論 文 の 内 容 の 要 旨

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論文題目

Studies on isolation, structure elucidation, and biosynthesis of secondary metabolites from the thermophilic bacterium *Thermosporothrix hazakensis*

（好熱性細菌 *Thermosporothrix hazakensis* 由来二次代謝産物の単離、構造解析、生合成に関する研究）

Microbial natural products have been excellent sources for drug discovery and development over the past three decades, but the isolation of known compounds is a common challenge that hinders the discovery of novel bioactive compounds. Because of this problem, new sources are needed for the discovery of novel natural products. One such source, the class of microorganisms known as extremophiles, has attracted the attention of natural product chemists because microorganisms under harsh conditions are likely to be subjected to substantial levels of competition, which could lead them to develop unique chemical arsenals, such as antibiotics. However, there have been few reports describing bioactive compounds from thermophiles. In addition, there have been few biosynthetic studies of secondary metabolites isolated from extremophiles.

Thermosporothrix hazakensis SK20-1^{T} is a novel thermophilic bacterium that has been isolated from ripe compost produced by a field-scale composter. The strain SK20-1^{T} is an aerobic and Gram stain-positive bacterium, and it grows at 31–58 °C, with its optimum growth at 50 °C. More interestingly, this strain forms multiple exospores per mother cell by budding in branched aerial mycelia, a unique characteristic of this thermophilic bacterium. These unique characteristics led me to consider that the thermophilic bacterium could produce unprecedented metabolites. I thus decided to

exhaustively analyze the metabolites produced by this strain.

Chapter I Bioinformatics analysis of *T. hazakensis* genome sequence

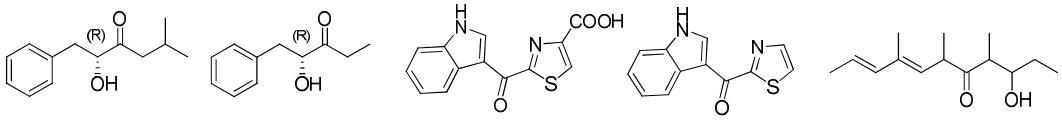
During last three decades, many biosynthetic pathways of a number of secondary metabolites have been elucidated mostly from microorganisms. Accumulated knowledge on the biosynthesis of secondary metabolites makes microbial genome sequences more valuable. In the field of natural product chemistry, various novel compounds appeared in the world by the genome-guided discovery and isolation. Therefore, this new methodology to expand chemical diversity would be applicable to as-yet unexplored organisms as well as well-studied microorganisms such as terrestrial actinomycete.

To verify the genetic potential of *T. hazakensis* for production of secondary metabolites, I subjected the *T. hazakensis* genomic DNA to draft sequencing with an Illumina DNA sequencer and analysed the genome sequence using various bioinformatics tools. Annotation of coding sequences revealed that many genes occupying two-third of all genes were hypothetical. Next, I carried out web-based investigation of gene loci that may be involved in the biosynthesis of secondary metabolites. Analysis of the genome sequences indicated the presence of several gene clusters including hybrid NRPS/PKS and ribosomal peptide synthase. Moreover, extensive analysis of each putative gene function suggested corresponding plausible chemical products. For example, the hybrid PKS/NRPS in the “cluster 17” was supposed to synthesize a lipohexapeptide compound. In addition, the “cluster 22” displayed very similar gene organization to that of the lantipeptide cinnamycin biosynthetic gene cluster. The core peptide sequence suggested the “cluster 22” encodes the biosynthetic genes responsible for a cinnamycin-like lantipeptide with a molecular weight of 1,888 Da. However, I could not predict secondary metabolites biosynthesized by other gene loci because of irregular gene organization or insufficient conserved domains to assume biosynthetic precursors. Nevertheless, these irregularities suggested high possibility to produce novel chemical skeletons.

Chapter II Isolation, structure elucidation, and biological activity of the secondary metabolites from *T. hazakensis*

After the identification of gene clusters that may be involved in secondary metabolites biosynthesis through the genome analysis of *T. hazakensis*, chemical studies including the fermentation, isolation, structure elucidation were executed to discover new

secondary metabolites with biological activity. At first, the fermentation condition was determined in small scale cultivations using several culture media. Next, from culture extracts of the mass fermentation using a jar fermenter, several compounds were isolated through the fractionation and isolation procedures. Extensive analysis of spectral experiments including HR-ESI-MS and NMR revealed chemical structures of the isolated compounds. Although the molecules expected from the genome sequence were not discovered, five new natural products including two acyloins (**1**, **2**), two indolecarbonyl thialzoles (**3**, **4**), and a polypropionate (**5**) were isolated along with six known compounds. Interestingly, the absolute configurations of **1** and **2** were assigned to be opposite that of previous reported acyloin compounds. Although *O*-methylated **3** was isolated from porcine lung, an indolecarbonyl thiazole moiety is an unusual scaffold in natural products isolated from microorganism. The isolated compounds (**1**−**4**) were also evaluated about biological activity including antibacterial, antifungal activity, and cytotoxicity. Of four compounds tested, **1**, **2,** and **3** displayed slight cytotoxicity against only a T lymphoma Jurkat cell line.



(-)-sattabacin (**1**) hazakacin (**2**) compound **3** compound **4** compound **5**

Figure 1. New compounds isolated from *T. hazakensis*

Chapter III Biosynthesis of the new compounds isolated from *T. hazakensis*

After the isolation and structure determination, I initiated the biosynthetic studies about the isolated compounds. First, two acyloin compounds (**1**, **2**) were supposed to be biosynthesized by a TPP-dependent enzyme on the analogy of the biosynthesis of other acyloins. Subsequently, the five TPP-dependent enzyme homologs, which were retrieved from the genome sequence by local blast search, were cloned and expressed in *Escherichia coli*. According to in vitro enzyme reaction, Thzk0150 was determined as the active enzyme involved in the biosynthesis of **1** and **2**. Further detailed experiment including LC-MS and NMR revealed two biosynthetic substrates for the TPP enzyme; phenylpyruvate as a donor substrate and 4-methyl-2-oxovalerate as a receptor substrate. From these results, I proposed the Thzk0150-catalyzed reaction mechanism via α-hydroxy-β-keto acid. In vitro enzyme reaction, sattabacin was identified as a racemic

mixture. Therefore, I supposed that unidentified decarboxylase in *T. hazakensis* is necessary for the production of (−)-sattabacin (**1**) and hazakacin (**2**) from an α-hydroxy-β-keto acid intermediate.

In case of indolecarbonyl thiazole (**3**, **4**), early speculation about the biosynthesis was the formation of a thiazoline/thiazole heterocycle from tryptophan and cysteine by the action of NRPS. However, I could not find proper NRPS encoding the gene cluster that consists of the adenylation domains for tryptophan and cysteine. Bioconversion experiment in the crude protein extract of *T. hazakensis* suggested that indole-3-pyruvate (IPA) is a precursor of compounds **3** and **4**. In fact, I demonstrated that Thzk3800 aminotransferase catalyzes deamination of tryptophan to give IPA. Whereas fungal tryptophan-dependent pigment was reported to be non-enzymatically generated from IPA, the spontaneous generation did not appear to be applied to the biosynthesis of **3** and **4** based on observation of no utilization of intact cysteine.

Conclusion

In summary, I identified the five new compounds (**1**−**5**) from the thermophile *T. hazakensis* SK20-1^T and revealed the presence of several biosynthetic gene loci related to secondary metabolite by the genome analysis. It should be noted that the absolute configurations of **1** and **2** were opposite those of the previously reported natural acyloin compounds. Indolecarbonyl thiazole (**3**, **4**) is also unusual scaffold in microbial natural products. Biochemical experiments demonstrated that the TPP-dependent enzyme Thzk0150 catalyzes the acyloin formation. In addition, I identified aminotransferase Thzk3800 for the formation of IPA, which is a precursor for the indolecarbonyl thiazole.

My study is the first to describe bioactive natural products and biosynthetic enzymes from *T. hazakensis*. Finally, I anticipate that my chemical and biochemical studies can contribute to the production of unprecedented compounds using α-keto acid substrates or possessing unusual indole-thiazole moiety, and that the thermophilic bacteria can be used as sources of novel natural products.

Reference

Park J.-S, Kagaya N, Hashimoto J, Izumikawa M, Yabe S, Shin-ya K, Nishiyama M, and Kuzuyama T. Identification and Biosynthesis of New Acyloins from the Thermophilic Bacterium *Thermosporothrix hazakensis* SK20-1^T, *Chembiochem*, DOI: 10.1002/cbic.201300690