Natural Product Biosynthesis Through Biotechnological and Fermentation Approaches

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Background

- Natural products as a source of medicine have long been attractive due to the huge structural diversity and promising biological activities such as antimicrobial, anticancer, antifungal, antiviral, antiparasitic as well as antioxidant properties.
- Researchers have come up with various solutions: Heterologous expression, co-cultivation, fermentation engineering, isolation of new species, and strain engineering/improvement for natural products production and for improving their properties.
- *o*-Coumaric acid and *p*-coumaric acid are phenolic antioxidants found in various plant sources. These also serve as the precursors for the biosynthesis of various natural products.
- The *Streptomyces* species are the rich source for discovery of bioactive natural products and novel biosynthetic enzymes.
- In this study, *Streptomyces* strains were screened for the ability to make novel derivatives of p-coumaric and o-coumaric acid.

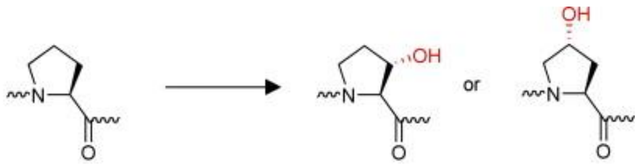

Figure 1: Hydroxylation of natural products

Objectives

- Discovery of novel derivatives of *p*-coumaric and *o*-coumaric acid
- Natural products isolation, analysis and characterization.
- Improve production and properties of natural products

Methodology

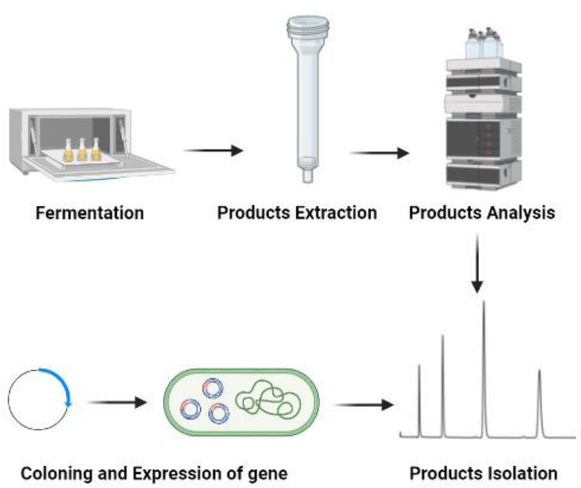

Figure 2: Summarized flow sheet of methodology

Results and Discussion

Among five strains, *Streptomyces* sp. NRRL B1677 was found potential strain for bio-transforming *p*-CA into products. Product-1, which is characterized as a caffeic acid.

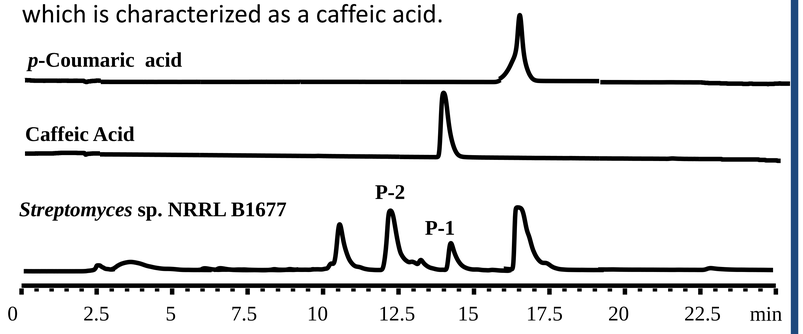

Figure 3: *Streptomyces* sp. NRRL B1677 ability to bio-transform *p*-CA into products

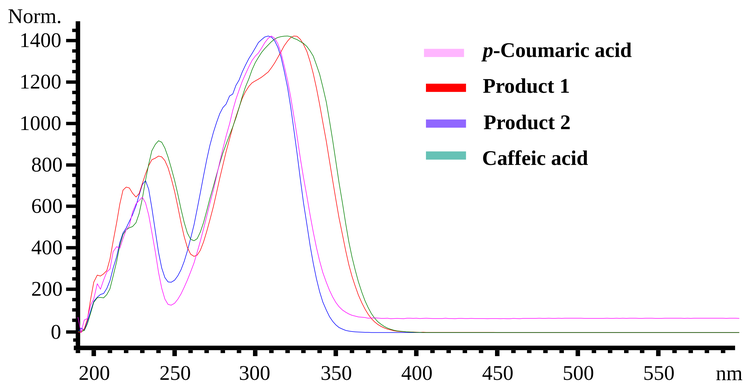

Figure 4: The UV spectrum comparison of substrate, products 1 & 2 and caffeic acid, hydroxylated derivative of *p*-Coumaric acid

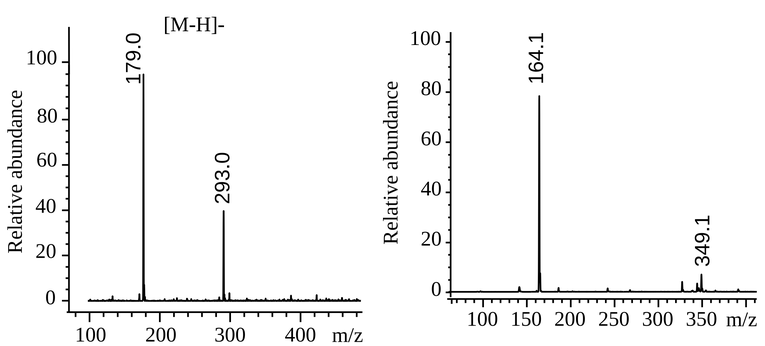

Figure 5: ESI-MS (-) spectrum of HSP-1 (left) and HSP-2 (right)

We cloned two putative 3-monooxygenase genes of *Streptomyces* sp. NRRL B1677 into pET28a and expressed the resulting plasmids (pHS8 and pHS10) in *E. coli* BL21(DE3). Results indicates that only pHS10 can bio-transform *p*-CA into caffeic acid, but production titer is low.

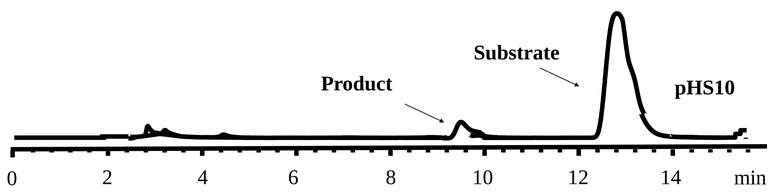

Figure 6: HPLC analysis of biotransformation of *p*-CA by engineered *E. coli* strains harboring pHS10.

These genes have also been cloned into pRM5 to yield pHS13 and pHS14, which will be tested for the function in *Streptomyces lividans* K4 to improve the production titer.

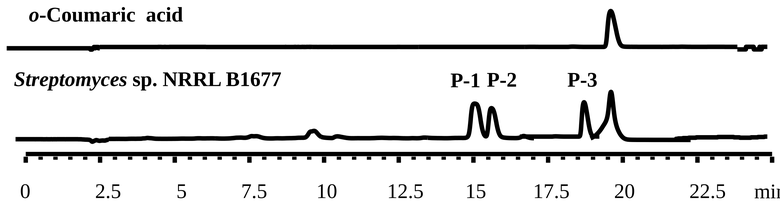

Figure 7: *Streptomyces* sp. NRRL B1677 ability to bio-transform *p*-CA into products

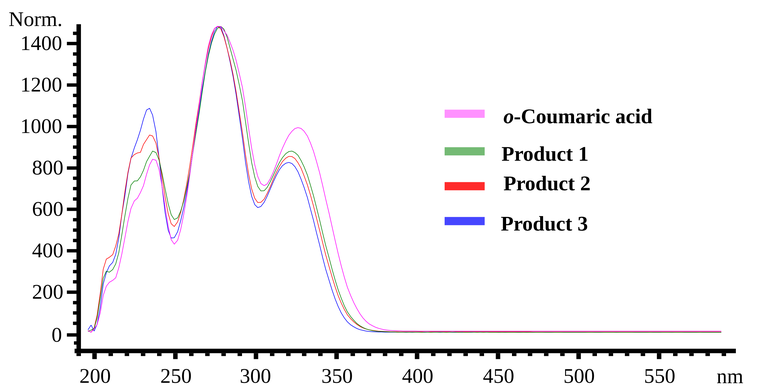

Figure 8: The UV spectrum comparison of *o*-coumaric acid with products 1-3

Conclusions and future work

- Among the five strains, *Streptomyces* sp. NRRL B1677 was found to bio-transform the two substrates into various products.
- One of the products from *p*-coumaric acid was identified as caffeic acid.
- A 3-monooxygenase was discovered from the *Streptomyces* sp. NRRL B1677 strain and heterologously reconstituted in *E. coli*.
- Results indicate that only the strain harboring pHS10 can bio-transform *p*-CA into caffeic acid, but production titer is low.
- pHS13 and pHS14, which will be tested for the function in *Streptomyces lividans* K4. Future work also includes the identification of other products and kinetic studies on the monooxygenase from *Streptomyces* sp. NRRL B1677.

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