Synthetic biology: Heterologous production of bioactive agents

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Synthetic biology provides powerful tools to design innovative products and technologies. In the medical field, examples include the elucidation of disease mechanisms, identification of potential targets, discovery of new chemotherapeutics or design of novel drugs [1]. Additionally, it enables the development of economically attractive microbial production processes for complex natural products.

Plants secondary metabolites are considered high-value chemicals exhibiting interesting biological activities. However, they are present in plants in low amounts and accumulate during long growth periods. Their extraction is often problematic since their purification is difficult originating low yields, which are also consequence of environmental and regional factors. In addition, chemical synthesis is complex and environmentally unfriendly. Therefore, the biosynthesis of these high-value chemicals in engineered organisms has emerged as a competitive alternative compared to chemistry-based methods.

Curcuminoids and coumarins are polyphenolic compounds produced in plants that exhibit very interesting pharmacological properties. Under this scope, we designed and constructed an artificial pathway using codon-optimized enzymes for the production of these compounds in *Escherichia coli* [2]. Both types of polyphenolic compounds can be produced from tyrosine or hydroxycinnamic acids as precursors. To produce curcumin, the most studied curcuminoid for therapeutic purposes, 4-coumaroyl-CoA ligase (4CL) from *Arabidopsis thaliana*, curcuminoid synthase from *Oryza sativa* or diketide-CoA synthase and curcumin synthase from *Curcuma longa* were used. Using this pathway 354 mg/L of curcumin was produced, which corresponds to the highest concentration obtained so far using a heterologous host. Curcumin was also produced for the first time using tyrosine as precursor and caffeic acid as an intermediate. Other curcuminoids, such as bisdemethoxycurcumin and demethoxycurcumin were also produced using as precursors tyrosine or hydroxycinnamic acids (p-coumaric acid or a mixture of p-coumaric and ferulic acids, respectively). Based in this pathway, a similar pathway was designed and constructed to produce coumarins. The enzymes 4CL and p-coumaroyl-CoA 2′-hydroxylase from *Ipomoea batatas* were used to produce the coumarins umbelliferone, scopoletin and esculetin from p-coumaric acid, ferulic acid and caffeic acid, respectively. Approximately 20-50 mg/L of each coumarin was produced. The optimization of coumarins production from tyrosine is being conducted.

Saccharomyces cerevisae, which is also an interesting host, has only been used to produce other polyketides (e.g. resveratrol) [2]. However, it presents some unique advantages over *E. coli* for the design and construction of biosynthetic pathways to produce curcuminoids or coumarins. Hence, we are also exploring *S. cerevisae* as a potential chassis for the production of these valuable chemicals.

References

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