学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　　博士（工学）　　氏名　申 寧

学 位 論 文 題 名

Development of *Escherichia coli* platform for tyrosine-derivative production using aromatic amino acid hydroxylases

（芳香族アミノ酸水酸化酵素を用いたチロシン関連化合物生産のための大腸菌の構築）

Aromatic compounds derived from tyrosine (Tyr) are important and diverse chemicals used for industrial and commercial applications. Although these compounds can be obtained by extraction from natural producers, their growth is slow, and their content is low. To overcome these problems, many of them have been chemically synthesized from petroleum-based feedstocks. However, because of the environmental burden and depleting availability of feedstock, microbial cell factories are attracting much attention as sustainable and environmentally friendly processes.

To develop microbial cell factories for Tyr derivative production, I constructed simple and convenient Tyr-producing *Escherichia coli* platforms with phenylalanine (Phe) hydroxylase (PheH), which converted Phe to Tyr with O_2 and tetrahydromonapterin (MH4) as a cofactor, by engineering their genes with plasmid-based and chromosome-integrated methods. For effective Tyr production from Phe, the platforms required MH4 regeneration system that reduced the oxidized form of the cofactor, quinonoid dihydromonapterin, with NADH by pterin-4α-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR). The Tyr titer of the plasmid-based *E. coli* platform expressing the genes encoding PheH, PCD and DHPR was 25.5 mM (4.63 g/L) in a medium containing 30.3 mM (5.00 g/L) Phe with a test tube. The strain was successfully used to produce industrially attractive compound, tyrosol, with a yield of 11.5 mM (1.58 g/L) by installing additional Tyr decarboxylase (TDC) and tyramine oxidase (TYO) genes on a plasmid.

Chromosomal engineering of *E. coli* has an advantage over the use of plasmids because it increases genetic stability without antibiotic feeding to the culture media and relieves the metabolic burden associated with plasmid maintenance. Therefore, I constructed a prototype *E. coli*, in which *tac* promoter controlled the PheH gene and *lac* promoter controlled

the PCD–DHPR-encoding operon were integrated into the chromosome. However, the productivity was quite low, likely because of the low copy number of the genes compared with the plasmid-based platform. Then, I utilized a stronger promoter. I constructed a Tyr-producing platform with a yield of 28.6 mM (5.19 g/L) in a medium containing 30.3 mM (5.00 g/L) Phe by using *T7* promoter and eliminating bottleneck steps in the pathway. Then, the platform strain was converted to a tyrosol-producer strain by integrating genes encoding a selected TDC and TYO under the control of the *T7* promoter on the chromosome. Unexpectedly, a melanin-like pigment, probably derived from an intermediate 4-hydroxyphenylacetaldehyde, was formed as a byproduct and the tyrosol productivity was still low. However, the tyrosol productivity was improved to 17.5 mM (2.42 g/L) with a concomitant decrease of the pigment by introducing a gene encoding medium-chain dehydrogenase/reductase catalyzing reduction of 4-hydroxyphenylacetaldehyde to tyrosol and by increasing the glycerol concentration in the medium.

In conclusion, I developed *E. coli* platforms for production of Tyr from Phe at multi-gram-per-liter levels in test-tube cultivation by plasmid-based and chromosome-engineered methods. The platforms would be useful for production of Tyr-derivatives at multi-grams-per-liter levels in test tubes.