SUSTAINABLE PRODUCTION OF β-XANTHOPHYLLS IN *SACCHAROMYCES CEREVISIAE*

Vicente F. Cataldo, Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.
vfcataldo@puc.cl
Natalia Arenas, Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.
Javiera López, Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.
Conrado Camilo, Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.
Eduardo Agosin, Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.

Key Words: Metabolic Engineering, Carotenoids, Xanthophylls, Zeaxanthin, Saccharomyces cerevisiae.

Xanthophylls are a group of C_{40} pigments that belong to the carotenoids family. β-Xanthophylls, such as zeaxanthin, violaxanthin and neoxanthin are derived from β-carotene metabolism, and play a central role in the protection of photo-oxidative damage in plants and algae. These molecules have interesting applications as precursors of commercially relevant natural aromas, like safranal and damascenone. Furthermore, zeaxanthin is also widely used as a nutraceutical to improve ocular health. In this study, we engineered the yeast *Saccharomyces cerevisiae* to biosynthesize zeaxanthin and violaxanthin from glucose. We used integrative vectors to construct a genetic stable β-xanthophylls pathway in a β-carotenogenic yeast strain. To find an effective zeaxanthin biosynthetic enzyme, we compared the titers achieved by bacterial, plant and algal β-carotene hydroxylases. Additionally, we evaluated the effect of the chloroplast transit peptide of plant and algal enzymes on zeaxanthin biosynthesis. The strain that expressed truncated version of *Solanum lycopersicum* β-carotene hydroxylase showed the best performance, reaching up to 4.7 mg/g DCW of zeaxanthin after 72 h cultivation in shake-flasks. Zeaxanthin producing strains were transformed with zeaxanthin epoxidase genes to further extend the pathway to violaxanthin, which was measured by UPLC-MS. To the best of our knowledge, this work presents the highest titer of zeaxanthin in *S. cerevisiae* reported to date, the first zeaxanthin cell factory using β-carotene hydroxylase from plants, and the first heterologous biosynthesis of violaxanthin.

Financial support of FONDECYT grant No.1170745 is greatly acknowledged. Vicente F. Cataldo acknowledges CONICYT for receiving graduate scholarship.