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ARTICLE

Increased carotenoid content of *Xanthophyllomyces dendrorhous* cultivated in plant oil supplemented media

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ABSTRACT **Carotenoid pigments (particularly astaxanthin) of the red yeast** *Xanthophyllomyces dendrorhous (Phaffia rhodozyma)* **have economical importance as food and feed colouring additives. Application of nutrients stimulating astaxanthin synthesis would improve the pigment production of the fungus. Vegetable oils contain various unsaturated fatty acids and isoprenoids, among them different precursors of the carotenoid biosynthesis. The effect of seven different, commercially available vegetable oils (sesame seed oil, corn seed oil, wheat germ oil, palm oil, pumpkin seed oil, coconut oil and olive oil) on the carotenoid production of two strains representing the teleomorph** *X. dendrorhous* **and the anamorph** *P. rhodozyma* **was examined. The two strains responded to the presence of the oil additives distinctly. Sesame seed and coconut oil stimulated the pigment production in the** *X. dendrorhous* **isolate only, whereas palm oil increased the production of both tested strains.**
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KEY WORDS

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Astaxanthin (3,3'-dihydroxy-P,P-carotene-4,4'-dione) is a very effective flesh pigmenter and has great economical importance as feed colouring additive in salmon, trout and crustacean, as well as poultry, farming (Nelis and De Leenheer 1991; Dufossé 2006). Astaxanthin is closely related to other well-known carotenoids, such as P-carotene, zeaxanthin and lutein, so that they share many of the metabolic and physiological functions attributed to carotenoids. It exhibits strong free radical scavenging activity and protects against lipid peroxidation and oxidative damage of LDL-cholesterol, cell membranes, cells, and tissues; it also has an anti-cancer activity and enhances the immune system (Guerin et al. 2003). All these properties of astaxanthin share it an importance in the human diet which could lead to extended commercial applications. Today, most of the industrial astaxanthin production is performed by chemical synthesis, but serious efforts are made to develop strains and techniques for achievement of the microbial production. The red pigmented basidiomycetes yeast, *Xanthophyllomyces dendrorhous (Phaffia rhodozyma)* is one of the best candidates as a natural source of astaxanthin and other carotenoid compounds. The original taxonomic description defined this red yeast as an anamorphic species and it was named as *Phaffia rhodozyma* (Miller et al. 1976). Later, Golubev (1995) discovered the sexual cycle of the yeast and introduced the new species *Xanthopyllomyces dendrorhous* (Basidiomycetes). *P. rhodozyma* was considered to be con-specific with *X. dendrorhous* and the latter became the commonly accepted species name. However, a number of data have accumulated later on suggesting that the teleomorph *X. dendrorhous* and the anamorph *P. rhodozyma,* would be separate species (Kucsera et al. 1995; Fell and Blatt 1999). For this reason, two isolates representing *X. dendrorhous* and *P. rhodozyma* were involved in the present study and their responses to the application of different plant oils were compared.

In the last decade, strain improvement studies were carried out to elevate the pigment production of *'Xanthophyllomyces* to an economic level, among others by mutagenesis and screening (Fang and Cheng 1993; Sun et al. 2004; Palágyi et al. 2006), protoplast fusion (Chun et al. 1992) or by means of metabolic pathway engineering (Visser et al. 2003). Besides development of carotenoid overproducing strains, application of nutrients stimulating carotenoid synthesis, through enzyme induction or by exerting a general positive effect on the fungal growth, could also improve the carotenoid production. Vegetable oils may contain various unsaturated fatty acids and isoprenoids, among them precursors of the astaxanthin biosynthesis. In earlier studies, several lipids and lipid derivatives were used successfully to increase P-carotene synthesis in the Zygomycetes fungi, *Phycomyces blakesleeanus* and *Blakeslea trispora* (Ciegler and Arnold 1959 a,b; Lampila et al. 1985). The aim of the present work was to investigate the effect of different, commercially available vegetable oils on the carotenoid pigment production of *X. dendrorhous/P. rhodozyma.*

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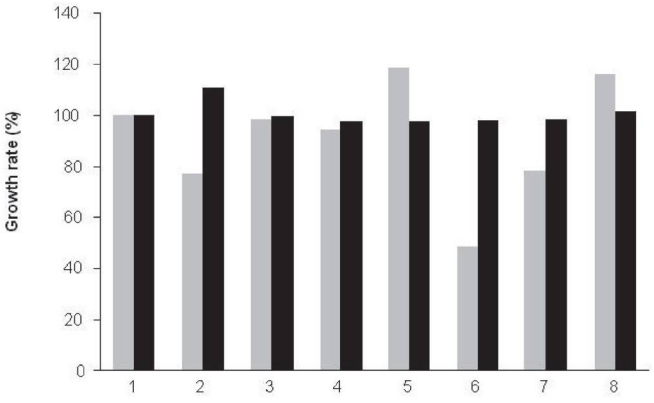

Figure 1. Effects of different vegetable oils on the astaxanthin production of *P. rhodozyma* and*X. dendrorhous.* : *P. rhodozyma* CBS 5905; **B:X.** *dendrorhous* CBS 6938.1: control; 2: sesame seed oil; 3: corn germ oil; 4: wheat germ oil; 5: palm oil; 6: pumpkin seed oil; 7: live oil; 8: coconut oil. Media contained 1% of the oils in every case.

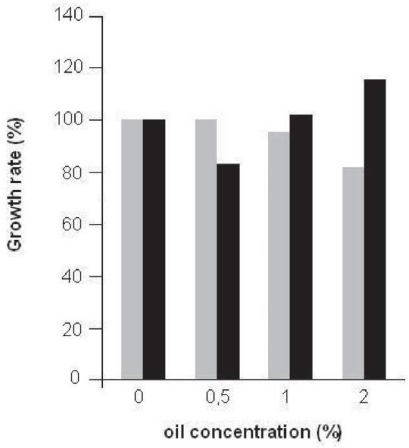

Figure 3. Effect of coconut oil (grease) on the astaxanthin production of P. *rhodozyma* and *X. dendrorhous.* : P *rhodozyma* CBS 5905; B: X. *dendrorhous* CBS 6938.

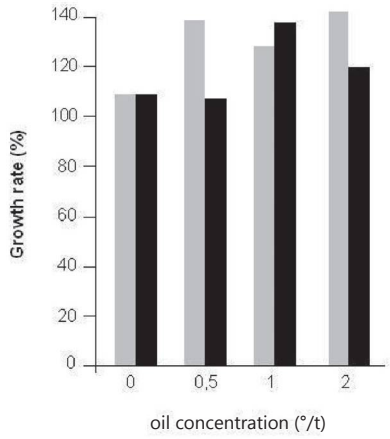

Figure 2. Effect of palm oil on the astaxanthin production of P. *rhodozyma* and X. *dendrorhous.* : P *rhodozyma* CBS 5905; ■: X. *dendrorhous* CBS 6938.

Materials and Methods

Strains and culture conditions

P. rhodozyma CBS 5905 and *X. dendrorhous* CBS 6938 were cultured in 25 ml of yeast-peptone-glucose (YPG: 1% glucose, 0.25% peptone, 0.25% yeast extract) liquid medium supplemented with the appropriate vegetable oil for 4 days at 20°C under continuous shaking (200 rpm). The tested plant oils were as follows: sesame seed oil (Sigma); com seed oil (Sigma); wheat germ oil (Sigma); palm oil (cooking oil); pumpkin seed oil (Sigma); coconut oil (cooking oil) and olive oil (extra virgin). The latter two were commercially available standard brands.

Measurement of the pigment content

After cultivation, cells were collected from 1 ml culture by centrifugation (5 min, 10 000 rpm) and washed twice with distilled water. The cell pellet was freeze-dried and treated with 1 ml of pre-heated dimethyl sulfoxide (DMSO) for 10 min at 55°C. Samples were centrifuged again (5 min, 12 000 rpm), and the total carotenoid content was measured in the supernatant by recording the absorbance at 492 nm. Dry-weight of cells harvested by centrifugation from 1 ml of each the cultures was also determined and the pigment content was related to dry cell mass. The carotenoid contents of the control extracts obtained from cultivation on YPG without oil supplements were regarded as 100%.

Thin layer chromatography (TLC) analysis

Pigment samples were obtained by the modihed method of Sedmack et al. (1990). To 3 ml carotenoid extract in DMSO, equal volume of diethyl ether was added in a separator funnel. It was placed on ice for 3 min and then 0.5 ml of water was added. The lower phase was removed and 5 ml of acetone and then 5 ml of 10% (v/v) ether-petrol were added. To achieve the separation of phases, 10 ml of water was added. The upper phase was decanted, washed with water (10 ml) and dried in a stream of N_2. Samples were redissolved in ethyl acetate and subjected on silica gel ($60F_{254}$, Merck) for TLC, which was developed with acetone-petroleum ether (20:80).

Results

In a primer experiment, effects of 1% of 7 plant oils (sesame seed oil, com seed oil, wheat germ oil, palm oil, pumpkin seed oil, coconut oil and olive oil) added into the cultivation media were tested (Fig. 1). Elevated carotenoid production

was detected in *X. dendrorhous* with the application of sesame seed oil; the total pigment content increased by 11%. At the same time, sesame seed oil and olive oil caused about 20% decrease in the carotenoid content of *P. rhodozyma.* Palm and coconut oil, in contrast, stimulated the pigment production of *P. rhodozyma:* an increment of 20% and 16% was observed, respectively. These compounds did not affect significantly the pigment production of *X. dendrorhous.* Pumpkin seed oil had no effect on *X. dendrorhous,* but decreased the carotenoid production of *P. rhodozyma* by 48%. The other oils tested seemed to have no effec on the carotenoid production in either organism.

After this preliminary experiment, the effect of coconut and palm oil was studied in more detail (Fig. 2-3), applying the oil supplements in 3 different concentrations (0.5; 1 and 2%). Palm oil stimulated the carotenoid production in both strains, but the effect of the same palm oil concentration in the two tested strains was different (Fig. 2). Carotenoid content of *P. rhodozyma* was increased by 20-29% depending on the oil concentration; the best result was observed if 2% of oil was added. In case of *X. dendrorhous,* a 25% increment of the total pigment content was observed on adding 1% palm oil, while 2% oil led to a lower increment (10%). The application of coconut oil exerted an opposite effect on the pigment production of *P. rhodozyma* and *X. dendrorhous.* Coconut oil decreased the carotenoid production in *P. rhodozyma* proportionally to the applied concentration. At the same time, the production in *X. dendrorhous* was increased (Fig. 3). The highest production (115%) was observed at 2% coconut oil concentration.

Samples extracted from the cultures containing sesame seed, palm and coconut oil were examined also with TLC (results not shown). Although the analysis detected the changes in the carotenoid - mainly astaxanthin - content, alterations in the carotenoid spectra were not observed.

Discussion

Phaffia rhodozyma and *Xanthophyllomyces dendrorhous* are the most promising fungal sources of the carotenoid astaxanthin. The commercial demand for astaxanthin increases, and although the biological production is still not economic, there is a continuous progress in improving strains as well as and fermentation methods. In our study on the effect of seven different oil extracts (all of them produced industrially in high quantity) as potential stimulators of the pigment production of these fungi, it was found that sesame seed, palm and coconut oils exerted significant effect on the pigment production. However, the two involved strains, representing the anamorph *(Phaffia)* and the teleomorph *(Xanthophyllomyces)* states, reacted to the applied oils very differently. This result supports the suggestion, based mainly on DNA sequence analysis data, that *Phaffia* is notjust an anamorph state of *'Xanthophyllomyces,* but a separate species (Kucsera et. al. 1998; Fell and Blatt; 1999, Lukács et al. 2006).

Extracts of palm oil generally contain several isoprenoid derivatives, such as P-carotene, different, cyclic and acyclic carotenoids and sterols, in high amounts (Lo and Choo 2003). Beta-carotene can be rapidly oxidised on exposure to light and in the presence of oxygen, but it is supposed that the lipid compounds of the palm oil can protect and conserve it. Together with other carotenoids, P-carotene is a precursor of astaxanthin, so the presence of these compounds in the oil additive could be one of the explanations of its stimulating effect on the astaxanthin production.

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