# 論 文 の 内 容 の 要 旨

論文題目: Physiological and genetic characteristics of deep-sea bacteria
(深海細菌の生理的、遺伝的特性に関する研究)

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The deep sea is referred to as the ocean below 1,000 m depth and is characterized by high hydrostatic pressure, low temperature, low nutrients and the absence of sunlight. Although it is regarded as one of the extreme environments on the Earth, generally $10^{3-4}$ cells/mL of prokaryotes are found in entire deep seawater. It has been expected that those microorganisms might have community structures and characteristics that are unique to the environment and make them survive and grow under such conditions. Recent application of molecular techniques to deep-sea microorganisms made it possible to clarify the community structures and genetic characteristics without depending on culture techniques. However, such genetic information does not offer meaningful information unless we have actual cultured strains. Our knowledge on such physiological characteristics, their functions or ecological implications is quite limited, primary due to the paucity of cultures isolated from the deep sea. Therefore, most works have been conducted with very few groups, typically, *Gammaproteobacteria*. It is critical to apply some new technique to isolate more diverse groups of microorganisms and obtain information with those strains. It is then possible to clarify how they respond to the deep-sea environmental conditions and what kind of gene is involved in actual microbial processes for the growth and survival in the extreme environment.

The purpose of this thesis was to clarify physiological and genetic characteristics of deep-sea bacteria in comparison with their surface-sea relatives by using culture-dependent and independent approach, physiological examination and genetic analyses. In order to expand our knowledge, newly isolated strains were used. For this purpose, first, the isolation of deep-sea prokaryotes in diverse phylogenetic groups was tried using newly designed culture media. Second, culture independent approach was taken to investigate the vertical community structures in the north-western Pacific Ocean. This clarified the phylogenetic and distributional position of my new isolates. Third, the physiological and genetic characteristics of the deep-sea isolates were investigated in comparison with phylogenetically relatives isolated from the surface environments. Whole genome of 7 strains were sequenced and used for the analyses.

The major contents of each chapter are as follows. In chapter 2, total 681 isolates were obtained from the deep-seawater in north-western Pacific Ocean using 1/5 marine agar 2216, 1/10 R2A agar and natural seawater liquid medium. 16S rDNA sequences of them revealed their phylogenetic positions. All the deep-sea isolates belonged to the domain *Bacteria* and none for *Archaea*. Among the isolates, strains of phyla *Verrucomicrobia* and *Lentisphaerae* were the first isolates in the phyla from the deep sea. Strains of orders *Arenicellales*, *Thiotrichales*, *Cellvibrionales*, *Kiloniellales* and *Acidimicrobiales* were also the first isolates within the orders. Strains affiliated

to 22 genera were considered as novel deep-sea species. Among them, *Rubrivirga marina*, *Rubrivirga profundi*, *Aurantivirga profunda*, and *Lentisphaera profundi* were validated after taxonomical investigations and reported as novel deep-sea species. Approximately 90 % of the identified isolates showed the similarity to the strain isolated from the surface with more than 99% 16S rRNA sequence similarity, suggesting that the majority of the deep-sea bacterial isolates may have closely related strains in the surface layer.

In chapter 3, the vertical community structures of bacteria in two water columns were investigated using pryoseqeuncing technique for clarifying the presence of depth related groups and also differentiating particle associated (PA) and free living (FL) state. Among the phylotypes affiliated with the deep-sea isolates, *Erythrobacter* phylotypes were detected in all depths. *Sulfitobacter*, *Paracoccus*, *Sphinogomonas*, *Colwellia*, *Alcanivorax*, *Marinobacter*, *Alteromonas*, *Moritella* and *Rubritalea*-like phylotypes were more retrieved from the deeper layers than the surface layer. Most of the phylotypes affiliated with the deep-sea isolates showed preference toward PA state. PA state suggests the tendency to attach particles and/or to colonize easily. Also, it suggests the possibility to attach sinking particles that are originating in upper water column. In addition, SAR11 and *Sphingomonadales* of *Alphaproteobacteria*, and *Bacteroidetes* were vertically cosmopolitan. *Deltaproteobacteria*, *Deferribactere*, *Planctomycetes*, *Actinobacteria* and *Nitrospirae* were confirmed as specific bacterial lineages in the deep layers. SAR11, *Chromatiales* of *Gammaproteobacteria*, SAR324 of *Deltaproteobacteria*, *Nitrospirae* and *Deferribactere* were found to be more as FL state in the deep sea. *Sphingomonadales*, *Alteromonadales* of *Gammaproteobacteria*, *Planctomycetes*, *Bacteroidetes*, *Lentisphaerae* and *Verrucomicrobia* were more as PA state in the deep sea.

In chapter 4, growth characteristics, cellular membrane composition and hydrolytic enzymes of eight strains within phyla *Proteobacteria*, *Verrucomicrobia* and *Bacteroidetes* were tested in combination with their "surface relatives" to clarify the characteristics of deep-sea bacteria. All the isolates showed decreasing growth at a higher pressure than atmospheric pressure, indicating that they are non-piezophiles. Of the 8 strains, only *Rubritalea* sp. SAORIC-165 of the phylum *Verrucomicrobia* showed optimum growth at 10˚C and no growth above 20˚C, indicating that the strain is psychrophilic and probably staying in the deep-sea for long time. *Erythrobacter* sp. SAORIC-644 and *Limnobacter* sp. SAORIC-580 showed optimum NaCl concentration at 1 and 0 %, suggesting the origin of low salinity environment. The deep-sea strains commonly contain higher numbers of phospholipids, compared to their surface-relatives. The additional phospholipids may allow the deep-sea strains to maintain the fluidity of cellular membrane under high pressure.

In chapter 5, whole genome of 7 strains within phyla *Proteobacteria* and *Verrucomicrobia* were sequenced and their genetic features were examined in comparison with those of the surface relatives. Comparisons with metagenome data were also made for genes that appeared unique to the deep-sea. The strains, of which group prefer PA state, contained genes encoding for pili assemble or adherence proteins (FAS1 and von Willebrand A domain), suggesting that the genes are supportive in attachment processes. Some deep-sea strains (more than 3 strains) showed the unique presence or more than 1.5 folds abundance in the numbers of the following genes (51 genes), compared to their surface-derived counterparts. These genes were related to respiration, stresses response, cellular structure, metabolism of in- and organic substrates, replication and transcription. Of 51 genes, 39 genes were over-represented in deep-sea metagenomic data, compared to surface-sea metagenomics data. Some of the genes were related with response in high pressure and low temperatures. Although further works are required, genetic (pili, flagella, adhesion proteins and abundant 51 genes) characteristics of the deep-sea isolates appear to

support growth and survival in the-deep sea environment.

In conclusion, bacteria from diversified phylogenetic groups were obtained from the deep sea for the first time. Some of them were investigated taxonomically, physiologically and genetically by recent molecular techniques. In addition, their preference to either PA or FL life style was investigated. Their physiological and genetic characteristics allowed to consider their ecology and evolutionary processes as well. Further investigation on the isolation and characterization of more deep-sea bacteria will offer clues to better understand the nature of the deep-sea prokaryotes.