨

論文題目     **Distribution and Characterization of  
Halophilic Archaea in Marine Environments**

(海洋環境における高度好塩古細菌の分布と特性  
に関する研究)

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Haloarchaea are extremely halophilic, aerobic members of *Archaea*, classified within the family *Halobacteriaceae*. For them, seawater salinity (*ca.* 3.5%) has been regarded to be too low for normal maintenance of the cell wall. However, recent studies have shown the presence of the haloarchaeal 16S rRNA gene in non-hypersaline environments. According to the knowledge, a hypothesis is derived. That is to say, “haloarchaea is certainly being in marine environments.” The purpose of this study is to examine this hypothesis. By clarifying distribution, phylogeny, and physiological property of haloarchaea in marine environments, this study show that haloarchaea the extremophile have more fluidity in the respect of ecology and evolution on the Earth.

In the Chapter I, the hypothesis was examined by culture-dependent and independent methods. In the way of culture dependent method, selective pressure by high salinity was applied for isolation. Isolation of haloarchaea from marine environments

provided direct demonstration for the hypothesis. In the way of culture independent method, PCR-DGGE (denaturing gradient gel electrophoresis) was applied for local existence of haloarchaea, and Q-PCR (quantitative polymerase chain reaction) was applied for isolation quantitative determination. As a result, altogether 69 strains were isolated from the the ORI aquarium, Saroma Lagoon, the Arabian Sea, Sagami Bay, and the Japan Sea. Sequence analyses of 16S rRNA gene showed that they are divided into 9 genera, *Halococcus*, *Halogramum*, *Halovivax*, *Halorubrum*, *Halostagnicola*, *Natrinema*, *Haloferax*, *Halalkalicoccus*, and presumed novel genus. DGGE analyses of the filter samples indicated that some strains belong to the family *Halobacteriaceae* were associated with macroaggregates. On the other hand, halophilic group was not detected in the free-living fraction. At the Q-PCR analyses, wide trend showed that haloarchaea were found in deeper zone than Marine Group IV. Attach fractions tend to have smaller number of 16S rRNA gene regardless of target, in contradiction to DGGE result.

In the Chapter II, physiological properties of marine isolates were described. First, the strain KeC-11 which was presumably belong to a novel lineage in the Chapter I, was examined in detail for physiological properties. Subsequently, it was classified as novel genus in family *Halobacteriaceae*. In addition, hence these isolates were come from relatively low salinity environments, reaction to various salinity conditions were at the center of attention. As a result of examination of growth speed of all 69 isolates by

each NaCl concentrations, it was indicated that the optimum salinity for growth of all strains was substantially higher than that of sea water (*ca.* 3.5%), but was considerably lower than that of *Natrinema pallidum*, typical obligately halophilic haloarchaeon (30%).

In the Chapter III, whole genome sequencing analysis of *Halomarina oriensis* KeC-11<sup>T</sup>, which was described as novel lineage in the Chapter II, was carried out. Genomic information provides potential metabolic ability, hence it is one answer how extreme halophiles adapt to low salinity. Followed by annotation using the BLAST (Basic local alignment search tool) search and the Clusters of Orthologous Groups (COGs) of proteins, comparative analysis of complete haloarchaeal protein sets were carried out. As a result of analysis, it became apparent that the genome of strain KeC-11<sup>T</sup> is 4,072,440 bp long and comprises one main circular chromosome of 3.1 Mbp with a 67.7% G+C content and nine plasmids (p1 to p9) that have a total of 0.96 Mb with 59.1 to 67.9% G+C contents. This situation of multiple plasmid possession is presumed as a result of environmental pressure to acquire genes for adaptivity in seawater. Curiously strain KeC-11<sup>T</sup> has osmoprotectant transport pathway which is prevailing mechanism among only other moderate halophiles. The accumulation of compatible solutes as known as “organic-solutes-in strategy” is a rare occurrence for haloarchaea. “Organic-solutes-in strategy” and “high salt-in strategy” are the two principal strategies to re-establish turgor pressure and to circumvent the detrimental consequences of the water loss when exposed

to increasing osmolality. *Halomarina oriensis* may separately use two strategies depending on the environmental salinity with dexterity.

This study provided new classification of halophiles on the base of osmoadaptation strategy, and redefined “euryhaline” as dual strategy holders of “high-salt-in cytoplasm strategy” and “organic-solutes-in cytoplasm strategy.” Although it is remarkably rare strategy, it is thought to be important on the boundary area between hypersaline and seawater environments. Development and acquisition of extremely halophilic property is important factor to reaffirming the origin of Marine Group IV. Previous study has indicated that Marine Group IV arose after establishment of the sister-grouping of class *Halobacteria* and class *Candidatus* “Nanohaloarchaea,” which belong to a new major *Euryarchaea* lineage, distantly related to well-known haloarchaea. Accordingly, Marine Group IV might be evolved from extremely high salinity environment into sea environment.

In conclusion, this thesis presented here provides the evidences of haloarchaeal existence in marine environments, and in part, physiological and genomic property of marine isolates. It would be an epoch-making data for reevaluation of basic biological phenomena such as adaptation, evolution and dispersal mechanism of haloarchaea on a global scale. To my knowledge, this thesis is the first systematic report of microorganisms on the boundary area between hypersaline and marine environments.