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THE EFFECT OF $ZnSO_4$ IN THE CULTIVATION MEDIUM ON *RHODOTORULA GLUTINIS* CCY 020-002-033 YEAST BIOMASS GROWTH, β-CAROTENE PRODUCTION AND ZINC ACCUMULATION

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ABSTRACT

The goal of this work was to evaluate the effect of zinc sulphate heptahydrate on growth rate and β-carotene production and to obtain zinc-enriched biomass using selected yeast strain *Rhodotorula glutinis* CCY 020-002-033. The batch cultivation was performed on synthetic basal medium supplemented with various concentrations of $ZnSO_4 \cdot 7H_20$ (0.025%, 0.035%, and 0.050%), in shake flasks and aerobic conditions, at 30^0C, for five days. Total zinc accumulated in yeast cells after fermentation period was performed using ICP-OES. The identification and yield of β–carotene were investigated by chromatographic analysis (HPLC). It was found that the amount of total zinc accumulated in yeast cells depends mainly on the zinc concentration in the culture medium. The highest zinc content of 7.030 mg/g dry biomass was obtained at optimal concentration of 0.025%, with an incorporation efficiency of 87%. High concentration of 0.050% was not tolerated by yeast. Zinc salt in concentration of 0.025%, acted as stimulant for volumetric β–carotene but not improve yeast growth biomass. β-Carotene maximum content in this strain's cell mass accounted 7.1 mg/L, when the dry cell biomass (1.89 g/L) was smaller than control (1.94 g/L), although there was no significant difference observed in final dry cell biomass versus control. The obtained results showed that when $ZnSO_4 \cdot 7H_20$ concentration was increased up to 0.050% a decrease in yeast growth rate, β–carotene production and zinc accumulation was observed.

Keywords: zinc sulphate, *Rhodotorula glutinis*, yeast biomass, β-carotene, zinc accumulation

INTRODUCTION

Yeasts are able to bioaccumulate essential mineral salts from aqueous solutions through mechanisms as production of metalloproteins, mineralization and capture of metals into vacuoles (**Eide, 1997**). *Saccharomyces cerevisiae* has become a model microorganism for studying trace metals uptake, transporters and their accumulation in the cells (**Nelson, 1999**). There are several reports (**Bhosale, 2004; Buzzini *et al.*, 2005; Marova *et al.*, 2011**) regarding the effect of supplementation of metal ions (Mg, Zn, Cu, Ca) in culture medium on metals-enriched biomass obtaining and stimulation of yeast growth and production of carotenoids. *Saccharomyces* strains were investigated for their ability to accumulate high concentrations of Cu, Zn and Mn salts to obtain ions enriched biomass (**Barbulescu *et al.*, 2009; Šillerová *et al.*, 2012; Azad *et al.*, 2014**) and the "pink yeast" *Rhodotorula glutinis* to demonstrate the stimulatory effect of Ba, Fe, Mg, Ca, Zn, Co divalent cations on growth rate and volumetric production of carotenoids (**Bhosale *et al.*, 2001; Buzzini *et al.*, 2005; Martínez *et al.*, 2006; Dai *et al.*, 2007**). This unicellular yeast, wide-spread in nature, is well known to biosynthesize, in low (less than 100 µg/ g dry biomass) (Besarab et al., 2018), medium (101 up to 500 µg/g) and high (more than 500 µg/g dry biomass) (**Buzzini *et al.*, 2000; Issa *et al.*, 2016**) proportions, β-carotene, torulene and torularhodin, specific pigments of high economic value and great biotechnological importance.

Thus, due to the capacity to accumulate metals, *Rhodotorula* biomass can be used as a supply both for minerals and carotenoids for functional food products. Among minerals, $zinc^{2+}$ is an essential micronutrient which plays an important role in yeast growth, in metabolism of proteins, nucleic acids, carbohydrates, lipids, and in the control of gene transcription due to stable association with macromolecules and coordination flexibility (**Rebar *et al.*, 2004; Valee *et al.*, 1993**). International Union of Biochemistry and Molecular Biology (IUBMB) established 6 enzymes classes in which zinc participates as cofactor: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (**Valee *et al.*, 1993**). Also, zinc is associated with oxidant defense system, maintaining an adequate level of metallothionein, and is integral part of Cu/Zn superoxide dismutase (SOD), the primary cellular defense against oxidative stress in most organisms (**Bogani *et al.*, 2013**). To obtain zinc-enriched biomass, *Rhodotorula* yeast has to be grown in culture medium supplemented with optimal concentration of zinc.

This work aims to examine the impact of different zinc sulphate concentrations on *Rhodotorula glutinis CCY 020-002*-033 yeast biomass growth and β-carotene production and to evaluate the enrichment of biomass with divalent zinc ions. This yeast strain was isolated from willow leafs, grown in synthetic medium at batch scale level, at optimal growth conditions, in order to assess its application as feed additive. Very little published information is available on the production of zinc enriched biomass and β-carotene by *R. glutinis CCY 020-002*-033.

MATERIALS AND METHODS

Materials

All reagents used in the experiments were of analytical grade. Yeast extract, malt extract and peptone were purchased from OXOID (UK), dextrose and agar from FLUKA (Sigma-Aldrich, Germany) and $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4.7H_2O$ and $ZnSO_4 \cdot 7H_20$ from CHIMREACTIV SRL (Romania). The 99.8% pure β-carotene standard, batch no. 133111, was purchased from Dr. EHRENSTORFER GmbH (Germany).

Microorganism

Rhodotorula glutinis CCY 020-002-033, isolated from willow leafs, was purchased from Culture Collection of Yeast (CCY), Bratislava, Slovakia and was maintained on YMA (Yeast Malt Agar) (g/L: 70 malt extract, 15 agar) or YPD (Yeast extract Peptone Dextrose) (g/L: 10 yeast extract, 20 peptone, 20 dextrose, 15-20 agar) slant, transferred monthly, and stored at 4 °C, in darkness.

Inoculum preparation

Four loop-full of *Rhodotorula glutinis CCY 020-002-033* strain were transferred from YPD agar slants into 100 mL flasks containing each 20 mL of sterile liquid ATCC 200 medium (g/100 mL: yeast extract 0.3, malt extract 0.3, peptone 0.5, dextrose 1) and incubated in orbital shaker at 30^0 C, 150 rpm. For each sample,

an approximate cell density was calculated, assuming, that an OD_{600} of 1.0 corresponds to exponential phase (about $1.3x10^7$ cells/mL). This concentration of yeast cells in suspension was confirmed by plate count (data not shown). The inoculum was used for the production medium.

Cultivation of red yeast in medium with zinc salt

In present work, **Azad *et al.* (2014)** method was used for cultivation of yeast with Zn divalent cations (Zn^{2+}). The cultivation of *R. glutinis* CCY 020-002-033 was carried out on liquid basal medium (g/100 mL: glucose 2, yeast extract 1, $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.5, $MgSO_4.7H_2O$ 0.05), used as control for growth of red yeast and liquid basal medium supplemented with various concentrations of $ZnSO_4 \cdot 7H_20$ (0.025%, 0.035%, and 0.050%). The addition of sterilized $ZnSO_4$ solutions was made at the beginning of inoculation (time 0).
The pH of basal solution was adjusted to 6 prior to autoclaving and inoculation. The yeast was grown in batch system. The batch processes were performed in 250 mL Erlenmeyer flasks, containing 50 mL of basal medium and basal medium supplemented with $ZnSO_4.7H_2O$ salts, respectively, which were inoculated with a 10% aliquot of inoculum reported to total volume of the fermentation medium, and incubated at optimal growth conditions (chosen based on previous work): aerobically, on a rotary shaker, at 150 rpm and 30 °C, for 120 h. The experiment was carried out in triplicate.
The viability of yeast cells developed both in experimental growth media containing $ZnSO_4.7H2O$ concentrations ranged between 0.025% - 0.050% and in medium without supplementation (control) was determined by counting colony-forming units (CFU/mL of cell culture), after 48 - 72 h of incubation at 30 ^{0}C on ATCC agar plates.

Analytical methods

Yeast biomass growth rate determination

Yeast cell growth rate during batch cultivation was monitored based on spectrophotometer Specord M42 UV-VIS, measuring optical density of the culture at 600 nm wavelength (OD_{600}) at 24 h intervals. The samples with optical density greater than 1.0 were diluted 1:10 with distilled water and optical density read again. At the end of cultivation period, the yeast biomass was collected by centrifugation (5000 rpm, 15 min), the cells sediment washed twice with sterile distilled water to remove minerals and zinc sulfate residues from the medium, centrifuged two times and drying at 60 °C to constant weight to determinate the dry biomass, which was expressed in $g.L^{-1}$ of culture. In order to characterize yeast growth, biomass concentration was calculated as follows:

$$\text{Biomass concentration (X g L}^{-1}) = \frac{X}{V}$$

where X is the weight of dried biomass (g/L), V is the sample volume (mL).
The dry biomass was used for analysis of zinc and β-carotene content.

Quantification of the total zinc accumulated in yeast cells

Total zinc accumulated in yeast cells was performed using ICP-OES (Optima 2100 DV Perkin Elmer), after fermentation period. Briefly, the ground dry biomass samples were roasted in the oven at 550^{0}C in a platinum crucible to obtain ash, which, after weighing and calculating the percentage of ash relative to dry matter, is disaggregated with HNO_3 (65%) and H_2O_2 (30%) in the Multiwave 3000 microwave digester (Anton Parr GmbH, Austria) the equipment having a combined pressure - temperature sensor and also an IR based temperature sensor. It is filtered through filter paper. For the determination of zinc content in biomass the Optima 2100 DV ICP-OES System (Perkin Elmer) with dual view optical system was used. It combines the radial and axial view of the plasma in a single sequence and functions as a transistor based radiofrequency generator with 40 MHz frequency. The system consists of a nebulizator PEEK Mira Mist® coupled with Baffled Cyclonic spraying chamber. The spectrometer consists of an optical module which includes an Echelle mono-chromator with bi-dimensional, charged coupled device, detector. The spectral domain is between 165 and 800 nm. Determination of zinc content was done after the appropriate dilutions to fit on the calibration curves, at λ = 213,857 nm. The total amount of zinc was calculated in mg/g dry weight and accumulation efficiency of zinc in yeast biomass was determined using the formula
% accumulation = m/M x 100
where m= zinc content (mg) determinate in cell biomass, M= the amount of zinc from $ZnSO_4 \cdot 7H_20$ initially incorporated in medium (mg)

Extraction and quantification of β-carotene

Pigmented cells of *Rhodotorula glutinis* CCY 020-002-033 were collected by centrifugation of 50 mL samples of culture medium. In all samples, the β-carotene pigment extraction process was achieved by DMSO and acetone followed by an extraction with light petroleum with the protocol described by **Weber *et al.* (2007**). After rotary-evaporated concentration to dryness at 40^{0}C the residue was taken up with acetone, filtered through a 0.45 μm filter and 10 μL was injected into HPLC.
Chromatographic analysis was performed on an Agilent 1100 system equipped with a diode array detector (DAD), connected to a ChemStation software. The separation was performed with a Zorbax SB-C18 column (150 mm×4.6 mm, 5μm). A gradient system was used: eluent A was water and eluent B was acetone. The flow rate was 1 $mL \times min^{-1}$ and the injection volume was 10 μL. The elution conditions applied were: 0 min. 70% B, 0-15 min., linear gradient from 70 to 100 % B, 15-20 min. 100% B. Detection was performed at 450 nm. A stock solution containing 200 $mg.L^{-1}$ β-carotene standard was prepared and diluted with acetone to an appropriate concentration in the range of 0.2-120 $mg.L^{-1}$.

Statistical analysis

All experiments were conducted in a randomized pattern. Average values of triplicates and standard deviations were evaluated.

RESULTS AND DISCUSSION

This study looked at the effect of $ZnSO_4 \cdot 7H_20$ added in the culture media on *Rhodotorula glutinis* CCY 020-002-033 strain growth rate, β-carotene production and zinc accumulation in cells. The results are presented in the table 1 and were obtained from triplicate experiments. Yeast strain was exposed to different concentrations of $ZnSO_4 \cdot 7H_20$ and compared with non-exposed yeast cells (control).

Table 1 Effect of $ZnSO_4 \cdot 7H_20$ on *Rhodotorula glutinis CCY 020-002*-033

Parameters	Control	$ZnSO_4 \cdot 7H_20$ concentration, %		
		0.025	0.035	0.050
Dry biomass g/L	1.94	1.89	0.69	0.16
Volumetric β-carotene mg/L	2.4	7.1	3.1	0.92
Total zinc accumulated mg/g dry matter	2.083	7.030	3.173	0.120

Yeast growth rate test

The monitoring of *Rhodotorula glutinis CCY 020-002-033* growth rate in the presence of $ZnSO_4 \cdot 7H_20$ in culture medium was performed daily by measuring the optical density at 600 nm (OD_{600}) over 120 h of incubation. Absorbance measurements showed an increase in absorbance with time, the growth rate being higher in the first 24 hours after inoculation, but lower compared to control (Fig 1).

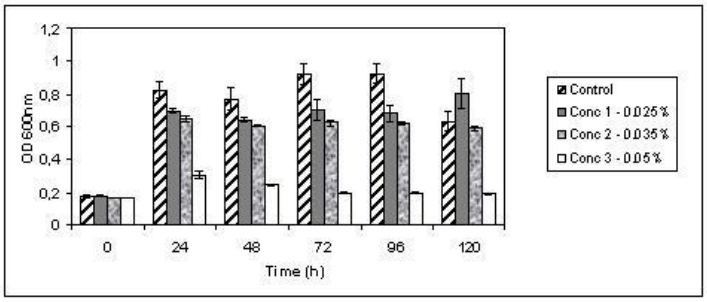

Figure 1 The influence of three different $ZnSO_4 \cdot 7H_20$ concentrations (C1-C3) on the growth rate of *Rhodotorula glutinis CCY 020-002-033*.**Legend**: control sample is without addition of $ZnSO_4 \cdot 7H_20$. The results are presented as the mean obtained. Error bars show standard deviation (SD)

The absorbance measurements showed that growth rate of control samples (without supplementation with Zn salt) increases with the incubation time, reaching the maximum at about 72 h and afterwards remained constant, decreasing at the end of the incubation period, according to normal phases of yeasts growth curve. In the experimental samples during the incubation period of 120 hours, the cultures revealed a dose-dependent progressive delay of growth until inhibition. In the presence of 0.25% $ZnSO_4 \cdot 7H_20$, growth of culture was readily inhibited compared to control. Some authors (**Bromberg *et al.*, 1997**) observe that zinc concentrations do not affect cell growth rate, but affect fermentation performance. **Azad et al. (2014**) report an improvement in cell growth rate of yeast *Saccharomyces cerevisiae* under the action of $ZnSO_4$ in concentration of 5 $mg.L^{-1}$ added to the culture medium, due to its involvement in a variety of enzymatic reactions and structural and functional roles in cell metabolism (**Hosiner *et al.*, 2014**). As the concentration of $ZnSO_4$ increases, there was a decrease in the growth rate of yeast culture. The culture exposed to the 0.035% concentration showed a decrease in growth rate during cultivation, compared to the lowest concentration and the control. By increasing the $ZnSO_4 \cdot 7H_20$ concentration to 0.050% inhibition of yeast growth was sever. The

percentage of viable cells decreased with increasing concentrations of Zn^{2+} in the medium. Excessive metal ions alter cellular functions. Zinc ions, in high concentration in culture medium, may be toxic because can compete with other metal ions for the active sites of enzymes or intracellular transport proteins causing inactivation of cells and loss of viability with decrease in yeast growth (**Liu *et al*., 1997; Colin et al., 2003**). Also, **Konopka et al. (1999)** report that the yeast biomass development decreases as the concentration of the heavy metal increases. The toxicity may be due to lack or low levels of manganese ions required for yeast to tolerate high levels of zinc (**Jones *et al*., 1994).**

Yeast biomass dry weight

Referring to effect of zinc ions on yeast biomass dry weight, it was observed that at the lowest concentration of $ZnSO_4 \cdot 7H_20$ in the culture medium (0.025%), biomass production was not affected. In contrast, as the zinc concentration increased up to 0.050%, the amount of biomass decreased (Fig. 2).

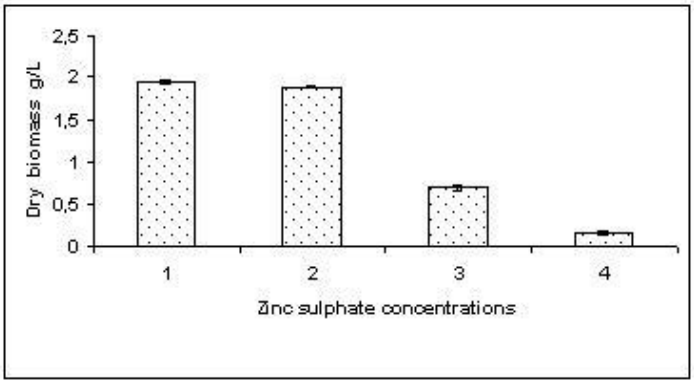

Figure 2 The effect of zinc sulfate on *Rhodotorula glutinis CCY 020-002-033* biomass yield. **Legend**: 1-control without addition of $ZnSO_4 \cdot 7H_20$ in culture medium; $ZnSO_4 \cdot 7H_20$ concentrations: 2 - 0.025%, 3 - 0.035%, 4-0.050%. The results represent the mean obtained. Error bars show standard deviation (SD)

The highest biomass production of 1.94 g/L was recorded in control and the lowest of 0.16 g/L in medium containing a $ZnSO_4 \cdot 7H_20$ concentration of 0.050% (tab. 1). So, $ZnSO_4 \cdot 7H_20$ in low concentration do not promote yeast growth, but the excess level of concentrations, above 0.025%, had significant toxicity leading to inhibition of cell growth and ultimately to cell death. Similar results have obtained **Sillerova *et al*. (2012**) on *Saccharomyces cerevisiae*, using different zinc salts in fixed concentrations of 0, 25, 50 and 100mg.100 mL^{-1}, without changing the amount of dry yeast biomass. Some investigators (**Boshale *et al*., 2001**) observe that divalent cations act as stimulants for biomass produced by wild strains of *Rhodotorula glutinis* and *Rhodotorula rubra.*

Evaluation of total zinc accumulation

After fermentation, the concentration of zinc ions in yeast cells was performed. In the figure 3 is presented the total quantity of Zn accumulated by *Rhodotorula* yeast cells.

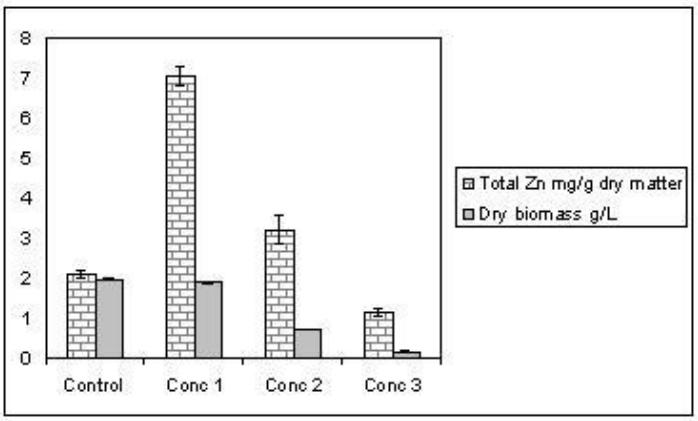

Figure 3 Total zinc accumulation in *Rhodotorula glutinis CCY 020-002-033* dry biomass. **Legend**: control-without addition of $ZnSO_4 \cdot 7H_20$ in culture medium; different $ZnSO_4 \cdot 7H_20$ conc.:1 - 0.025%, 2 - 0.035%, 3 - 0.050%. The results represent the mean obtained. Error bars show standard deviation (SD)

After 120 h of incubation, the highest intracellular zinc content in the yeast cells was obtained in the medium supplemented with 0.025% $ZnSO_4 \cdot 7H_20$, and yeast biomass and the zinc content in yeast biomass reached 1.89 g/L and 7.030 mg/g dry matter, respectively, without any reduction of growth rate or viability. The accumulation efficiency of zinc in biomass was 87% of the total zinc in medium (Fig. 4). In contrast, the control samples had a low amount of intracellular zinc, namely 2.083 mg/g dry matter. In response to higher $ZnSO_4 \cdot 7H_20$ concentrations of 0.035% and 0.050%, Zn uptake in yeast cells severe decreased to 3.173 mg/g and 0.120 mg/g, respectively with simultaneous decline in efficiency.

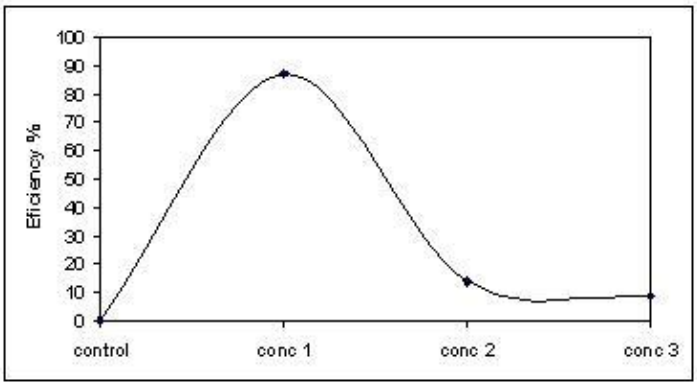

Figure 4 Efficiency of zinc ions accumulation in *Rhodotorula glutinis CCY 020-002*-033 dry biomass. **Legend**: control-without addition of $ZnSO_4 \cdot 7H_20$ in culture

Sensitive yeast strains are known for their ability to uptake metal ions from aqueous solutions. Because of the cell wall structure and the metabolic state of the cell, the composition of nutritive medium influences the amount of metal uptake, creating other binding sites or diverse enzymatic systems within the cells (**Norris et al., 1977**). Uptake of zinc ions by yeast cells is typically biphasic, involving passive bio-sorption, a metabolism-independent process which proceeds rapidly by metal binding mechanisms such as coordination, complexation, ion exchange, physical adsorption (e.g. electrostatic) or inorganic micro-precipitation, followed by active bio-sorption, a transporter phase of zinc inside the cell (metabolism-dependent on the activity of a proton-pumping ATP-ase) and its storage in vacuolar system (**Mowll *et al*., 1983; Brady *et al*., 1994; Nilanjana *et al*., 2008**). Strains of *Rhodotorula glutinis* bound the majority of Zn^{2+} ions to the fibrillar part of the cell walls (**Breierova et al., 2018**) and their bio-sorption depends on concentrations of $ZnSO_4 \cdot 7H_20$ in culture medium (Fig. 3). This trend is in accordance to **Azad *et al*. (2014)** who, using 30 $mg.L^{-1}$ ZnSO4, obtains *Saccharomyces cerevisiae* biomass enriched with a content in Zn increased by 24-fold, to 4132.34 $\mu g.g^{-1}$, after 72 h of incubation, the control culture having the lowest concentration of Zn of 171 $\mu g.g^{-1}$ in its biomass. **Barbulescu *et al*. (2009)** obtained, by optimizing media factors, *Saccharomyces cerevisiae* ICCF 349 yeast biomass enriched with 1143.4 $mg.L^{-1}$ zinc. The variation of the amount of zinc in the biomass may be due to type and size of strain and the different pH values of the culture medium. According to **Failla *et al*. (1976)** at pH = 6.8 in soil zinc forms complexes with poly-phosphates, carbonates and hydroxides (insoluble precipitates) and at pH=7 in sea water forms colloids. Below these pH values higher amounts of zinc ions accumulates, presumably by modulating membrane ATP-ase activity. **Chen *et al* (2007)** consider that pH=5.8 is the optimal value for zinc accumulation by the *Saccharomyces* yeast.

Identification and quantification of β-carotene

For quantification of β-carotene the external standard method was the technique used. Peak areas from HPLC chromatogram were plotted against the known concentrations of stock solutions of varying concentrations. Figure 5 presents the equation of the regression line for β-carotene. The identification of β-carotene in the samples was performed by comparing the retention time with the analytical standard. It is known that the relative retention time of the analyte shall correspond to that of the calibration solution at a tolerance of ± 2.5% (Official Journal of the European Communities, Directive 96/23/EC, 2002). In the *Rhodotorula glutinis CCY 020-002-033* medium the retention time found was <1.0% to the standard, confirming the presence of *β*-carotene in samples. The quantification was of the peak using the external standard method.

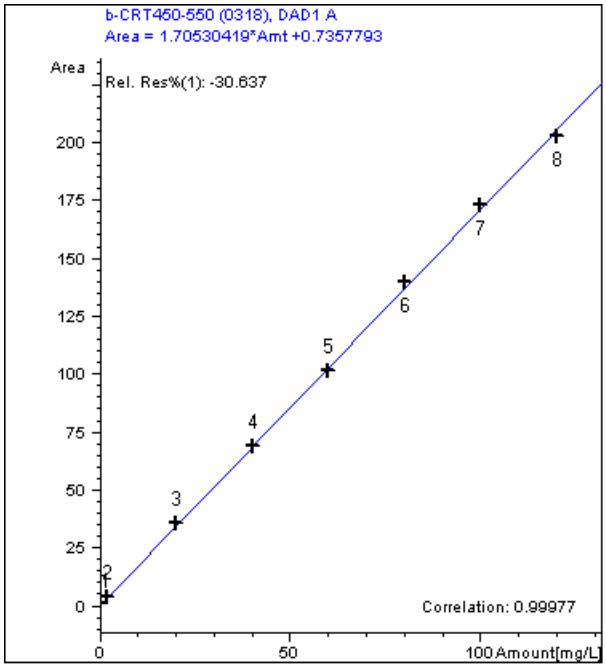

Figure 5 Calibration curve for the concentration range 0.2mg.L^{-1} to 120 mg.L^{-1} β-carotene medium; $ZnSO_4 \cdot 7H_20$ concentration:1 - 0.025%, 2 - 0.035%, 3 - 0.050%.

The calculated amounts of determined β-carotene were given in mg/L culture medium. Figure 6 shows the chromatographic carried out by the integration profile (HPLC) of the *β*-carotene produced by *R. glutinis* CCY 020-002-033 in basal medium.

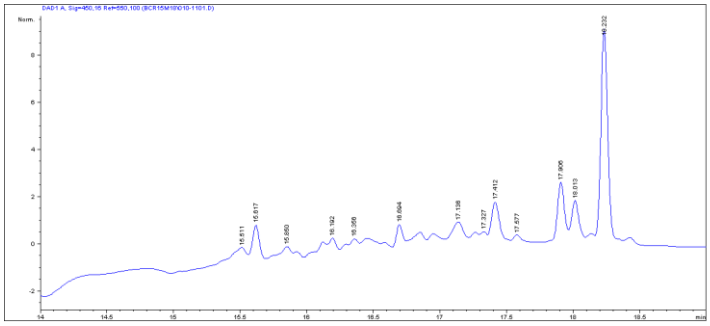

Figure 6 Chromatogram of *β*-carotene produced by *Rhodotorula glutinis* CCY 020-002-033**:** *β*-carotene (time retention =18.232min.)

The repeatability was studied by calculating the relative standard deviation (RSD) in %, for 2 determinations performed under the same experimental conditions. β-carotene production/quantification in selected *Rhodotorula* strain cultivated in basal medium supplemented with zinc ions is presented in Table 2. These levels of RSD (<30%) are typical for the biological products which show significant batch-to-batch variability (Official Journal of the European Communities, Directive 96/23/EC, 2002).

The yield of *β*-carotene varied 2.4 mg.L^{-1} in control to 7.1 mg.L^{-1} in medium containing 0.025% $ZnSO_4 \cdot 7H_20$. Supplementing the culture medium with zinc ions can stimulate carotenoid biosynthesis by *R. glutinis* CCY 020-002-033, being in agreement with **Bhosale *et al*. (2001)**, who obtained yeast biomass with 2.2 mg.L^{-1} *β*-carotene in 72 h. **El-Banna *et al.* (2012)** reported almost 2-fold increase in carotenoid content in yeast biomass, compared to the control medium, under the influence of zinc sulphate. It is assumed a stimulatory effect of divalent cations on carotenoid-synthetizing enzymes, namely, on specific desaturases involved in carotenoid biosynthesis **(Buzzini *et al.*, 2005)**. Addition of at 0.050% ZnSO4 in the growth medium of *Rhodotorula glutinis* CCY 020-002-033 inhibited the pigmentation. It could be noticed that there was no direct proportional relationship between *β*-carotene synthesis and cellular concentration. This outcome is in contrast to previous studies of **Bhosale (2001)** who obtained considerable β-carotene production in late logarithmic phase when the cell mass was maximum, but in agreement with **Maldonade *et al*. (2012)**, who consider that yeast growth and carotenoid production don't have a direct correlation and propose two-stage batch fermentation for the production of carotenoids by *Rhodotorula mucilaginosa*. **Naghavi *et al.* (2013)** assumed that the maximum content of total carotenoid was not directly associated with the maximum amount of cell biomass, their relative amount depending on genus of yeast.

Table 2 Effect of zinc sulphate concentrations on the production of *Rhodotorula glutinis* CCY 020-002-033 biomass and β-carotene

$ZnSO_4 \cdot 7H_20$ concentration, %	Dry cell weight, g/L	Total volumetric β-carotene, mg/L,	Standard deviation (s) of the production process, n=2,	RSD, %
Control	1.94	2.4	0.09	3.7
0.025	1.89	7.1	0.82	11.5
0.035	0.69	3.1	0.21	6.7
0.050	0.16	0.92	0.08	8.6

In the present study, the analysis of the obtained results highlight that zinc cations have stimulatory effect on volumetric production (mg/L) of carotenoids in biomass and that the higher yields of β-carotene were obtained at the low concentration of zinc.

CONCLUSIONS

The experimental results showed that *Rhodotorula glutinis CCY 020-002-033* cultures exposed to $ZnSO_4 \cdot 7H_20$ concentrations up to 0.050%, for 120 h, exhibit a dose-dependent decrease in cell growth rate, up to an inhibitory effect at the highest test concentration. Also, at high concentrations of zinc, there is a decrease in cell viability, evidenced by the method of determining CFU/mL. A slightly increase in cell concentration, close to that of the control and a Zn^{2+} ions bio-sorption efficiency of 87% were recorded at low $ZnSO_4 \cdot 7H_20$ concentration of 0.025% in culture medium. For the separation of β-carotene found in *Rhodotorula glutinis* CCY 020-002-033 yeasts the HPLC developed method was applied. The proposed method is simple, sensitive, rapid and could be applied for monitoring β-carotene from these complex matrices. The composition and the preparation of culture media are very important to improve the yield of *β*-carotene production. In general, greater carotenoids production was accomplished by lower cell growth. When the zinc source concentration is increased up to 0.050% a decrease in yeast growth, β–carotene and zinc accumulation was observed. These results confirm that this strain is susceptible to ZnSO4 concentration, compared with other yeasts of genus *Rhodotorula.*

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REFERENCES

ABEROUMAND, A. 2011. A Review article on Edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World Journal of Dairy & Food Sciences*, 6 (1), 71-78.

AKSU, Z., AYSE, T.E. 2007. Production of carotenoids by the isolated yeast of *Rhodotorula glutinis*. *Biochemical Engineering Journal*, 35, 107–113.

AZAD, S.K., FARID, S., MOHAMMAD, A., KARIMI, T. 2014. Production of zinc-enriched biomass of *Saccharomyces cerevisiae*. *J. Elem.*, 313–326. http://dx.doi.org/ 10.5601/jelem.2014.19.2.655

BARBULESCU, I.D., RUSU, N., RUGHINIS, R., POPA, O., STEFANIU, A., CASARICA, A. 2010. Obtaining yeast biomass enriched with copper, zinc and manganese. *Romanian Biotechnological Letters*, 15(1), 5008-5016.

BESARAB, N.V., GERASIMOVICH, K.M., KANTEROVA, A.V., NOVIK, G.I. 2018. Biosynthetic production of carotenoids using yeast strains of genus *Rhodotorula* on the cheap beer wort substrate. *Journal of microbiology, biotechnology and food sciences*, 7(4), 383-386, http://dx.doi.org/10.15414/jmbfs.2018.7.4.383-386

BHOSALE, P. 2001. Studies on yeast *Rhodotorula*, its carotenoids and their applications. Thesis submitted to the University of Pune for the degree of doctor of philosophy in microbiology.

BHOSALE, P., GADRE, R. 2001. Production of β-carotene by a mutant of *Rhodotorula glutinis*. *Applied Microbiology and Biotechnology*, 55(4), 423- 427.

BHOSALE, P. 2004. Environmental and cultural stimulants in the production of carotenoids from microorganisms. *Applied Microbiology and Biotechnology*, 63, 351–361, ISSN 0175-7598

BOGANI, D., MORGAN, M.A. , NELSON, A.C. , COSTELLO, I., MCGOURAN, J.F. , KESSLER, B.M. 2013. The PR/SET domain zinc finger

protein Prdm4 regulates gene expression in embryonic stem cells but plays a nonessential role in the developing mouse embryo. *Mol Cell Biol*, 33 (19), 3936-3950.
BRADY, D., DUNCAN, J.R. 1994. Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Appl. Microbiol. And Biotechnology*, 41, 149–154.
BREIEROVA, E., CERTIK, M., MAROVA, I., VADKERTIOVA, R. 2018. The effect of Zn (II) ions and reactive oxygen on the uptake of zinc and production of carotenoids by selected red yeasts. *Chem.Biodivers*,. http://dx.doi.org/10.1002/cbdv.201800069
BROMBERG, S.K., BOWER, P.A., DUNCOMBE, G.R., FEHRING, J., GERBER, I., LAU, V.K., TATA, M. 1997. Requirements for zinc, manganese, calcium and magnesium in wort. *J. Am. Soc. Brew.Chem*., 55, 123-128.
BUZZINI, P., MARTINI, A. 2000. Production of carotenoids by strains of *Rhodotorula glutinis* cultured in raw materials of agro-industrial origin. *Bioresource Technology*, 71(1), 41-44.
BUZZINI, P., MARTINI, A., GAETANI, M., TURCHETTI, B., PAGNONI, U.M., DAVOLI, P. 2005. Optimization of carotenoid production by *Rhodotorula graminis* DBVPG 7021 as a function of trace element concentration by means of response surface analysis. *Enzyme Microb.Technol*, 36(5–6), 687–692.
CHEN, C., WANG, J. 2007. Characteristics of Zn2+ biosorption by *Saccharomyces cerevisiae*. In *Biomedical and Environmental Sciences*, 20(6), 478-482.
COLIN, W.M., MILANICK, M.A., EIDE, D.J. 2003. Induction of the ZRC1 metal tolerance gene in Zinc-limited yeast confers resistance to zinc shock. *The Journal of Biological Chemistry*, 278(17), 15065–15072. http://dx.doi.org/10.1074/jbc.M300568200
DAI, C., TAO, J., XIE, F., DAI, Y. J., ZHAO, M. 2007. Biodiesel generation from oleaginous yeast *Rhodotorula glutinis* with xylose assimilating capacity. *Afric. J. Biotechnol*, 6, 2130-4.
EIDE, D. 1997. Molecular biology of iron and zinc uptake in eukaryotes. *Curr. Opin. Cell Biol*., 9, 573-577.
FAILLA, M.L., BENEDICT, C.D., WEINBERG, E.D. 1976. Accumulation and storage of Zn2+ by *Candida utilis*. *J Gen Microbiol*., 94(1), 23-36.
HOSINER, D., GERBER, S., LICHTENBERG-FRATÉ, H., GLASER, W., SCHU, C., KLIPP, E. 2014. Impact of Acute Metal Stress in Saccharomyces cerevisiae. *PLoS One*, 9, 1–14.
ISSA, S., ALHAJALI, A., ALAMIR, L. 2016 Improving carotenoid pigments production in *Rhodotorula mucilaginosa* using UV irradiation. *International Food Research Journal*, 23(2), 873-878.
JONES, R.P, GREENFIELD, P.F. 1994. A review of yeast ionic nutrition, I.: Growth and fermentative requirements. *Process Biochemistry*, 4, 48-59.
LIU, F.X, SUPEK, F., NELSON, N., CULOTTA, V.C. 1997. *J. Biol. Chem*., 272, 11763–11769.
MALDONADE, I.R, RODRIGUEZ-AMAYA, D.B., SCAMPARINI, A.R.P. 2008. Carotenoids of yeasts isolated from Brazilian ecosystem. *Food Chem*, 107(Suppl 1), 145–150.
MALDONADE I.R., RODRIGUEZ-AMAYA D.B., SCAMPARINI, A.R.P. 2012. Statistical optimisation of cell growth and carotenoid production by *Rhodotorula mucilaginosa*. Brazilian Journal of Microbiology, 43(1), 109-115. http://dx.doi.org/10.1590/S1517-83822012000100012
MAROVA, I., CERTIK, M., BREIEROVA, E. 2011. Production of Enriched Biomass by Carotenogenic Yeasts - Application of Whole-Cell Yeast Biomass to Production of Pigments and Other Lipid Compounds, in book Biomass - Detection, Production and Usage, 496 p. ISBN 9533074922, 9789533074924.
MARTÍNEZ, C., GERTOSIO, C., LABBE, A., PÉREZ, R., GANGA, M.A. 2006. Production of *Rhodotorula Glutinis*: A Yeast that Secretes α-Larabinofuranosidase. *Electron. J. Biotechnol*., 9, 407-13.
MATA-GÓMEZ, L.C., MONTAÑEZ, J.C., MÉNDEZ-ZAVALA, A., AGUILAR, C.N. 2014. Biotechnological production of carotenoids by yeasts: an overview. *Microbial Cell Factories*, 13, 1-11.
MOWLL, M.L., GADD, G.M. 1983. Zinc uptake and toxicity in the yeasts *Sporobolomyces roseus* and *Saccharomyces cerevisiae*. *J. Gen. Microbiol*., 129(11), 3421-3425.
NAGHAVI, F.S, PARICHEHR, H., SOUDI, M.R., AZRA, S., GHORBANI, A. 2013. Evaluation of the relationship between the incubation time and carotenoid production in *Rhodotorula slooffiae* and *R. mucilaginosa* isolated from leather tanning wastewater. *Iran J Basic Med Sci*, 16(10), 1114-1118.
NELSON, N. 1999. Metal ion transporters and homeostasis. *EMBO J*., 18(16), 4361-4371.
NILANJANA, D., VIMALA, R., KARTHIKA, P. 2008. Biosorption of heavy metals–An overview. *Indian Journal of Biotechnology*, 7, 159-169.
NORRIS, P.R., KELLY, D.P. 1997. Accumulation of cadmium and cobalt by *Saccharomyces cerevisiae*. *J. Gen. Microbiol*., 99, 317– 324.
OSGANIAN, S.K., STAMPFER, M.J., RIMM, E., SPIEGELMAN, D., MANSON, J.E., WILLETT, W.C. 2003. Dietary carotenoids and risk of coronary artery disease in women. *American Journal of Clinical Nutrition*, 77(6), 1390–1399.
REBAR, E.J., MILLER, J.C. 2004. Design and applications of engineered zinc finger proteins. *Bio Tech Int*., 6(2), 20–24.
ŠILLEROVÁ, S., LAVOVÁ, B., URMINSKÁ, D., POLÁKOVÁ, A., VOLLMANNOVÁ, A., HARANGOZO, L. 2012. Preparation of zinc enriched yeast (*Saccharomyces cerevisiae*) by cultivation with different zinc salts, *Journal of Microbiology, Biotechnology and Food Sciences*, 1 (February Special issue), 689-695.
SIMPSON, B.K., SOOTTAWAT, B., SAPPASITH, K. 2012. Natural Food Pigments, in Food Biochemistry and Food Processing, 876 p. ISBN: 9780813808741|Online ISBN:9781118308035.| http://dx.doi.org/10.1002/9781118308035
VACHALI, P., BHOSALE, P., BERNSTEIN, P.S. 2012. Microbial carotenoids from fungi. *Methods Mol Biol*, 898, 51-59.
VALEE, B., FALCHUK, K.H. 1993. The biochemical basis of zinc physiology. *Physiol Rev*., 78, 79–118.
VÍLCHEZ, C., FORJÁN, E., CUARESMA, M., BÉDMAR, F., GARBAYO, I., VEGA, J.M. 2011. Carotenoids: biological functions and commercial applications. *Mar Drugs*, 9, 319-333.
WEBER R.W.S., ANKE, H., PAOLO, D. 2007. Simple method for the extraction and reversed-phase high-performance liquid chromatographic analysis of carotenoid pigments from red yeasts (Basidiomycota, Fungi). *Journal of Chromatography A, 1145*, . 118–122.