



NOVEL TRYPTOPHAN METABOLISM BY A POTENTIAL GENE CLUSTER THAT IS WIDELY DISTRIBUTED AMONG ACTINOMYCETES

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Introduction. The characterization of potential gene clusters is a promising strategy for the identification of novel natural products and the expansion of structural diversity. However, there are often difficulties in identifying potential metabolites because their biosynthetic genes are either silenced or expressed only at a low level. In this study, we focused on a selected group of putative gene clusters including an indole prenyltransferase *lptA* homolog because it is widely distributed among actinomycetes. We classified the gene clusters into two types on the basis of their constituent genes; in addition to the indole prenyltransferase gene, one gene cluster contained a tryptophanase gene, whereas the other contained a flavin-dependent monooxygenase (FMO) gene. Takahashi *et al.* have demonstrated that the former type of gene cluster in *Streptomyces* sp. SN-593 is responsible for the biosynthesis of 6-dimethylallylindole-3-carbaldehyde (1). No metabolic pathways involving the latter type of gene clusters have yet been elucidated, though FMO gene clusters are also widely distributed among actinomycetes.

The objective of our work is identification of a metabolite that is synthesized by a potential gene cluster including the *lptA* homolog SCO7467 and the FMO SCO7468, which were mined from *Streptomyces coelicolor* A3(2). Biochemical characterization of SCO7467 and SCO7468 is also the objective.

Methods. We introduced the SCO7467 and SCO7468 genes into the closely related *Streptomyces lividans* TK23 using a plasmid vector and analyzed the culture broth of the transformant on an LC-MS/MS system. The transformant culture was incubated in a 500-ml baffled flask containing 100 ml of TSB *tsr* (3% Tryptone soya broth and 30 µg/mL thiostrepton) medium for 4 days at 27°C on a rotary shaker at 160 rpm. The structure of an unidentified metabolite detected in the culture broth was determined on the basis of NMR and high-resolution MS spectral data.

We purified the recombinant SCO7467 and SCO7468 from *Escherichia coli* BL21(DE3) to elucidate each biochemical function of these enzymes.

Results. The heterologous expression of SCO7467 and SCO7468 in *S. lividans* TK23 allowed us to identify a novel metabolite, 5-dimethylallylindole-3-acetonitrile (5-DMAIAN) (Fig. 1) (2). Biochemical characterization of the recombinant SCO7467 and SCO7468 demonstrated the novel L-tryptophan metabolism leading to 5-DMAIAN (2). SCO7467 catalyzes the prenylation of L-tryptophan to form

5-dimethylallyl-L-tryptophan (5-DMAT). This enzyme is the first actinomycetes prenyltransferase known to catalyze the addition of a dimethylallyl group to the C-5 of tryptophan. SCO7468 then catalyzes the conversion of 5-DMAT into 5-dimethylallylindole-3-acetaldoxime (5-DMAIAOx) in the presence of NADPH. An aldoxime forming reaction catalyzed by the FMO enzyme was also identified for the first time in this study. Finally, dehydration of 5-DMAIAOx presumably occurs to yield 5-DMAIAN.

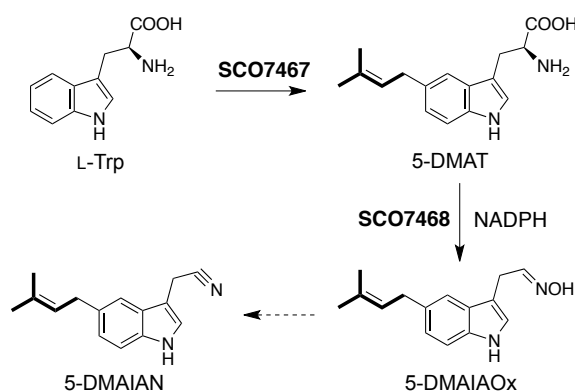


Fig. 1 Proposed pathway for 5-DMAIAN biosynthesis.

Conclusions. A potential gene cluster containing an indole prenyltransferase gene (SCO7467) and an FMO gene (SCO7468), which were mined from the genome of *S. coelicolor* A3(2), was revealed to be responsible for 5-dimethylallylindole-3-acetonitrile biosynthesis. The biosynthetic route leading to 5-dimethylallylindole-3-acetonitrile represents a novel tryptophan metabolism (Fig. 1). The present study provides insight into the biosynthesis of prenylated indole derivatives that have been purified from actinomycetes.

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