

## Molecular cloning and functional characterization of the recombinant ATP:Citrate lyase of *Phaffia rhodozyma*

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Astaxanthin is a secondary metabolite and the main carotenoid produced in *Phaffia rhodozyma*. It is a powerful antioxidant commonly found in marine animals, and used in humans to prevent a number of degenerative diseases including cancer. Biosynthesis of astaxanthin requires acetyl-CoA as initial precursor. ATP-citrate lyase (ACL) is the key cytoplasmic enzyme which catalyses the formation of acetyl-CoA and oxaloacetate from citrate and CoA with a concomitant hydrolysis of ATP. ACL supplies acetyl-CoA for fatty acid biosynthesis in oleaginous yeasts. The biosynthesis of fatty acids and carotenoids had positive correlation with ACL activity in *P. rhodozyma*, consequently it has been suggested that both pathways have a common source of acetyl-CoA, although this has not been studied at the molecular level.

Firstly, we isolated and cloned the ACL gene of *P. rhodozyma* in *E. coli*. An *in silico* analysis showed a 4,117 pb gene sequence, with 3,341bp open reading frame that encodes a 120.9 kDa protein. The ACL gene from *P. rhodozyma* had 82.2% identity with that of *Postia placenta*, and 85.6% similarity with that of *Cryptococcus gattii*. Additionally, the ACL enzyme from *P. rhodozyma* has the two multi domains PNL02235 and PLN02522, characteristic in all ACL enzymes. Secondly, the ACL ORF was subcloned in pPICZB, and the resultant construction used to transform the yeast *Pichia pastoris* X-33 for expression. The expressed enzyme shows ACL activity and has a tag-His fusion which serves to purify the enzyme by nickel attachment.

### Biography

Cipriano Chavez Cabrera is a 38 years old Ph.D. student at Research Center for Advanced Studies of the National Polytechnic Institute (CINVESTAV-IPN), Mexico City. He has co-authored two papers on *Phaffia rhodozyma* and previously worked in the food industry and is a teacher at College of Science and Technology Studies of the Michoacan state (CECyTE Michoacan).

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