Poster

Improving carotenoids biosynthesis pathway in the unicelullar microalgae *Chlamydomonas reinhardtii*

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ABSTRACT

Introduction: Carotenoids are a wide group of isoprenoids synthesized by photosynthetic organisms and some non-photosynthetic yeast and bacteria (1).They are indispensable in light harvesting and energy transference during photosynthesis and in the protection of the photosynthetic apparatus against the photooxidative damage. Mammals cannot synthesize them and must include them in their diet as precursors for essential compounds. The important colorant, antioxidant and provitamin properties of carotenoids, have made of them an important group of high-added value compounds, massively commercialized (1,2).There is an increasing demand of natural carotenoids and microalgae can be an excellent natural source of carotenoids.

Results and conclusions: In this work, we describe the subcloning of two genes from the carotenoid biosynthesis pathway in a microalgal expression vector: The *PSY* gene from *Dunaliella salina*, encoding phytoene synthase, a key enzyme in the pathway, which catalyzes the formation of phytoene, and *CRTI* gene encoding for fitoene desaturase from *Erwinia Uredowa*. The bacterial *CRTI* gene catalyzes the conversion of phytoene to lycopene, replacing the function of two microalgal enzymes phytoene desaturase (PDS) and chis-carotene desaturase (ZDS) (1,3). *PSY* and *CRTI* genes were fused by a short DNA fragment which encodes a self-cleaving peptide and fused to the selective marker gene *APHVIII* from *Streptomyces rimosus*, encoding for an aminoglycoside 3'phosphotransferase that confers resistance to the antibiotic paromomycin. All genes were placed under the control of the strong constitutive promoters RBCS2 and HSP70A and terminated by the 3'untranslated region of RBCS2 (plasmid 4-75).

C. reinhardtii was nuclear transformated with plasmid 4-75. Obtained transformants were analysed by PCR to check the insertion of the *PSY*, *CRTI* and *APHVIII* genes into the genome of *C. reinhardtii* and tested for expression at mRNA level. The phenotype of some of the transformants was also analyzed studying their carotenoid composition.

The obtained data show that the designed vector allows the insertion of all genes into the genome, although we have detected unexpected DNA cleavage and rearrangements during the integration process, which leads to discontinuous insertion of the genes, as they are placed in the 4-75 plasmid. This has made impossible to isolate any transformant with significant phenotypical changes so far and it is subject of current investigation.

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