Functional analysis of *rub*P1 homologous to the hydroxyphenylpropionate hydroxylase gene from the rubradirin producer *Streptomyces achromogenes* var. *rubradiris* NRRL3061

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Abstract

The ansamycin antibiotic rubradirin isolated from *Streptomyces achromogenes* var. *rubradiris* NRRL3061 consists of rubransarol, 3-amino-4-hydroxycoumarin, dihydroxydipicolinic acid and 2,6-dideoxynitrosugar. Rubransarol is synthesized through naphthoquinone ring formation in its biosynthetic pathway. *rub*P1 exists in the rubradirin biosynthetic gene cluster and is similar to hydroxyphenylpropionate (HPP) hydroxylase gene. We expect that the product of *rub*P1 is involved in naphthoquinone ring formation. The *rub*P1 gene was deleted from chromosome and expressed in *E. coli*. The mutant containing the inframe deleted *rub*P1 gene could not produce rubradirin. When the C-14 labeled substrate 3-amino-5-hydroxybenzoic acid (AHBA) was fed into the mutant an unknown metabolite different from rubradirin was detected. To identify in vitro function of RubP1, we expressed *rub*P1 gene in *E. coli* and purified, then tried to do conversion reaction after addition of NADH and compounds structurally analogous to the anticipated substrate. Although we couldn't detect the conversion of the compounds we found the conversion of NADH to NAD^+. This results suggests that RubP1 is related to biosynthesis of rubradirin and its function as a hydroxylase.

Reference

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