Engineering the production of major catechins by Escherichia coli carrying metabolite genes of Camellia sinensis.

Abstract

A mimicked biosynthetic pathway of catechin metabolite genes from C. sinensis, consisting of flavanone 3 hydroxylase (F3H), dihydroflavonol reductase (DFR), and leucoanthocyanidin reductase (LCR), was designed and arranged in two sets of constructs: (a) single promoter in front of F3H and ribosome-binding sequences both in front of DFR and LCR; (b) three different promoters with each in the front of the three genes and ribosome-binding sequences at appropriate positions. Recombinant E. coli BL (DE3) harbouring the constructs were cultivated for 65h at 26°C in M9 medium consisting of 40g/L glucose, 1mM IPTG, and 3mM eriodictyol. Compounds produced were extracted in ethyl acetate in alkaline conditions after 1h at room temperature and identified by HPLC. Two of the four major catechins, namely, (−)-epicatechin (0.01) and (−)-epicatechin gallate (0.36mg/L), and two other types ((+)-catechin hydrate (0.13mg/L) and (−)-catechin gallate (0.04mg/L)) were successfully produced.

Keyword: Catechins; Flavanone 3 hydroxylase; Dihydroflavanol reductase; Leucoanthocyanidin reductase; Camellia sinensis; Escherichia coli.