学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Exploration of lanthanide-dependent methylotrophic bacteria
ランタノイド依存メタノール資化性細菌の探索

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Plants release large amount of methane and methanol into the atmosphere as by-products of their metabolism. Methane and methanol are important C1 compounds widespread in nature and they are essential intermediates in the global carbon cycle. These compounds can be utilized by methylotrophs, which are defined as a group of microorganisms capable of utilizing C1 compounds as the sole carbon and energy source. In Gram-negative methylotrophs, oxidation of methanol is catalyzed by methanol dehydrogenase (MDH). It has been found that two types of MDHs exist. One is well-studied Ca^{2+}-dependent MDH, encoded by *mxaF* gene. MxaF-MDH contains Ca^{2+} in its active site. The function of another MDH, XoxF, encoded in all methylotrophs, had been a mystery for a long time. Recently it was found to be a lanthanide (Ln^{3+})-dependent MDH, sharing 50% amino acid sequence identity with that of MxaF. The unexpected dependency of XoxF on Ln^{3+} has not only shed light on the unexplored bacterial methylotrophy but also expanded the importance of the new metal in biology. Although (meta)genomic analyses suggested the existence of large number of methylotrophs, the members so far isolated are very limited, and in general, soil microorganisms are known to be un-culturable. In this study, in order to explore the variety and function of as-yet unisolated methylotrophs, I aimed to isolate and characterize Ln^{3+}-dependent methylotrophs.

A new aerobic facultative methylotrophic and diazotrophic strain $SM30^{T}$ was isolated from rice rhizosphere by Dr. Sachiko Masuda with nitrate mineral salts (NMS) medium containing 20%

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methane and 30 μM lanthanum (La^{3+}). Its growth on methanol was not dependent on Ln^{3+} but enhanced by Ln^{3+}. The strain was most closely related to *Pleomorphomonas oryzae* DSM 16300^T, with low 16S rRNA gene similarity of 94.17%. Due to its low 16S rRNA gene identity, strain SM30^T was considered to belong to a novel genus. Thus, I characterized the phenotypes of the strain and published it as a new genus and new species, and the name *Oharaeibacter diazotrophicus* gen. nov., sp. nov. was proposed (type strain SM30^T = NBRC 111955^T = DSM 102969^T). Further, I determined the complete genome of the strain using PacBio Sequencer. The genome consists of one chromosome and two plasmids, comprising a total of 5,004,097 bp, and the GC content was 71.6 mol%. A total of 4497 protein-coding sequences (CDSs), 67 tRNA, and 9 rRNA were encoded. Typical alpha-proteobacterial methylotrophy genes were found: pyrroloquinoline quinone (PQQ)-dependent MDH (*mxaF* and *xoxF1-4*), methylotrophy regulatory proteins (*mxbDM* and *mxcQE*), PQQ synthesis, tetrahydrofolate (H_4F) pathway, tetrahydromethanopterin (H_4MPT) pathway, formate oxidation, serine cycle, and ethylmalonyl-CoA pathway. SDS-PAGE and subsequent LC-MS analysis, and qPCR analysis revealed that MxaF and XoxF1 were the dominant MDH in the absence or presence of La^{3+}, respectively. The growth of MDH gene-deletion mutants on alcohols indicated that *mxaF* and *xoxF1* were involved in the oxidation of methanol, ethanol, and propanol, and *xoxF2* and *xoxF3* were partly engaged in the growth on methanol and ethanol. Four Ln^{3+} such as La^{3+}, cerium (Ce^{3+}), praseodymium (Pr^{3+}), and neodymium (Nd^{3+}) served as cofactors for XoxF1 by supporting *ΔmxaF* growth on methanol. Recently, *Mongoliimonas terrestris* from desert soil and *Chthonobacter albigriseus* from grass-field soil showing 96.3% and 96.28% 16S rRNA gene identity to that of strain SM30^T, respectively, have been published as new genus and new species after the publication of *Oharaeibacter diazotrophicus* SM30^T. The phylogenetic ananlysis based on 16S rRNA gene and multilocus sequence analysis (MLSA) combined with the digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values indicated that strain SM30^T was totally different from the two new genus and species and confimed the novel phylogenetic location of strain SM30^T. Additionally, strain SM30^T can fix nitrogen, which may offer nitrogen source for plants to promote the growth of plants.

On the other hand, I myself also tried to isolate novel Ln^{3+}-dependent methanotroph and methylotroph with NMS medium supplemented with 20% methane as the sole carbon source and 30

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μM La^{3+} or 30 μM Ho^{3+} as an essential growth factor. About 300 isolates were isolated. Two isolates (Ho311 and Ho312) could grow on methane, however, their growth on methane was not Ln^{3+}-dependent. The growth of strain La2-4^T on methanol, which was isolated from rice rhizosphere soil, was strictly Ln^{3+}-dependent. Its 16S rRNA gene sequence showed only 93.4% identity to that of *Methylophilus luteus* Mim^T, and the name *Novimethylophilus kurashikiensis* gen. nov. sp. nov. was proposed (type strain La2-4^T = NBRC 112378^T = KCTC 62100^T). Its draft genome (ca. 3.69 Mbp, G+C content 56.1 mol%) encodes 3579 putative CDSs and 84 tRNAs. The genome harbors five *xoxF*s but no *mxaFI*. XoxF4 was the major MDH in the cells grown on methanol and methylamine, evidenced by protein identification and quantitative PCR analysis. Methylamine dehydrogenase gene was absent in the La2-4^T genome, while genes for the glutamate-mediated methylamine utilization pathway were detected. The genome also harbors those for the tetrahydromethanopterin and ribulose monophosphate pathways. Additionally, as known species, isolates of *Burkholderia ambifaria*, *Cupriavidus necator*, and *Dyadobacter endophyticus* exhibited Ln^{3+}-dependent growth on methanol.

In this research, new Ln^{3+}-dependent methylotrophs were obtained from rice rhizosphere by the addition of Ln^{3+} via enrichment cultivation. I also measured methanol emission from rice roots and Ln^{3+} concentration in the field soil of the institute. The data suggested that Ln^{3+}-dependent methylotrophy takes place in the agricultural environment. This study contributes to understanding the bacterial methylotrophy in which Ln^{3+} plays an important role in MDH activity and regulation, and lets us imagine that Ln^{3+} participate in life activities much more actively beyond our envision. The discovery of the two new bacteria enriches the varity of life in the world.