Degree in Biochemistry

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Curcuma longa cell culture

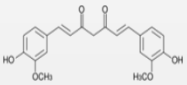
Introduction

In vitro production of plant secondary metabolites

Most known secondary metabolites come from plants, many of them useful for mankind. Since cultivable land is shrinking, in vitro production is increasingly seen as an alternative. In vitro production ensures a uniform yield, under perfectly controlled culture conditions.

Curcuma longa and curcumin

Turmeric (*Curcuma longa*) has been used for centuries as a spice, a dye and in traditional medicine. The bioactive compounds in *Curcuma* are polyphenols called curcuminoids. The main curcuminoid, curcumin, has been Studied recently for its medicinal properties.



Objectives

- Establishment of a stable in vitro culture of *C. longa* at an experimental level
- Maximisation of curcumin yield
- In the long term: Scale-up to an industrial level

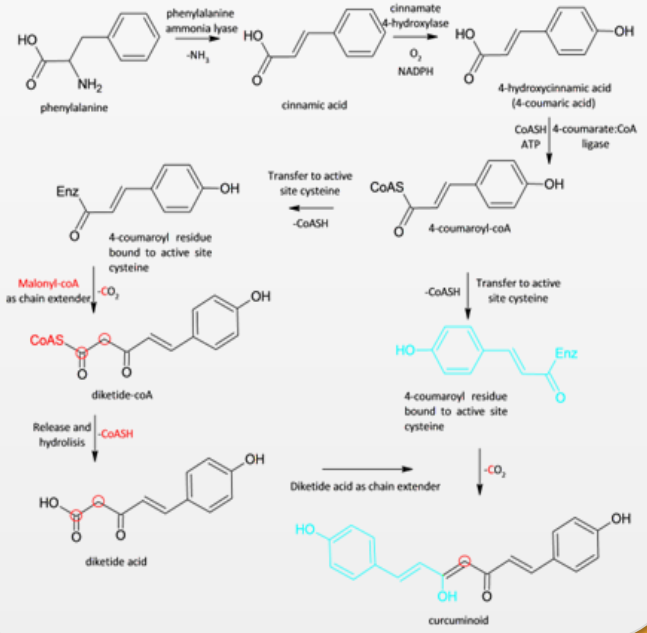
Establishment of the culture

Sterilization of shoots → Incubation for 30 days, No growth regulators → **CALLUS INDUCTION**: Incubation in the dark, 2.0 µM BA, 1.2 µM 2,4-D, 2.5 µM TDZ → Calli are subcultured at 30 day intervals → **ESTABLISHMENT OF A SUSPENSION CELL CULTURE**: 0.3 mg/L 2,4-D, 0.1 mg/L NAA (enhance cell proliferation), Ascorbic acid and citric acid (antioxidant agents), Glutamine (nitrogen source), Biotin (cofactor to many enzymes) → **CULTURE CONDITIONS**: The cells are incubated on a shaker at 25ºC, Indirect light, 18 h photoperiod, The cells are subcultured every 10 days

Precursor feeding

In order to enhance curcumin production, two of its precursors are added to the culture.

- **Phenylalanine**, the substrate of phenylalanine ammonia lyase (PAL). PAL is a key regulating enzyme in phenylpropanoid metabolism, and the first step in the synthesis of polyphenols, one of the main groups of secondary metabolites. None of the cinnamic acids are incorporated into curcumin as well as phenylalanine.
- **Malonate** provides the central carbon atom that joins the cinnamyl units of curcumin.



Elicitation

Elicitors are biotic or abiotic factors which are perceived as stressful by plants. In order to ensure survival, plants respond with a series of changes, one of which is the **activation of secondary metabolism pathways**. Some common elicitors are fungal carbohydrates, yeast extract, methyl jasmonate, and chitosan. Elicitors must be added to the culture during the exponential phase of growth.

Conclusions

- As there is little research about this subject done on *C. longa*, most of this project is adapted from articles about other related plants.
- If it is put to practice, all of the steps and conditions must be tested and optimised to suit this plant.
- It is essential to experiment with various elicitors and precursors to find the most efficient ones, as their effects vary greatly among species.

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