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REGULAR ARTICLE

PRODUCTION OF ENRICHED BIOMASS BY RED YEASTS OF *SPOROBOLOMYCES* SP. GROWN ON WASTE SUBSTRATES

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ABSTRACT

Carotenoids and ergosterol are industrially significant metabolites probably involved in yeast stress response mechanisms. Thus, controlled physiological and nutrition stress including use of waste substrates can be used for their enhanced production. In this work two red yeast strains of the genus *Sporobolomyces* (*Sporobolomyces roseus, Sporobolomyces shibatanus*) were studied. To increase the yield of metabolites at improved biomass production, several types of exogenous as well as nutrition stress were tested. Each strain was cultivated at optimal growth conditions and in medium with modified carbon and nitrogen sources. Synthetic media with addition of complex substrates (e.g. yeast extract) and vitamin mixtures as well as some waste materials (whey, apple fibre, wheat, crushed pasta) were used as nutrient sources. Peroxide and salt stress were applied too, cells were exposed to oxidative stress (2-10 mM H_2O_2) and osmotic stress (2-10 % NaCl). During the experiment, growth characteristics and the production of biomass, carotenoids and ergosterol were evaluated. In optimal conditions tested strains substantially differed in biomass as well as metabolite production. *S.roseus* produced about 50 % of biomass produced by *S.shibatanus* (8 g/L). Oppositely, production of pigments and ergosterol by *S.roseus* was 3-4 times higher than in *S.shibatanus*. *S.roseus* was able to use most of waste substrates, the best production of ergosterol (8.9 mg/g d.w.) and beta-carotene (4.33 mg/g d.w.) was obtained in medium with

crushed pasta hydrolyzed by mixed enzyme from *Phanerochaetae chrysosporium*. Regardless very high production of carotenes and ergosterol, *S.roseus* is probably not suitable for industrial use because of relatively low biomass production.

Keywords: *Sporobolomyces* sp., red yeasts, carotenoids, ergosterol, waste substrates, stress

INTRODUCTION

Red yeasts are ubiquitous microorganisms. They occur in soil, fresh and marine water, on plants, are commonly associated with animals and are also found frequently in man-made habitats such as foods **(Rosa and Péter, 2005**). The environment presents for yeasts a source of nutrients and forms space for their growth and metabolism. On the other hand, yeast cells are continuously exposed to a myriad of changes in environmental conditions (reffered to as environmental stress). These environmental changes may be of a physical or chemical nature: temperature, radiation, concentrations of solutes and water, presence of certain ions, toxic chemical agents, pH and nutrient availability. In nature, yeast cells often have to cope with fluctuations in more than one such growth parameter simultaneously **(Hohmann and Mager, 2003)**. Understanding yeast requirements is important for successfull cultivation of yeast in the laboratory but also for optimization of industrial fermentation process (**Walker, 1998**).

Carotenogenic yeasts are a diverse group of unrelated organisms (mostly *Basidiomycota*) and the majority of the known species are distributed in four taxonomic groups: the *Sporidiobolales* and *Erythrobasidium* clade of the class *Urediniomycetes*, and *Cystofilobasidiales* and *Tremellales* of the class *Hymenomycetes* (**Libkind *et al.*, 2005**). The genus *Rhodotorula* includes three active species; *Rhodotorula glutinis*, *Rhodotorula minuta* and *Rhodotorula mucilaginosa* (formerly known as *Rhodotorula rubra*) (**Hoog *et al.*, 2001**). Colonies are rapid growing, smooth, glistening or dull, sometimes roughened, soft and mucoid. They are cream to pink, coral red, orange or yellow in color. *Rhodotorula* is well known for its characteristic carotenoids: torulene, torularhodin and β-carotene. *Rhodotorula glutinis* is also reported to accumulate considerable amount of lipid (**Perrier *et al.*, 1995**).

The genus *Sporobolomyces* contains about 20 species. The most common one is *Sporobolomyces roseus* and *Sporobolomyces salmonicolor* (**Hoog *et al.*, 2001**). *Sporobolomyces* colonies grow rapidly and mature in about 5 days. The optimal growth temperature is 25-30 °C. The colonies are smooth, often wrinkled, and glistening to dull. The

bright red to orange color of the colonies is typical and may resemble *Rhodotorula* spp. *Sporobolomyces* produces yeast-like cells, pseudohyphae, true hyphae, and ballistoconidia. The yeast-like cells are the most common type of conidia and are oval to elongate in shape (**Hoog *et al.*, 2001**).

Carotenoids are the most pronounced, naturally occurring pigments. They are of great interest in many scientific disciplines because of wide distribution and diverse functions. (**Britton *et al.*, 2008**). Carotenoids have the ability to act as antioxidants and thus protect cells against photooxidation. The ability of carotenoids to quench singlet oxygen is well known and reactions with radical species have also been studied (**Edge *et al.*, 1997**). Dietary carotenoids inhibit onset of many diseases in which free radicals are thought to play a role in initiation, such as atherosclerosis, cataracts, age-related macular degeneration, multiple sclerosis and most importantly cancer **(Bhosale, 2003**). Antioxidant properties of carotenoids are exploited also in cosmetics. A cosmetic preparations comprising the carotenoids were reported to be effective in preventing various kinds of damage resulting from oxidation and exposure to UV light (**Britton *et al.*, 2008**). Carotenoids are popular also in food industries as colourants and vitamin A sources. Carotenoid-containing preparations are also playing important role as feed additive. Astaxanthin is the major carotenoid used for pigmentation of fishes and salmons **(Nelis and Leenheer, 2008**).

Ergosterol, one of the most important components in fungal membranes, is involved in numerous biological functions, such as, membrane fluidity regulation, activity and distribution of integral proteins and control of the cellular cycle. Ergosterol pathway is fungal-specific; plasma membranes of other organisms are composed predominantly of other types of sterol (**Tan *et al.*, 2003)**. Biosynthesis of ergosterol similarly to carotenoids and other isoprenoid compounds (e.g. ubiquinone), is derived from acetyl-CoA in a three-stage synthehtic process (**Metzler, 2003)**. It should be noted that isoprenoid pathway is of great importance in secondary metabolism.

Carotenogenic yeasts are considered to be ubiquitous due to its world-wide distribution in terrestrial, freshwater and marine habitats, and to its ability to colonize a large variety of substrates. They can assimilate various carbon sources, such as glucose, xylose, cellobiose, sucrose, glycerol, sorbitol, etc. For this reason, various waste materials can be used as cheap substrates for its cultivation. The red yeast is able to grow under a wide range of initial pH conditions from 2.5 to 9.5 and over a wide range of temperatures from 5 to 26°C **(Frengova and Beshkova, 2009; Libkind *et al.*, 2008**).

Under stress conditions the red yeast accumulates higher quantity of carotenoids (**Marova *et al.*, 2004)**. This is of increased interest to the biotechnology. The use of this stressed biomass in feed industry could have positive effect not only in animal and fish feeds because of high content of physiologically active substances, but it could influence nutritional value and organoleptic properties of final products for human nutrition. Knowledge of molecular mechanism of the carotenoid production stimulation can then lead to improvement of such biotechnological process **(Latha *et al.*, 2005)** .

Recently, some studies focused on production of carotenoids and enriched biomass mainly by *Rhodotorula* strains was presented **(Marova *et al.*, 2010; 2011)**. In presented work two of non-traditional red yeast strains of the genus *Sporobolomyces* were studied. Production properties of tested strains were influenced by exogenous stress, nutrition stress and by waste substrates used as alternative carbon and nitrogen sources.

MATERIAL AND METHODS

Yeast and fungal strains

Yeast strains *Sporobolomyces roseus* CCY 19-4-8 and *Sporobolomyces shibatanus* CCY 19-20-3 were purchased from Collection Culture of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia.

Fungal strains *Alternaria alternata* CCM 8326, *Aureobasidium pollulans* CCM F-148 and *Phanerochaete chrysosporium* CCM 8074 were obtained in Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno.

Cultivations

Red yeasts were cultivated in a simple glucose medium aerobically at 28 °C. Physiological stress was induced by nutrition components (C and N source) and by addition of 2 and/or 5 mM peroxide and 2 % and/or 5 % NaCl.

Three series of cultivations were realized with each strain. Two-step inoculation was done. All strains were firstly inoculated into a medium containing yeast extract (7 g), $(NH_4)_2SO_4$ (5 g), glucose (40 g), KH_2PO_4 (5 g), $MgSO_4$ (0.34 g) per liter (INO I) and cultivated at 28 °C for 24 hours at permanent shaking and lighting. Second inoculum (INO II)

was prepared similarly, in 1st series and 3rd series the same medium as INO I was used, in 2nd series lyophilized whey was added (7 g/L). Cultivation in INO II undergo at 28 °C for 24 hours at permanent shaking and lighting. Production media contained $(NH_4)_2SO_4$ (5 g), glucose (40 g), KH_2PO_4 (5 g), $MgSO_4$ (0.34 g) per liter. Several waste substrates were added and cultivation was done for 80 hours at 28 °C under permanent lighting and shaking. Production media were prepared according to following scheme:

a) 1st series: INO I---INO II --- production: sample Nr. 1 – control, Nr. 2 – 5 mM peroxide, Nr. 3 – 2 % NaCl, Nr. 4 – 5 % NaCl, Nr. 5 – lyophilized whey non-processed (7 g/L), Nr. 6 – lyophilized whey processed by deproteination agent (7 g/L), Nr. 7 – potato extract (Hi Media; 7 g/L)
b) 2nd series: INO I --- INO II (+ whey, 7 g/l)--- production: sample Nr. 1 – control, Nr. 2 – 5 mM peroxide, Nr. 3 – 2 % NaCl, Nr. 4 – 5 % NaCl, Nr. 5 – lyophilized whey non-processed (7 g/L), Nr. 6 – lyophilized whey processed by deproteination agent (7 g/L)
c) 3rd series: INO I ---INO II --- production: Hample Nr. 1 – control; Nr. 2 –5 apple fiber; symplex Nr. 6 – 9 pasta crush; symplex Nr. 10 – 13 wheat; to each waste medium 3 types of mixed hydrolytic enzyme preparative obtained from lyophilized cell medium of: E1... *Alternaria alternata* CCM 8326, E2… *Aureobasidium pollulans* CCM F-148; E3… *Phanerochaete chrysosporium* CCM 8074.

Whey waste substrate was obtained from dairy industry (Pribina Ltd., Pribyslav, Czech Republic) and its composition was as followed: water (94 %), dry weight 60 g/L; ash 31 g/L; lactose 40 g/L; glucose 0.4 g/L; phosphorus 63 mg/L; soluble proteins 2 g/L; total nitrogen 0.12 %. Whey substrate was either lyophilized without processing or processed by deproteination. Whey was acidified by 0.1 mol/L H_2SO_4 to pH 4.6, proteins were precipitated by boiling for 20 min and removed by centrifugation (5 000 rpm; 10 min). Before cultivation was pH adjusted to neutral by 1 mol/L NaOH. Cereal waste substrates were purchased in local markets. Media with waste substrates (without enzymes) were autoclaved for 20 min at 121 °C. Fungal extracellular mixed enzyme preparatives were obtained by lyophilization of cell-less media after 5-day cultivation at CCM recommended conditions for individual strains. Three parallel experiments were carried out with each strain and each substrate combination. Average values and standard deviations were evaluated.

Extraction and analysis of carotenoids and other metabolites

Cells were collected by centrifugation (3000 rpm; 30 min). For the subsequent isolation of carotenoids, the whole biomass obtained from 250 ml of medium was used. Yeast cells were disintegrated using a mechanical disruption by shaking with glass beads (70 – 100 U.S sieve). A mixture of pigments, sterols and other organic compounds was extracted from the cell homogenate using 50 ml of acetone. After saponification of the extract by ethanolic KOH, carotenoids were extracted twice with 50 ml of diethyl ether. The diethyl ether extracts were collected and dried under vacuum. After evaporation, the residue was dissolved 1-2 ml of methanol (gradient grade) and used for HPLC chromatographic analysis.

Carotenoid pigments extracted from yeast cells were individually identified and quantified by RP-HPLC using a chromatographic system described previously **(Marova *et al.*, 2004; 2010).** Samples (10 microliter valve) were filtered through PTFE filters and injected onto Zorbax EclipsePlus C18 column (150 x 4.6 mm, 5 μm; Agilent Technologies) that had been equilibrated with a mobile phase (methanol/water; 95:5). Isocratic elution was carried out at 45 °C by a flow rate of 1.0 ml/min. Detection of carotenoids was achieved at 450 nm. Data processing of analyses was assessed using Clarity software. Individual carotenoids were verified by on-line LC/MS/ESI analysis (Mass spectrometer LCQ Advantage Max, Thermo Finnigan).

RESULTS

Characterization of red yeasts

In order to study yeast physiology under different conditions, it is important to know so called “reference parameters” which these yeasts possess under optimal condition. Red or carotenogenic yeasts are well known producers of valuable carotenoids. On agar plates they form characteristic yellow, orange and red coloured colonies. Red yeast can be of ellipsoidal or spherical shape (Fig 1). Under optimal conditions (28°C, 100 rpm, permanent lighting) they are able to grow up in 5 to 7 days. The growth curve of carotenogenic yeast is characterized by two-stepped course with long stationary phase (Fig 2).

Growth characteristics in stress conditions

Comparison of presented growth characteristics (Figs 2-5) led to some partial conclusions about growth of red yeasts. Both tested *Sporobolomyces* strains reached stationary phase after about 50 hours of cultivation. All strains also exhibited prolonged stationary phase with at minimum one, more often with several growth maxima. First growth maximum was observed in all strains after about 80 hours of growth.

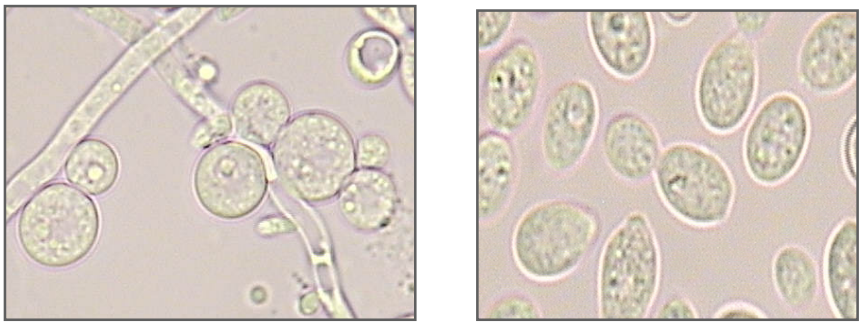

Figure 1 Microscopic image and streak plate of *Sporobolomyces roseus (A) and Sporobolomyces shibatanus (B)*

In strains followed for longer time than 100 hours additional local growth maxima was observed after 105 – 140 hours. Carotenogenic yeasts probably utilize some endogenous substrates accumulated at the beginning of stationary phase. Growth maxima are mostly accompanied with carotenoid production maxima, mainly in first 90 hours of cultivation **(Marova *et al.*, 2004; 2010)**. For all stress and waste experiments cultivation in production media was carried out for 80 hours (to first production maximum) to eliminate potential growth inhibition caused by nutrient starvation or toxic effect of stress. Longer cultivation can be also complicated by higher ratio of dead and living cells and in semi-large-scale and large-scale experiments also with higher production costs.

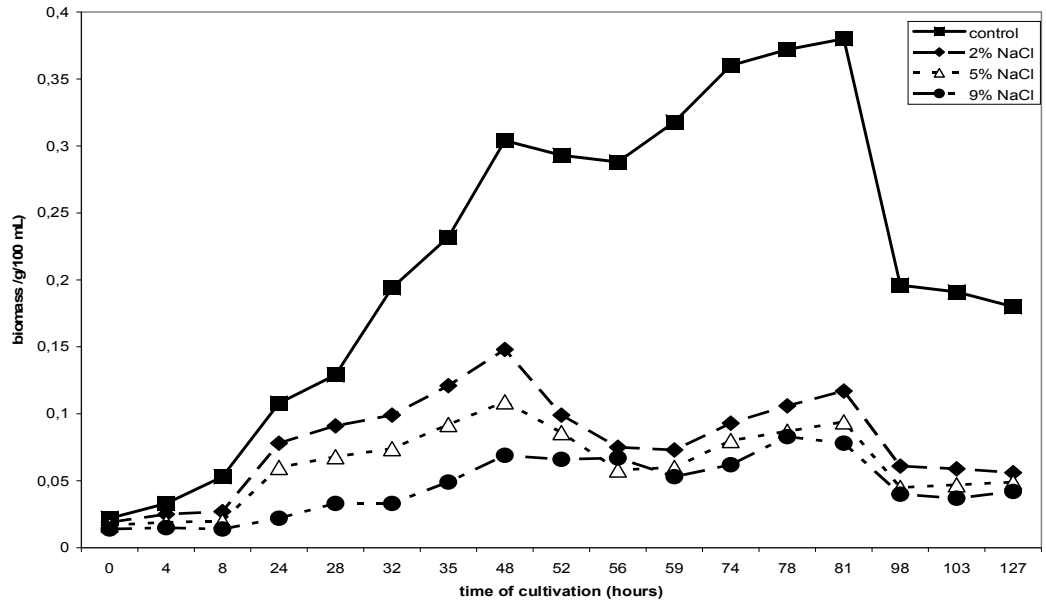

Figure 2 Growth of *Sporobolomyces roseus* under osmotic stress

According to our results, tested *Sporobolomyces* strains differed in growth characteristics under oxidative as well as osmotic stress. Nevertheless, growth diferences in both types of stress were very similar. In *Sporobolomyces shibatanus* used relatively low concentrations of oxidative and osmotic stress, which can under specific conditions induce carotenogenesis, have no significant effect on yeast growth (Figures 3, 5). Oppositely, in *S.roseus* addition of 2 mM peroxide or 2 % salt led to dramatic decrease of biomass (Figures 2, 4). Further increase of stress factor concentration exhibited minimal effect on biomass production decrease.

Production characteristics in optimal conditions

Production properties of both tested strains at optimal conditions are compared in Figure 6. Although *S.shibatanus* produced about 2 times higher amount of biomass (8.08 g/L) when compared with *S.roseus*, production of metabolites was in *S.roseus* substantially higher. The highest difference was observed in beta-carotene production, which was in *S.roseus* about 20 times higher than in *S.shibatanus* (2112 ug/g and 94 ug/g, respectively). Simultaneously, about 3x higher production of total carotenoids and ergosterol was observed in *S.roseus*.

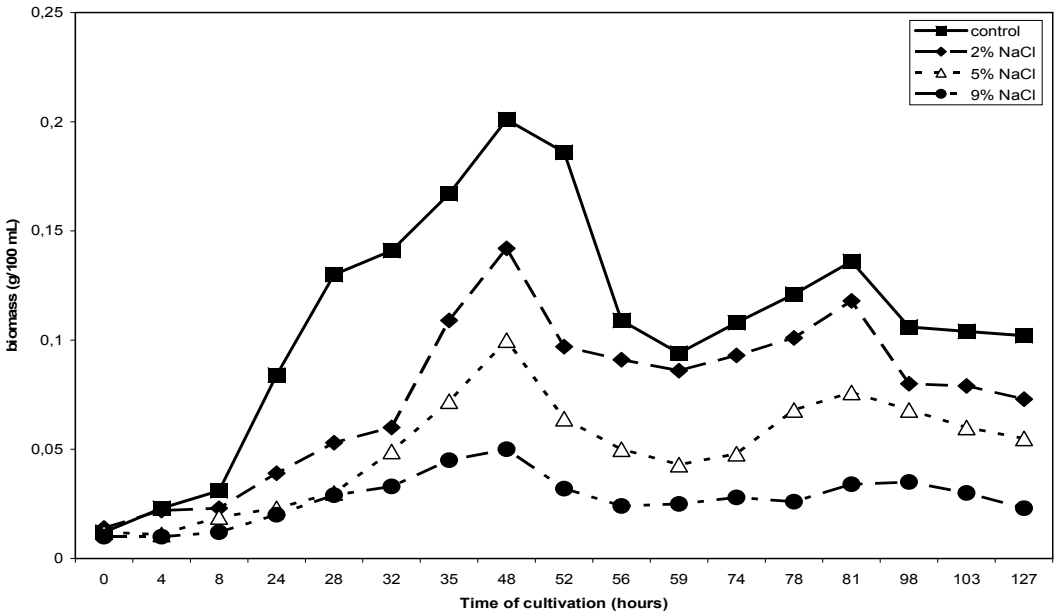

Figure 3 Growth of *Sporobolomyces shibatanus* under osmotic stress

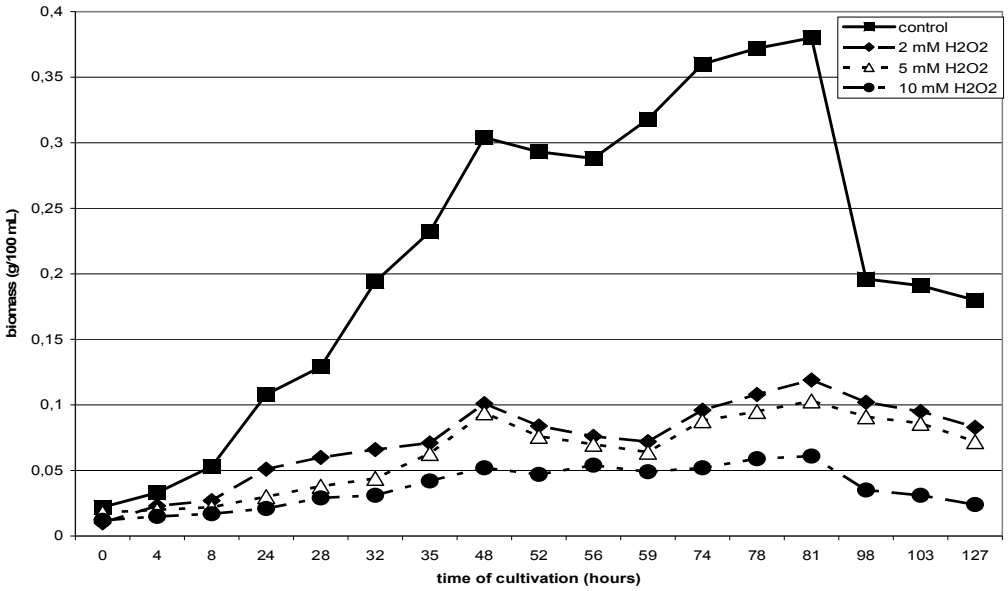

Figure 4 Growth of *Sporobolomyces roseus* under oxidative stress

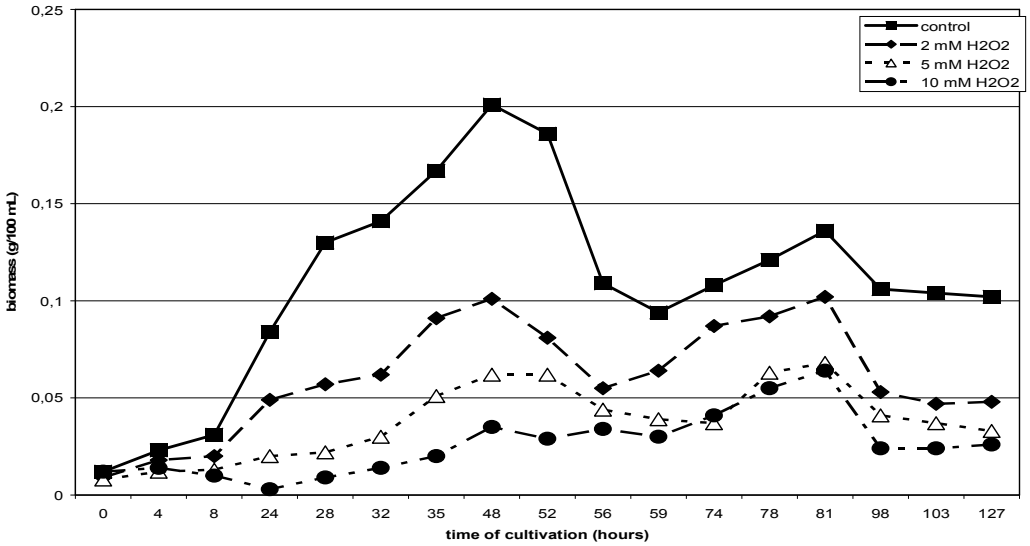

Figure 5 Growth of *Sporobolomyces shibatanus* under oxidative stress

Production properties using waste substrates

Because of extremely low production of carotenoids and ergosterol in *S.shibatanus* strain, further experiments focused on cultivations on waste substrates combined with stress influence were performed only using *S.roseus* cultures. As waste substrates whey (liguid, processed and lyophilized), apple fibre, wheat and crushed pasta were used.

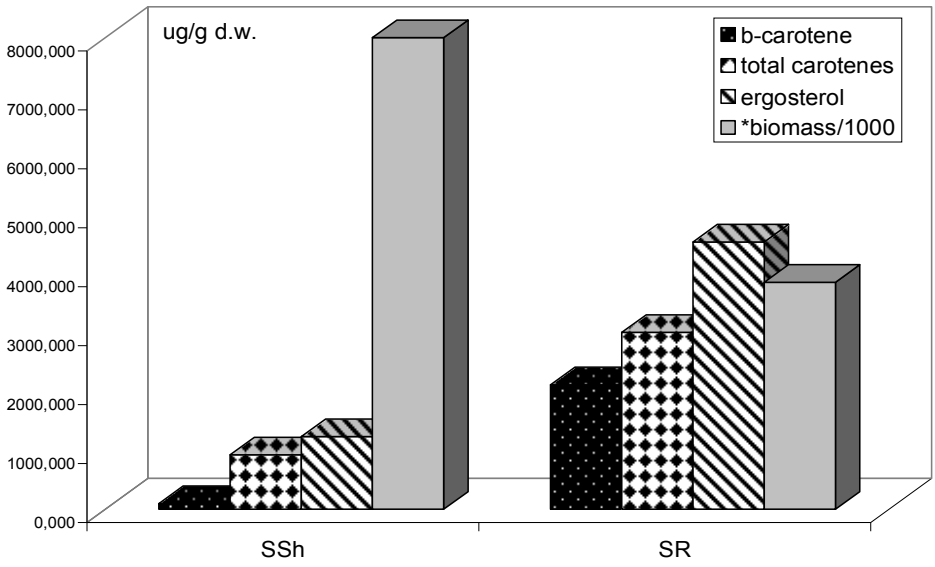

Figure 6 Production properties of analyzed *Sporobolomyces* strains in optimal conditions

Sporobolomyces roseus (Table 1) exhibited significant changes in biomass:carotene ratio dependent on whey substrate addition. 20-fold increase of β-carotene production when compared with control (wildtype) was observed in yeast *Sporobolomyces roseus* cultivated on non-processed lyophilized whey added into inoculation and production media (11.47 mg of β-carotene/l culture fluid; 2580.8 μg of β-carotene/g CDW). High intracellular accumulation of β-carotene was achieved also on deproteined lyophilized whey added either only in production media (2780.5 μg of β-carotene/g CDW) or in both, inoculation and production media (1710.20 μg of β-carotene/g CDW) (Tab 1).

We can conclude that in whey medium substantial biomass decrease in presence of lyophilized whey in INO II (under 5 g/L) was accompanied by very high beta-carotene yield. Nevertheless, total production of biomass by *S.roseus* was about 4 – 5 g/L, which is at minimum 2-times lower as in *R.glutinis* (**Marova *et al.*, 2010)**. So, this is the reason why *S.roseus* CCY 19-4-8 cells is less suitable to enriched biomass production

Table 1 Production of biomass and pigments on whey medium including stress and adaptation in INO II - Erlenmeyer flasks; *Sporobolomyces roseus* CCY 19-4-8.

Medium composition INO I	INO II	production	**Biomass** (g/L)	**beta-carotene** (μg/g d.w.)
Nr. 1/Control	Control	Control	5.82 ± 0.93	65.60 ± 23.27
Nr. 2/Control	Control	Peroxide 5 mmol/L	4.84 ± 1.36	440.80 ± 120.00
Nr. 3/Control	Control	Salt 5 %	4.26 ± 1.05	1500.60 ± 123.40
Nr. 4/Control	Control	Whey lyophilized	5.20 ± 0.80	2520.30 ± 225.36
Nr. 5/Control	Control	Whey deprotein.	5.25 ± 1.15	2780.50 ± 331.12
Nr. 6/Control	Whey deprotein.	Control	5.37 ± 0.89	625.50 ± 147.20
Nr. 7/Control	Whey deprotein.	Peroxide 5 mmol/L	4.92 ± 0.66	930.00 ± 220.10
Nr. 8/Control	Whey deprotein.	Salt 5 %	3.86 ± 0.65	1350.80 ± 369.82
Nr. 9/Control	Whey deprotein	Whey lyophilized	4.71 ± 0.59	2580.80 ± 258.80
Nr. 10/Control	Whey deprotein	Whey deprotein.	5.35 ± 1.11	1710.20 ± 450.10

Legend: INO I …inoculum I, INO II …inoculum II; 1-10...sample number

In yeast strain *Sporobolomyces roseus* grown in whey media also dramatic ergosterol accumulation was observed in cells cultivated in liquid whey (3.13 μg/g dry cells; not shown).

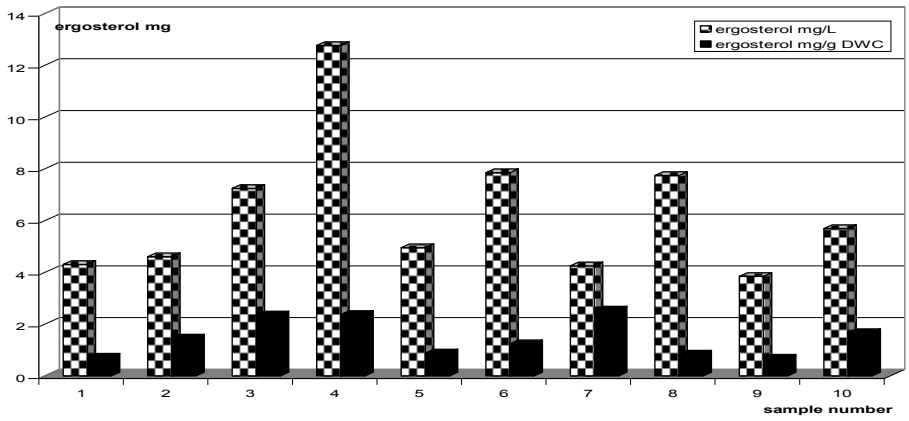

Figure 7 Ergosterol production by *Sporobolomyces roseus* growing on whey substrates

In Figure 7 prodution of ergosterol in mg/L of culture and mg/g DWC is documented. Similarity of values of these two parameters is further evidence for very low biomass production. The best result of ergosterol production was achieved in media with lyophillized, non-processed whey (12.76 mg/l culture fluid). Appropriate biomass production is illustrated in Tab 1.

In Table 2 results of biomass, carotenoids and ergosterol production by *S.roseus* cells in media with some cereal waste substrates are introduced. In some cultivations waste substrates were processed by orientational 24 hour hydrolysis of lyophilized mixed raw preparations of fungal enzyme preparatives. These preparatives were sterilized by ultrafiltration before addition to media. Enzyme activites of individual hydrolases were not measured in this experiment.

When compared non-processed waste substrates, the highest production of biomass was observed in medium with crushed pasta. Addition of fungal enzymes led to slightly increased production of biomass in aple fiber and wheat medium. Production of carotenoids and ergosterol was significantly influenced by type of waste substrate and type of enzyme preparative. The best production was obtained in medium with crushed pasta processed by enzyme mix from *P.chrysosporium*.

Table 2 Production of biomass and beta-carotene by *S.roseus* CCY 19-4-8 cultivated on cereal substrates processed by hydrolytic enzymes

Waste substrate	**Enzyme**	**Biomass** (g/L)	**Ergosterol** (μg/g d.w.)	**Beta-carotene** (μg/g d.w.)
Control	0	3.84 ± 0.90	5434.5± 666.70	2119.86 ± 356.70
Apple fiber	0	3.73 ± 0.56	5158.2± 885.12	1822.17 ± 120.00
Apple fiber	E1	4.51 ± 1.00	2890.5± 302.16	3209.03 ± 425.40
Apple fiber	E2	4.04 ± 0.76	7128.2± 883.84	2544.87 ± 189.12
Apple fiber	E3	3.35 ± 0.95	6494.7± 877.10	3438.45 ± 402.80
Crushed pasta	0	4.29 ± 0.84	7449.6± 496.58	4328.92 ± 272.60
Crushed pasta	E1	4.21 ± 0.80	3106.2± 286.86	1914.50 ± 158.20
Crushed pasta	E2	3.82 ± 0.58	5188.2± 472.12	2988.23 ± 319.00
Crushed pasta	E3	3.80 ± 0.98	8890.8± 448.86	4319.16 ± 408.44
Wheat	0	4.35 ± 0.72	5989.7± 925.44	2478.40 ± 324.80
Wheat	E1	3.93 ± 0.92	1676.2± 382.10	546.72 ± 80.50
Wheat	E2	3.97 ± 0.64	7290.2± 558.44	3736.74 ± 464.40
Wheat	E3	4.46 ± 1.01	6721.8± 662.22	2103.39 ± 198.24

Legend: E1...mixed enzyme preparative from *Alternaria alternata* CCM 8326, E2… mixed enzyme preparative from *Aureobasidium pollulans* CCM F-148; E3… mixed enzyme preparative from *Phanerochaete chrysosporium* CCM 8074

DISCUSSION

The number of red yeasts species *Rhodotorula, Rhodosporidium, Sporidiobolus, Sporobolomyces, Cystofilobasidium* and *Phaffia* are known as producers of carotene pigments. Among yeasts, *Rhodotorula* species is one of main carotenoid-forming microorganisms with predominant synthesis of β-carotene, torulene and torularhodin (**Davoli et al., 2004**). Nevertheless, although there are many strategies for stimulation of carotene biosynthetic machinery in yeasts, attention is still focused on unexplored yeast's habitats for selection of hyper-producing strains what is the important step towards the design and optimization of biotechnological process for pigment formation (**Libkind and van Broock, 2006; Maldonade *et al.*, 2008**).

In *Sporobolomyces* species, the main produced pigment is beta-carotene. Production of torulenes was not clearly confirmed, nevertheless, some oxidized carotenoid derivatives are produced in both studied *Sporobolomyces* strains. In most of red yeasts oxidative and osmotic stress was reported to induce carotenoid production probably as a part of stress response. Phenotypic profiling of the oxidative and osmotic stress responses demonstrates genetic susceptibility of yeast to environmental stress. Measurements of cell viability and growth provide versatile and sensitive assays for characterization of cytotoxic effect of environmental stress. Yeast cells exhibit a graded concentration-dependent response to chemically induced stress: continued growth, cellular adaptation, checkpoint arrest/growth delay, apoptosis, and necrosis. Standardized assay conditions are required to quantify yeast growth curves. In our work strong differences in growth properties under stress were confirmed in tested strains. Different sensitivity to stress may be related to character of natural environment and adaptation mechanisms activated in individual strains dependent on environmental effects. However, the mentioned results are just pivot because the adaptation and growth delay responses are difficult to measure reproducibly in shake flask cultures. This problem can be alleviated by an automated cell growth assay in a microculture **(Weiss *et al*., 2004)**. Yeast cells in the microculture format maintain uniform growth conditions that enable highly precise and reproducible assays of chemical sensitivity.

In recent preliminary experiments with *Sporobolomyces roseus* CCY 19-4-8 cultivated in laboratory fermentor substantially higher production of biomass was obtained when compared with cultivation in flasks. Mainly in whey medium about 3-times biomass increase (about 12 g/L) was reached and production of beta-carotene was mostly higher than in *R.glutinis*. Because of low biomass production, total yields were in *S.roseus* mostly lower than in *R.glutinis* cells. The best beta-carotene production (29.4 g/L) was obtained on whey medium **(Marova *et al*., 2011)**.

In order to improve the yield of carotenoid pigments and subsequently decrease the cost of this biotechnological process, diverse studies have been performed by optimizing the culture conditions including nutritional and physical factors. Factors such as nature and concentration of carbon and nitrogen sources, minerals, vitamins, pH, aeration, temperature, light and stress have a major influence on cell growth and yield of carotenoids. Numerous sources have been considered as potential carbon sources for biotechnological production of carotenoids (**Bhosale and Gadre, 2001; Lukacs *et al*., 2006**). Work of **Tinoi *et al.*** (2005) demonstrates the effectiveness of using a widely available agro-industrial waste product as

substrate and the importance of the sequential simplex optimization method in obtaining high carotenoid yields.

In our work possibility to use waste substrates and, moreover, to increase the biomass and pigment production in waste/stress medium was confirmed. Carotenoid yields obtained in this work appeared comparable with those reported in the literature **(Frengova and Beshkova, 2009)**. Also ergosterol production by carotenogenic yeasts is significant. Mentioned compounds increase biological value and so exploitation of the yeast. This carotene-enriched biomass can be used as source of carotenoid pigments or as animal feed. Unfortunately, in *Sporobolomyces* strains with excellent production of pigments and other metabolites relatively low biomass production was observed.

In the future, other types of waste materials (for instance from winemarket) are intended to be tested as carbon sources for carotenogenesis in red yeasts. Moreover, application of an environmental stress in combination with waste materials can lead to overproduction of carotenoids and lipids and decrease cost of their production. Such strategies could result into production of yeast biomass rich not only in carotenoids and other provitamins, but also in other nutrition components (proteins, PUFA, metal inos etc.) that originate both from yeast cells and from cultivation substrates. This is the way to production of complex food additives based on naturally enriched yeast biomass.

CONCLUSION

In this work production of biomass enriched by carotenoids and ergosterol by two strains of *Sporobolomyces* – *S.roseus* and *S.shibatanus* were studied. Both strains were cultivated in optimal conditions. Production properties of these strains were substantially different. *S.shibatanus* produced about 2-times higher amount of biomass than *S.roseus*, which was accompanied by about 20x lower production of beta-carotene and 3x lower ergosterol production. With regard to low production activity of *S.shibatanus*, further experiments with waste substrates were performed by *S.roseus* only.

Changes in medium composition can lead to substantial changes in biomass as well as carotenoid production. Waste substrates can be used as medium component, which can in particular strains and conditions induce carotenoid as well as biomass production. Thus, waste substrates could be used industrially for carotenoid-rich biomass production. In this work, S.*roseus* CCY 19-4-8 was able to use most of waste substrates, the best production of ergosterol (8.9 mg/g d.w.) and beta-carotene (4.33 mg/g d.w.) was obtained in medium with

crushed pasta hydrolyzed by mixed enzyme from *Phanerochaetae chrysosporium*. Regardless very high production of carotenes and ergosterol, *S.roseus* could be probably not recommended for industrial use neither enriched biomass, nor pigments alone, because of very low biomass production.

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