

PIGMENTED YEASTS AS SOURCES OF SUBSTANCES WITH ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

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Abstract: The purpose of this work was to evaluate the biochemical, antioxidant and antimicrobial potential of eight strains of pigmented yeasts from the genus *Rhodotorula* and *Sporobolomyces*. Biomass productivity, protein, carbohydrate, carotenoid contents, total antioxidant activity, catalase-type enzymes and antifungal activity of the strains cultivated on the YPD nutrient medium under identical conditions were determined. The obtained results allow us to conclude that the biomass of pigmented yeasts, especially *Rhodotorula gracilis*, is an excellent source of proteins of 64.53 ± 9.10 - 74.93 ± 7.41 % d.w. and carotenoids of 190.1 ± 19.1 - 293.23 ± 24.84 $\mu\text{g} \cdot \text{g}^{-1}$, the content of which varies depending on the strain. At the same time, of the eight studied strains, the *S. pararoseus* CNMN-Ys-01 showed maximum carotenoid synthesis potential of 341.26 ± 51.29 $\mu\text{g} \cdot \text{g}^{-1}$. The studied yeast strains possess high antioxidant activity up to 85.48 ± 3.93 % of inhibition and of CAT-type enzymes at the level of 91.87 ± 8.18 - 738.0 ± 81.88 $\text{mmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein. The antimicrobial test established that the studied yeast strains showed antagonism towards some strains of phytopathogenic fungi. Thus, pigmented yeasts present a valuable source of biologically active substances with antioxidant, enzymatic and antimicrobial effects with enormous potential for use in agriculture, animal husbandry, veterinary medicine, the cosmetic industry, etc.

Keywords: antimicrobial activity, antioxidant activity, biochemical composition, pigmented yeasts

INTRODUCTION

The fungi and pigmented yeasts *Blakeslea*, *Rhodospiridium*, *Sporobolomyces*, *Monascus*, *Ashbya*, *Phycomyces*, *Candida*, *Xanthophyllomyces*, *Phaffia*, *Rhodotorula* are recognized as producers of various biologically active substances and have the ability to synthesize carotenoids [1, 2].

Carotenoids are a class of pigmented tetraterpenoid compounds naturally produced by different organisms, plants, algae, numerous fungi and bacteria, which are widely used in various sectors of industry for the production of food, nutraceuticals, feed, pharmaceuticals, cosmetics and dyes [3].

These compounds have been and are extensively studied and verified for different beneficial effects on living organisms, especially human health, because they serve as precursors of vitamin A, possess anti-inflammatory effect, antimicrobial and antioxidant activities [4 – 7].

The biosynthesis of carotenoids by red yeasts has attracted commercial interest because yeasts are more convenient than algae or fungi for wide use due to their high growth rate and unicellular nature. Also, the carotenoids production by yeasts is characterized by the use of simple sources of nitrogen and carbon for their growth, and they are easily adapted for growth on cheap substrates. Moreover, many of the potential producers are considered generally recognized as safe (GRAS) [8]. At the same time, yeasts are considered an important source of proteins, carbohydrates, lipids, minerals, etc. [9].

Currently, about 90 % of commercial carotenoids are produced by chemical synthesis. At the same time, many researchers consider carotenoid production based on yeasts is better to chemical synthesis because it is natural, organic, with short duration of production cycle, the possibility of optimizing nutrient media and cultivation parameters, conducting the biotechnological process. Being one of the famous carotenoid producer groups, *Rhodotorula* strains may play an important role in the production of natural carotenoids in the future [10].

The qualitative composition and quantity of carotenoid pigments from numerous natural isolates from the genus *Rhodotorula/Rhodospirium* and *Sporobolomyces/Sporidiobolus* have been studied in detail [11]. The profile of carotenoids produced by pigmented yeasts is mainly represented by γ -carotene, β -carotene, torulene and torularhodin, although other compounds have also been identified and described [12].

For cultivation of yeasts and production of carotenoids, it is necessary to determine the producer, the nutrient medium and the optimal cultivation conditions to exploit the maximum potential of the selected strain [8].

Taking into account the global population growth and environmental challenges, it is essential to find new ecological, sustainable sources of biologically active substances, including carotenoids, with high nutritional value, antioxidant and antimicrobial activities, in addition to those obtained from agriculture.

Thus, this research aimed to evaluate the biochemical, antioxidant and antimicrobial potential of the eight strains of pigmented yeasts from the genus *Rhodotorula* and *Sporobolomyces*, in order to identify the potential producers of various biologically active substances of microbial origin.

MATERIALS AND METHODS

Object of the study

As the object of study the eight red pigmented yeast strains from the Fungal Biotechnology Laboratory of the Institute of Microbiology and Biotechnology of the Technical University of Moldova, stored in the National Collection of Nonpathogenic Microorganisms named *Sporobolomyces pararoseus* CNMN-Ys-01, *Rhodotorula gracilis* CNMN-Ys-03, *Rhodotorula gracilis* CNMN-Y-06, *Rhodotorula glutinis* CNMN-Ys-08, *Rhodotorula rubra* CNMN-Ys-09, *Rhodotorula mucilaginosa* CNMN-Ys-10, *Rhodotorula gracilis* CNMN-Y-45, *Rhodotorula gracilis* 1/15, were served.

Media and fermentation conditions

The inoculum was obtained by cultivating yeast strains in YPD liquid nutrient medium, for 48 hours, on a stirrer (200 rpm), at the temperature of +27 - 28 °C. The inoculum (2×10^6 cells·mL⁻¹) constituted 5 % of the volume of the nutrient medium. Depth cultivation was carried out in Erlenmeyer flasks containing 0.2 L YPD nutrient medium with the following composition: 1 % yeast extract (Sigma Aldrich, France), 2 % fermentative peptone (HIMedia RM1892, India), 2 % glucose (Sigma Aldrich, Germany), 1 L distilled water, pH 5.5 (benchtop pH meter inoLab® pH 7110, Germany) [13], on a stirrer with a rotation speed of 200 rpm, at the temperature of +27 - 28 °C, for 120 hours.

Methods of achieving research

The yeast biomass productivity was determined gravimetrically prepared by centrifugation (laboratory centrifuge Janetzki, MLW K70 D, Leipzig, Germany), at 4000 rpm for 15 min., dry biomass was determined by drying a known weight of the yeast biomass at 105 °C to constant weight.

The protein content was determined spectrophotometrically according to the Lowry method, with Folin&Ciocalteu's phenol reagent (Sigma Aldrich, USA) and crystalline albumin from bovine serum as the standard [14].

The total carbohydrate content was determined spectrophotometrically with the Anthron reagent (Sigma Aldrich, Germany) and D-glucose (Sigma Aldrich, Germany) as standard. The absorption was recorded at 620 nm [15].

The carotenoid content was determined spectrophotometrically by extracting carotenoids using 96 % ethanol (ElaDum Pharma, Moldova) [16].

Antioxidant activity was determined spectrophotometrically with the use of 2,2 azinobis 3-ethylbenzothiazoline-6-sulfonic acid Radical Cation (ABTS⁺) (Sigma Aldrich, Germany) method [17].

Catalase activity (CAT) was determined by the spectrophotometric method based on the ability of hydrogen peroxide (Sigma Aldrich, Germany) to interact with ammonium molybdate tetrahydrate salt (Sigma Aldrich, USA) to form a stable-colored complex [18]. All spectrophotometric assays were performed on the Shimadzu UV-1280 spectrophotometer (Japan).

Antimicrobial activity was performed by the diffusimetric method (Kirby-Bauer), determining of the inhibition zone diameter of the test strain [19]. As the test strains served the plant phytopathogen fungi: *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* and *Fusarium solani* offered by the National Collection of Nonpathogenic Microorganisms of Moldova.

Statistical analysis

Statistical processing of the results was performed using the Microsoft Office Excel and Statistics 9.0 software suite. The results were expressed by calculating the mean, standard deviation and confidence interval for an average of three repetitions of the entire process. All differences were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

In order to satisfy the increasing demand of biologically active substances, including natural carotenoids, the selection, growth and utilization of red yeast strains with high yield is still a current research problem.

The biomass productivity of yeast strains depends on the composition of the nutrient medium, cultivation conditions (temperature, pH, aeration, duration) and is an important index, which emphasizes their potential use in various biotechnologies. In the present research was established that the studied strains, grown on YPD medium under identical conditions (pH 5.5, +28 °C, 120 hours, 200 rpm), have biomass productivity ranging from 7.48 ± 0.10 to 8.31 ± 0.63 g·L⁻¹. Maximum productivity was exhibited by strains *R. glutinis* CNMN-Ys-08, *R. gracilis* CNMN-Y-06 and *R. rubra* CNMN-Ys-09 of 8.16 ± 0.31 , 8.28 ± 0.40 and 8.31 ± 0.63 g·L⁻¹ respectively. Lower productivity of 7.48 ± 0.10 - 7.81 ± 0.22 g·L⁻¹ was recorded in the other 5 studied strains, of which *S. pararoseus* CNMN-Ys-01 recorded the lowest biomass productivity (Table 1).

Table 1. Biomass productivity, protein and carbohydrate contents of the yeast strains

Strains	Productivity [g·L ⁻¹] (drew biomass)±SD	Proteins [%] d.w.±SD	Carbohydrates [%] d.w.±SD
<i>R. rubra</i> CNMN-Ys-09	8.31±0.63	56.56±5.31	33.60±2.24
<i>R. glutinis</i> CNMN-Ys-08	8.16±0.31	46.49±0.64	29.75±1.22
<i>R. mucilaginosa</i> CNMN-Ys-10	7.81±0.22	48.75±4.65	29.64±2.21
<i>S. pararoseus</i> CNMN-Ys-01	7.48±0.10	49.15±2.41	15.75±1.63
<i>R. gracilis</i> CNMN-Y-45	7.81±0.17	64.83±2.65*	26.05±0.89
<i>R. gracilis</i> CNMN-Ys-03	7.53±0.33	65.67±3.06*	25.97±0.51
<i>R. gracilis</i> 1/15	7.55±0.27	64.53±9.10	30.42±2.57
<i>R. gracilis</i> CNMN-Y-06	8.28±0.40	74.93±7.41*	27.31±1.30

* $P \leq 0.05$

The obtained results are comparable to those reported in the specialized literature. For example, Dias Rodrigues *et al.* [20] established that the strain *R. mucilaginosa* CCT 7688 cultivated in the optimized nutrient medium, composed of 70 g·L⁻¹ sugar cane molasses and 3.4 g·L⁻¹ corn steep liquor at temperature of +25 °C and 180 rpm in 168 hours, accumulates up to 7.9 g·L⁻¹ biomass.

Ribeiro *et al.* [21] and others determined that the strain *R. glutinis*, intended for the production of carotenoids and fatty acids, accumulates significant amount of biomass ($10.28 \text{ g}\cdot\text{L}^{-1}$), as well as high amount of carotenoids and lipids cultivated in nutrient medium based on cassava wastewater as the only source of nutrients, at $+30^\circ\text{C}$, agitation at 200 rpm for 120 hours.

The *R. mucilaginosa* CCT3892 strain cultivated on 3 different nutrient media, synthetic one, medium based on hydrolyzed sugarcane molasses and hydrolyzed molasses supplemented with yeast extract, for 120 hours, at $+30^\circ\text{C}$ and 200 rpm, accumulates respectively 16.42 ± 0.32 , 8.28 ± 0.03 and $7.15 \pm 0.29 \text{ g}\cdot\text{L}^{-1}$ dry biomass [22].

Kot *et al.* [23] established that *R. glutinis* LOCKR13, *R. mucilaginosa* ATCC 66034 and *R. gracilis* ATCC 10788 strains cultivated on YPD nutrient medium at $+28^\circ\text{C}$ for 120 hours at 200 rpm accumulate 11 - 13 $\text{g}\cdot\text{L}^{-1}$ dry biomass.

According to Marova *et al.* [24], biomass productivity of red yeasts from the genus *Sporobolomyces* cultivated on nutrient medium containing yeast extract of $7 \text{ g}\cdot\text{L}^{-1}$, $(\text{NH}_4)_2\text{SO}_4$ of $5 \text{ g}\cdot\text{L}^{-1}$, glucose of $40 \text{ g}\cdot\text{L}^{-1}$, KH_2PO_4 of $5 \text{ g}\cdot\text{L}^{-1}$, MgSO_4 of $0.34 \text{ g}\cdot\text{L}^{-1}$, supplemented with oxygen peroxide, NaCl, whey waste, potato extract, apple fiber and various enzymes in different concentrations and combinations, at $+28^\circ\text{C}$ for 80 hours varied significantly depending on the strain. Thus, the strain *S. shibatanus* CCY 19-20-3 produced of $8.08 \text{ g}\cdot\text{L}^{-1}$ biomass, while *S. roseus* CCY 19-4-8 of $3.86 \pm 0.65 - 5.82 \pm 0.93 \text{ g}\cdot\text{L}^{-1}$ biomass. Considering the low biomass productivity of *S. roseus* CCY 19-4-8, the authors do not recommend the strain for industrial use [24]. In our case, the strain *S. pararoseus* CNMN-Ys-01 with the biomass productivity of $7.48 \pm 0.10 \text{ g}\cdot\text{L}^{-1}$ and other studied strains, possess high potential for industrial use.

Yeasts of various species, including some of the pigmented ones, are considered and are excellent sources of safe natural proteins GRAS, which determine the nutritional value of biomass and products obtained from it. The main component of yeast cells, which determines the nutritional value of the biomass are proteins.

In our investigations, the protein content in yeast biomass varied significantly depending on genus and species. Thus, the biomass of *R. gracilis* 1/15, *R. gracilis* CNMN-Ys-03, *R. gracilis* CNMN-Y-06 and *R. gracilis* CNMN-Y-45 strains can be considered as an excellent source of protein, which constitutes $64.83 \pm 2.65 - 74.93 \pm 7.41 \%$ d.w., *R. rubra* CNMN-Ys-09 strain synthesizes up to $56.56 \pm 5.31 \%$ d.w. proteins. In the biomass of strains *R. glutinis* CNMN-Ys-08, *R. mucilaginosa* CNMN-Ys-10 and *S. pararoseus* CNMN-Ys-01 the protein content ranged from 46.49 ± 0.64 to $49.15 \pm 2.41 \%$ d.w. (Table 1).

Research carried out by De La Cruz-Noriega *et al.* [25] using the *R. mucilaginosa* strain determined that protein and carotenoid yields varied in function of pH and substrate concentration. The mentioned strain achieved maximum protein content of $20.22 \text{ mg}\cdot\text{g}^{-1}$ in nutrient medium, based on asparagus waste (pH 5) and $25.98 \text{ mg}\cdot\text{g}^{-1}$ – in medium based on artichoke waste (pH 7.1) [25].

According to Hu *et al.* [26], the *R. mucilaginosa* strain TZR2014 cultivated on PDA (potato dextrose agar) medium comprised 200 g of potato, 20 g of glucose, 20 g of agar powder, and distilled water accumulates of $37.8 \pm 0.52 \%$ d.w. proteins.

The strain *R. mucilaginosa* 111 grown on nutrient medium based on pea whey in optimal conditions of $+16.9^\circ\text{C}$, pH 7.8, inoculum 1.85 % accumulates up to $81 \text{ g}\cdot\text{dm}^{-3}$ biomass with the protein content of $58.90 \pm 3.03 \%$ d.w. [27].

Red yeast strains, in addition to producing significant amounts of carotenoids and proteins, are also a rich source of carbohydrates, which provide biomass and high energy value. Evaluation of the carbohydrate content established that the maximum carbohydrate content of $29.64 \pm 2.21 - 33.60 \pm 2.24$ % d.w. contain strains of *R. rubra* CNMN-Ys-09, *R. glutinis* CNMN-Ys-08 and *R. mucilaginosa* CNMN-Ys-10, which accumulated minimal amounts of protein. At the same time, the strains of *gracilis* species with high protein contents, accumulated lower amounts of carbohydrates of $25.97 \pm 0.51 - 27.31 \pm 1.30$ % d.w., exception being the *R. gracilis* 1/15 with content of 30.42 ± 2.57 % d.w. (Table 1).

The research carried out by Tikhomirova [28] established that the total carbohydrate content of the cell biomass of *R. rubra* VKM Y-341, producer of rhodoxman, at the end of fermentation on Harada medium was 54.3 ± 4.1 % d.w. Mannose, glucose, galactose and fucose in cell hydrolysates were identified. *Cryptococcus laurentii* 1803-K cells during krilan production, at the end of the cultivation process on Golubev's medium, contained 42.8 ± 2.1 % carbohydrates, which included mannose, xylose, glucuronic acid, galactose and glucose. The cells of these strains grown on agarized beer wort had a higher carbohydrate content (*R. rubra* - by 11 %, *C. laurentii* - by 35 %) and similar qualitative composition of monosaccharides [28].

The *Rhodotorula glutinis* DBVPG 3853 strain, selected as the carotenoid producer, cultivated on the nutrient medium with pH 5.5, which contains as the unique carbon source concentrated grape must, yeast autolyzed and various mineral salts, for 120 hours at $+30$ °C and 160 rpm, has the ability to accumulate from 40.4 ± 3.7 to 71.9 ± 7.1 % d.w. total carbohydrates, depending on the duration of cultivation [29].

According to Park *et al.* [30], the biomass of the yeast strain *Sporobolomyces holsaticus* FRI Y-5, cultivated on nutrient medium containing starch, urea, yeast extract and mineral salts, contains 33.08 % total carbohydrates, 45.63 % proteins and 20.01 % lipids.

Thus, we can mention that the red yeast strains used in our research synthesize significant amounts of proteins, which quantitatively prevail over those published in the specialized literature, instead with the lower carbohydrate content, compared to those reported by other researchers.

Carotenogenesis is one of the basic indicators of the biomass quality of pigmented yeasts. Thus, in equivalent conditions the studied strains showed different carotenogenic activity. The analysis of the results presented in Figure 1 allows us to conclude that all strains of the *R. gracilis* have accumulated larger amounts of carotenoids in the biomass, their concentration being $190.10 \pm 19.13 - 293.23 \pm 24.84$ $\mu\text{g} \cdot \text{g}^{-1}$, compared to those of *rubra*, *glutinis* and *mucilaginosa* species, these indices constituted of $78.27 \pm 7.47 - 133.75 \pm 10.63$ $\mu\text{g} \cdot \text{g}^{-1}$. However, the most active in this sense was the *S. pararoseus* CNMN-Ys-01 strain, which accumulates up to 341.26 ± 51.29 $\mu\text{g} \cdot \text{g}^{-1}$ carotenoids. Taking into account the biomass productivity of the strains, the total productivity of carotenoids per volume of nutrient medium of the *R. gracilis* strains species was $1.43 \pm 0.08 - 2.43 \pm 0.31$ $\text{mg} \cdot \text{L}^{-1}$, and in those of the *rubra*, *glutinis* and *mucilaginosa* species $0.65 \pm 0.05 - 1.09 \pm 0.06$ $\text{mg} \cdot \text{L}^{-1}$. Maximum productivity of carotenoids showed the *S. pararoseus* CNMN-Ys-01 strain, in which this index was 2.55 ± 0.42 $\text{mg} \cdot \text{L}^{-1}$ (Figure 1).

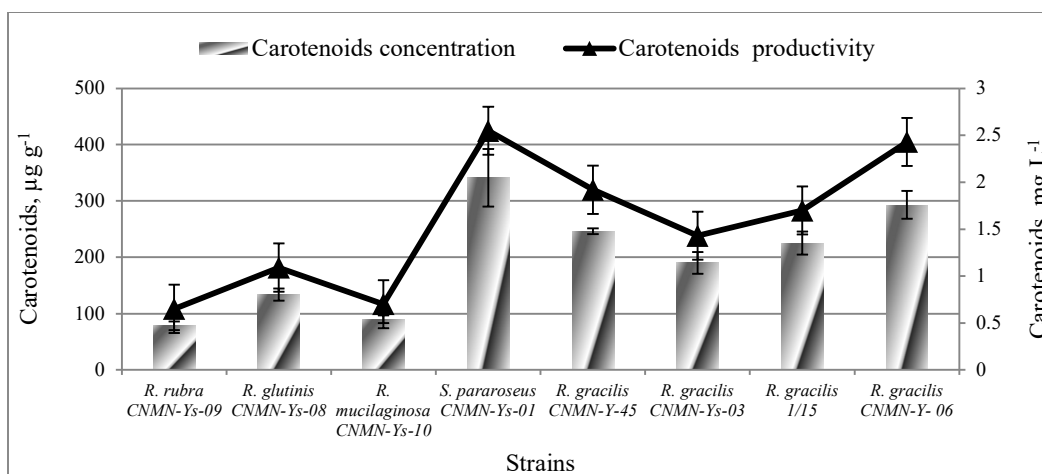


Figure 1. Concentration and productivity of carotenoids of the yeast strains

Ueno *et al.* [31] isolated 40 yeast strains from various aquatic sources in Japan capable of carotenoid synthesis. These yeast strains were identified as *Rhodotorula* spp. and *Rhodospiridium* spp. and synthesize carotenoids in amounts higher than $200 \mu\text{g}\cdot\text{g}^{-1}$ of their dry cell mass [31].

The *R. mucilaginosa* AJB01 strain, isolated from food samples, collected in Barranquilla, Colombia, subjected to different stress conditions, including osmotic stress, physical stress by ultraviolet light and light stress, using different photoperiods, synthesizes up to $118.3 \mu\text{g}\cdot\text{g}^{-1}$ carotenoids in optimal growth conditions [32].

The *R. mucilaginosa* CRUB 0138, isolated from the Lake Tonck (Patagonia), produces carotenoids at the level of $234 \pm 7 \mu\text{g}\cdot\text{g}^{-1}$ after cultivation for 96 h on nutrient medium with an initial glucose concentration of 1 % [33].

The red yeast *Rhodotorula* spp. RY1801, isolated by Chinese researchers from the Yellow Sea (Jiangsu province, China), in optimal cultivation conditions of temperature $+28^\circ\text{C}$, pH 5.0, $10 \text{ g}\cdot\text{L}^{-1}$ glucose and $10 \text{ g}\cdot\text{L}^{-1}$ yeast extract, produces maximum concentration of total carotenoids of $987 \mu\text{g}\cdot\text{L}^{-1}$. The authors indicate that the studied yeast strain can be used as a potential carotenoid producer on an industrial scale [34].

Four isolates, obtained from the soils of the forest parks in Tehran (Iran), identified as *R. mucilaginosa* and selected as potential carotenoid producers, cultivated in optimal conditions of 120 hours duration, at the temperature of $+28^\circ\text{C}$, pH 6.0 and exposure to light white, on YPG nutrient medium synthesizes up to $223.5 \mu\text{g}\cdot\text{g}^{-1}$ d.w. carotenoids. These isolates cultivated on YPD medium showed more modest results, the concentration of carotenoids being $90.29 \pm 0.045 - 91.78 \pm 0.03 \mu\text{g}\cdot\text{g}^{-1}$ d.w. [35], results practically similar to those obtained for *R. mucilaginosa* CNMN-Ys-10 (Figure 1).

Strains *R. glutinis* ATCC 26085 and *S. roseus* D99040, cultivated under yeast extract-dextrose (YED) medium containing $30 \text{ g}\cdot\text{L}^{-1}$ glucose, $4 \text{ g}\cdot\text{L}^{-1}$ yeast extract, $1 \text{ g}\cdot\text{L}^{-1}$ KH_2PO_4 and $0.5 \text{ g}\cdot\text{L}^{-1}$ $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, at $+24^\circ\text{C}$, 120 rpm, for 108 hours and different aeration regimes, showed differential responses in carotenoid content. Thus, at optimal aeration the strains accumulate 206 and $412 \mu\text{g}\cdot\text{g}^{-1}$ d.w. carotenoids respectively [36].

In this context, we can conclude that the strains *R. gracilis* CNMN-Y-45, *R. gracilis* CNMN-Y-06, *R. gracilis* 1/15, *R. gracilis* CNMN-Ys-03 and *S. pararoseus* CNMN-Ys-

01 possess a high carotenoid synthesis potential, comparable to that of other yeast strains reported in the specialized literature, and in some cases even surpassing it.

Yeasts, especially pigmented ones and various ones derived from their biomass possess high antioxidant activity [37 – 39]. The energetic benefit of aerobic metabolism is associated with the generation of reactive oxygen species ("oxidative stress"), capable of damaging DNA molecules, proteins and lipids, which various organisms face due to the aerobic way of life. Carotenoids have the ability to act as antioxidants and thus protect cells against oxidation. The ability of carotenoids to quench singlet oxygen is well known and widely studied. Pigments of biological origin are rich in biofunctional compounds with high antioxidant capacity [40, 41], which are due to their terpenoid nature and multiple conjugated double bonds [42, 43].

In our research, evaluation of the antioxidant activity of the biomass of the pigmented yeasts revealed four strains with high antioxidant activity, which in the *R. rubra* CNMN-Ys-09, *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-03 strains was from 62.18 ± 5.60 to 66.95 ± 3.72 % of inhibition, and in the *S. pararoseus* CNMN-Ys-01 strain it was 85.48 ± 3.93 % of inhibition. The rest of the strains showed lower antioxidant activity of 21.46 ± 2.43 - 45.40 ± 4.24 % of inhibition (Figure 2).

Microbial cells possess two defense systems against oxidative damage: enzymatic and non-enzymatic. The first is mainly constituted by the enzymes superoxide dismutase and catalase, which is involved in the direct removal of ROS or in the recycling of oxidized compounds such as ascorbate, glutathione, α -tocopherol and carotenoids [44].

CAT activity of the biomass of the studied strains varied within wide limits, depending on the taxonomic variety. Thus, *R. mucilaginosa* CNMN-Ys-10 and *R. glutinis* CNMN-Ys-08 strains showed minimal CAT activity of 91.87 ± 8.18 , respectively 94.55 ± 4.17 mmol $\cdot$ min $^{-1}\cdot$ mg $^{-1}$ protein. At the same time, significantly higher values were recorded in the strains *S. pararoseus* CNMN-Ys-01, *R. gracilis* CNMN-Y-06 and *R. gracilis* CNMN-Ys-03, which varied from 553.55 ± 23.67 to 738.00 ± 81.88 mmol $\cdot$ min $^{-1}\cdot$ mg $^{-1}$ protein (Figure 2).

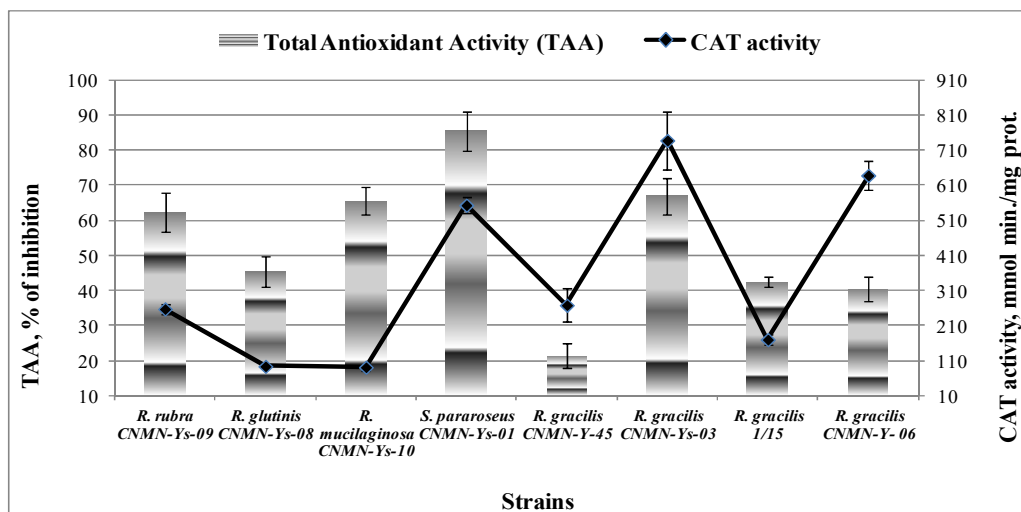


Figure 2. Total antioxidant and CAT activities of yeast strains

Excellent antioxidant capacity was established by authors in the *R. slooffiae* strain of 57.91 ± 7.34 % inhibition (DPPH test), cultivated on MMS broth, at temperature +30 °C,

150 rpm, for 72 hours and which was associated with a high capacity to produce intracellular carotenoids. Under the same conditions, the *R. mucilaginosa* strain recorded a lower antioxidant activity of 39.32 ± 5.85 % inhibition and lower carotenoid content [45]. The high antioxidant activity of carotenoids of yeasts is mentioned by several researchers and is mainly due to the presence of toruline, torularhodin and β -carotene [46].

According to Liu *et al.* [47], toruline exhibits the strongest antioxidant effect *in vivo*, which protects SK-HEP-1 cells from damage caused by hydrogen peroxide, but torularhodin had a strong effect on oxidative damage caused by H_2O_2 in AML12 cells. Also, *Sporidiobolus pararoseus* carotenoids show high antioxidant activity *in vitro* (DPPH and ABTS tests) [48].

The maximum antioxidant activity of the carotenoids of the *R. glutinis* R12 strain of 52.09 ± 0.4 % inhibition, established by the DPPH tests, was recorded when the strain was cultivated on the medium with $20 \text{ g}\cdot\text{L}^{-1}$ glucose, $4 \text{ g}\cdot\text{L}^{-1}$ yeast extract, $1 \text{ g}\cdot\text{L}^{-1}$ KH_2PO_4 and $0.5 \text{ g}\cdot\text{L}^{-1}$ $MgSO_4\cdot 7H_2O$ and MS3 medium with $30 \text{ g}\cdot\text{L}^{-1}$ glucose, $1.5 \text{ g}\cdot\text{L}^{-1}$ yeast extract, $5 \text{ g}\cdot\text{L}^{-1}$ NH_4NO_3 , $1 \text{ g}\cdot\text{L}^{-1}$ KH_2PO_4 , $0.4 \text{ g}\cdot\text{L}^{-1}$ $MgSO_4\cdot 7H_2O$, $0.4 \text{ g}\cdot\text{L}^{-1}$ NaCl and $0.4 \text{ g}\cdot\text{L}^{-1}$ L-alanine [49]. The comparative analysis of our results with those of other researchers allows us to conclude that some strains, from those researched, could become a biosource of carotenoids and natural antioxidants for use in the pharmaceutical industry, cosmetics, food, animal husbandry and other industries.

Microbial products are among the natural compounds with various medicinal properties, including antimicrobial, antitumor and wound healing [50].

Yeasts may represent novel source of antimicrobial metabolites and may allow the development of pharmacologically, food preservatives in food industry for improving human health [51].

Yeasts are widespread in most living environments and have developed a series of strategies for survival and response to stress conditions that allow them to compete for occupation of ecological niches. They possess various mechanisms of antagonistic action through which they cause the death of competing microorganisms or limit their growth. The most common mechanisms of antagonistic action are competition for nutrient sources, modification of the composition of the culture medium or its physico-chemical characteristics, and the synthesis of secondary metabolites that inhibit the growth of other microorganisms. Thus, the identification and selection of yeast strains with antimicrobial potential is current and of scientific and practical interests.

The antimicrobial test established that the strains of pigmented yeasts, used in this research, possess antifungal activity. Thus, all 4 strains from the species *R. gracilis*, *R. mucilaginosa* CNMN-Ys-10 and *S. pararoseus* CNMN-Ys-01 showed antagonism towards *F. oxysporum*, the growth inhibition zones of pathogen varying among 12.3 ± 2.5 and 23.3 ± 2.89 mm. The most active strains are *S. pararoseus* CNMN-Ys-01 and *R. mucilaginosa* CNMN-Ys-10. Towards to *F. solani*, the *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-06 showed antagonistic activity, the growth inhibition zones of this pathogen being 10.0 ± 0 and 21.7 ± 2.89 mm, respectively (Table 2). The *R. rubra* CNMN-Ys-09 strain showed antagonism towards *B. cinerea*, and *R. glutinis* CNMN-Ys-08 towards *A. alternata* with zones of inhibition of 10.0 ± 0 and 11.3 ± 1.15 mm, respectively (Table 2).

Table 2. Antifungal activity of pigmented yeast strains

Strains	Test-strains, the diameter of the inhibition zone [mm]			
	<i>A. alternata</i>	<i>B. cinerea</i>	<i>F. solani</i>	<i>F. oxysporum</i>
<i>R. rubra</i> CNMN-Ys-09	-	10.0±0	-	-
<i>R. glutinis</i> CNMN-Ys-08	11.3±1.15	-	-	-
<i>R. mucilaginosa</i> CNMN-Ys-10	-	-	10.0±0	23.3±2.89
<i>S. pararoseus</i> CNMN-Ys-01	-	-	-	22.7±2.5
<i>R. gracilis</i> CNMN-Y-45	-	-	-	13.3±2.89
<i>R. gracilis</i> CNMN-Ys-03				20.0±5.0
<i>R. gracilis</i> 1/15	-	-	-	20.7±1.15
<i>R. gracilis</i> CNMN-Ys-06	-	-	21.7±2.89	12.3±2.5

The antibacterial activity of products derived from pigmented yeasts is reported by several researchers. In this context, Pothayi *et al.* [52] established that the carotenoids of the *R. mucilaginosa* strain possessed strong antimicrobial activity against pathogenic bacteria *Staphylococcus aureus*. Also, the exopolysaccharides produced by the *R. mucilaginosa* UANL 001 L strain possess significant antimicrobial properties against various strains of pathogenic bacteria, *S. aureus* being the most sensitive to their action [53]. According to Yolmeh *et al.* [54], carotenoids of the *R. glutinis* inhibit the growth of some pathogenic bacteria, including *Bacillus cereus* and *Salmonella enteritidis*, which showed high sensitivity to the extract, but Gram-positive bacteria were more sensitive than Gram-negative ones to the action of pigments of *R. glutinis*. Poorniammal *et al.* [55] established that *R. glutinis*, in addition to being a producer of different pigments, can serve as a biological control agent against post-harvest fruit decay. The *Rhodotorula* sp. Amby109 possesses antimicrobial activity against a wide spectrum of pathogenic bacteria from the genus *Escherichia*, *Enterococcus*, *Vibrio*, *Salmonella* sp. and *Shigella* sp. [56]. According to the authors carotenoids of *Sporobolomyces* sp. showed high antimicrobial activity against *E. coli* and *S. aureus*, the inhibition zones being 2.9 and 2.6 cm, respectively [57]. Carotenoid pigments from *Rhodotorula* spp. yeasts also have the ability to inhibit the growth of *Klebsiella pneumonia*, the diameter of inhibition zones varying from 12 to 26 mm, depending on the carotenoid concentration [58].

Regarding the antifungal activity of pigmented yeasts and products derived from them, there are fewer reports in specialized literature. For example, the cell suspension and filtrate of the *R. glutinis* Has.AA-44 has demonstrated its effectiveness in controlling gray mold rot disease of strawberry caused by the fungus *B. cinerea*. The isolate stimulated plant productivity improved vegetative characteristics, and reduced infection severity before and after harvest by inducing systemic resistance of plant [59]. The effectiveness of *R. mucilaginosa* in reducing of stress caused by *F. oxysporum* in tomato plants is also known [60].

In this context, the results regarding the antimicrobial properties of the yeast strains presented in this article supplement the existing scientific results regarding the antifungal activity of pigmented yeasts and highlight the high potential of the strains studied by us for practical use.

CONCLUSIONS

The biomass of pigmented yeasts is an excellent source of proteins, carotenoids and carbohydrates, the concentration of which varies depending on the taxonomic variety of the strain. From those of the eight studied strains, *R. gracilis* CNMN-Ys-06 and *S. pararoseus* CNMN-Ys-01 showed maximum carotenoid synthesis potential of up to $2.55 \pm 0.42 \text{ mg} \cdot \text{L}^{-1}$.

The biomass of pigmented yeast strains possesses high antioxidant activity up to $85.48 \pm 3.93 \%$ of inhibition and of CAT-type enzymes at the level of $91.87 \pm 8.18 - 738.00 \pm 81.88 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}$.

The antimicrobial test established that pigmented yeast strains show antagonism towards *Fusarium oxysporum* and *Fusarium solani* with inhibition zones of up to $23.3 \pm 2.89 \text{ mm}$. These results allow us to conclude that the pigmented yeasts of the genus *Rhodotorula* and *Sporobolomyces* could serve as a biosource of proteins, carbohydrates, carotenoids and other biologically active substances with antioxidant and antimicrobial properties for use in agriculture, functional food, pharmaceutical, cosmetics, etc.

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