CELL FACTORY CONSTRUCTION BASED ON 4-HYDROXYBENZOIC ACID AS A PRECURSOR IN *PSEUDOMONAS CHLORORAPHIS*

ABSTRACT

Microbial-based synthetic biology is a powerful and eco-friendly approach for the production of high-value products from sustainable carbon sources. A series of commercial biological products have been successfully developed through the design and construction of artificial biosynthesis pathways in conventional microorganisms. *Pseudomonas chlororaphis* HT66 has been well engineered as a phenazine-producing platform organism due to well-characterized physiology and genetics, and fast growth rate using glycerol as a carbon source, it can also be used as the cell factory and for synthetic biology design based on the high-efficient shikimate pathway. In our study, a potential endogenous strong promoter P_{phz} was screened on the basis of transcriptomic and proteomic analysis of different phenazine-producing strains. And 1.6 g/L 4-hydroxybenzoic acid (4-HBA) was synthesized through the natural synthetic pathway derived from chorismate using the native promoter P_{phz} after the phenazine synthetic pathway was blocked. Then three plasmid-free biosynthetic pathways were constructed in *P. chlororaphis* for enhanced muconic acid (MA), arbutin and 2-pyrone-4,6-dicarboxylic acid (PDCA) biosynthesis from the promising intermediate 4-HBA, and the titer of MA, arbutin and PDCA production reached 3.4 g/L, 6.79 g/L and 0.58 g/L, respectively. MA, arbutin and PDCA biosynthetic genes were installed to the chromosome of *P. chlororaphis* under the control of endogenous strong promoter P_{phz}. For muconic acid is a platform chemical and an important intermediate in the degradation process of a series of aromatic compounds, arbutin is a plant-derived glycoside compound with potential antioxidant, antibacterial and anti-inflammatory activities. In conclusion, *P. chlororaphis* cell factory based on 4-HBA as a precursor revealed a broad application perspective. The biosynthesis of value-added products

were produced from precursor 4-HBA when the designed biosynthetic gene cluster was inserted into the genome of *P. chlororaphis* HT66 under the control of the endogenous strong promoter P_{phz} independent of plasmid and inducer. This study may provide an important basis for the construction of *P. chlororaphis* cell factory and production of biochemicals on the basis of shikimate pathway.

KEY WORDS: *Pseudomonas chlororaphis* HT66, Shikimate pathway, 4-Hydroxybenzoic acid, Cell factory