報告番号	※　　第　　号

主　論　文　の　要　旨

論文題目　Biosynthetic study of miuraenamide A, an antifungal antibiotic of a slightly halophilic myxobacterium
（亜好塩性粘液細菌の抗真菌抗生物質 miuraenamide A の生合成に関する研究）

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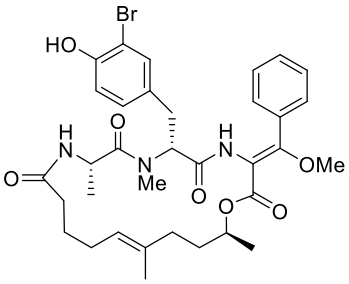
論　文　内　容　の　要　旨

Bioactive molecules (mainly secondary metabolites) are closely related to human life as they are used as lead compounds for drug discovery, biocontrol agents in agriculture, etc. Myxobacteria, gliding and fruiting body-forming rare bacteria, are recently attracting attention as new microbial factory in these areas. Among them, halophilic strains are extremely rare and difficult to isolate and culture, but they have shown great potential for producing secondary metabolites with novel structures and bioactivity. *Paraliomyxa miuraensis* SMH-27-4 is one of the 11 halophilic strains isolated to date and produces the potent antifungal antibiotic miuraenamide A (**1**). In this thesis, the potential of the secondary metabolite production of this strain and the biosynthetic machinery for **1** were illustrated.



miuraenamide A (**1**)

(1) Genomic analysis of *P. miuraensis* SMH-27-4

The draft genome of *P. miuraensis* SMH-27-4 was sequenced and de novo assembled into 11.8 Mbp consisting of 164 contigs. The analysis suggested a high degree of completeness of the genome assembly (93% coverage of the complete genome). The result of genome sequence-based phylogenetic analysis supported the taxonomy of the strain as representing a novel genus, *Paraliomyxa*, in the family Nannocystaceae. Seventeen biosynthetic gene clusters (BGCs) were found in the genome. One of them, type I polyketide synthase/nonribosomal peptide synthase hybrid type (PKS/NRPS), was estimated to be the BGC for miuraenamide A based on gene functional analyses. The 16 other BGCs contained two PKS/NRPS hybrids, four terpenoid biosynthetic gene clusters, etc., which showed low or no similarity

with the BGCs for the previously reported products, revealing the great potential of the strain to produce novel secondary metabolites.

(2) BGC for miuraenamide A (*miu* cluster)

The *miu* cluster, consisting of 36 orfs (85.9 kbp, Fig. 1A), was successfully cloned and heterologously expressed in the well-known terrestrial myxobacterium *Myxococcus xanthus*. However, the constructed transformant produced **1** in a low yield (0.06 mg/L, 6% of that of SMH-27-4). The products of three core genes *miuA*–*miuC*, MiuA/MiuB (PKSs) and MiuC (NRPS), recruit and sequentially couple five C_2/C_3 carboxylic acids and three amino acids to generate the early intermediate **2** (Fig. 1B). The products of four genes *miuD*–*miuG* are estimated to be modification enzymes. The MiuE (*O*-methyltransferase) and MiuG (halogenase) were readily confirmed by the facts that the Δ*miuE* and Δ*miuG* mutants produced the known congener miuraenamide E (**3**) and a new congener, debromomiuraenamide A (**4**) (Fig. 1B). The disruption of *miu*D (cytochrome P450 gene) and *miuF* (thioesterase gene) did not affect the production of **1**, and the estimated early intermediate **2** was detected. The gene responsible for the oxidation of **3** to **1** could be outside the *miu* cluster. The removal of the *orf25-29* and *orf19-23* regions resulted in a substantial increase of the yield of **1**, and feeding the mutant on 3-bromo-L-tyrosine promoted the production of **1** at a little higher level (1.2 mg/L) than that of the original SMH-27-4 strain (1 mg/L). Considering the growth speed of this mutant (4 days of culture time), the productivity was five times as effective as that of SMH-27-4 (18 days).

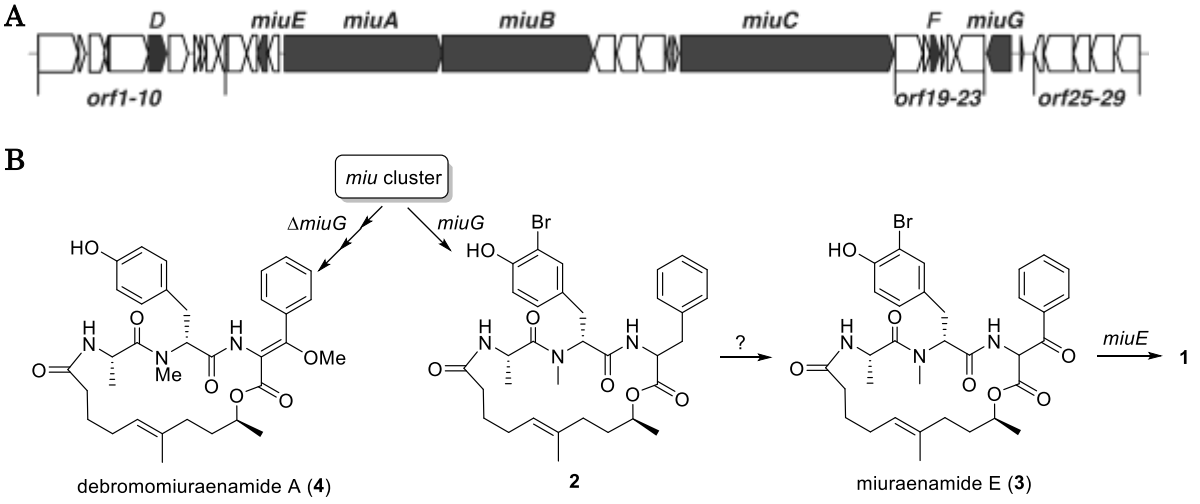


Figure 1. Organization of *miu* cluster (A) and a plausible biosynthetic route for miuraenamide A (**1**) (B).