Biol Res 41: 93-108, 2008

Genomic organization of the structural genes controlling the astaxanthin biosynthesis pathway of *Xanthophyllomyces dendrorhous*

MAURICIO NIKLITSCHEK[1], JENNIFER ALCAÍNO[1], SALVADOR BARAHONA[1], DIONISIA SEPÚLVEDA[1], CARLA LOZANO[1], MARISELA CARMONA[1], ANDRÉS MARCOLETA[1], CLAUDIO MARTÍNEZ[2], PATRICIA LODATO[1], MARCELO BAEZA[1] and VÍCTOR CIFUENTES[1*]

[1] Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.
[2] Departamento de Ciencia y Tecnología de Alimentos, Universidad de Santiago de Chile.

ABSTRACT

The cloning and nucleotide sequence of the genes (*idi*, *crtE*, *crtYB*, *crtI* and *crtS*) controlling the astaxanthin biosynthesis pathway of the wild-type ATCC 24230 strain of *Xanthophyllomyces dendrorhous* in their genomic and cDNA version were obtained. The *idi*, *crtE*, *crtYB*, *crtI* and *crtS* genes were cloned, as fragments of 10.9, 11.5, 15.8, 5.9 and 4 kb respectively. The nucleotide sequence data analysis indicates that the *idi*, *crtE*, *crtYB*, *crtI* and *crtS* genes have 4, 8, 4, 11, and 17 introns and 5, 9, 5, 12 and 18 exons respectively. In addition, a highly efficient site-directed mutagenesis system was developed by transformation by integration, followed by mitotic recombination (the double recombinant method). Heterozygote *idi* (*idi*$^{+}$/*idi*$^{-}$::*hph*), *crtE* (*crtE*$^{+}$/*crtE*$^{-}$::*hph*), *crtYB* (*crtYB*$^{+}$/*crtYB*$^{-}$::*hph*), *crtI* (*crtI*$^{+}$/*crtI*$^{-}$::*hph*) and *crtS* (*crtS*$^{+}$/*crtS*$^{-}$::*hph*) and homozygote mutants *crtYB* (*crtYB*$^{-}$::*hph*/*crtYB*$^{-}$::*hph*), *crtI* (*crtI*$^{-}$::*hph*/*crtI*$^{-}$::*hph*) and *crtS* (*crtS*$^{-}$::*hph*/*crtS*$^{-}$::*hph*) were constructed. All the heterozygote mutants have a pale phenotype and produce less carotenoids than the wild-type strain. The genetic analysis of the *crtYB*, *crtI* and *crtS* loci in the wild-type, heterozygote, and homozygote give evidence of the diploid constitution of ATCC 24230 strains. In addition, the cloning of a truncated form of the *crtYB* that lacks 153 amino acids of the N-terminal region derived from alternatively spliced mRNA was obtained. Their heterologous expression in *Escherichia coli* carrying the carotenogenic cluster of *Erwinia uredovora* result in trans-complementation and give evidence of its functionality in this bacterium, maintaining its phytoene synthase activity but not the lycopene cyclase activity.

Key terms: astaxanthin biosynthesis, *Xanthophyllomyces dendrorhous*, *Phaffia rhodozyma*.

INTRODUCTION

In nature, carotenoids are produced on a large scale: over 100 million tons per year. In addition, these pigments are synthesized in a wide variety of structures by plants, algae, bacteria and fungi. One of them, astaxanthin, is the principal carotenoid responsible for the orange-red color of marine invertebrates, fish, and birds and is primarily produced by phytoplankton and the red basidiomycetous yeast *Xanthophyllomyces dendrorhous* (Andrewes et al., 1976; Johnson and Lewis, 1979; Miller et al., 1976; Johnson, 2003).

In this yeast, astaxanthin, like other carotenoids, is a terpenoid pigment that is produced by the terpenoid biosynthetic pathway from the basic C5-isoterpene unit isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP), produced by the isopentenyl pyrophosphate isomerase enzyme encoded by the *idi* gene. In a following step, DMAPP and IPP are condensed to geranyl pyrophosphate, farnesyl, and geranylgeranyl pyrophosphate (GGPP), possibly through the action of farnesyl pyrophosphate synthase and geranylgeranyl pyrophosphate synthase encoded by the *fps*

* Corresponding Author: Víctor Cifuentes, Casilla 653, Santiago, Chile; Tel: 56-2-9787346; Fax: 56-2-2727363 ; E-mail: vcifuentes@uchile.cl

Received: June 26, 2007. In Revised form: April 28, 2008. Accepted: May 5, 2008