GROWTH ASSOCIATED ASTAXANTHIN SYNTHESIS BY *PHAFFIA RHODOZYMA* INDUCED BY COPPER DEFICIENCY

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Key words: Carotenoid, copper, electron transport chain.

Introduction. Astaxanthin is the main carotenoid produced by the yeast *P. rhodozyma*. Due to its powerful antioxidant activity, greater than of vitamin E, it has found several uses, such as protecting from ultraviolet radiation and in cancer prevention [1]. There are several factors that affect the production of astaxanthin. These include dissolved oxygen tension and deficiency of nutrients such as nitrogen, phosphates and copper [2]. It is known that copper deficit in the culture medium inhibits the flow of electrons through the electron transport chain leading to oxidative stress. This was reported to cause the activation of astaxanthin synthesis and increase in the specific carotenoid content [3]. The aim of this work was to prove that carotenoid synthesis can occur in *P. rhodozyma* either growth-associated or not growth-associated, depending on the concentration of Cu^{+2} in the culture medium.

Methods. *P. rhodozyma* NRRL-Y-10922 was used throughout this work. Cultures were performed in a 2.5-liter Applikon system under the following conditions: operating volume 2 liters, 1 vvm airflow, 22 ºC and 700 rpm. A C8 HPLC column with methanol-MTBE as mobile phase at flow rate 1.2 µL/min was used for analysis of carotenoid. The detector used was a UV lamp at 450 nm. Astaxanthin/β-carotene standard (SIGMA) was used.

Results. Fig. 1A shows the production of carotenoid by *P. rhodozyma* in a culture with copper deficit. It is noted that the carotenoid synthesis was growth-associated starting from the beginning of the exponential growth phase and throughout the culture. However, in the culture without copper-deficit (Fig. 1B), the synthesis was not associated with growth *i.e.*, took place mainly when the culture was at the stationary phase (as do many wild-type strains).

Table 1. End carotenoid content in *P. rhodozyma* cultures with and without copper (< 0.12 µM).

Culture	µg/mL	µg/ g yeast
7µM copper	2.5	354
< 0.12 µM copper	4.3	442

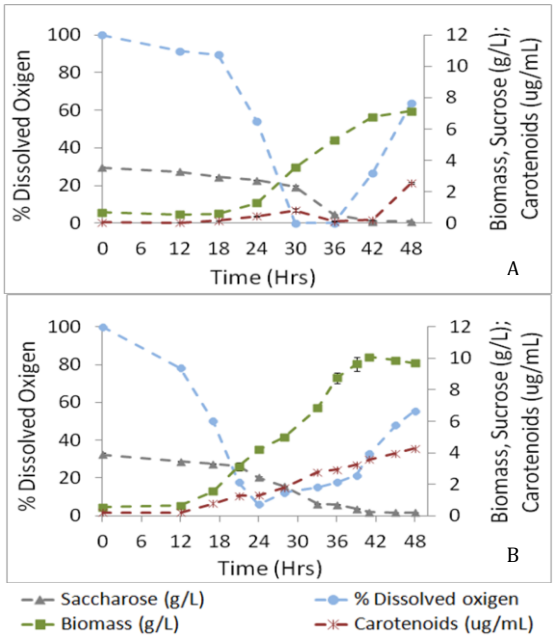

Fig.1 Growth curve, sucrose consumption, dissolved oxygen and carotenoid production by *P. rhodozyma*. A: with copper deficit, B: without copper deficit.

Moreover, the end concentration of carotenoid in the culture with Cu^{+2}-deficit was significantly higher (>70%) than the culture without Cu^{+2}-deficit (table 1). Also outstandingly, the astaxanthin/total carotenoid ratio was much higher under Cu^{+2}-deficit than without Cu^{+2}-deficit.

Conclusions. Copper removal from the culture medium, promotes the synthesis of carotenoid by *P. rhodozyma* in a growth-associated mode. The volumetric and specific content of carotenoid was 1.7 and 1.25 times higher in the culture with Cu^{+2}-deficit than without Cu^{+2}-deficit.

Acknowledgements. I appreciate the CONACYT scholarship to carry out this project.

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