OBTAINING POTENT ANTIOXIDANTS FROM MULBERRY CELL CULTURES

PATENTED TECHNOLOGY

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ABSTRACT

The *Plant Proteomics and Functional Genomics group* of the University of Alicante, in collaboration with the *Institute of Integrative Systems Biology* of the University of Valencia, has developed the technology of plant cell cultures of the genus *Morus* to obtain stilbenes. The innovation lies in the simultaneous use of two elicitor compounds to promote their production. With this technology, trans-resveratrol and trans-oxyresveratrol are obtained in high quantities, with the possibility of using them in the pharmaceutical, cosmetic and food industries thanks to their powerful antioxidant character. Companies interested in acquiring this technology for its commercial exploitation through patent licensing agreements are sought.

INTRODUCTION

Stilbenes are biologically active phenolic compounds with a broad spectrum of antibiotic and pharmacological activity. However, the production of this group of compounds in nature is restricted to a small number of plant species such as grapevine (*Vitis* sp.) mulberry (*Morus* sp.) or peanut (*Arachis hypogaea*), as an adaptive mechanism in response to stress (UV irradiation, microbial infection, exposure to heavy metals or ozone treatment). Within this group of compounds (stilbenes), trans-resveratrol (hereinafter t-resveratrol), piceatanol and trans-oxyresveratrol (hereinafter t-oxyresveratrol) are of particular note.

t-Resveratrol and piceatanol are found in grapevine and peanut, while t-resveratrol and t-oxyresveratrol are found in mulberry, indicating that stilbene synthesis is species-dependent.

Epidemiological and laboratory studies have shown that stilbenes in general, and t-resveratrol and t-oxyresveratrol in particular, have favourable health effects, making their inclusion in the human and animal diet desirable.

t-Resveratrol is very effective in the prevention and therapy of atherosclerosis, as an anti-inflammatory agent and as an anti-hyperoxidative agent, while t-oxyresveratrol is notable for its pharmacological properties as an anticancer, anti-inflammatory, antioxidant, neuroprotective, antibiotic and antiviral agent, as well as exhibiting potent tyrosinase inhibitor activity, which gives it interesting cosmetic properties.

Given the beneficial role of t-oxyresveratrol on human and animal health, it is important to have a suitable biological source to obtain it.

The stimulation of stilbene synthesis has been explored with a variety of "elicitor" type compounds, such as pieces of fungal cell walls, cyclodextrins, methyl jasmonate, etc.

The combined treatment of these elicitors produces a synergistic effect in grapevine cell culture. However, there is very little knowledge on how to produce t-oxyresveratrol.

With current knowledge, the production of t-oxyresveratrol by biotechnological means requires the implementation of two processes:

a) On the one hand, the production of mulberroside A (double glycosylated form of t-oxyresveratrol) using mulberry (*Morus alba*) cell cultures elicited with salicylic acid, followed by extraction and purification.

b) On the other hand, the bioconversion of mulberroside A into t-oxyresveratrol by enzymatic treatment.

TECHNICAL DESCRIPTION

In order to solve the biotechnological problems described above, it is necessary to have a **more efficient method for obtaining t-oxyresveratrol in a single process**.

In this sense, a **method has been developed to obtain stilbenes** by the **combined addition of cyclodextrins and methyl-jasmonate** to a culture medium of **plant cells of the genus *Morus***. Specifically, large extracellular quantities of t-reveratrol and, above all, t-oxyresveratrol are obtained (*see Figure 1*):

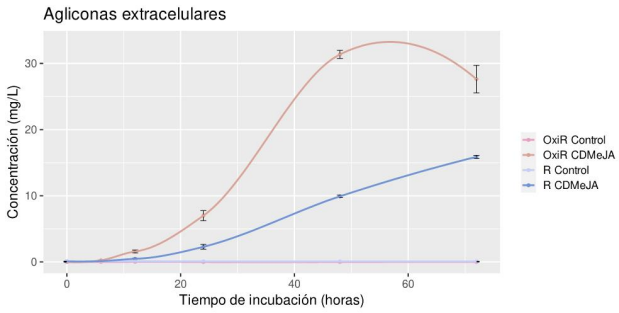

Figure 1: Kinetics of extracellular accumulation of the aglycone (non-glycosylated) forms of t-resveratrol and t-oxyresveratrol in milligrams per litre of extracellular medium, from a cell culture of Morus alba red clone elicited with the combination of cyclodextrins and methyl jasmonate, versus untreated control.

Any cell line of a plant of the genus *Morus* is capable of producing these compounds, either naturally or after genetic modification.

The method of production comprises the following **steps**:

1) Addition of cyclodextrins and methyl-jasmonate to a culture medium of plant cells of the genus *Morus*.

2) Incubation of the cell culture medium obtained in the previous step.

3) Separation of t-resveratrol and t-oxyresveratrol (obtained in step 2) from the culture medium.

4) Purification of the t-resveratrol and t-oxyresveratrol separated in the previous step.

Some images of the blackberry plant cells obtained can be seen below:

Picture 1: Development of the growth of mulberry plant cells ("white" and "red" clones) from the solid state to the state in liquid suspension. The name "red" clone is due to the color of the fruits of the specimen from which it originates (the plant cells of both clones, "white" and "red", have the same color).

TECHNOLOGY ADVANTAGES AND INNOVATIVE ASPECTS

ADVANTAGES OF THE TECHNOLOGY

The developed method has the following **advantages**:

1) It is **more efficient** than current biotechnological methods.

2) It is carried out **in a single process**, which simplifies the production process.

3) The **accumulation** of t-resveratrol and t-oxyresveratrol is **mostly extracellular**.

4) The **extraction and purification process is simplified** (no need to break the plant cells and then remove the cellular remains).

5) It is possible to use the plant cells in suspension for **new t-resveratrol and t-oxysveratrol synthesis cycles**.

6) The **trans- forms** of both compounds (which are the biologically active forms, as opposed to the cis- isomers) are mainly generated.

7) **High amounts** of stilbenes are obtained:

- In the **red clone**:
  - **124 mg/L t-oxyresveratrol**.
  - 24 mg/L t-resveratrol.
- In the **white clone**:
  - **114 mg/L t-resveratrol**.
  - 81 mg/L de t-oxyresveratrol.

8) t-Oxyresveratrol and t-resveratrol **production is stable** and **independent of environmental and socio-economic factors**.

9) The **quality** of the final product is **improved**.

10) The process is **sustainable and environmentally friendly**.

11) The technology allows large quantities of stilbenes to be obtained at a **lower cost** than other similar techniques currently available on the market, which increases its **accessibility for different industrial applications**.

INNOVATIVE ASPECTS OF THE TECHNOLOGY

Surprisingly and unexpectedly, the **combined use of cyclodextrins and methyl jasmonate** in plant cell cultures of the genus *Morus* results in the production of two stilbenes not anticipated in the state of the art, namely t-resveratrol and, especially, t-oxyresveratrol.

The cumulative concentrations of both stilbenes are higher in the combined treatment than the sum of the individual treatments, hence the **synergistic effect** of both elicitors.

The **accumulation** of both stilbenes occurs mostly in the **extracellular** medium, which simplifies the extraction and purification process and reduces production costs.

The technology makes it possible to obtain **large quantities of t-resveratrol** and, above all, **t-oxyresveratrol**.

CURRENT STATE OF DEVELOPMENT

The described technology has been developed at **laboratory scale** (Technology Readiness Levels: **TRL = 4**).

Laboratory tests have shown the synergistic effect of the use of methyl jasmonate and different types of cyclodextrins on the production of t-resveratrol and t-oxyresveratrol in mulberry cell cultures.

When *Morus Alba* cell culture is elicited with methylated (or randomly hydroxypropylated) cyclodextrins, an extracellular accumulation of:

t-Resveratrol:

- **60-140 times higher** (in the white clone) compared to the control.
- 30-60 times higher (in the red clone) compared to the control.

t-Oxyresveratrol:

- **600-800 times higher** (in the red clone) than the control.
- 30-60 times higher (in the white clone) than the control.

And when the cell culture is elicited with the combination of cyclodextrins and methyl jasmonate, an additional extracellular accumulation of t-resveratrol and t-oxyresveratrol is **3 times higher**, compared to the sum of the levels accumulated in the single treatments, hence the **synergistic effect of the joint elicitation** (both in the white and red clones).

The glycosylated forms inside the cells do not change significantly over time due to elicitation, whereas the aglycones (non-glycosylated forms), both intracellular and extracellular, exhibit a cumulative pattern over time. This change is more intense for t-oxyresveratrol than for t-resveratrol in the case of the red clone cell line.

MARKET APPLICATIONS

This novel technology belongs to the field of **plant biotechnology**.

The main sectors of application are:

- Pharmaceutical.
- Cosmetics.
- Food.
- Nutraceutical.

COLLABORATION SOUGHT

Companies interested in acquiring this technology for **commercial exploitation** through **patent licensing** agreements are sought.

INTELLECTUAL PROPERTY RIGHTS

The present invention is protected through **patent application**:

- *Patent title: "Método de obtención de estilbenos por cultivos celulares de una planta del género Morus".*
- *Application number: P202231084.*
- *Application date: 20th December 2022.*

MARKET APPLICATION (3)

Molecular Biology and Biotechnology
Pharmacology, Cosmetics and Ophthalmology
Medicine and Health

TECHNICAL IMAGES (1)

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