Fermentation of flavonoids by microorganisms that contain an artificial biosynthetic gene cluster

Sueharu HORINOUCHI

Department of Bioechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo

Research aims

We have so far studied the regulation of antibiotic production and morphological differentiation by the genus *Streptomyces*. This genus produces most of the biologically active substances, including antibiotics and immunosuppressors, and includes industrially important strains. We have proposed a complex A-factor regulatory cascade and a signal transduction pathway that lead to secondary metabolism and morphogenesis. During these studies, we discovered a chalcone synthase (CHS) in *Streptomyces griseus*, which had been believed to be present only in plants, and revealed its surprising catalytic mechanism. The CHS in *S. griseus,* named RppA, is the first type III polyketide synthase in bacteria and catalyzes the synthesis of 1,3,6,8-tetrahydroxynaphthalene (THN) by selecting malonyl-CoA as the starter substrate, carrying out four successive extensions of malonyl-CoA, and finally Claisen condensation for the ring closure that is presumably accompanied by decarboxylation of the carbonyl group of the malonyl-CoA used as the starter. CHS catalyzes the synthesis of chalcone, which is the key internediate for a variety of flavonoids. Flavonoid and isoflavone compounds show a variety of biological activities. We have therefore started, as first step, to produce flavonoids in *Streptomyces* and other microorganisms by assembling the plant genes necessary for the synthesis of chalcone, an intermediate of flavonoids. In the present research period, four genes were placed under the control of strong promoters in *Streptomyces* and *Escherichia coli* and expressed to produce chalcone from phenylalanine or tyrosine.

Methods

An artificial gene cluster containing four plant genes under the control of a strong promoter in pIJ6021 for *Streptomyces* and pET26 for *E. coli* were constructed and introduced in the respective hosts (Fig. 1). Characteristic features of theis gene cluster were (i) the use of strong promoters, *PtipA* for *Streptomyces* and the *lac* promoter for *E. coli*, (ii) the attachment of a strong ribosome-binding sequence (GGAGG) in front of the first gene, (iii) fusion of the termination codon (TGA) of the preceding gene with the initiation codon (ATG) of the following gene, and (iv) the use of *S. lividans* and *S. griseus*, both of which we have studied for a long time. For expression of these genes in *E. coli*, the gene cluster was placed in the multi-linker site of pET26.

Results

The artificial gene cluster on pET26 designed for production of chalcone in *E. coli* was introduced in strain BL21. The transformant was grown in M9 medium containing tyrosine as a substrate at 30°C for 2 days. The culture broth was extracted with 80% acetone and ethylacetate and the solvent layer was evaporated to dryness. The residual material was dissolved in methanol containing 0.1% acetic acid and analyzed by HPLC equipped with an ODS column. Two peaks, 1 and 2, were detected, both of which were not present in the culture broth prepared similarly from *E. coli* harboring only the vector plasmid. The retension time of peak 2, as well as its UV spectrum, were the same as those of authentic naringenine. Peak 1 was sup-

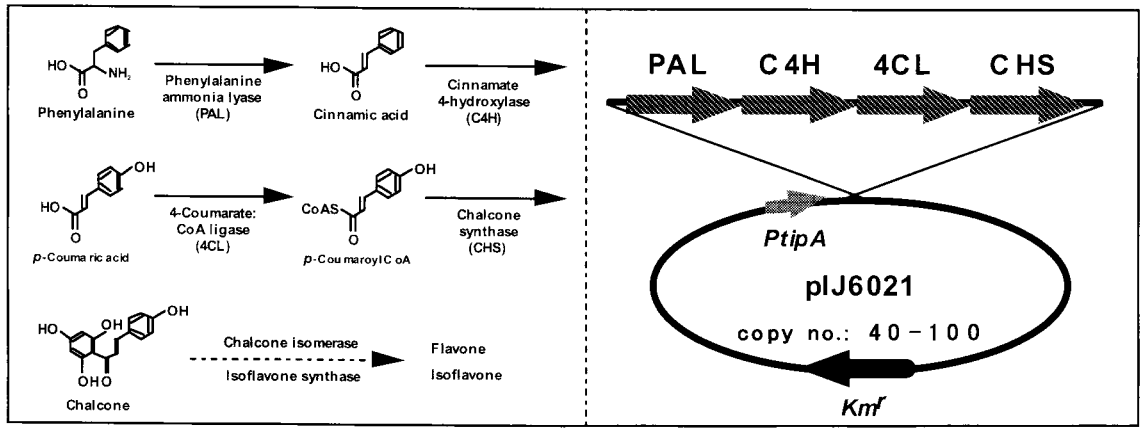

Fig. 1. Structure of expression plasmid for *Streptomyces*.

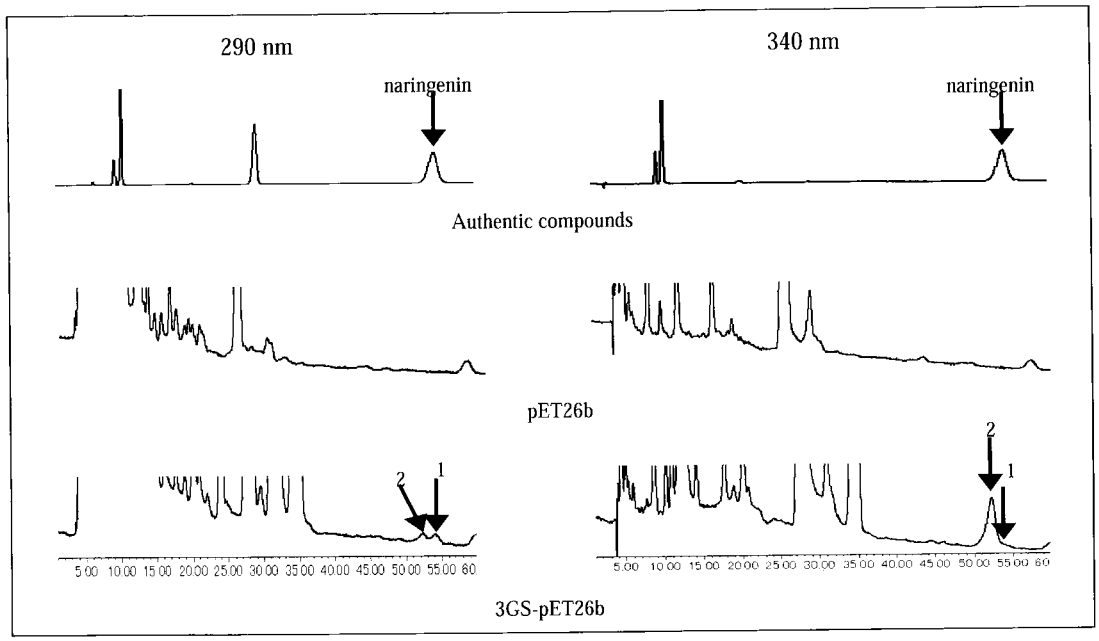

Fig. 2. HPLC analysis of the culture broth of *E. coli* containing an artificial biosynthetic gene cluster.

posedly chalcone. Naringenine is readily converted non-enzymatically from chalcone. We are now trying to determine the chemical structures of compounds 1 and 2 by LC-MS. An attempt to produce chalcone in *Streptomyces* is also being tested.

Conclusions

We have succeeded in producing chalcone and naringenine in *E. coli* harboring an artificial biosynthetic gene cluster. The yield is still low and some improvement in cultural conditions is apparently necessary. Also promising is to produce chalcone in *Streptomyces*.