

Enhanced vanillin production from *Escherichia coli* mutant with improved resistance vanillin toxicity

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Abstract

Vanillin is one of the most important aromatic flavor compounds used in the food and cosmetics industries. The most intensively studied process for producing vanillin by biotransformation, which then can be designated "natural," is based on the substrate ferulic acid.⁽¹⁾ The genes consisting of the CoA-dependent path way, *fcs* and *ech*, encoding feruloyl-CoA-synthetase and enoyl-CoA-hydratase/ aldorase respectively, can catalyze the conversion ferulic acid to vanillin.⁽³⁾ In this study, both of the genes were cloned from *Amycolatopsis* HR104 by PCR, and transferred to pTrc99A expression vector, resulted in pTAHEF. Vanillin is highly toxic substance for most bacteria, and there was a notable inhibition of normal *E.coli* at a vanillin concentration of 0.1%.⁽²⁾ Therefore, we made the *E.coli* mutant which could grow at a vanillin concentration of 0.2% through NTG mutation which increased the tolerance of cells to vanillin. The optimal yield of vanillin was obtained with the *E.coli* mutant harboring pTAHEF when it was cultivated for 48h in 2YT media at 37°C. Under this cultivation condition, 3.35g/l of vanillin was produced from 6g/l of ferulic acid.

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Referances

1. Achterholt. S., Priefert. H., Steinbuchel. A.(2000), Identification of *Amycolatopsis* sp. strain HR167 genes, involved in the bioconversion of ferulic acid to vanillin. *Appl. Microbiol. Biotechnol.* 54:799-807.
2. Muheim. A., Lerch. K.(1999), Towards a high-yield bioconversion of ferulic acid to vanillin. *Appl. Microbiol. Biotechnol.* 51:456-461.
3. Michael J. Gasson., Yoshie Kitamura.(1998), Metabolism of ferulic acid to vanillin. *J. Biol., Chem.* 273(7):4163-4170.