Heterologous production of plant natural polyphenolic compounds in *Escherichia coli*

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Introduction

Plant natural polyphenolic compounds → High-value chemicals

Curcuminoids

- Present in the rhizome of turmeric (*Curcuma longa*)
- Used as food additives (especially as a curry spice) and in traditional medicine due to their several therapeutic properties, including anti-cancer activity [1]

Furanocoumarins

- Produced in different plants as a defense mechanism against various types of predators
- Potential therapeutics for the treatment of skin disorders (e.g. vitiligo, psoriasis)

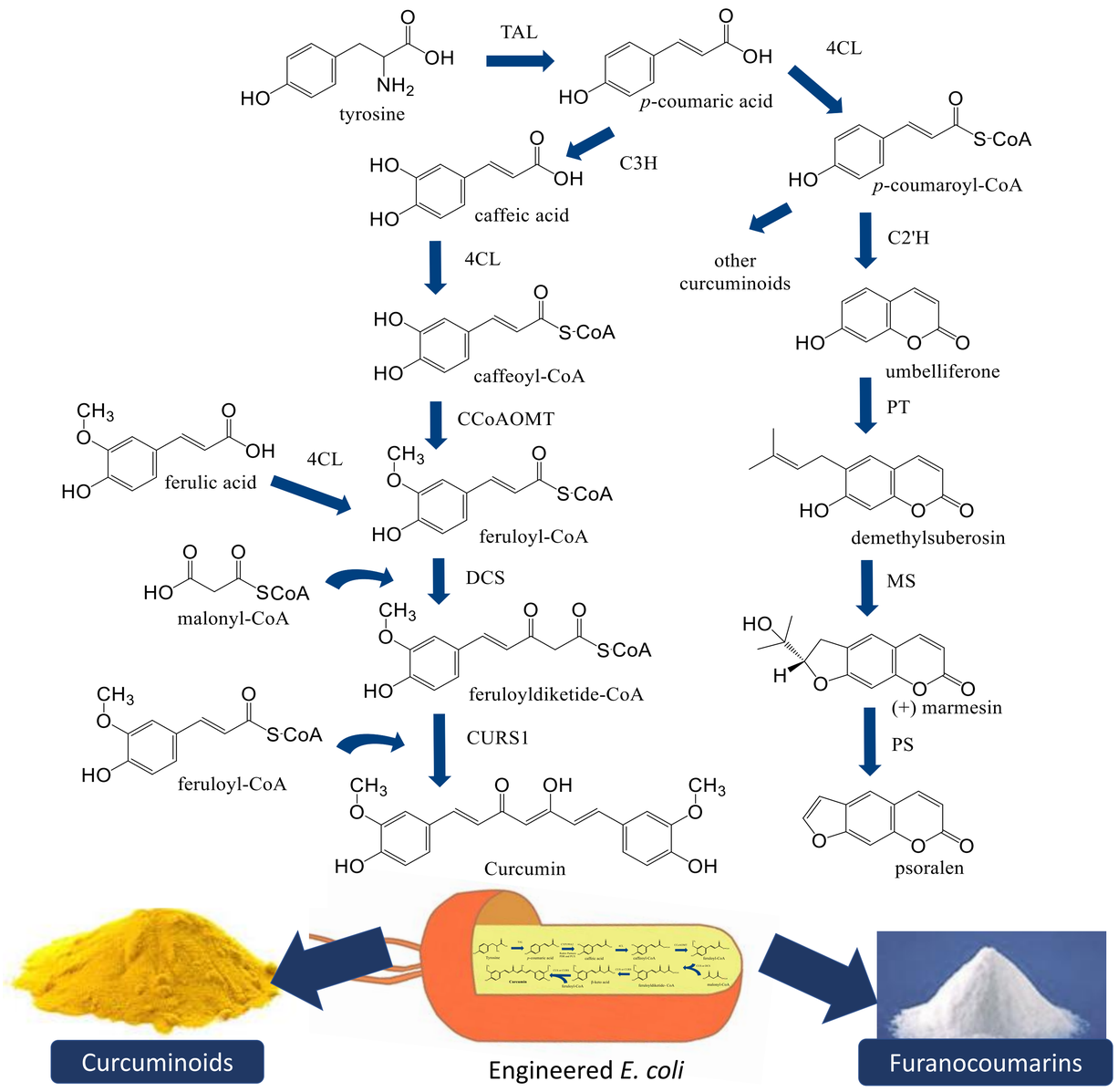
Why a synthetic biology approach?

Extraction from plants:
- Low purity
- Low yields
- Limited by seasonality
- Environmentally unfriendly
- Too complex
- Expensive

→ Chemical synthesis

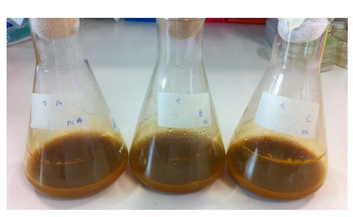
Objective

Engineer artificial biosynthetic pathways for curcuminoids and furanocoumarins production by *E. coli.*

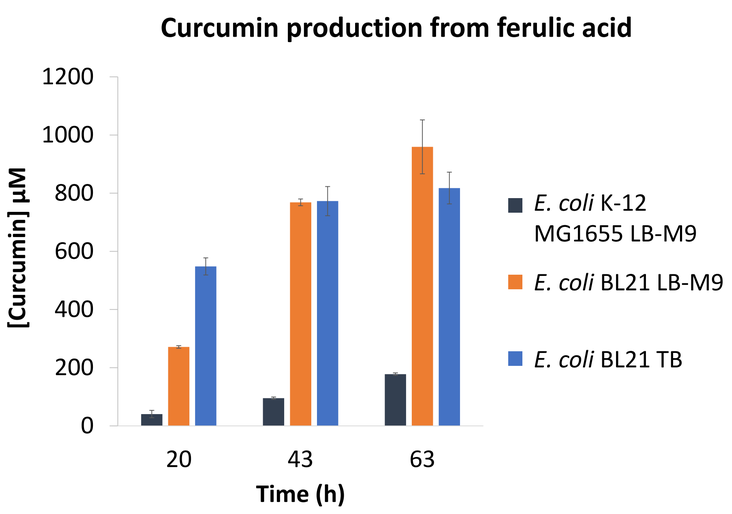


Results & Discussion

Coumaric and caffeic acid were efficiently produced using TAL and C3H [2,3]. 4CL1, DCS and CURS1 enzymes were used to produce curcumin (188 µM) from ferulic acid. Bisdemethoxycurcumin and demethoxycurcumin were also produced, but in lower concentrations (≤ 6 µM), by feeding coumaric acid or a mixture of coumaric and ferulic acids, respectively. Additionally, curcuminoids were produced from tyrosine through the caffeic acid pathway (≤ 1 µM). CCoAOMT was used to convert caffeoyl-CoA to feruloyl-CoA [4]. To increase curcuminoids production in *E. coli* different strains and operational conditions were tested. *E. coli* BL21 allowed to obtain the highest concentrations of curcumin reported so far in a heterologous organism (959 µM).

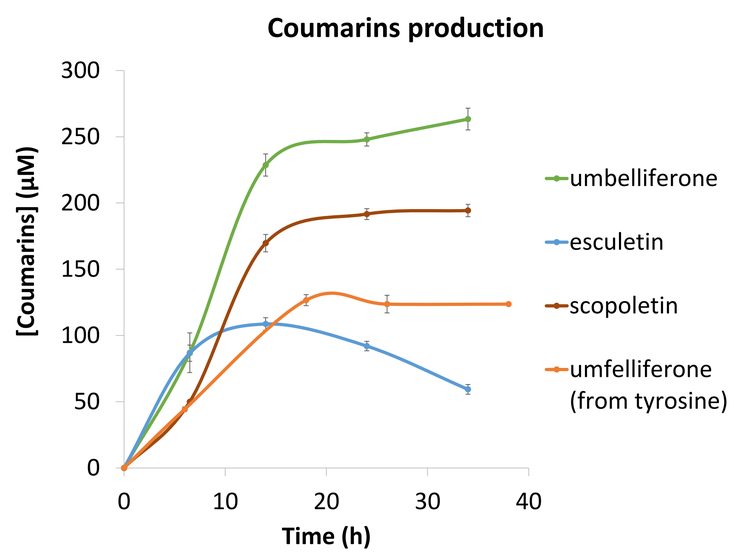


Curcumin production by *E. coli* K-12 MG1655 and by *E. coli* BL21



In addition, it was found that TB is a great medium to produce curcumin since it also allowed to produce high concentrations (818 µM). The use of this single fermentation medium represents an advantage at industrial scale and although the final production is lower than the one obtained with LB-M9 combination, it allows to obtain a significantly higher production in the first 24 h of fermentation.

For the furanocoumarins pathway, the enzyme C2'H was tested. It allowed to produce 263 µM of umbelliferone from coumaric acid and 126 µM from tyrosine. It was also verified that it was possible to produce esculetin and scopoletin by adding other substrates (caffeic and ferulic acids, respectively).



Conclusions & Future Perspectives

The curcumin concentrations achieved in this study from ferulic acid were the highest reported so far. However, additional research is still needed to increase the productions from tyrosine. Optimizations at genetic level include testing other potential curcuminoid synthases and alternative 4CLs. In addition, a different pathway that converts caffeic acid to ferulic acid could be tested. Regarding furanocoumarins production, the assembly of the first part of the pathway (until umbelliferone) was already achieved with success. In the future, it is important to study the other steps of the pathway and to optimize the operational conditions.

Nomenclature

4CL: 4-coumaroyl-CoA ligase from *Arabidopsis thaliana*
C2'H: coumaroyl-CoA 2' hydroxylase from *Ipomoea batatas*
C3H: 4-coumarate 3-hydroxylase from *Saccharothrix espanaensis*
CCoAOMT: caffeoyl-CoA O-methyltransferase from *Medicago sativa*
CURS1: curcumin synthase from *Curcuma longa*
DCS: diketide-CoA synthase from *C. longa*
MS: marmesin synthase
PS: psoralen synthase
PT: prenyltransferase
TAL: tyrosine ammonia lyase from *Rhodotorula glutinis*
TB: Terrific Broth

References

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[3] **J. L. Rodrigues**, R. G. Araújo, K. L. J. Prather, L. D. Kluskens, L. R. Rodrigues. Heterologous production of caffeic acid from tyrosine in *Escherichia coli*, Enzyme and Microbial Technology 71, 2015, 36-44.
[4] **J. L. Rodrigues**, R. G. Araújo, K. L. J. Prather, L. D. Kluskens, L. R. Rodrigues. Production of curcuminoids from tyrosine by a metabolically engineered *Escherichia coli* using caffeic acid as an intermediate, Biotechnology Journal 10, 2015, 599-609.

Acknowledgments

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