

The Production, Regulation and Extraction of Carotenoids from *Rhodospiridium toruloides*

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Abstract

Carotenoids are valuable pigments of commercial interest for various health benefits. There is rising demand for natural carotenoids from microorganisms, although the majority of industrially produced carotenoids are currently chemically synthesized. *Rhodospiridium toruloides* is oleaginous red yeast, which can produce large amounts of carotenoids and lipids simultaneously. It is also able to assimilate waste substrates as a carbon source. In this review we propose that *Rhodospiridium toruloides*'s high lipid accumulating ability, is linked to its ability to also produce carotenoids. We provide details of the production and regulation of carotenoids and lipids biosynthesis in this yeast, which appear to be in sync. We attribute this to the fact that the simultaneous production of carotenoids and lipids is advantageous for the cells to maintain proper structure, function and membrane homeostasis. We also highlight the need for further developments in carotenoids extraction methods to advance microbial bioprocess technology. In addition, we provide information on recent carotenoids extraction technology using two phase media, developed in an engineered strain of *Rhodospiridium toruloides*. The in depth understanding of this yeast would help future developments to increase the yield and extraction of carotenoids and lipid.

Keywords: Carotenoids; Lipid; Regulation; Extraction; *Rhodospiridium toruloides*

Carotenoids from a Microbial Source

Carotenoids are a diverse class of C₄₀ pigments. Currently, there are over 700 different types. Carotenoids are valued for their antioxidant, anti-carcinogenic and coloring properties. For example, β-carotene is a pro-vitamin A molecule as it can be cleaved to produce two vitamin A molecules. Vitamin A is essential for proper macular function and maintenance of epithelial tissue [1,2]. Hence carotenoids are in high demand across the cosmetics, food, poultry, and pharmaceutical industries [3].

Carotenoids can be obtained from various fruits and vegetables such as carrots and tomatoes. However due to their slow growing rates, large land requirements and susceptibility to weather conditions, the majority of industrially produced carotenoids are chemically synthesized. A small portion is extracted from naturally carotenoid producing microorganisms, such as micro algae, bacteria and yeasts. In recent years there is an increasing market demand for natural sources of carotenoids, from plants and microorganisms, due to negative health concerns over chemically synthesized carotenoids. As such, researches to increase yields of carotenoids in microorganism are gaining importance [4].

The current industrial carotenoid producer is the microalgae, *Dunaliella salina* (*D. salina*). However, micro algae are prone to contamination and require sunlight for growth. In comparison, yeasts are much more robust and less susceptible to contamination during the fermentation process. Robust yeasts such as *Rhodospiridium toruloides* (*R. toruloides*) also have an added advantage as it can grow on various waste substrates such as whey and palm oil mill effluent [5,6] besides the use of glucose as the traditional carbon source.

Although the cost of fermentation is reduced by utilizing waste substrates as a carbon source, the high cost of extraction and recovery of carotenoids from microbial cells remains a daunting challenge [1]. This is due to the cell wall of microorganisms which needs to be broken. This is usually carried out by physical methods such as sonication or bead milling which are difficult to scale up, or by enzymatic treatments which are costly. In addition, carotenoids are extracted using organic solvents such as hexane, which are toxic and environmentally unfriendly. A recent technology known as supercritical fluid extraction, has been shown to be rapid, efficient and non-toxic. However it is still more costly as compared to the use of organic solvents [7]. Hence more studies are required to develop a low cost, safe and efficient carotenoid extraction in order for microbial bioprocess to be economically viable.

Rhodospiridium toruloides's Ability to Produce Large Amounts of Lipids may be Linked to its Ability to Produce Carotenoids

Rhodospiridium toruloides (*R. toruloides*) is yeast well studied for its oleaginity [8]. It can accumulate up to 60% of its dry cell weight in lipids, which can be used for biodiesel [9]. This yeast also has the natural ability to produce the valuable carotenoids β-carotene, torulene and torularhodin [6,10]. The entire genome of this yeast has been elucidated recently [11,12], which helped shed light on the reason behind this yeast's unique ability to produce large amounts of lipids. The mechanism for its oleaginity is due to the fact that it contains the mitochondrial β-oxidation pathway, a novel fatty acid synthase system and the ATP citrate lyase enzyme. In addition, another contributing factor to *R. toruloides*'s oleaginity is linked to the presence of its carotenoid biosynthetic pathway [13,14], although more studies are

needed to understand the relationship between its carotenoid and lipid production.

Carotenoid Production may be Closely Linked with Lipid Biosynthesis as it may be Beneficial for the Organism

Carotenoid metabolism and its regulation is well studied in plants such as horticultural crops [14], but is not well understood in yeast at the moment. A study using the yeast *Rhodotorula glutinis* showed that both carotenoids and lipids were stimulated to accumulate in high amounts, when a culture condition of high carbon to nitrogen ratio was used [15]. This suggests that carotenoids and lipids are regulated in a similar fashion. Using omics technology, a study found that in a nitrogen limited condition which stimulated high lipids accumulation, four genes related to the carotenoid synthesis pathway were also up regulated. These genes include ERG10, ERG12, ERG20 and BTS1 [12]. In a recent metabolomic study, the authors showed that the reason behind a strain of *R. toruloides* with high lipid and carotenoid production was due to the acetyl CoA being redirected from entering the TCA cycle, into the carotenoid and lipid synthesis pathway. Taken together, these findings indicate a trend that the carotenoid synthesis pathway tends to be regulated in sync with the lipid synthesis pathway.

In light of this, we hypothesize that the concurrent production of carotenoids and lipid may be because it is beneficial for the microorganism. The presence of the long polyene backbone of a carotenoid molecule interacts with the hydrophobic moieties of lipids in the lipid membrane bilayer [16] and this serves as photo protection for the cell against oxidative damage [17]. Structurally, carotenoids also help to stabilize membrane proteins by acting as co-factors [16]. Another study also showed that the interaction of carotenoids and lipid help to maintain membrane homeostasis of the cell by controlling its fluidity [18,19]. Therefore it seems that a concurrent production of carotenoids and lipids is beneficial and required, in order to maintain the structure and function of the cell.

Two Phase Media for *in situ* Extraction of Carotenoids

The two phase system was developed to enable *in situ* extraction in a bid to overcome the step of breaking the microorganism's cell wall prior to carotenoid extraction. One of the first studies to demonstrate *in situ* extraction of β -carotene from the cells into the extracellular media was using the microalgae *D. salina*. In this study, the two phase media used was composed of an aqueous and organic phase.

The organic phase is required in order to dissolve the carotenoid molecules, which are hydrophobic. The use of dodecane as the organic phase was shown to be able to selectively extract β -carotene out from the cells, although the exact mechanism is unknown [19]. Sunflower oil has also been tested as the organic phase for the successful *in situ* extraction of β -carotene in *S. cerevisiae* [20]. It is suggested that the linoleic acid in sunflower oil, is the responsible component for the extraction of β -carotene [21]. Recently a study developed a Pdr10 strain, by engineering *R. toruloides* with a membrane transporter. The Pdr 10 strain could export carotenoids out of the cell using a two phase media. Interestingly, the fatty acids *R. toruloides* accumulated reflected the type of fatty acids in the organic phase, which was grape seed oil [22]. This is the first study which indicates the ability of this yeast to consume hydrophobic substrates. It also suggests that the diet of *R. toruloides* should be a factor to be taken into consideration, depending on the desired types of fatty acids.

Conclusion

Microbial derived carotenoids are gaining importance and demand. The development of a two phase system using vegetable oils such as sunflower oil to extract carotenoids, is a greener technology, as compared to organic solvents, and it also eliminates the need to break the cell wall. However, more studies are needed to develop other green and low cost technologies to extract carotenoids, for microbial derived carotenoids to be industrially viable. *R. toruloides* is a potential biotechnological producer of carotenoids and lipids, and it can grow on waste substrates. It would be beneficial to further understand the relationship between carotenoid and lipid biosynthesis in, to enable future genetic and metabolic engineering work to increase carotenoid and lipid yields.

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