学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Studies on alkane degradation and its molecular mechanisms of *Geobacillus kaustophilus* HTA426 isolated from the Mariana Trench

（マリアナ海溝から単離された *Geobacillus kaustophilus* HTA426のアルカン分解と
その分子機構に関する研究）

Introduction, the genus *Geobacillus* is a Gram positive thermophilic spore-forming bacterial group, which has gained attention due to its high-temperature tolerant nature and its benefits in the industrial production of thermostable enzymes. Several *Geobacillus* strains are reported to be able to degrade hydrocarbons. During a previously study on *Geobacillus thermoleovorans* B23 (Boonmak et al., 2014), a broad range alkane (C11 – C32) degrader, it was suggested that *Geobacillus kaustophilus* HTA426 can also degrade alkane. However, genome analysis of *G. kaustophilus* HTA426 suggested that none of the known alkane monooxygenase gene existed in the genome, e.g. AlkB-type, Alm-type, CYP-type, and Lad-type which can be found in other alkane degrading Geobacilli. I found a candidate gene, *GK2771*, in the HTA426 genome that encodes an isolated ribonucleotide reductase small subunit (RNR2) located in a cluster of genes responsible for alkane degradation. Recently, GK2771 has been proposed as a member of new protein group, R2-like ligand binding oxidase (R2lox), that resembles to RNR2 class Ic but lacks ribonucleotide reductase activity. The function and substrate for R2lox are yet unknown. I hypothesized it could function as an alkane monooxygenase based on its structural high similarity to soluble methane monooxygenase (sMMO). **Chapter 1**, it was verified that *G. kaustophilus* HTA426 can degrade alkane (C11 – C17) in nutrient medium LB at 60℃. Moreover, it was found to degrade wider range C10 – C24 alkane in minimal medium LBM condition. **Chapter 2,** a recombinant *E. coli* (pET28a-GK2771) was prepared in which *GK2771* is overexpressed under the control of T7-promoter. As expected, the recombinant GK2771 crude enzyme degraded alkane and its activity was not much different between 40℃ and 60℃. This finding demonstrates that an isolated RNR2, GK2771 (*Gk*R2loxI), is a novel alkane monooxygenase, and answers questions about the function and substrate of the R2lox protein group. **Chapter 3,** GK2772, a putative aldehyde dehydrogenase gene, was overexpressed in *E. coli* (pET28a-GK2771), purified and examined its activity. Recombinant GK2772 showed significantly higher activity against acetaldehyde at 60℃ compared to 30℃, 40℃ and 50℃ conditions. It was also suggested that GK2771 has dehydrogenase activity against a fatty aldehyde, decanal C10, at 60℃. **Chapter 4,** describes general discussion including distribution of *GK2771* gene homologues. This study expands our understanding of the vast diversity and new evolutionary lineage of the bacterial alkane monooxygenase family.