involved in the biosynthesis thereof in microbial systems or in plants with high productivity. The biosynthesis of such compounds starts with the symmetrical cleaving, in positions 7,8 and 7'8', of zeaxanthin by means of a dioxygenase (CCD, Carotenoid Cleavage Dioxygenase) (Fig. 1). The two products of the cleavage of zeaxanthin, i.e. 3-OH-β-cyclocitral and crocetin dialdehyde, are dehydrogenated and glycosylated in order to produce, respectively, picrocrocin and crocins. The glucosyltransferases responsible for the glycosylation reaction have been described in saffron and *Gardenia* (5) (6). With regard to the first biosynthetic step, some authors have reported that an enzyme called ZCD cleaves zeaxanthin symmetrically at positions 7,8/7',8', producing crocetin dialdehyde (7), which is an observation contradicted by subsequent works (8). The modelling of the three-dimensional structure of ZCD has shown that ZCD is an incomplete enzyme, devoid of blade VII of the conserved *β-propeller* structure (9), consistent with the absence of enzymatic activity of ZCD (8). Consequently, it is not currently possible by biotechnological methods to produce the apocarotenoid compounds of saffron, and in particular crocetin and crocins, since the identification of the first enzyme of the biosynthetic pathway, i.e. CCD, which is responsible for the symmetrical cleaving in positions 7,8 and 7',8' of zeaxanthin, is still uncertain.

The object of the present invention is to provide a new carotenoid dioxygenase and methods for the biological production in microorganisms and plants of compounds of high added value derived from saffron.

Summary

Also disclosed is a nucleotide sequence of SEQ ID No. 1 coding for a carotenoid dioxygenase enzyme able to cleave carotenoids symmetrically in positions 7,8 and 7,'8' with the production of crocetin dialdehyde.

Also disclosed is an enzyme coded from the nucleotide sequence as described herein, having the amino acid sequence of SEQ ID No. 2.

In one aspect, there is provided a method for the production of crocetin dialdehyde and/or crocetin in microorganisms that accumulate zeaxanthin, comprising a step of expression in said microorganisms of the enzyme coded by the nucleotide sequence of SEQ ID No. 1.

In another aspect, there is provided a method for the production of crocetin in vegetable cells that express zeaxanthin, comprising a step of expression in said cells of the enzyme coded by the nucleotide sequence of SEQ ID No. 1.

Also disclosed is use of an enzyme coded by the nucleotide sequence of SEQ ID No. 1 or of an enzyme having SEQ ID No. 2 to cleave symmetrically the

CLAIMS

1. A method for the production of crocetin dialdehyde and/or crocetin in microorganisms that accumulate zeaxanthin, comprising a step of expression in said microorganisms of the enzyme coded by the nucleotide sequence of SEQ ID No. 1.

2. The method according to Claim 1, wherein said microorganisms are bacterial cells of E.coli.

3. A method for the production of crocetin in vegetable cells that express zeaxanthin, comprising a step of expression in said cells of the enzyme coded by the nucleotide sequence of SEQ ID No. 1.

4. The method according to Claim 3, in which said step of expression is carried out by transforming said cells with the binary vector pBI121, said vector comprising said nucleotide sequence.