Diatom-based genetic engineering system methodology for the eco-sustainable production of ovothiols

Abstract

The invention relates to the field of biotechnology with the development of an eco-sustainable protocol that, through a genetic engineering system of of the diatom *Phaeodactylum tricornutum,* enables the stimulation of the production of methylated through a genetic engineering system 5-thiohistidines - and in particular ovothiol B, one of the most powerful natural antioxidants - that are receiving increasing attention for their chemical, biological and pharmacological properties. The protocol is eco-sustainable because it uses cells and nutrients for biosynthesis and does not produce toxic side compounds. The use of *P. tricornutum* cultures accomplishes high productivity and allows easy scale-up of upstream and downstream processes.

State of the art

The protocols of ovothiols' chemical synthesis described so far are complex, involving many steps, and too expensive for a convenient commercial production of these molecules, while the precursor of ovothiols, desmethylated 5-thiohistidine, can be prepared by a more feasible chemical synthesis. On the other side, ovothiol A, 5(Nπ)-methyl thiohistidine, can be purified by sea urchin eggs, which represent one of the richest source of these metabolites. However, sea urchins are not an eco-sustainable source for these compounds and cannot provide sufficient amounts of ovothiols for extensive in vitro and in vivo studies, also considering the necessity to preserve marine resources. Among microalgae, diatoms can be an alternative natural source for ovothiol biosynthesis. However, up to date, no protocols have been described for the sustainable production of these compounds in these organisms.

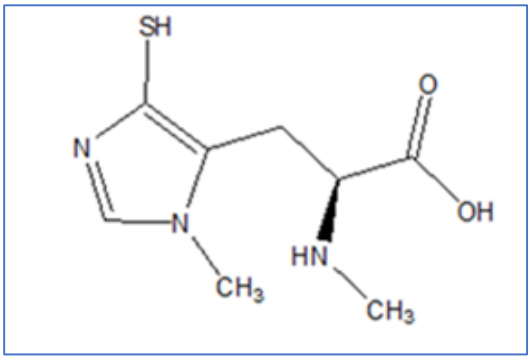

Figure no. 1 – Ovothiol B

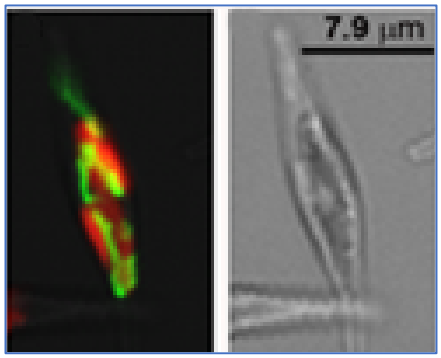

Figure no. 2 – Fluorescent signal in the mytochondrium - the green signal indicates the PtOvoA-YFP, the red signal indicates the autofluorescence chlorophyll

Invention description

The present invention concerns the production of an engineered diatom capable to biosynthesize increased amount of ovothiol B (Fig. 1), which compared to ovothiol A displays an additional methyl group on the lateral chain of 5-thiohistidine.

P. tricornutum has been transformed through two alternative methods: the traditional biolistic approach and the recently developed bacterial conjugation, both allowing the introduction in the cell of a construct containing an overexpression cassette driving the expression of the of the ovothiol-biosynthetic enzyme, sulfoxide synthase OvoA fused to the Yellow Fluorescent Protein under the guidance of an endogenous strong promoter. Both procedures have been successful and produced cells with a strong fluorescent signal in the mytochondrium (Fig. 2). The metabolite was extracted from the modified strains and identified as Ovothiol B with a yield increase ranging from 2 to 4.5 folds compared to the wild type strain.

The figure no. 3 shows the pipeline of the procedure starting from the creation of the overexpressing clone to the production of ovothiol B.

Industrial Property

Italian Patent Application no. IT 102021000026639 filed on 18/10/2021

Applicant

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Advantages

The invention has the following advantages:

- *P. tricornutum*, with a fully sequenced genome and a number of tools for genetic engineering, is easily cultivated and allows high biomass yields.
- The developed protocol is eco-sustainable because of the use of photosynthetic cells for ovothiol biosynthesis and does not produce toxic side-compounds.
- Microalgal biomass moreover can be exploited for additional uses after ovothiol extraction, making the production process more cost-effective.

Applications

This invention, allowing the ecosustainable production of ovothiol molecules, finds application in the following areas:

- Pharmaceutical field, because the ovothiol can be used to prepare pharmaceutical formulations aimed to prevent and ameliorate inflammation conditions associated with pathologies like diabetes, cardiovascular diseases and liver dysfunction.
- Nutraceutical and Cosmeceutical fields in relation to the production of nutrition and cosmetics formulations

Development stage

Current TRL: 4

Development of a protocol for ovothiol B purification for testing biological activities and improvement of the genetic engineered system to produce other ovothiol derivatives by changing substrate availability and growth conditions.

Perspective TRL: 5

The technology will be convalidated in industry by the use of bioreactors to obtain high diatoms mass and final metabolite.

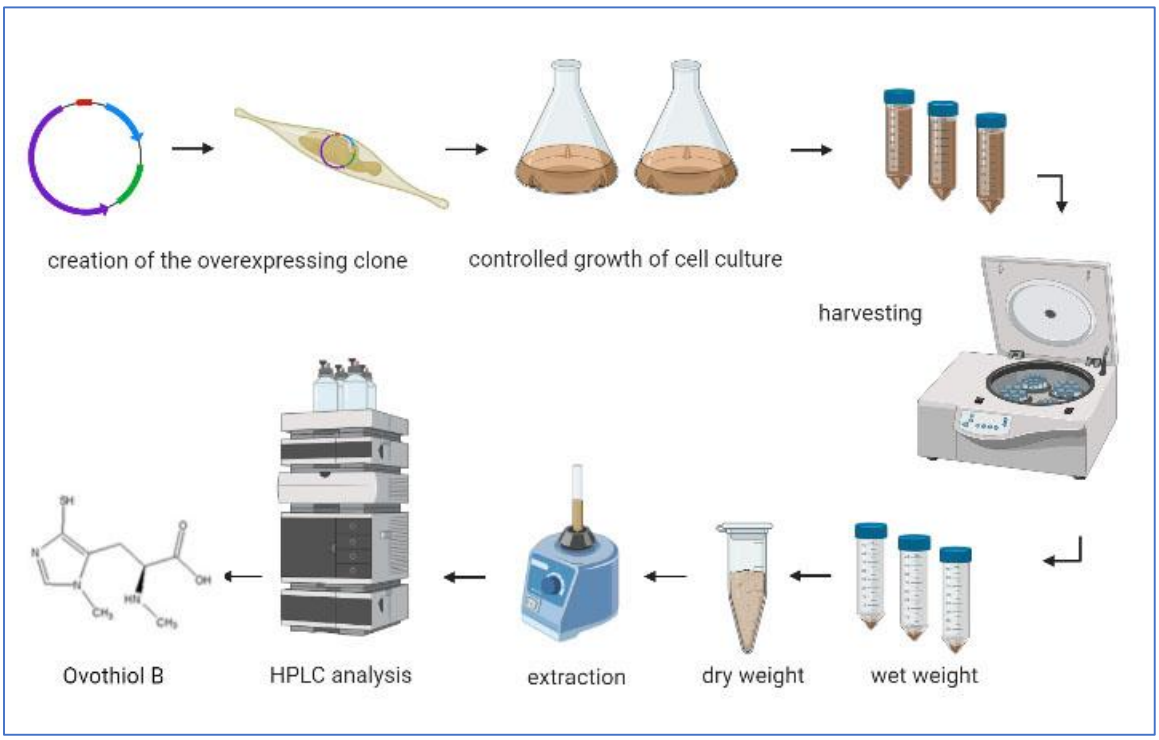

Figure no. 3 – Pipeline of the procedure starting from the creation of the overexpressing clone to the production of ovothiol B

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