JOURNAL OF ENGINEERING annals OF FACULTY OF ENGINEERING HUNEDOARA

BETA-CAROTENE PRODUCTION BY MUCORALEAN FUNGI

Tamás PAPP[1], Gábor NAGY[1], Árpád CSERNETICS[1],
András SZEKERES[2], Csaba VÁGVÖLGYI[1]

[1]University of Szeged, Faculty of Sciences and Informatics, Department of Microbiology, Szeged, [2]FumoPrep Ltd, Mórahalom, HUNGARY

Abstract

Although some fungi belonging to the order Mucorales (Zygomycetes), such as *Phycomyces blakesleeanus*, *Blakeslea trispora* and *Mucor circinelloides*, have been traditionally involved in the study of the fungal carotenoid biosynthesis, the majority of the related species have not been studied from this aspect. As morphological observations indicates that a number of other species also seems to be promising producers, the main objective of the present study was to investigate the beta-carotene production ability of several Mucoralean fungi belonging to the genera *Mucor*, *Backusella* and *Gilbertella*. After cultivation under different conditions, the total carotenoid level and the beta-carotene content in the mycelia were measured by an HPLC method. Pigment production of *Gilbertella persicaria* was worth to mention only if it was cultured as a mixture of isolates with opposite mating types. Some *Mucor* and *Backusella* strains produced beta-carotene in significantly higher amounts than the *M. circinelloides* reference strain or the wild-type *B. trispora*, a model organism of the carotenogenic studies. Effects of the illumination, the carbon source and the growth temperature on the carotene production were examined.

Keywords

pigment production, carotenoid, Zygomycetes, *Mucor*

1. INTRODUCTION

Carotenoids are one of the most important groups of natural pigments. They are used in the food, pharmaceutical and cosmetic industry and as feed colour additives. Carotenoids recently attracted great attention, due to their beneficial effects on human and animal health; for example, their antioxidant property linked with a preventive action on different types of cancer [5] and the enhancement of the immune system [2]. Most of the carotenoid production is performed by chemical synthesis and only a few natural compounds can be obtained from cheap plant sources [1]. Currently there is an increasing interest in sources of carotenoids from microbial origin, especially in cases of the β-carotene and its oxygenated derivatives.

In Zygomycetes fungi β-carotene is the predominant carotenoid species. Traditionally three Zygomycetes, e.g. *Blakeslea trispora*, *Phycomyces blakesleeanus* and *Mucor circinelloides*, have been involved in the study of the carotene biosynthesis.

The aim of the present study was to obtain information on the carotenoid production, especially on the β-carotene content of some Mucoralean fungi in order to determine new producer strains potentially applicable in further analyses and developments.

2. THE STUDY

Strains and growth conditions.

The 21 fungal strains involved in this study are listed in Table 1. Strains were cultured on plates containing malt extract medium (5 % malt extract, 0.5 % yeast extract, 1% D-glucose, 1.5 % agar), grown for 4 days under continuous light.

Carotenoid extraction and analysis.

Carotenoids were extracted from 500 mg mycelial powder with 500 µl acetone and vortexing. This extraction step was repeated until the pellet was found to be devoid of

pigments. Extracts were combined and then partitioned with an equal volume of 10% diethyl ether in petroleum ether. To facilitate the separation and to remove dissolved acetone, 1 ml distilled water was added. The petroleum ether fractions were combined and dried under nitrogen gas [6].

For high-performance liquid chromatography (HPLC), samples were analyzed by using a modular Shimadzu low-pressure gradient HPLC system equipped with an UV-Vis detector. The dried samples were dissolved in 100 µl tetrahydrofuran supplemented with butylated hydroxytoluene (100 µg/ml) directly before the analysis and 3 µl was subjected to HPLC analysis on a Phenomenex Prodigy column (4.6 x 250, ODS 3 µm). The separation was performed with a gradient (where min/solvent A%/solvent B% was 0/99/1; 8/60/40; 13/46/54; 15/0/100; 18/0/100; 21/99/1; 25/99/1) using 4% water-96% methanol as solvent A and 4% water-96% methyl-*terc*-butyl ether as solvent B, at a flow rate of 1 ml/min. The detection wavelength was 450 nm. To identify the carotenoids, the following standards were used: astaxanthin, lycopene and β-carotene from Sigma, β-cryptoxanthin, zeaxanthin and canthaxanthin from Carl Roth, and echinenone from DHI Water and Environment.

For spectrophotometry, samples were dissolved in petroleum ether; total carotenoid content was measured at 450 nm.

3. ANALYSIS AND DISCUSSION

For the study, 21 fungal isolates were selected on the basis of morphological observations, e.g. of their colony colour (Table 1). These isolates represent 10 different species belonging to the genera *Mucor*, *Rhizopus*, *Backusella* and *Gilbertella*. Overall carotenoid content of the isolates tested are shown in Fig. 1. The carotene production showed high variability even among the isolates of a same species. The most promising producers were the isolates M19, M59, M76 and MH1 with a carotene production more than 400 µg/g dry mass; *M. circinelloides* (M20) and *B. lamprospora* (B1) also had remarkable production. Maybe the high production of *M. bainieri* strain M76 can be connected with the obligate azigospore forming nature of this fungus. Trisporic acids, substances with hormonal activity forming during the zygosporogenesis (e.g. the mating), have been shown to stimulate the β-carotene biosynthesis [5].

Gilbertella persicaria produced higher amounts of pigments only if it was plated as a mixture of the opposite mating types.

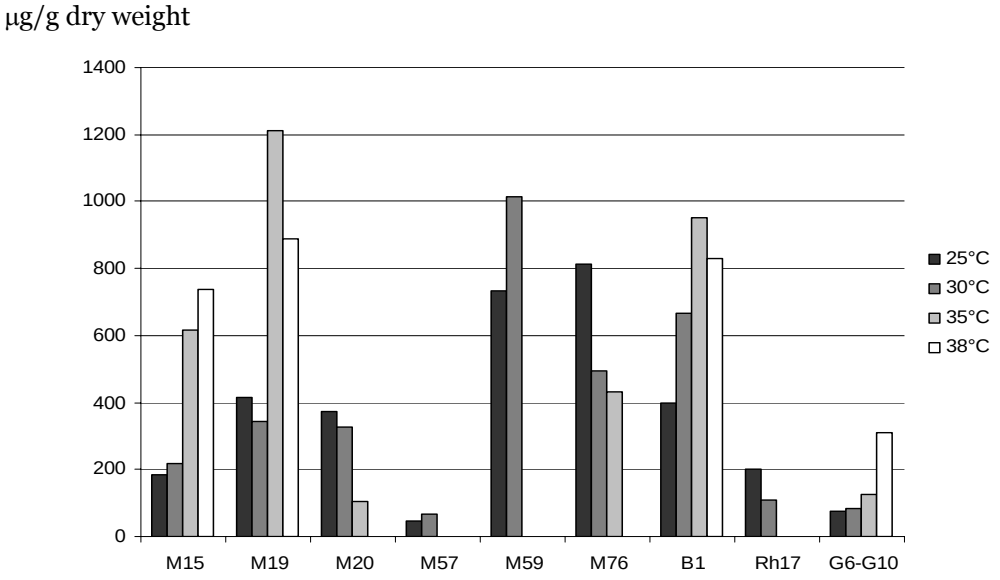

Figure 1. Total carotenoid production of Mucoralean fungi at different temperatures.
The averages were calculated from 3 different measures from independently cultured mycelia.

Ten strains were selected for further analysis (G6 and G10 examined in mixed cultures to achieve higher carotenoid production). Effect of the growth temperature on the carotenoid production was examined (Fig. 1). In an earlier study, three-times higher carotenoid content was observed in *M. rouxii* when the culturing temperature was increased from the optimum growth temperature (28°C) to 37°C [4].

Table 1. Investigated fungal strains and their overall carotenoid content
[a]These codes where used throughout the paper for clarity. [b]Strains were cultured at 25°C under continuous light; averages were calculated measuring 3 independent extracts.

Species	Code of isolate[a]	Total carotenoid content[b]
Mucor albo-ater	M30	20
M. bainieri	M51	36
M. bainieri	M76	825
M. circineloides	M20	378
M. circineloides	M50	98
M. hiemalis	MH1	570
M. hiemalis	M18	135
M. hiemalis	M12	25
M. hiemalis	M22	105
M. hiemalis f. *hiemalis*	M55	24
M. hiemalis f. *luteus*	M57	46
M. hiemalis f. *hiemalis*	M59	740
M. inequisporus	M58	35
M. mucedo	M19	420
M. rouxi	M15	192
Backusella lamprospora	B1	400
Rhizopus stolonifer	Rh17	200
Rhizopus stolonifer	Rh5	57
Gilbertella persicaria	G10	29
Gilbertella persicaria	G5	28
Gilbertella persicaria	G6	29
Gilbertella persicaria	G5-G6	151
Gilbertella persicaria	G6-G10	127

μg/g dry weight

In our experiments, higher growth temperature also stimulated the production in the majority of the strains. Elevation of the growth temperature led to the highest carotenoid production in the strains M59 (*M. hiemalis*) and M19 (*M. mucedo*), where the total carotenoid contents exceeded 1 mg/g dry weight at 30 and 35°C, respectively. It is worth to mention that all fungi showed more or less restricted growth at temperatures higher than 30°C. The only exception was the mating culture of *G. persicaria* (G6-G10) retaining its growth intensity even at 38°C where it produced about 4 times more carotenoids than at 25°C. Carotenoid production of the strains M20, M79 and Rh17 (*M. circinelloides*, *M. bainieri* and *Rhizopus stolonifer*, respectively) decreased at higher temperatures.

ACKNOWLEDGEMENTS This research was supported by ETT grants (214/2006; 261/2006) and the J. Bolyai Research Scholarship.

REFERENCES

[1] Bhosale, P. Environmental and cultural stimulants in the production of carotenoids from microorganisms. Appl. Microbiol. Biotechnol. 63: 351–361, 2004
[2] Hughes, D.A. Effects of carotenoids on human immune function. Proc. Nutr. Soc. 58: 713–718, 1999
[3] Lampila, L.E., Wallen, S.E., Bullerman, L.B. A review of factors affecting biosynthesis of carotenoids by the order Mucorales. Mycopathologia 90: 65-80, 1985
[4] Mosqueda-Cano, G., Gutierez-Corona, J.F. Environmental and developmental regulation of carotenogenesis in the dimorphic fungus *Mucor rouxii*. Curr. Microbiol 31: 141-145, 1995

[5] Nishino, H., Murakosh, M., Ii, T., Takemura, M., Kuchide, M., Kanazawa, M., Mou, X.Y., Wada, S., Masuda, M., Ohsaka, Y., Yogosawa, S., Satomi, Y., Jinno, K. Carotenoids in cancer prevention. Cancer Metastasis Rev. 21: 257–264, 2002

[6] Papp, T., Velayos, A., Bartók, T., Eslava, AP., Vágvölgyi, Cs., Iturriaga, E.A. Heterologous expression of astaxanthin biosynthesis genes in *Mucor circinelloides*. Appl. Microbiol. Biotech. 67: 526-531, 2006

[7] Vágvölgyi, Cs., Magyar, K., Papp, T., Palágyi, Zs., Ferenczy, L., Nagy, Á. Value of substrate utilization data for characterization of *Mucor* isolates. Can. J. Microbiol. 42: 613-615, 1996