学位論文内容の要旨

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学位論文題名

Molecular mechanisms of rare earth element utilization by methane-oxidizing bacteria and protease-producing bacteria

（メタン酸化細菌およびプロテアーゼ生産菌のレアアース元素利用機構）

Rare earth elements (REEs) form a chemically uniform group and include scandium (Sc), yttrium (Y), and 15 lanthanides. Despite the name, their abundance in the earth's crust is not low. Their bioavailability had long been overlooked until their discovery as a co-factor in the active site of alcohol dehydrogenases, such as XoxF-type methanol dehydrogenase (REE-MDH), whereas their well-characterized counterparts MxaF-type (Ca-MDH) are Ca-dependent. Since then, the response in some methane/methanol-oxidizing bacteria to REEs has been researched and it showed REEs can readily enhance and suppress the expressions of REE-MDH and Ca-MDH, respectively. However, this regulation, so called REE switch, has only been investigated in a limited number of methane/methanol-oxidizing species. Furthermore, most of REEs exist in the natural environment as insoluble oxide forms. However, the utilization mechanism of such REEs is remaining unclear. In addition, there are no known cases of REEs being used for enzymes other than alcohol dehydrogenases.

In this study, REE switch and the differences from other species was confirmed in one of the model strains of gamma-proteobacterial methane-oxidizing bacterium *Methylococcus capsulatus* Bath. Moreover, the molecular mechanisms of their utilization to insoluble REE were investigated. Besides, I focused on proteases as a candidate for REE-containing enzymes, the effects of REEs on protease-producing bacteria is examined.

1. REE switch in *Methylococcus capsulatus* Bath

REE switch in *M. capsulatus* Bath was investigated by measuring its MDH gene expressions by quantitative RT-PCR and transcriptome analysis. The expressions of the REE-MDH and Ca-MDH were up- and down-regulated, respectively, by supplementation of Ce chloride, one of the most abundant REEs. Other than Ce, results

also showed the REE switch was also activated in *M. capsulatus* Bath by light REEs (e.g., La) and slightly by Nd, but not by non-lanthanide REEs (Sc, Y) or heavy REEs like Dy. Compared to other reported species, *M. capsulatus* Bath can respond to lower concentrations of REEs as 10 nM.

2. Putative molecular mechanism of insoluble REE utilization by Bath

Bath responds to not only soluble REE chlorides but insoluble forms of REE oxides. When insoluble REE oxides (CeO_2) were suspended with Bath culture supernatant, elution of Ce ions was observed, indicating the presence of REE chelators in the culture supernatant. The production of REE chelators was also confirmed by the Chrome Azurol Sulfonate (CAS) assay, which changes color depending on the presence of metal chelator compounds. It was also found that Bath produces the REE chelators only under conditions of insufficient REEs (<0.1 μM soluble $CeCl_3$). Further, gene expression was compared under chelator-producing and non-chelator-producing conditions. Twenty-six genes were highly expressed in the chelator-producing condition. Among them, we found two genes that may be related to chelator production, namely, a non-ribosomal peptide synthetase gene and a c-type cytochrome gene. Knockout mutants of these genes are now under construction and their functions are being analyzed.

3. Effects of REEs on protease-producing bacteria

Since REEs are showing chemical properties similar to Ca, REE dependence in other enzymes containing Ca such as protease was hypothesized. Enrichment cultures of protease-producing bacteria on media with and without REE chlorides resulted in dominance of different types of microorganisms. Three strains belonging to genus *Stenotrophomonas* and *Chryseobacterium*, which were predominated in REE enrichment cultures, were isolated as protease producing bacteria. In which, *Stenotrophomonas* strains showed higher protease activities, and their extracellular proteases were detected by activity staining on casein-containing SDS-polyacrylamide gels. Further identification and the metal content analysis of those proteases is currently underway.