Pathway expression optimization using the Ribosome Binding Site (RBS) Calculator tool

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Hydroxycinnamic acids and curcumin are plant metabolites with great therapeutic potential, including anti-inflammatory and anticancer activities. In this study, *p*-coumaric acid, caffeic acid and curcumin were produced in *Escherichia coli* using an artificial biosynthetic pathway [1]. Their production was induced by heat using the *dnaK* and *ibpA* heat shock promoters [2]. The ribosome binding sites (RBSs) used were tested and further optimized for each gene to assure an efficient translation. To optimize the RBSs we used the bioinformatic design tool RBS Calculator (v1.1) developed by Salis Lab (Penn State University) [3]. This tool predicts the translation initiation rate (TIR) of mRNAs and designs synthetic RBS with specific TIRs. This allows to improve the translation efficiency and to reach a desired response and therefore obtain the expected production using novel genes or biosynthetic pathways.

Tyrosine ammonia lyase from *Rhodotorula glutinis* was used to produce *p*-coumaric acid from tyrosine. *p*-Coumaric acid was converted to caffeic acid using 4-coumarate 3-hydroxylase from *Saccharothrix espanaensis* or cytochrome P450 CYP199A2 from *Rhodopseudomonas palustris*. Curcumin was produced from ferulic acid using 4-coumarate-CoA ligase from *Arabidopsis thaliana*, diketide-CoA synthase and curcumin synthase from *Curcuma longa*. The optimization of the RBSs lead to an increase in the production of *p*-coumaric acid, caffeic acid and curcumin up to 97.8, 11.7 and 14.4 times, respectively. The highest *p*-coumaric acid, caffeic acid and curcumin production obtained were 2.5 mM, 370 µM and 17 µM, respectively. These results demonstrate that it is of utmost importance to consider the strength of the RBS when designing a biosynthetic pathway and user-friendly bioinformatic tools such as RBS Calculator can be very useful for that purpose.

References:

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