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Combinatorial approaches to achieve de novo production of prenylated flavonoids in *Escherichia coli*

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Prenylflavonoids are characterized by the presence of a lipophilic prenyl side-chain in the flavonoid skeleton exhibiting a wide range of bioactivities. They are present only in residual amounts in nature and their extraction is difficult and environmentally unfriendly. Using microorganisms as microbial cell factories is an interesting alternative to produce prenylflavonoids in an efficient and cheaper way. In this work, we designed, constructed, and validated a biosynthetic pathway to produce prenylnaringenins in *Escherichia coli* for the first time. Firstly, tyrosine ammonia-lyase, 4-coumarate-CoA ligase, chalcone synthase and chalcone isomerase were expressed to produce the intermediary naringenin. An optimized *E. coli* strain was able to produce 689.5 mg/L of naringenin. Then, a prenyltransferase (PT) was expressed to produce prenylnaringenins. Four different PTs were first tested in vitro to evaluate their ability to convert naringenin into a prenylated compound. From the tested PTs, two were derived from plants (PT from *Humulus lupulus* (HlPT) and PT from *Sophora flavescens* (SfPT)). Since plant PTs are membrane-bound enzymes, two alternative soluble PTs from fungi (CdpC3PT and AnaPT from *Neosartorya fischeri*) were also tested. All the PTs showed in vitro ability to convert naringenin into a prenylated compound. Then, these PTs were expressed in the previously constructed and optimized *E. coli* strain. In vivo production experiments were carried out to produce prenylnaringenins using only glucose as substrate and it was demonstrated that prenylnaringenins were produced but only in residual amounts. To overcome this limitation, an optimized *E. coli* strain able to produce high amounts of the extender substrates of the pathway (malonyl-CoA and dimethylallyl pyrophosphate) will be constructed sorting to synthetic biology approaches. This work represents a step- forward to achieve for the first time de novo production of prenylflavonoids in *E. coli*.