Bioprocess design to produce resveratrol

Part 2: Strain, Upstream and Reaction

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INTRODUCTION: Characteristics of resveratrol and its growing demand have been previously stated. In this poster, the selected recombinant strain (*Escherichia coli*) and its metabolism involved in resveratrol production are explained, as well as the molecular engineering techniques used. Due to the high cost of the main raw material (p-coumaric acid), alternative ways to produce it are analyzed. Furthermore, reactor operation with its different phases are described.

STRAIN

Organism selection

Organism	Productivity ($mg \cdot L^{-1} \cdot day^{-1}$)	Observations
Vitis vinifera	11.6	Difficulties in culturing Inducers are needed
Saccharomyces cerevisiae	1.2	Suitable for human consumption (GRAS)
Aspergillus niger & yeast	1390	Complicated manipulation (co-culture)
Escherichia coli [1]	**1640**	**Widely studied organism**

The selected *E. Coli* BW27784[1] recombinant strain converts 97% of p-coumaric acid (2.4 g/L) into resveratrol (2.3 g/L).

Cellular metabolism

Resveratrol is synthesized by the reaction of **p-coumaric acid** with **malonyl-CoA**.

- 4-coumaroyl-CoA ligase (4CL) activates p-coumaric acid, binding it to coenzyme A.
- Stilbene sinthase (STS) synthesizes resveratrol, condensing p-coumaric-CoA acid and malonyl Co-A18.

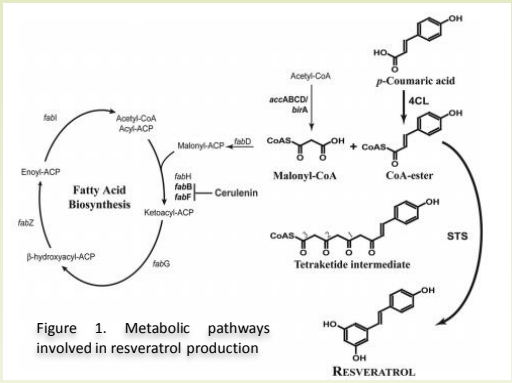


Figure 1. Metabolic pathways involved in resveratrol production

Molecular engineering

Genes *4CL* (from *Arabidopsis thaliana*) and *STS* (from *Vitis vinifera*) have been cloned into a pUC18 plasmid and expressed in a bicistronic transcript regulated by GAP constitutive promoter.

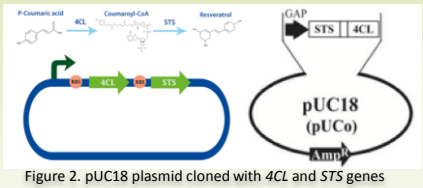


Figure 2. pUC18 plasmid cloned with *4CL* and *STS* genes

This strain includes genes *fabB* and *fabF* (involved in anabolic metabolism) from *E. coli* K12-DM86 (a temperature-conditional mutant for the expression of these genes). At higher temperatures than 40-42ºC, *fabB* and *fabF* show no expression.

UPSTREAM

Alternative ways to produce p-coumaric acid

Due to the high cost of this raw material, the viability of the process can be greatly altered with an increase of the market price (it represents 55% of the raw materials cost). Several ways for p-coumaric acid production have been studied. The design and calculations were performed using SuperPro Designer V8.5.

Extraction from lignocellulosic biomass

- P-coumaric acid is the main component in lignin.
- Using bibliographic resources [2], a design and an analysis of its extraction has been made.
- The extraction process consists in an alkaline hydrolysis.

Conclusions

- P-coumaric acid content in lignocellulosic biomass is too low (1.67%).
- This process requires large quantities of NaOH. Thus making the production cost too high.

Conversion from L-tyrosine using immobilized *E. coli*

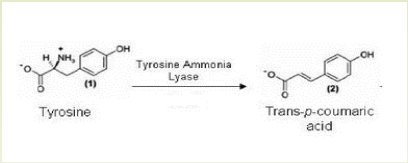


Figure 3. Tyrosine Ammonia Lyase converts tyrosine into p-coumaric acid

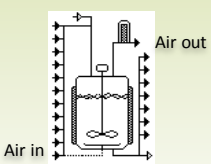
- *E. coli* cells that overexpress TAL (around 40% of total cell protein)
- Immobilization is achieved by entrapment to a calcium alginate matrix.
- For designing this alternative, sizing of the equipment has been calculated, and an economical analysis has been performed.
- With this alternative the p-coumaric acid cost would be 135 $/kg

Market price

Market price is around 80 $/kg. An analysis of the sensitivity of the process to p-coumaric acid price show that the economy of the process could resist increases up to 550 $/kg.

The results aforementioned have been extracted from the economical analysis in "Part 4: Sustainability analysis" of this same project.
In conclusion, it has been decided that the best option currently available is to buy p-coumaric acid directly in the market. If the price should rise and exceed 135 $/kg the alternative using L-tyrosine as source for p-coumaric acid production is to be considered.

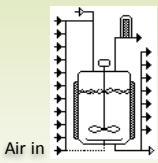
REACTION



Cellular growth phase: 15 hours

M9 medium (glycerol)
Aerobical conditions
Ends when DO is 0.8

Reactor volume: 30 m^3



Transformation phase: 20 hours

P-coumaric acid is charged (2.4 g/L)
Temperature is increased: 40-42ºC
2.3 g/L resveratrol is obtained

REFERENCES

1 - Lim, C. G., Fowler, Z. L., Hueller, T., Schaffer, S. & Koffas, M. A. G. High-yield resveratrol production in engineered Escherichia coli. *Appl. Environ. Microbiol.* **77,** 3451–60 (2011)
2 - Ou, S. Y., Teng, J. W., Zhao, Y. Y. & Zhao, J. p -coumaric Acid Production from Lignocelluloses. (2012)