# Metabolic engineering of *Synechocystis* sp. PCC 6803 for astaxanthin production

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**BACKGROUND:** Astaxanthin (Asx) is the highest value carotenoid produced by microalgae, with a global annual market estimated to exceed $ 1.5 billion for 2020 [1]. It possesses a wide range of applications in the nutraceutical, pharmaceutical and cosmetic sectors, due to its well-known antioxidant activity [2]. Moreover, Asx is considered as an essential feed additive in aquaculture for imparting the pinkish-red coloration to the flesh of salmons, shrimp, trouts and ornamental fish. Thus, the demand for this pigment is constantly increasing as a consequence of the expansion of aquaculture industry [3]. The richest natural source of Asx is the green alga *Haematococcus pluvialis*, however the biological process for Asx production from *H. pluv*ialis is limited by slow growth times and low cell density, while the chemical synthesis process is expensive and the synthetic Asx is not approved for human consumption [1]. Several attempts have been made in order to produce Asx from other biological sources [4], but the commercial applications are still poorly developed.

## AIM and EXPERIMENTAL STRATEGY:

**Here we present an alternative strategy based on the metabolic engineering of the cyanobacterium *Synechocystis sp*. PCC6803, a model species commonly employed for biotechnological applications, which is naturally able to accumulate zeaxanthin (Zea), the metabolic precursor of Asx, and is easy to be cultured and harvested.**

The genes encoding for two enzymes (a β-carotene ketolase, CrtW, and an hydroxylase, CrtZ) that lead to the biosynthesis of Axt from β-carotene (**Fig. 1**), isolated from a naturally Asx accumulating proteobacterium, have been cloned under the control of an inducible promoter [5].

Firstly, the two genes were transformed individually, obtaining the Syn CrtW and Syn CrtZ mutant strains, and subsequently, in a double construct, obtaining the Syn CrtW+ CrtZ mutant. The three engineered strains were assessed for their ability to produce and accumulate Asx after the induction of transgene expression.

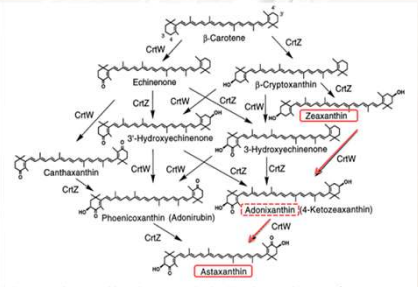


**Figure 1**: Astaxanthin biosynthetic pathway from β-carotene and the catalytic functions of CrtW and CrtZ (modified from [6]).

## RESULTS and PERSPECTIVES:

Upon induction, total carotenoids and chlorophyll have been extracted and characterized by means of absorption spectroscopy, HPLC (**Fig. 3 A-B-C** ) and TLC analysis (**Fig.4**). The mutant strain carrying only CrtW has been shown to produce a small amount of Asx and to accumulate the precursor canthaxanthin (Canta) at a very high level, while -as expected- the Syn CrtZ strain accumulated mainly Zea. On the other side, the engineered strain Syn CrtW+CrtZ, carrying both genes, have been proved to produce significant amounts of Asx, at a level comparable to the β-carotene content in Syn WT (**Fig. 3B-C**). This strain has proved to be a good Axs producer, being able to accumulate Asx up to 30% of total carotenoids (**Fig. 3C**). Moreover the growth of the mutant strain seems not to be negatively affected by the production of the exogenous carotenoid Asx.

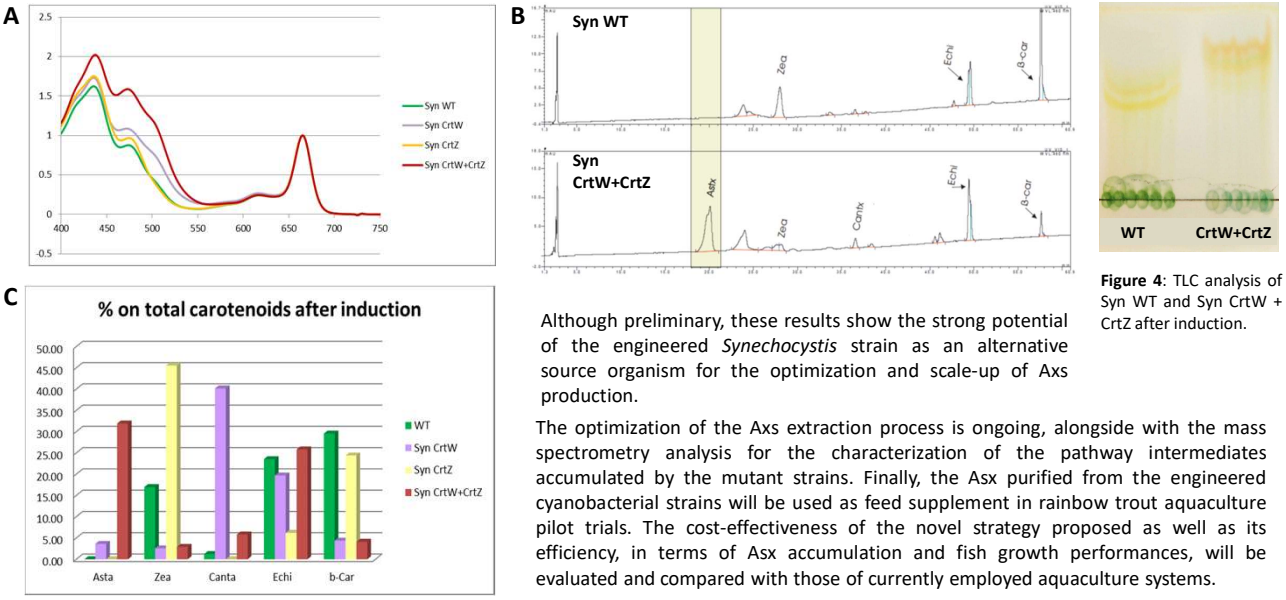





**Figure 4**: TLC analysis of Syn WT and Syn CrtW + CrtZ after induction.

Although preliminary, these results show the strong potential of the engineered *Synechocystis* strain as an alternative source organism for the optimization and scale-up of Axs production.

The optimization of the Axs extraction process is ongoing, alongside with the mass spectrometry analysis for the characterization of the pathway intermediates accumulated by the mutant strains. Finally, the Asx purified from the engineered cyanobacterial strains will be used as feed supplement in rainbow trout aquaculture pilot trials. The cost-effectiveness of the novel strategy proposed as well as its efficiency, in terms of Asx accumulation and fish growth performances, will be evaluated and compared with those of currently employed aquaculture systems.

**Figure 3: 3A**- Absorption spectra (400-760 nm) of total carotenoids and Chlorophyll a in Syn WT (green) and mutant strains Syn CrtW (purple) and Syn CrtZ (yellow) and CrtW+CrtZ (red) after induction; **3B**- HPLC analysis of WT and CrtZ/W mutant after induction, the orange box highlights the Asx production, other abbreviations: Zea (zeaxanthin), Canta (canthaxanthin), Echi (echinenone), β-car (β-carotene); **3C**- Evaluation by HPLC of Asx, Zea, Canta, Echi and β-car production expressed as percentage on total carotenoids from WT (green) and mutant strains Syn CrtW (purple) and Syn CrtZ (yellow) and CrtW+CrtZ (red) after induction.

**References:** [**1**] Panis, G. and Carreon, J.R., Algal Research (2016) 18:175-190; [**2**] Higuera-Ciapara, I. *et al*., Crit Rev Food Sci Nutr. (2006) 46: 185-96. [**3**] Li, K.C. *et al*., Reviews in Aquaculture (2017) 0:1–36. [**4**] Mao, X. *et al*., Biotechnology Advances (2017) 35:1004-1021. [**5**] Mermet-Bouvier, P. and Chauvat, F., Current Microbiology (1994) 28:145-148. [**6**] Choi, S.K. *et al*., Mar Biotechnol, (2005) 7(5):515-522.

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