Screening and functional analysis of enzymes from secondary metabolism

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Secondary metabolites produced by microorganisms are important for agricultural as represented by kasugamycin which is used as an agricultural fungicide and an antiparasitic agent, milbemycin. The ability of microorganism to produce secondary metabolite with various activities and structures indicates that the microorganisms have developed wide variety of enzymes with different catalytic activity during their evolution. Therefore, studying the biosynthesis of secondary metabolites should result in discovery of enzymes with unique activities.

Cremeomycin is a secondary metabolite with a diazo group. By studying its biosynthetic pathway, we discovered a novel nitrous acid biosynthetic pathway consists of two enzymes CreE and CreD.[1] Nitrous acid produced by these enzymes was revealed to be the source of the diazo group. Interestingly, CreE and CreD homologs were widely distributed among actinobacteria. By analyzing the function of these homologs, we discovered novel desferrioxamine derivatives, desferrioxamine I from *Streptomyces davawensis*. In addition, we elucidated the reaction mechanism of CreD which catalyzes elimination of nitrous acid from nitrosuccinate and proposed its reaction mechanism in which arginine is acting as a catalytic acid.

Nonribosomal peptides are important class of peptide which is synthesized independent from ribosome. One of the important features of these peptides is that it can be synthesized from various nonproteinogenic amino acids. We focused on two nonribosomal peptides, rufomycin and JBIR-34 and elucidated the biosynthesis of nonproteinogenic amino acids involved in their biosynthesis. As a result, RufO, a cytochrome P450 catalyzing nitration of tyrosine using nitric oxide and FmoH, hydroxymethyltransferase catalyzing α-methyl-L-serine synthesis from D-alanine was identified.

Benzastatins are class of secondary metabolites synthesized from *p*-aminobenzoic acid and geranyl group. By elucidating the biosynthetic pathway of benzastatins, we discovered the unique cytochrome P450 (BezE), which catalyzing nitrenoid formation and transfer to synthesize indoline and tetrahydroquinoline scaffold.

By analyzing the biosynthetic pathway of ishigamide, polyene discovered by genome mining, we discovered a novel class of type II polyketide synthase which is responsible for polyene biosynthesis.

In conclusion, we have discovered several enzymes with unique and important activities. Further study on biosynthesis of secondary metabolites should result in discovery of enzymes with unique function and industrial applications.

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2) Tomita H., Katsuyama Y., Minami H., Ohnishi Y.: ***J. Biol. Chem.*** 292: 15859-15869 (2017).
3) Tsutsumi H., Katsuyama Y., Izumikawa M., Takagi M., Fujie M., Satoh N., Shin-Ya K., Ohnishi Y.: ***J. Am. Chem. Soc.*** 140: 6631-6639 (2018).