THE BIOSYNTHESIS OF PORPHYRIN COMPOUNDS IN AMYCOLATOPSIS ORIENTALIS

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Introduction. Porphyrin compounds are important small-molecular cofactors widespread in organisms. In our study, the correlation between the level of porphyrin compounds and the yield of important secondary metabolites such as vancomycin and ECO-0501 was first found in *Amycolatopsis orientalis* HCCB-10007 when the bacterial culture conditions was changed. To illustrate this correlationship, clarification of the biosynthetic pathway of porphyrin compounds will be necessary.
Here, the biosynthesis pathway of porphyrins in this strain was predicted based on the whole genome bioinformatics analysis. Two key enzymes HemA (glutamyl-tRNA reductase) and HemN (COPROGEN oxidase) were studied in this abstract.

Methods. Analysis of the biosynthesis pathway of the porphyrins was performed by KEGG. Gene disruption was carried out by homologous recombination. Metabolites were analyzed by HPLC.

Results. In most organisms, the biosynthetic pathway of heme were highly conserved(1). Based on the genome information of *A.orientalis* (CP003410）, the biosynthetic pathway was predicted (Fig.1). Two ALA (5-aminolevulinic acid) biosynthesis routes were found, the C5 pathway and the Shemin (C4) pathway. To confirm which pathway used in the biosynthesis of heme, *hemA1* of C5 pathway was disrupted. As a result, the mutants can only survive with heme or ALA supplement in the media. Analysis of the *hemA2* revealed that the gene was located in the ECO-0501 gene cluster, which means the ALA formed by the Shemin pathway serves as a precursor of the amino-hydroxy-cyclopentenone compounds(2), which was similar to the occurrence in *Streptomyces nodosus* subsp. *asukaensis*(3).
In our previous study, the porphyrin compounds, especially coproporphyrinogen compounds accumulated massively when the bacterial culture conditions were changed. It was speculated that the transformation from coproporphyrinogen III to protoheme was probably disturbed(4). Therefore, the *hemN* gene was knockout to imitate this condition. The growth of the disruptants almost was not affected and the coproporphyrinogen compounds accumulated simultaneously. The yields of ECO-0501 and vancomycin were decreased, which can be recovered partly through heme supplement. Additionally, the results revealed that like its relative strain *A.mediterranei*(5), there was likely another isoenzyme exist to catalyze the reaction from coproporphyrinogen III to protoporphyrinogen IX which is not yet discovered.

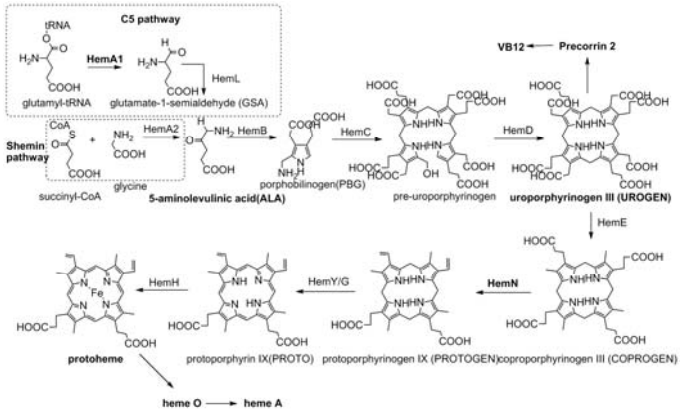

Fig.1 The biosynthesis pathway of heme predicted in *A.orientalis*.

Conclusions. In *A.orientalis*, the C5 route produces ALA for tetrapyrrole biosynthesis, and the C4 route forms ALA for ECO-0501 producing. Although *hemF* was not detected in *A.orientalis* genome, another isomenzyme exist likely. Heme and its analogues can obviously influence the secondary metabolites yielding.To illustrate the complete biosynthetic pathway of heme, there remains a lot to be studied.

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