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CARBOHYDRATE COMPONENTS OF CULTURE MEDIA AS DETERMINANTS OF THE *RHODOSPORIDIUM DIOBOVATUM* IMB Y-5023 YEAST METABOLISM

SUBSTRAT WĘGLOWODANOWY W PODŁOŻU JAKO DETERMINANT METABOLIZMU DROŻDŻY *RHODOSPORIDIUM DIOBOVATUM* IMB Y-5023

Abstract

Background. The basidiomycete *Rhodosporidium diobovatum* is an oleaginous yeast capable of carotenoid synthesis. Scientific research on this yeast indicates that it can be a promising industrial producer of this pigment. The yeast is capable of assimilating numerous carbon substrates and it can grow under various, often extreme, environmental conditions. Wild strains can be isolated from soil, the depths of the sea and the surface of plants. They can grow both in the dark and in the presence of light. The aim of this study was to determine the capability of the *Rh. diobovatum* IMB Y-5023 yeast strain to produce and accumulate carotenoids when cultured on media with different carbohydrate concentration and composition and with low monosaccharide levels. The yeast was cultured at different illuminances. This research is of great theoretical and practical importance, because it may enable the use of various organic by-products from the agri-food industry as substrates for culturing *Rh. diobovatum* and for microbial production of carotenoids. This research also provides an opportunity to reduce the costs of the microbiological process by choosing optimal illuminance of the culture.

Material and methods. *Rhodosporidium diobovatum* IMB Y-5023 was cultured on three test media: CM (carrot medium), BYP (wheat bran extract), and the YM medium with higher concentration of malt extract as the main source of oligosaccharides and with maltotriose added. Cultures were carried out at 22°C for 5 days at illuminance ranging from 0 to 5,000 lx. The carbohydrate composition of the media was determined before and after the culturing of *Rh. diobovatum* IMB Y-5023 by means of UHPLC coupled with tandem mass spectrometry (MS). The following parameters were also measured after culturing: biomass yield (DCW – dry cell weight, g/l), the concentration of carotenoids in the yeast biomass (mg/g DCW), the biomass yield coefficient ($Y_{X/S}$)

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and the carotenoid yield coefficient ($Y_{C/S}$). The samples were compared by means of cluster analysis (CA) and principal component analysis (PCA), which were used to visualise the relationships between the study variables and the samples.

Results. The *Rh. diobovatum* IMB Y-5023 yeast strain grew and produced carotenoids on all the media. It assimilated the DP3 and DP4 oligosaccharides contained in the media. Regardless of the illuminance during culturing, the consumption of sugars in the test media remained at 90% or more. However, the concentration and type of carbohydrates in the test medium affected the course of their metabolism. Regardless of the illuminance, the highest concentration of pigments in the cells and the lowest yield of biomass were noted with YM. The initial concentration of sugars in that medium was three–four times greater than in the other test media, and their type was considerably diversified. The data clustering (CA) and principal component analysis (PCA) showed that the BYP and CM media (with similar initial sugar concentrations of 11–15 g/l) had similar influence on the yeast metabolism, whereas the measured variables were completely different in the cultures with the YM medium.

Conclusions. The *Rh. diobovatum* IMB Y-5023 yeast strain is a promising industrial producer of carotenoids. It can effectively assimilate both monosaccharides and DP3 and DP4 oligosaccharides. It does not require illumination to grow and produce pigments effectively, especially when it is grown on media that have high sugar concentrations and are rich in oligosaccharides. The cost of industrial carotenoid production with *Rh. diobovatum* IMB Y-5023 can be reduced by using inexpensive by-products from the agri-food sector as a culture medium, and by eliminating the illumination of cultures.

Keywords: *Rhodosporidium diobovatum,* carbohydrates assimilation, carotenoids, cultivation, medium composition, illumination

Introduction

Yeasts hold a leading position among biotechnological resources as main producers of biomass rich in proteins, vitamins, various enzymes, and carotenoid pigments (Čarnecká, 2009; Latha and Jeevaratnam, 2010). They are chemoorganotrophic organisms, capable of using a wide variety of substrates as nutrients (Marova et al., 2011). Yeasts can be used in organic recycling processes to produce biomass rich in valuable substances (Buzzini and Martini, 1999).

For this reason, the Basidiomycetes carotene-synthesising yeast *Rhodosporidium diobovatum* is of great biotechnological interest. It is known that the growth and carotenogenesis of yeasts can be affected by many factors: temperature, pH, aeration, light, as well as the nature and concentration of sources of carbon, nitrogen, minerals and vitamins (Libkind et al., 2008; Simova et al., 2004). The type and concentration of carbon sources is one of the main factors limiting not only the growth of yeast cells, but also the synthesis of carotenoids. It is caused by the fact that carotenoid biosynthesis is regulated by various levels and activity of enzymes involved in full carbon metabolism through the system of carotenoid synthesis (Frengova and Beshkova, 2009).

Rhodosporidium diobovatum can assimilate carbon sources such as: glucose, galactose, sucrose, maltose, cellobiose, trehalose, raffinose, inulin, xylose, arabinose, d-ribose, ethanol, and glycerol (Newell and Hunter, 1970). It can grow not only on various media, but also under various environmental conditions. Its wild strains are found in the depths of the sea (Seshadri et al., 2011), in soil and on the surface of plants i.e. in various envir-

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onments differing in exposure to sunlight (Guo et al., 2014). Thereby, it is very interesting to investigate the effect of light intensity on the yield of biomass and carotenoid synthesis as well as the carotenoid content during the growth of *Rh. diobovatum* on media with different compositions. It is known that there is dependence between light intensity and carotenogenesis (Stachowiak, 2013). However, the microorganisms that are capable of synthesising these pigments differ in this dependence according to the conditions in their natural habitat and the function of carotenoids in the microorganism (Walter and Strack, 2011).

The research on the capability of *Rh. diobovatum* IMB Y-5023 to grow and accumulate carotenoids on media with different concentration and composition of sugars and with low monosaccharide content is of great theoretical and practical interest. It may extend the range of organic by-products used as substrates for yeast culturing. Studies proved that *Rh. diobovatum* IMB Y-5023 could grow on waste produced by the alcohol industry, in particular on maize kernels (Goltvianskiy et al., 2015).

This study presents how the carbohydrate concentration and composition of the culture medium and the illuminance (0–5,000 lx) affect the biomass and carotenoids produced by the *Rh. diobovatum* IMB Y-5023 yeast.

Material and methods

Microorganism and media

Rhodosporidium diobovatum IMB Y-5023 yeast was obtained from the Microorganism Depository of the D. K. Zabolotny Institute of Microbiology and Virology (Kiev, Ukraine). The stock culture was maintained on CM (carrot medium) agar slants (10% carrot extract, 10 g/l glucose, 10 g/l peptone, 1 g/l NaCl, 20 g/l agar; Stachowiak, 2012) at 4°C until use. The yeast was cultured on the following test media: (1) liquid CM medium, (2) modified YM medium, consisting of 10 g/l glucose, 5 g/l maltotriose, 5 g/l peptone, 3 g/l yeast extract and 25 g/l malt extract, and (3) BYP medium (Goltvianskiy et al., 2015). The BYP medium was obtained as follows. The mycelia of *Pleurotus ostreatus* HK-35 (provided by the Department of Vegetable Crops, Poznań University of Life Sciences, Poland) were transferred into 300 ml of 3% offal extract enriched with 10 g/l yeast extract in a one-litre flask. The offal extract was obtained by thermal treatment of a mixture of offal and water at 121°C for 90 min (in an autoclave). The culture was kept at 25°C without shaking. After 2 days, the mycelium was removed by filtering. Next the culture medium was heated for 5 min at 100°C. The resulting filtrate was the BYP medium.

The pH of all media was adjusted to 5.0.

Cultivation

Yeast grown on a CM agar slant was washed with 10 ml of the test medium and the resulting suspension was transferred to 50 ml of fresh test medium in a 300 ml flask. The culture was kept in the dark at 22°C for 2 days. Next, 5 ml of the inoculum culture was transferred to 45 ml of an appropriate test medium. The cultures were grown in

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a thermostat chamber with a phytotron (ST 700 Fit, Pol-Eko-Aparatura, Poland) at 22°C for 5 days, in orbital shakers (agitation speed 150 rpm) at illuminance ranging from 0 to 5,000 lx. Lamps (Philips, Netherlands) emitting light close to natural daylight were used as light sources. Four Master TL5 HO 24W/840 lamps were used in a sequence in overhead racks. The light intensity was controlled using a TES 1335 luxmeter (TES Electrical Electronic Corp., Taipei, Taiwan).

Carbohydrates in test media

The concentration and composition of carbohydrates in the test media were determined at the beginning and at the end of *Rh. diobovatum* IMB Y-5023 cultivation. The following method was applied: 10 ml of the culture was centrifuged (15 min, 3,500 g), methanol was added (1 : 4) to the supernatant and the sample was left in the refrigerator overnight. Then it was filtered through a 0.45 μm membrane Millipore filter (Merck Millipore, MA, USA) to separate large molecules and cell aggregates. The content of simple carbohydrates (monosaccharides and oligosaccharides) in the resulting samples was determined using ultrahigh-performance liquid chromatography with tandem mass spectrometry (LCMS). The carbohydrates were separated using an UltiMate 3000 UHPLC (Dionex Softron GmbH, part of Thermo Fischer Scientific Inc., Germany) with a column of RCM-Monosaccharide Ca^{+2} (8%) at 80°C (Phenomenex). The analysis was continued with a maXis impact mass spectrometer (Bruker Daltonik GmbH, Germany). The details of chromatographic separation and the ESI-MS settings were described in detail by Gumienna et al. (2016).

Carotenoid extraction and analysis

Carotenoid pigments were extracted from the yeast cells using the method described by Sedmak et al. (1990), as modified by Stachowiak (2012). 10 ml of the culture was centrifuged (15 min, 3,500 g) and the pellet was resuspended in 5 ml of DMSO (Sigma-Aldrich, USA) and preheated to 55°C. After centrifuging for 30 s, 5 ml of the hexane fraction of petroleum (POCh, Gliwice, Poland) was added. The samples were centrifuged for 30 s again and 20% NaCl aqueous solution was added in aliquots of 0.5 ml. The upper hexane fraction with the extracted pigments was separated by centrifugation (15 min, 3,500 g). Total carotenoid concentrations were quantified spectrophotometrically at 450 nm, where β-carotene (Sigma-Aldrich, USA) was used as the standard.

The dry cell weight (DCW) was determined (grams per 1 l of growth medium), and the carotenoids were expressed as milligrams per 1 g DCW.

Statistical analysis

All the experiments were performed in triplicates. Hierarchical cluster analysis (CA) was applied (the Ward rule with the Euclidean distance measure was used for amalgamation). The data were presented in the new coordinate system and principal component analysis (PCA) was applied. Statistica version 10 (StatSoft Inc., OK, US) was used for data processing.

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Results

The composition of carbohydrates in the initial test media

The media differed in the total content of carbohydrates and their profiles (Table 1). The YM medium contained three times more sugar than the CM medium, and about four times more than the BYP medium. The YM medium contained a wide variety of carbohydrates, but mostly trisaccharides (68.3%) and glucose (22%). The CM medium contained mainly glucose (66.5%), a small amount of sucrose (18%) and fructose (14.4%). The BYP medium mostly contained trisaccharides (82.9%) and tetrasaccharides (11.9%).

Table 1. Initial sugar content in the test media (g/l)

Sugar	Media		
	YM	CM	BYP
DP2	1.30 ±0.06	2.83 ±0.14	0.16 ±0.01
DP3	32.33 ±1.61	0.04 ±0.00	9.25 ±0.43
DP4	2.96 ±0.14	trace amount	1.33 ±0.06
DP5	0.11 ±0.00	0.00	0.08 ±0.00
DP6	trace amount	0.00	0.01 ±0.00
Rabinose	0.00	0.00	0.00
Fructose	trace amount	2.17 ±0.10	trace amount
Galactose	0.00	0.00	0.00
Glucose	10.60 ±0.53	10.00 ±0.47	0.33 ±0.01
Ribose	0.00	0.00	0.00
Xylose	0.00	0.00	0.00
Total	47.30 ±2.35	15.04 ±0.72	11.16 ±0.51

DP2 – disaccharides, DP3 – trisaccharides, DP4 – tetrasaccharides, DP5 – pentasaccharides, DP6 – hexasaccharides.

The composition of carbohydrates in the test media after the culturing of *Rhodosporidium diobovatum* IMB Y-5023 in the dark and under various illuminances

Table 2 shows the amount of total sugars and their composition after 5 days of *Rh. diobovatum* IMB Y-5023 culturing on the test media.

The sugars were totally consumed in the cultures grown on the BYP medium, irrespective of the illuminance.

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Table 2. Sugar content in the test media after 5 days of *Rhodosporidium diobovatum* IMB Y-5023 culturing at various illuminances (g/l)

Illuminance (lx)	Glc	Fru	Rib	DP2	DP3	DP4	DP5	DP6	∑S
YM medium									
0	t/a	0.00	t/a	0.01 ±0.00	0.55 ±0.02	0.09 ±0.00	t/a	t/a	0.65 ±0.03
300	0.01 ±0.00	t/a	t/a	0.03 ±0.00	0.41 ±0.01	0.03 ±0.00	t/a	t/a	0.47 ±0.02
750	t/a	t/a	t/a	0.05 ±0.00	0.37 ±0.01	2.24 ±0.09	0.07 ±0.00	t/a	2.73 ±0.11
2000	t/a	0.00	t/a	0.06 ±0.00	0.44 ±0.01	0.12 ±0.00	t/a	t/a	0.63 ±0.03
5000	t/a	t/a	t/a	0.05 ±0.00	0.39 ±0.01	0.28 ±0.01	t/a	t/a	0.72 ±0.03
CM medium									
0	0.00	0.10 ±0.00	t/a	0.33 ±0.01	0.08 ±0.00	t/a	t/a	t/a	0.51 ±0.02
300	0.00	0.03 ±0.00	t/a	0.43 ±0.01	0.03 ±0.00	t/a	t/a	t/a	0.49 ±0.02
750	1.21 ±0.05	0.04 ±0.00	0.00	0.77 ±0.02	0.04 ±0.00	t/a	t/a	0.00	2.06 ±0.10
2000	0.00	t/a	t/a	0.56 ±0.02	0.06 ±0.00	t/a	t/a	t/a	0.62 ±0.03
5000	0.00	0.00	t/a	0.33 ±0.01	0.03 ±0.00	t/a	t/a	t/a	0.36 ±0.01
BYP medium									
0	0.00	0.00	t/a	0.00	0.03 ±0.00	t/a	0.00	0.00	0.03 ±0.00
300	t/a	0.00	0.00	0.00	t/a	0.00	0.00	0.00	t/a
750	0.00	0.00	0.00	0.00	t/a	0.00	0.00	0.00	t/a
2000	0.00	0.00	t/a	t/a	t/a	t/a	0.00	0.00	t/a
5000	0.00	0.00	0.00	t/a	t/a	t/a	0.00	0.00	t/a

Glc – glucose, Fru – fructose, Rib – ribose, DP2 – disaccharides, DP3 – trisaccharides, DP4 – tetrasaccharides, DP5 – pentasaccharides, DP6 – hexasaccharides, ∑S – total sugars, t/a – trace amount.

The consumption of sugars in the cultures grown on the YM and CM media at 750 lx amounted to 94% and 87%, respectively. The YM medium chiefly contained DP4. The CM medium mostly contained glucose and sucrose. Sugars were almost completely consumed (97–99%) in the other cultures in both media, regardless of illuminance.

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The growth and production of carotenoids by *Rhodosporidium diobovatum* IMB Y-5023 in the test media in the dark and under different illuminances

Table 3 shows the biomass yield (DCW, g/l), the concentration of carotenoids in the yeast biomass (mg/g DCW), the biomass yield from the substrate coefficient ($Y_{X/S}$) and the carotenoid yield from the substrate coefficient ($Y_{C/S}$) after 5 days of cultivation on the test media.

Table 3. The effect of the medium composition and illuminance on the test parameters in 5-day cultures of *Rhodosporidium diobovatum* IMB Y-5023

Medium	Illuminance (lx)	Sugars consumed (%)	Dry cell weight, DCW (g/l)	Total carotenoids (mg/g DCW)	Biomass yield from substrate coefficient, $Y_{X/S}$ (g/g)	Carotenoid yield from substrate coefficient, $Y_{C/S}$ (mg/g)
YM	0	98.6	3.10 ±0.10	24.98 ±0.89	0.07	0.54
	300	99	3.40 ±0.20	20.67 ±0.72	0.07	0.44
	750	94.2	3.30 ±0.10	20.13 ±0.71	0.07	0.45
	2000	98.7	3.30 ±0.10	11.87 ±0.45	0.07	0.25
	5000	98.5	3.90 ±0.11	12.66 ±0.40	0.08	0.27
CM	0	96.6	7.60 ±0.10	4.99 ±0.26	0.52	0.34
	300	96.7	8.30 ±0.20	5.72 ±0.31	0.57	0.39
	750	86.3	8.80 ±0.10	5.24 ±0.41	0.68	0.40
	2000	95.9	8.60 ±0.10	5.50 ±0.30	0.60	0.38
	5000	97.6	8.20 ±0.20	7.09 ±0.31	0.56	0.48
BYP	0	97.2	6.90 ±0.30	3.35 ±0.24	0.62	0.30
	300	100	7.00 ±0.10	3.48 ±0.20	0.63	0.31
	750	100	4.90 ±0.20	7.99 ±0.32	0.44	0.75
	2000	100	4.20 ±0.10	7.79 ±0.55	0.38	0.68
	5000	100	3.80 ±0.10	5.65 ±0.34	0.34	0.51

The lowest DCW ranged from 3.1 to 3.9 g/l. It was noted in the cultures grown on the YM medium. The lowest value was noted for the cultures grown in the dark, whereas the highest values were observed for those grown at 5,000 lx. Simultaneously, the $Y_{X/S}$ values were very low (0.07–0.08), although the yeast consumed almost all sugars. The highest concentration of carotenoids in the yeast cells was noted in the cultures grown on this medium although the production was affected by the illuminance. The cultures grown at illuminances of 0–750 lx gave the highest cellular yields of carotenoids. They were about four–five times greater than those given by the cultures grown on the CM

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and BYP media under the same conditions. The carotenoid synthesis was significantly reduced in the cultures grown on the YM medium at high illuminance levels (2,000 and 5,000 lx). The $Y_{C/S}$ values in the cultures grown on the YM medium depended on the illuminance. When the illuminance increased, the $Y_{C/S}$ values decreased.

Simultaneously, regardless of the lighting conditions, the DCW in the *Rh. diobovatum* IMB Y-5023 cultures grown on the CM medium was two and a half times greater than in the cultures grown on the YM medium. Moreover, the $Y_{X/S}$ values in the cultures grown on the CM medium were approximately ten times greater than in those grown on the YM medium. The highest $Y_{X/S}$ value (0.68) was noted in a culture grown at an illuminance of 750 lx.

The highest cellular yield of carotenoids (7 mg/g DCW) and the highest $Y_{C/S}$ value (0.48) in the cultures grown on the CM medium was noted at an illuminance of 5,000 lx. The yields of carotenoids and $Y_{C/S}$ value were respectively lower or similar in the cultures grown at lower illuminances.

In the cultures grown on the BYP medium the biomass yield and $Y_{X/S}$ values were lower at higher illuminances. The DCW in the cultures grown on the BYP medium in the dark was almost twice as high as in the cultures grown at 2,000 and 5,000 lx. The highest cellular yield of carotenoids was noted in the culture grown at 750 lx. The $Y_{C/S}$ value was 0.75.

Discussion

The research showed that *Rh. diobovatum* IMB Y-5023 consumed different carbohydrates, including DP3 and DP4 oligosaccharides. The illuminance had almost no effect on the amount of sugars consumed during culturing. However, the composition of the medium and the amount of sugar it contained determined the course of sugar metabolism, affecting the yield of biomass and carotenoids as well as the $Y_{X/S}$ and $Y_{C/S}$ values (Table 3).

The CA and PCA were performed in order to illustrate the overall relationship between sugars, biomass and carotenoids after 5 days of culturing on the media.

The parameters of the samples were compared by means of CA and PCA and listed in Tables 2 and 3 (Fig. 1, 2B). The BYP and CM media, which had similar initial sugar contents of 11–15 g/l, had similar effects on the metabolism of *Rh. diobovatum* IMB Y--5023. However, the parameters were completely different in the cultures grown on the YM medium, where the sugar levels were three–four times greater (Fig. 1, 2B).

The results of the PCA (Fig. 2) show the dependence between the variables and their impact on individual samples. The maximum carotenoid concentration in the yeast cells grown on the YM medium was positively correlated with the assimilation of sugars (Fig. 2A). However, the conversion of sugars into DCW was the highest in the CM medium, whereas the conversion of sugars into carotenoids was the highest in the BYP medium at high illuminance.

It is known that the reaction of fungi to light depends on the source of carbon (Schuster et al., 2007; Tisch and Schmoll, 2010). Light is considered to be the main factor triggering fungal metabolic processes, especially the carotenoid synthesis. How-

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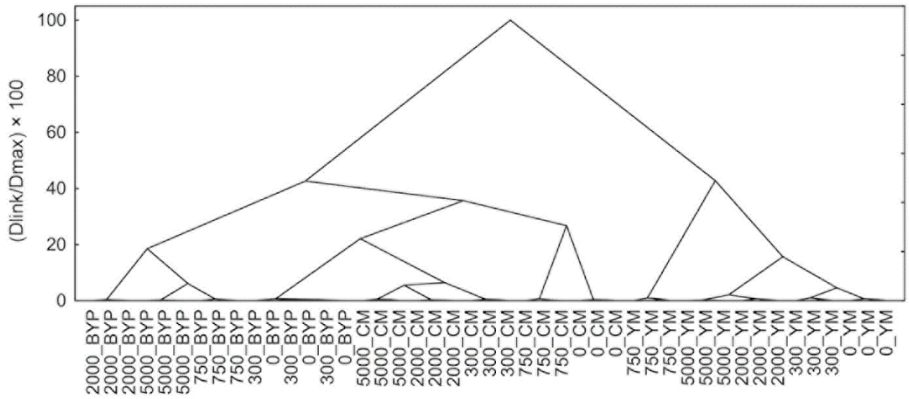

Fig. 1. The results of cluster analysis for the parameters shown in Table 3 obtained for 5-day *Rhodosporidium diobovatum* IMB Y-5023 cultures grown on the test media (BYP, CM, YM) at different illuminances (0–5,000 lx). Agglomeration rule: Ward's method, distance metric: Euclidean distance

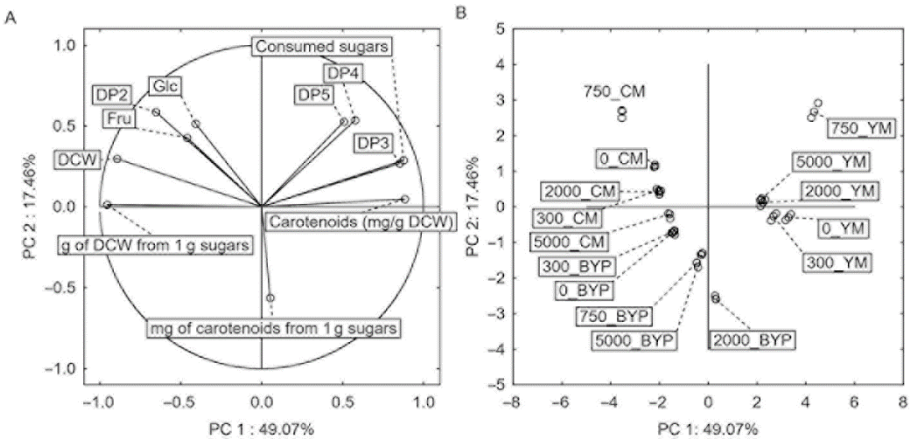

Fig. 2. The PCA of loadings plot (A) and score plots (B) for samples obtained from 5-day cultures of *Rhodosporidium diobovatum* IMB Y-5023 on CM, YM, and BYP media at various illuminances (0–5,000 lx); based on the parameters given in Tables 2 and 3. Clusters were identified using the k-means procedure with the V-fold cross-validation algorithm

ever, some metabolic processes primarily depend on the source of carbon, where light is a catalysing factor only (Friedl et al., 2008).

Table 3 shows the results of our research. As can be seen, light intensity affected the synthesis of cellular biomass and the amount of carotenoids synthesised in the *Rh. diobovatum* IMB Y-5023 cultures. However, cellular metabolism was chiefly influenced by the composition of the culture medium, especially by the concentration of sugars in the medium before fermentation.

The differences in the production of biomass and pigments by *Rh. diobovatum* IMB Y-5023 may have been caused by other components of the test media and by the development of different metabolic pathways in *Rh. diobovatum* IMB Y-5023 cells. The nitrogen content in the medium and the C/N ratio significantly affect the metabolism of oleaginous yeasts such as *Rh. diobovatum*. A high C/N ratio stimulates lipid storage, whereas a low C/N ratio results in significant overproduction of carotenoids (Taskin et al., 2011). *Rhodosporidium diobovatum* IMB Y-5023 may have produced some lipids in the cultures grown on the BYP medium, as indicated by the mucous consistency of the centrifuged yeast biomass pellets. The measurement of the C/N ratio in the test media seems to be a key issue in future studies on *Rh. diobovatum* IMB Y-5023.

Conclusions

1. *Rhodosporidium diobovatum* IMB Y-5023 yeast can grow and produce carotenoids in low-glucose media and it is capable of assimilating DP3 and DP4 oligosaccharides. The cost of industrial carotenoid production with *Rh. diobovatum* IMB Y-5023 can be reduced by using inexpensive by-products from the agri-food industry as growth media.

2. *Rhodosporidium diobovatum* IMB Y-5023 can be cultured in the dark. Illuminance was shown to have almost no effect on the amount of sugars consumed during the growth of yeast on the test media. The costs of illumination of the cultures can be eliminated.

3. The concentration of assimilable carbohydrates in the test media before culturing noticeably influenced the nature of metabolism of the sugars consumed by *Rh. diobovatum* IMB Y-5023. Regardless of the illuminance, the highest cellular yields of carotenoids and the lowest biomass yields were noted in the cultures grown on the YM, where the concentration of sugars was three–four times greater than in the other test media, and where the carbohydrates profile was considerably diversified. The data clustering analysis showed that the culture media with similar initial sugar contents of 11–15 g/l (BYP and CM) had similar effect on the metabolism of *Rh. diobovatum* IMB Y-5023, while the measured variables were completely different in the cultures grown on the YM medium.

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SUBSTRAT WĘGLOWODANOWY W PODŁOŻU JAKO DETERMINANT METABOLIZMU DROŻDŻY *RHODOSPORIDIUM DIOBOVATUM* IMB Y-5023

Abstrakt

Wstęp. Grzyb *Rhodosporidium diobovatum* z klasy Basidiomycetes jest zaliczany do drożdży olejogennych, zdolnych również do syntezy karotenoidów. Nieliczne publikacje naukowe na jego temat wskazują, że jest on obiecującym producentem tych barwników na skalę przemysłową. Jest on zdolny do asymilacji licznych substratów węglowych oraz do wzrostu w różnych, często ekstremalnych, warunkach środowiskowych. Szczepy dzikie są izolowane z gleby, głębin mórz, a także z powierzchni roślin. Mogą zatem rosnąć w ciemności, a także w obecności światła. Celem niniejszej pracy było zbadanie zdolności szczepu *Rh. diobovatum* IMB Y-5023 do wzrostu i kumulacji karotenoidów podczas hodowli na podłożach o różnej zawartości i różnym składzie węglowodanów oraz o małej zawartości monosacharydów. Hodowle prowadzono przy różnym natężeniu oświetlenia. Badania te mają duże znaczenie teoretyczne i praktyczne, ponieważ mogą umożliwić wykorzystanie różnych organicznych odpadów przemysłu rolno-spożywczego jako podłoży do hodowli *Rh. diobovatum* i mikrobiologicznej produkcji karotenoidów. Stwarzają również możliwość redukcji kosztów procesu mikrobiologicznego poprzez dobór optymalnego poziomu oświetlenia hodowli.

Materiał i metody. Hodowle drożdży *Rh. diobovatum* IMB Y-5023 prowadzono na trzech podłożach testowych: na podłożu CM (podłoże marchwiowe), na podłożu BYP (wyciąg z otrąb owsianych) oraz na zmodyfikowanym podłożu YM o zwiększonej zawartości ekstraktu słodowego jako źródła oligosacharydów i z dodatkiem maltotriozy, przy natężeniu oświetlenia w zakresie 0–5000 lx, przez 5 dni. Skład węglowodanów określano w podłożach testowych przed zakończeniem i po zakończeniu hodowli *Rh. diobovatum* IMB Y-5023 z wykorzystaniem chromatografii UHPLC sprzężonej ze spektrometrią mas (MS). Po zakończeniu hodowli kontrolowano również: wydajność biomasy (DCW, g/l), koncentrację barwników w biomasie drożdży (mg/g DCW) oraz współczynnik wydajności biomasy z substratu – $Y_{X/S}$ i wydajności karotenoidów z substratu – $Y_{C/S}$. W celu porównania próbek zastosowano analizę skupień (CA) i analizę składowych głównych (PCA), które wykorzystano do zobrazowania zależności pomiędzy badanymi zmiennymi a analizowanymi próbkami.

Wyniki. Drożdże *Rh. diobovatum* IMB Y-5023 rosły i produkowały karotenoidy na wszystkich podłożach testowych i były zdolne do asymilacji oligosacharydów DP3 i DP4 obecnych w tych podłożach. Bez względu na natężenie oświetlenia hodowli zużycie cukrów w podłożach testowych pozostawało na poziomie 90% i wyższym. Jednak stężenie węglowodanów w podłożu testowym i prawdopodobnie ich rodzaj wyraźnie wpływały na kierunek ich przemian. Niezależnie od poziomu naświetlenia największą koncentrację barwników w komórkach, a jednocześnie najmniejszy plon biomasy uzyskano w hodowlach na podłożu YM. W podłożu tym początkowe stężenie cukrów było trzy–czterokrotnie większe niż w pozostałych podłożach testowych, a ich rodzaj był bardzo zróżnicowany. Analizy skupień (CA) oraz składowych głównych (PCA) wykazały, że podłoża BYP i CM (o zbliżonej początkowej zawartości cukrów: 11–15 g/l) wywarły podobny wpływ na metabolizm drożdży, podczas gdy w hodowlach prowadzonych w podłożu YM badane zmienne różniły się.

Wnioski. Drożdże *Rh. diobovatum* IMB Y-5023 są obiecującym producentem karotenoidów na skalę przemysłową. Są zdolne do efektywnej asymilacji zarówno monosacharydów, jaki i oligosacharydów DP3 i DP4, a obecność światła nie jest wymagana do wzrostu i efektywnej produkcji przez nie barwników, szczególnie podczas wzrostu na podłożach o znacznej koncentracji cukrów, bogatych w oligosacharydy. Te fakty stwarzają możliwość obniżenia kosztów przemysłowej produkcji karotenoidów z udziałem *Rh. diobovatum* IMB Y-5023 poprzez wykorzystanie niedro-

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gich odpadów/produktów ubocznych z branży rolno-spożywczej jako podłoży hodowlanych oraz wyeliminowanie kosztów związanych z oświetleniem hodowli.

Słowa kluczowe: *Rhodosporidium diobovatum,* asymilacja węglowodanów, karotenoidy, hodowla, skład podłoża, oświetlenie

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