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ASTAXANTHIN SYNTHESIS BY *XANTHOPHYLLOMYCES DENDRORHOUS* DSM 5626 AND ITS ASTAXANTHIN OVERPRODUCING MUTANTS ON XYLOSE MEDIA UNDER DIFFERENT ILLUMINATION*

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ABSTRACT

Background. Astaxanthin is the most important and expensive carotenoid pigment used in aquaculture. Its commercial attractiveness is also related with its antioxidant potential. *Xanthophyllomyces dendrorhous* yeast is considered to be promising for commercial production of astaxanthin. The aim of this study was to investigate the possibility of the growth and astaxanthin production by *X. dendrorhous* strains on media containing xylose under different illumination.

Material and methods. *X. dendrorhous* DSM 5626 and its mutants: 10BE and 26UV were used in this study. The cultures were carried out on hydrolysed rye stillage (HS) and YM medium with xylose (YM-K). Cell concentration, total carotenoid and astaxanthin yields were assessed in 5-day cultures. The effect of illumination in the range of 0-5,000 lx on growth and on astaxanthin production of yeasts in cultures run on YM-K medium was also examined.

Results. For the tested yeast strains better growth parameters and astaxanthin yields were obtained on the YM-K medium, on which for all strains the highest pigment yields were recorded at 600-1,000 lx. The highest concentration of astaxanthin in cells was recorded for 26UV in a culture at 1,000 lx (0.51 $g \cdot kg^{-1}$ DCW). The volume yield of the pigment regardless of strain was highest in cultures at 600 lx. In this case 10BE was found to be the best astaxanthin producer with a yield of 2.15 $mg \cdot dm^{-3}$.

Conclusions. Astaxanthin synthesis in *X. dendrorhous* DSM 5626 and its mutants was better on YM-K medium comparing to hydrolysed rye stillage. Moreover, carotenogenesis in the studied yeast strains was subjected to marked photoregulation. Illumination within the range of 600-1,000 lx promotes carotenogenesis and astaxanthin production, while exceeding a certain light capacity results in microbial cell death.

Key words: astaxanthin, culture, xylose, illumination, *Xanthophyllomyces dendrorhous*

INTRODUCTION

Astaxanthin – 3,3'-dihydroxy-β,β-carotene-4,4'-dion – is a xanthophyll, with a chemical formula of $C_{40}H_{52}O_4$ and a molecular mass of 596 Da [Goswami et al. 2010]. From an economic viewpoint, astaxanthin is the second, next to β-carotene, most important carotenoid with a global market size of $219 million in 2007

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(29% of total carotenoid sales). It is estimated to reach $253 million by 2015, with an average annual growth rate of 1.8% [Schmidt et al. 2011].

Astaxanthin commercial application is connected first of all with the feed industry. At present, it is one of the most important and most expensive pigments used in aquaculture [Breithaupt 2007]. It is an obligatory component in feeds for salmon, trout and prawn, providing characteristic pigmentation of their tissues and thus influencing consumer preferences. This pigment is also an indispensable component of feed for aquarium fish and for large ornamental fish [Xu et al. 2006]. It was also shown that astaxanthin has an advantageous effect on coloration of egg yolks as well as skin and meat tissue in carcasses of broiler chickens [Takahashi et al. 2004].

Moreover, astaxanthin is thought to be responsible for the beneficial properties which prevent human diseases including cancer, certain neurodegenerative diseases such as Parkinson's and Alzheimer's disease, oculopathies (cataract, age-related macular degeneration), atherosclerosis and diabetes. It also exhibits anti-inflammatory and immunostimulatory action [Goswami et al. 2010, Pashkow et al. 2008, Yuan et al. 2011]. The beneficial effect of astaxanthin is related to the presence of coupled double bonds in the long hydrocarbon chain as well as polar hydroxyl and carbonyl groups in both ionone rings [Liu and Osawa 2007]. Due to its unique chemical structure: polar-nonpolar-polar, astaxanthin can react with phospholipid head groups or water in the aqueous environment, quenching radicals from the surface of or inside the lipid bilayer of cell membrane [Pashkow et al. 2008]. It protects membrane phospholipids and other lipids against peroxidation more effectively than β-carotene and lutein [McNulty et al. 2007] and shows higher scavenging capacity against peroxyl and hydroxyl radicals than that of α-tocopherol, lutein, lycopene and β-carotene [Rodrigues et al. 2012]. However, some literature data indicate that the antioxidant effect of astaxanthin depends on the adopted experimental model [Gramza-Michałowska and Stachowiak 2010]. Regardless of that fact, astaxanthin is proposed as a component of dietary supplements, functional food and cosmetic agents related to the prevention and control of oxidative stress. Testing results indicate that a daily dose of astaxanthin already at 5 $mg \cdot kg^{-1}$ body weight protects the human organism against oxidative stress [Karppi et al. 2007].

Chemical synthesis is currently acknowledged as the lowest-cost production process of astaxanthin and will very likely remain the main source of this compound in animal feed. The market price of synthetic astaxanthin is around $2,000 per kilogram, while the natural product is sold for more than $7,000 per kilogram [Schmidt et al. 2011]. The natural preparations of astaxanthin are demanded for the cosmetic and nutraceutical market.

Xanthophyllomyces dendrorhous (the perfect state of *Phaffia rhodozyma*) and its astaxanthin overproducing mutants, next to algae *Hematococcus pluvialis*, are the most promising sources of natural astaxanthin. They may utilise different carbon substrates: glucose, maltose, sucrose, cellobiose, xylose, arabinose, lactose and many other, frequently unconventional sources [Palágyi et al. 2001]. Literature data show that higher yields of astaxanthin with *X. dendrorhous* yeast are obtained on media containing xylose than on media containing glucose or other carbon substrates [Vazquez et al. 1997, Parajó et al. 1998, Ananda and Vadlani 2010].

Xylose is a pentose and it is produced commercially by acid hydrolysis of lignocellulose materials. For this reason many research papers concern the potential production of astaxanthin using hardwood or waste from the agri-food sector, containing that substrate. Cultures of *X. dendrorhous* were run on hydrolysates of *Pinus pinaster* [Parajó et al. 1997], *Eucalyptus globulus* [Cruz and Parajó 1998], waste from maize processing [Leathers 2003], barley straw and sugar cane pulp [Montanti et al. 2011].

The application of stillage as a substrate for the production of astaxanthin is considered to be highly promising, as it would be a chance to manage this troublesome waste. Liquid stillage may be a cheap and valuable source of nutrients required for microbial growth [Krzywonos et al. 2009]. In turn, a problem is connected with the low content of dry matter and thus readily available carbon substrate. Nevertheless, fiber may be a source of this substrate. For this reason stillage is used in bioprocesses and it is frequently hydrolysed previous to its use.

The aim of this study was to investigate the growth and synthesis of astaxanthin by *X. dendrorhous* DSM

5626 and its astaxanthin overproducing mutants 10BE and 26UV, obtained previously [Stachowiak 2013 a] on media containing xylose, including hydrolysed rye stillage. The influence of illumination on the production of biomass and astaxanthin in the studied yeasts in cultures on YM-K medium were also investigated.

MATERIAL AND METHODS

Microorganisms

The parental *Xanthophyllomyces dendrorhous* DSM 5626 (= ATCC 24202 and CBS 5905) strain from Deutche Sammlung von Mikroorganismen and Zellkulturen (Braunschweig), and its two astaxanthin hyperproducing mutants, 10BE and 26UV, generated at our laboratory, were used in this study. The mutant 10BE was isolated from medium with ethidium bromide at 1 mg·cm^{-3}. The mutant 26UV was obtained as result of exposure of parental yeast to UV radiation (λ = 254 nm) for 5 min [Stachowiak 2013 a]. Yeasts were maintained on YM agar slants (1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar for solid, pH 5.0) at 4°C until used.

Test media

- YM-K (YM medium with xylose): in the classical medium YM, glucose was replaced by xylose. The YM-K medium composition was as follows: malt extract (BTL, Łódź, Poland) – 3 g; yeast extract – 3 g (BTL, Poland); peptone (Difco Laboratories Inc., France) – 5 g; xylose (BTL, Poland) – 10 g, distilled water – 1 dm^3; pH – 5.0.
- HS (hydrolysed rye stillage): stillage from the Turew distillery (TOP FARMS, Poland) was treated with commercial cellulases/xylanases (OPTIMASH™ VR, Genencor International) at a dose recommended by the producer (1000:3 v/v) for 1 hour at 55°C, at pH = 4.5, under dynamic conditions (150 rpm). Solids were removed from hydrolysed stillage by centrifugation at 2,320 g for 20 min; pH – 5.0.

Cultivation

Yeast strains kept on agar slants were cultivated twice on a liquid test medium. Cultures were grown for 4 days at 22°C in 250-cm^3 Erlenmeyer flasks (working capacity of 80 cm^3, including 5% inoculum) with constant agitation (250 rpm) under illumination of 600 lx. Next, the cultures were run for 120 hours under identical conditions as those given above, and at different illumination levels of 0-5,000 lx.

Lamps emitting light close to natural (Osram, Germany) were used as light sources. Four 18W/77 lamps and four 18W/840 LUMILUX COOL WHITE lamps placed alternately in one frame were applied. Light intensity was controlled using a TES 1335 luxmeter (Tes Electrical Electronic Corp.).

Carotenoid extraction and analysis

Pigments from yeast cells were isolated as follows: 10 cm^3 of culture were centrifuged at 2,000 g for 15 min, the cell pellet was washed twice with distilled water and suspended in 5 cm^3 of DMSO (Sigma-Aldrich, France) preheated to 55°C. The entire volume was vortexed for 30 s and next 5 cm^3 of the hexane fraction from petroleum (POCh, Gliwice, Poland) was added. The volume was again vortexed for 30 s and 20% NaCl (BTL, Poland) aqueous solution was added in batches at 0.5 cm^3. The hexane phase with pigments was separated by centrifugation at 2,000 g for 15 min and recovered.

Total carotenoid, astaxanthin yields and sugar contents in samples were determined with a Waters Alliance HPLC System 2695 (Milford, MA, USA) with PAD detection (Waters Z690). Analysis conditions of pigments were described by Stachowiak [2013b] and sugar analyses were described by Szwengiel et al. [2007].

Biomass was measured by DCW (dry cell weight). Total nitrogen content was analysed by the Kjeldahl method and total carbon content was determined by Dr Lange cuvette test [Handbook... 2000].

Statistical analysis

All experiments were performed in triplicate. Statistical calculations were made in the Statistica 6.0 and MS Excel 2003 programs.

RESULTS AND DISCUSSION

Development of yeasts and production of carotenoids on test media with xylose

At first stage yeasts were cultured on YM-K and HS media at a constant illumination intensity of

600 lx. The detailed profile of sugars and their contents for YM-K medium were as follows: glucose (0.6 g·dm^{-3}), xylose (10 g·dm^{-3}), maltose (1.3 g·dm^{-3}); total – 11.9 g·dm^{-3}. In the case of HS medium, the contents of sugars were: glucose (5.3 g·dm^{-3}), xylose (11.5 g·dm^{-3}), maltose (3.7 g·dm^{-3}); total – 20.5 g·dm^{-3}. Therefore, for both media, xylose was the main carbon substrate. All identified sugars are readily metabolised by *X. dendrorhous* DSM 5626 and tested mutants [Palágyi et al. 2001, Stachowiak 2012].

For all yeasts better growth was recorded in cultures on the YM-K medium (Table 1). In this case the biomass yield was 15-20% higher than those recorded in cultures on hydrolysed stillage and values of $Y_{X/S}$ on YM-K medium were over two times higher. Upon completion of cultures in the YM-K medium total sugar consumption was 80% regardless of the strain and only the presence of xylose was found. Sugars were used in 100% in yeast cultures on hydrolysed stillage.

On YM-K medium, 50% higher cell yields (g·kg^{-1} DCW) and volume yields of astaxanthin (mg·dm^{-3}) were recorded for the parental strain (Table 1). In the case of both mutants the cell yield of astaxanthin was approx. 40% higher in comparison to the culture on stillage, while the volume yield amounted to 50%.

Table 1. Effect of medium composition on growth parameters and carotenoid production by *Xanthophyllomyces dendrorhous* DSM 5626 and its mutants

Parameter	DSM 5626	10BE	26UV
YM-K medium			
DCW, g·dm^{-3}	5.70 ±0.18	5.47 ±0.38	5.10 ±0.10
$Y_{X/S}$, g·g^{-1}	0.58 ±0.02	0.55 ±0.02	0.52 ±0.03
Carotenoids, mg·dm^{-3}			
– total	1.67 ±0.09	2.26 ±0.11	1.94 ±0.22
– astaxanthin	1.60 ±0.07	2.15 ±0.14	1.82 ±0.22
Carotenoids, g·kg^{-1} DCW			
– total	0.29 ±0.01	0.41 ±0.01	0.38 ±0.02
– astaxanthin	0.28 ±0.01	0.39 ±0.01	0.36 ±0.03
Astaxanthin, %	96 ±0.79	95 ±0.24	94 ±0.53
HS medium			
DCW, g·dm^{-3}	4.83 ±0.48	4.43 ±0.31	4.24 ±0.57
$Y_{X/S}$, g·g^{-1}	0.24 ±0.01	0.22 ±0.02	0.21 ±0.02
Carotenoids, mg·dm^{-3}			
– total	0.72 ±0.02	1.15 ±0.08	1.08 ±0.06
– astaxanthin	0.68 ±0.02	1.03 ±0.03	0.98 ±0.05
Carotenoids, g·kg^{-1} DCW			
– total	0.15 ±0.01	0.26 ±0.02	0.25 ±0.01
– astaxanthin	0.14 ±0.01	0.23 ±0.03	0.23 ±0.02
Astaxanthin, %	94 ±2.50	90 ±2.15	91 ±0.52

Astaxanthin was the main pigment produced by all tested yeasts. Its share in the total carotenoids was very high and amounted to 90-96% depending on the strain and composition of the medium. A slightly lower share of astaxanthin in total carotenoids was recorded in cultures run on stillage.

In the presented results hydrolysed rye stillage turned out to be a relatively poor medium for growth and astaxanthin production by tested yeasts. It may result from many factors, the most important being the initial C/N ratio, the initial sugar concentration and oxygen supply. Literature data indicate that a high initial C/N ratio promotes the production of carotenoids and fatty acids (both compounds are synthesized *via* the mevalonate pathway) however, it is accompanied by a limited protein synthesis, resulting in a low biomass yield [Hu et al. 2005]. It is suggested that excess C, ATP and NADPH formed as a result of limited protein synthesis is used by yeasts to produce carotenoids and fatty acids [Flores-Cotera et al. 2001]. On HS medium used in these experiments the C/N ratio was 6.78, while in the case of YM-K it was 1.54. A lower yield of yeast biomass was actually obtained on HS medium for all tested strains (at complete sugar consumption) when compared to YM-K medium (at 80% sugar consumption), although the differences were not spectacular. However, in yeast cultures on HS medium lower cell yields of total carotenoids were produced, with the yield of astaxanthin reduced by 50% depending on the strain. Vustin et al. [2004] indicated that the initial C/N ratio below 5 has a negative effect on the synthesis of carotenoids in *X. dendrorhous*, so poor growth of studied yeasts, low values of $Y_{X/S}$ and low yields of astaxanthin recorded in this study in cultures on HS might be the result from the deficit of some compounds, e.g. specific amino acids, macro- and microelements or vitamins. Some of them (e.g. vitamin B) are essential for the appropriate development of *X. dendrorhous.* Although vitamin B complex is present in the stillage [Krzywonos et al. 2009], following enzymatic hydrolysis stillage was centrifuged and all solids were removed, including also residual yeast, being a source of nitrogen and vitamins B. Such prepared medium was most probably too deficient in substrates other than carbon and in biostimulants in contrast to YM-K medium, which completely covered the nutritional requirements for yeast.

A decisive effect on carotenogenesis in the case of *X. dendrorhous* is also found for the initial sugar concentration and oxygen supply. *Xanthophyllomyces dendrorhous* is Crabtree-positive and a change from aerobic metabolism to aerobic fermentation occurs at a 5% concentration of sugars in the culture medium [Reynders et al. 1997]. In the tested media the initial sugar content varied. In YM-K medium it was approx. 1.2%, while in HS medium it was approx. 2.05%. Thus this factor should have no effects on primary metabolism in yeasts.

In turn, two-fold greater sugar concentration in HS medium could have resulted in a lower astaxanthin production in broth cultures. Ananda and Vadlani [2010] considered high viscosity of the culture medium, leading to lower diffusion of oxygen, to have been the cause of this result. When running 5-day cultures of *Phaffia rhodozyma* ATCC 24202 (the parental strain used in this study) on media containing whole maize stillage they obtained a very low yield of astaxanthin (0.026 $\mu g \cdot g^{-1}$ of freeze-dried stillage), while β-carotene was the primary pigment produced by *P. rhodozyma*. It accounted for as much as 75% total pigments, while typically astaxanthin constituted 83-90% or even 100% of the total carotenoids of *P. rhodozyma* [Schmidt et al. 2011]. Under microaerophilic conditions β-carotene is accumulated at the expense of astaxanthin.

Recorded experimental results indicate that stillage is not a profitable medium for the strains tested in this study. The composition of stillage may probably be optimised, e.g. by concentration, supplementation with an adequate source of nitrogen, minerals, vitamins or other nutrients and on its basis a medium may be prepared for profitable production of astaxanthin using yeasts *X. dendrorhous*. However, each such attempt generates costs. In this study spent stillage (contained 8.6% of dry matter) was only subjected to cheap, enzymatic hydrolysis in order to increase the pool of sugars assimilated by *X. dendrorhous*. Prior to hydrolysis it was 3.8 $g \cdot dm^{-3}$, while yeast growth was practically negligible.

However, it needs to be stressed that growth and astaxanthin production in cultures in stillage or media enriched with stillage depend on used yeast strain.

For example, Bon et al. [1997] in their experiments obtained a 2-fold higher yield of biomass in cultures of two wild strains of *P. rhodozyma* and mutant JB2 run on 70% TS (thin stillage) than on the conventional YM medium. However, only in the case of the mutant the carotenoid production was identical in TS and YM. For the wild strains a drastic reduction of pigment production on TS was observed.

The tested YM-K medium proved to be more suitable for the production of astaxanthin than stillage. For the tested strains on this medium better growth parameters and astaxanthin were obtained in comparison to YM medium with glucose [Stachowiak 2013 a]. Literature data indicate that the best results on growth parameters, astaxanthin proportion and carotenoid productivity were obtained in media containing xylose [Vazquez et al. 1997, Parajó et al. 1998, Montanti et al. 2011].

The effect of illumination intensity on development of tested yeasts and carotenogenesis in cultures on YM medium with xylose

The effect of illumination intensity on the controlled parameters in cultures run on YM-K medium is presented in Table 2. Light intensity had a significant effect on growth and carotenogenesis in the tested yeasts ($p < 0.05$). For all strains the highest yield of biomass was produced in cultures at illumination intensity of 600 lx. Both weaker and stronger illumination intensity inhibited yeast growth. A particularly drastic effect was observed in cultures at 5,000 lx, at which practically no growth of yeasts was detected.

In the yeast cultures the yield of astaxanthin as well as total carotenoids initially increased with an increase in illumination intensity. Generally the highest yields of pigments were recorded within the range of 600-1,000 lx. The highest cellular concentration of astaxanthin was detected for mutant 26UV cultured at 1,000 lx (Table 2). Unfortunately, the biomass yield in this case was low and eventually only a slight volume yield of the pigment was obtained.

Regardless of the strain the greatest amounts of astaxanthin per 1 dm^3 culture were produced at 600 lx. Mutant 10BE proved to be the best producer of the pigment with the yield of astaxanthin amounting to $2.15 \cdot dm^{-3}$. Illumination above 1,000 lx inhibited carotenogenesis. Similarly as in the case of biomass, 5,000 lx turned out to critical in this respect. Astaxanthin was the main pigment produced by the tested yeasts irrespective of the culture illumination level. With an increase in illumination intensity its share in the total carotenoids increased by as much as almost 100% at 1,000-5,000 lx.

Carotenoid production and accumulation are reported to be positively affected by illumination in many microorganisms. Light generates free oxygen radical and carotenogenesis is considered to be one of the basic photoprotection mechanisms [Bhosale 2004]. According to literature data, light stimulates carotenogenesis and the production of astaxanthin in *X. dendrorhous*. However, its level is very often differently specified. Improved volume yields of carotenoids were reported by Vázquez [2001] in *Phaffia rhodozyma* flask cultures run under illumination of 500 lx comparing to cultures run in the dark. De la Fuente et al. [2010] obtained very high yields of biomass and astaxanthin when illuminating flask fermentations from the beginning with ultraviolet and white light of 1,000 lx. In turn, An and Johnson [1990] described a situation when in plate culture strong light inhibited growth and carotenogenesis in yeast *X. dendrorhous*. Tropea et al. [2013] reported that astaxanthin yield of $0.97\,g \cdot kg^{-1}$ DCW was obtained after fed batch cultivation of *X. dendrorhous* in the conventional reactor and in the enlightened reactor lower values, at about $0.93\ g \cdot kg^{-1}$ DCW, were found.

Results of the presented experiments indicate that carotenognesis in *X. dendrorhous* DSM 5626 and its mutant is subjected to marked photoregulation and illumination within the range of 600-1,000 lx promotes carotenogenesis and their production of astaxanthin, while exceeding a certain light capacity results in microbial cell death. Thus at commercial synthesis of astaxanthin using *X. dendrorhous* yeast, the level of culture illumination seems to be a major factor affecting its profitability. Based on the results of this study and those reported in literature sources it may be stated that this parameter needs to be specifically adapted to each strain individually.

Table 2. Effect of illumination on growth and carotenoid production by *Xanthophyllomyces dendrorhous* DSM 5626 and its mutants in 5-days culture on YM-K medium

Parameter	Illumination, lx					
	0	300	600	1000	2000	5000
DSM 5626						
DCW, g·dm^{-3}	3.33 ±0.06	4.03 ±0.05	5.70 ±0.08	4.75 ±0.02	4.36 ±0.15	0.70 ±0.12
Carotenoids, mg·dm^{-3}						
– total	0.37 ±0.04	1.09 ±0.13	1.67 ±0.11	1.64 ±0.20	1.31 ±0.09	0.00
– astaxanthin	0.32 ±0.03	0.98 ±0.12	1.60 ±0.11	1.57±0.20	1.28 ±0.09	0.00
Carotenoids, g·kg^{-1} DCW						
– total	0.11 ±0.01	0.27 ±0.03	0.29 ±0.02	0.34 ±0.04	0.30 ±0.01	0.00
– astaxanthin	0.09 ±0.01	0.24 ±0.03	0.28 ±0.01	0.33 ±0.04	0.29 ±0.01	0.00
Astaxanthin, %	86	90	96	96	98	0
10BE						
DCW, g·dm^{-3}	4.80 ±0.01	4.77 ±0.31	5.47 ±0.06	4.57 ±0.05	4.11 ±0.10	0.87 ±0.05
Carotenoids, mg·dm^{-3}						
– total	1.28 ±0.12	1.67 ±0.25	2.26 ±0.16	1.69 ±0.11	1.27 ±0.04	0.00
– astaxanthin	1.22 ±0.11	1.59 ±0.24	2.15 ±0.15	1.62 ±0.11	1.22 ±0.04	0.00
Carotenoids, g·kg^{-1} DCW						
– total	0.27 ±0.02	0.35 ±0.03	0.41 ±0.03	0.37 ±0.03	0.31 ±0.01	0.00
– astaxanthin	0.25 ±0.02	0.33 ±0.03	0.39 ±0.02	0.36 ±0.03	0.30±0.01	0.00
Astaxanthin, %	95	95	95	96	96	0
26UV						
DCW, g·dm^{-3}	1.80 ±0.15	3.33 ±0.32	5.10 ±0.17	3.43 ±0.03	3.37 ±0.06	0.77 ±0.06
Carotenoids, mg·dm^{-3}						
– total	0.39 ±0.05	1.46 ±0.29	1.94 ±0.16	1.85 ±0.04	0.81 ±0.08	0.10 ±0.02
– astaxanthin	0.27 ±0.04	1.23 ±0.24	1.82 ±0.15	1.76 ±0.04	0.78 ±0.07	0.10 ±0.02
Carotenoids, g·kg^{-1} DCW						
– total	0.22 ±0.02	0.44 ±0.05	0.38 ±0.02	0.54 ±0.01	0.24 ±0.02	0.13 ±0.01
– astaxanthin	0.15 ±0.01	0.37 ±0.04	0.36 ±0.02	0.51 ±0.01	0.23 ±0.02	0.13 ±0.01
Astaxanthin, %	68	84	94	95	96	97

CONCLUSIONS

Presented results reveal that (1) astaxanthin synthesis in *X. dendrorhous* DSM 5626 and its mutants was better in YM-K medium comparing to hydrolysed rye stillage, (2) carotenogenesis in the studied yeast strains was subjected to marked photoregulation and (3) illumination within the range of 600-1,000 lx promotes carotenogenesis and astaxanthin production, while exceeding a certain light capacity results in microbial cell death.

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SYNTEZA ASTAKSANTYNY PRZEZ *XANTHOPHYLLOMYCES DENDRORHOUS* DSM 5626 I JEGO NADPRODUKUJĄCE ASTAKSANTYNĘ MUTANTY NA PODŁOŻACH Z KSYLOZĄ, PRZY RÓŻNYM NATĘŻENIU OŚWIETLENIA

STRESZCZENIE

Wstęp. Astaksantyna jest najważniejszym i najdroższym karotenoidem stosowanym w akwakulturze. Jej komercyjna atrakcyjność wynika również z wysokiego potencjału przeciwutleniającego. Obecnie drożdże *Xanthophyllomyces dendrorhous* wydają się najbardziej obiecującymi mikroorganizmami w przemysłowej produkcji tego barwnika. Celem pracy było zbadanie możliwości wzrostu i produkcji astaksantyny przez szczepy *X. dendrorhous* w hodowlach na podłożach z ksylozą oraz zbadanie wpływu oświetlenia na karotenogenezę.
Materiał i metody. W badaniach użyto szczep *X. dendrorhous* DSM 5626 oraz jego mutanty: 10BE i 26UV. Hodowle przeprowadzono na zhydrolizowanym wywarze żytnim (HS) i na podłożu YM z ksylozą (YM-K). Po pięciu dniach w hodowlach kontrolowano: wydajność biomasy oraz komórkową i objętościową wydajność karotenoidów i astaksantyny. W hodowlach na podłożu YM-K oceniano również wpływ natężenia oświetlenia w zakresie 0-5000 lx na wzrost drożdży i karotenogenezę.
Wyniki. Dla badanych szczepów drożdży lepsze parametry wzrostu i wydajności astaksantyny odnotowano w hodowlach na podłożu YM-K niż na HS. W hodowlach na podłożu YM-K najlepsze wydajności astaksantyny uzyskano przy natężeniu oświetlenia 600-1000 lx. Największą komórkową koncentrację

astaksantyny odnotowano dla szczepu 26UV w hodowli przy 1000 lx (0,51 $g \cdot kg^{-1}$ DCW), natomiast objętościową dla 10BE, w hodowli przy 600 lx (2,15 $mg \cdot dm^{-3}$).
Wnioski. Badane szczepy *X. dendrorhous* DSM 5626 i jego mutanty rosły lepiej i syntetyzowały więcej astaksantyny na podłożu YM-K niż na zhydrolizowanym wywarze żytnim. Ponadto u badanych drożdży karotenogeneza podlegała silnej fotoregulacji. Natężenie oświetlenia w zakresie 600-1000 lx sprzyjało syntezie karotenoidów, w tym astaksantyny.

Słowa kluczowe: *Xanthophyllomyces dendrorhous,* astaksantyna, synteza karotenoidów, natężenie oświetlenia, ksyloza

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