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Production of Carotenoid-/Ergosterol-Supplemented Biomass by Red Yeast *Rhodotorula glutinis* Grown Under External Stress

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Summary

The aim of this study is to compare the production of biomass enriched with carotenoids and ergosterol by yeast strain *Rhodotorula glutinis* CCY 20-2-26 grown under optimal growth conditions and in the presence of exogenous stress factors. *R. glutinis* cells were exposed to UV irradiation, oxidative stress (2–10 mmol/L H_2O_2) and osmotic stress (2–10 % NaCl). During the experiment, growth characteristics and the production of biomass, carotenoids and ergosterol were evaluated. Experiments were carried out in Erlenmeyer flasks and in laboratory fermentor. First, *R. glutinis* cells were exposed to higher concentration of stress factors added into the production medium. Further, low concentrations of NaCl and H_2O_2 were added to the inoculum medium or to both inoculum and production media. Exposure of red yeast cells to all tested stress factors resulted in higher production of carotenoids as well as ergosterol, while biomass production was changed only slightly. Under high stress, 2–3 times increase of β-carotene was observed. The addition of low salt or peroxide concentration into the inoculation media led to about 2-fold increase of carotenoid production. In Erlenmeyer flasks the best effect on the carotenoid and ergosterol production (3- to 4-fold increase) was exhibited by the combined stress: the addition of low amount of NaCl (2 %) into the inoculum medium, followed by the addition of H_2O_2 (5 mmol/L) into the production medium. The production of ergosterol in most cases increased simultaneously with the production of carotenoids. Cultivation of *R. glutinis* carried out in a 2-litre laboratory fermentor was as follows: under optimal conditions about 37 g/L of yeast biomass were obtained containing approx. 26.30 mg/L of total carotenoids and 7.8 mg/L of ergosterol. After preincubation with a mild stress factor, the yield of biomass as well as the production of carotenoids and ergosterol substantially increased. The best production of enriched biomass was obtained in the presence of peroxide in the inoculation medium (52.7 g/L of biomass enriched with 34 mg/L of carotenoids) and also under combined salt/peroxide or salt/salt stress (about 30–50 g/L of biomass enriched with 15–54 mg/L of total carotenoids and about 13–70 mg/L of ergosterol). *R. glutinis* CCY 20-2-26 could be used as a potential biotechnological producer of carotenoid-rich biomass.

Key words: *Rhodotorula glutinis*, carotenoids, β-carotene, ergosterol, exogenous stress

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Introduction

Carotenoids are naturally occurring lipid-soluble pigments, the majority being C_{40} terpenoids. They act as membrane-protective antioxidants that efficiently scavenge $^1O_2$ and peroxyl radicals; their antioxidative efficiency is apparently related to their structure (*1*). The most significant part in the molecule is the conjugated double bond system that determines their colour and biological action (*2*). Carotenoid pigments occur universally in photosynthetic systems of higher plants, algae and phototrophic bacteria. In non-photosynthetic organisms, carotenoids are important in protecting against photooxidative damage. Thus, many non-phototrophic bacteria and fungi rely on carotenoids for protection when growing in light and air (*3,4*).

Commercially, carotenoids are used as precursors of vitamin A, in nutrient supplementation, for pharmaceutical purposes, as food colorants and in animal feeds (*4*). There is an increased interest in carotenoids as natural antioxidants and free radical scavengers because of their ability to reduce and alleviate chronic diseases, various pathological stages and aging (*5*). However, the application of chemical synthetic methods to prepare carotenoid compounds as food additives has been strictly regulated in recent years. Therefore, attention is paid to the finding of suitable natural methods for its production. One possibility lies in biotechnological techniques employing the potential of microorganisms that are able to convert various substrates into carotenoid pigments, even if this approach is restricted by a number of useful species and also the carotenoid yield cannot compete with chemical synthesis (*6–8*).

In order to improve the yield of carotenoid pigments and subsequently decrease the cost of this biotechnological process, diverse processes have been performed by optimizing the culture conditions including nutritional and physical factors. In microorganisms, whose environment is highly variable, stress responses are of particular importance. Under stress, different classes of substances are overproduced (*9–11*). Carotenoids as well as another important metabolite, ergosterol, are also involved in stress responses of microorganisms (*12*). Thus, their production could be substantially influenced by several exogenous stress factors. Study of the molecular basis of these effects could make it possible to realize regulated overproduction of selected metabolites, above all in such industrial applications when it is not acceptable to use genetically modified strains. Moreover, biotechnological use of specific yeast strains takes advantage of the utilization of the whole biomass, efficiently enriched for particular metabolites (*13*).

The aim of the presented work is to study the influence of several types of exogenous stress factors on the production of yeast biomass enriched with carotenoids and ergosterol using yeast strain *Rhodotorula glutinis* CCY 20-2-26.

Materials and Methods

Yeast strain

The yeast strain CCY 20-2-26 of *Rhodotorula glutinis* was obtained from the Culture Collection of Yeasts (CCY), Bratislava, Slovakia. The strain was conserved in malt agar in darkness at 4 °C.

Cultivation of microorganism

Rhodotorula glutinis cells were inoculated into liquid medium containing (in g/L of distilled water): yeast autolysate 7, $(NH_4)_2SO_4$ 5, glucose 40, KH_2PO_4 5 and $MgSO_4$ 0.34. After two-step inoculation (inoculum I: 20–24 h at 28 °C, inoculum II: 24 h at 28 °C), inoculum II was transferred into minimal production medium containing (in g/L of distilled water): glucose 30, $(NH_4)_2SO_4$ 4, KH_2PO_4 4 and $MgSO_4$ 0.34. The cultivation of yeast cells in the production medium was performed at 28 °C for 50 h (exponential phase of growth) and/or for 80 h (stationary phase). During the whole growth, the cell suspension was permanently shaken under light. The growth of yeast culture was monitored using spectrophotometric detection of cell suspension turbidity at 630 nm. The amount of the produced biomass was determined using experimentally obtained relation between turbidity ($A_{630\,nm}$) and dry mass of yeast cells in gramms per litre (γ):

$$A_{630\,nm}=0.826\times\gamma+0.023, \quad R^2=0.990 \qquad /1/$$

Two parallel growth curves were monitored, so that statistical parameters could not be evaluated.

Induction of exogenous stress

UV irradiation

Two-step inoculation was performed as described previously. After the inoculation of production medium (100 mL), yeast suspensions were exposed to short-time UV irradiation (UV lamp LB-151.2, 253 nm, 30 W) for several times: 5, 10, 20, 35 and 55 min. Further cultivation was performed at 28 °C for 50 and 80 h. The control cultivation was not irradiated by UV light.

Application of chemical stress factors

In the first series of experiments, *R. glutinis* cells were exposed to individual stress factors added to the production medium at high concentration (10 mmol/L H_2O_2, 10 % NaCl). Furthermore, the influence of mild stress induced by the addition of stress factors into inoculum II was evaluated (2 mmol/L H_2O_2, 2 % NaCl). In the following experiments, combinations of stress factors were tested: yeast cells were preincubated in the presence of low concentration of one stress factor added into inoculum II and then cultivated in the production medium with higher concentration of the same or other stress factor according to the following scheme: (*i*) inoculum II – 2 mmol/L H_2O_2, production medium – 5 % NaCl; (*ii*) inoculum II – 2 % NaCl, production medium – 5 mmol/L H_2O_2. Cultivations in the production media under all types of stresses were performed in Erlenmeyer flasks at 28 °C for 50 and 80 h. Production medium volumes in all stress experiments were 100 mL. Except for UV irradiation, influence of all other types of stress factors was tested in three parallel experiments.

Cultivation of R. glutinis cells and stress experiments in a laboratory fermentor

Pilot experiments were carried out in a 2-litre laboratory fermentor Biostat B (B. Braun Biotech International, Germany). Two-step inoculation was performed in Erlenmeyer flasks in the optimal inoculation medium (see above). The first inoculum (50 mL) was cultivated for 24 h at 28 °C under permanent lighting and shaking.

Inoculum I was transferred into 240 mL of fresh inoculum II (INO II) with or without a stress factor, which was grown under the same conditions as inoculum I. After 24 h, INO II was transferred into a laboratory fermentor containing sterile minimal production medium (PROD). Fed-batch cultivation in a fermentor was carried out at 28 °C under permanent lighting, shaking (150–200 min^{-1}) and aeration (6 L of air/min). Several types of pilot stress experiments in a fermentor were performed according to the following scheme: (*i*) INO II (Erlenmeyer flask), 2 mmol/L H_2O_2, PROD (fermentor), no stress factor added; (*ii*) INO II (Erlenmeyer flask), 2 % NaCl, PROD (fermentor), no stress factor; (*iii*) INO II (Erlenmeyer flask), 2 mmol/L H_2O_2, PROD (fermentor), 5 mmol/L H_2O_2; (*iv*) INO II (Erlenmeyer flask), 2 % NaCl, PROD (fermentor), 5 mmol/L H_2O_2; (*v*) INO II (Erlenmeyer flask), 2 mmol/L H_2O_2, PROD (fermentor), 5 % NaCl; (*vi*) INO II (Erlenmeyer flask), 2 % NaCl, PROD (fermentor), 5 % NaCl. Two parallel control experiments as well as two parallel experiments with each stress factor were carried out.

Extraction and analysis of carotenoids and ergosterol

Cells were collected by centrifugation (3000 rpm, 30 min). For the subsequent isolation of carotenoids, the whole biomass obtained from 100 mL of medium was used. Yeast cells were disintegrated using a mechanical disruption by shaking with glass beads (150–212 μm). 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 in 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 (*9*). Samples (10-microlitre valve) were filtered through PTFE filters and injected onto Nucleosil 100 C18 column, 5 μm, 150×4.6 mm with guard column 30×4.6 mm, 5 μm, which had been equilibrated with a mobile phase (methanol/water, 95:5). Isocratic elution was carried out at 45 °C with a flow rate of 1.0 mL/min. Detection and quantitative analysis of lycopene and β-carotene was achieved at 450 nm. Ergosterol was analysed in the same filtered extract as carotenoids. Separation was carried out in the same column as carotenoids using a mixture of acetonitrile/methanol=95:5 as mobile phase and UV detection at 285 nm for identification. Data processing of analyses was assessed using Clarity software (DataApex, Prague, Czech Republic). Individual carotenoids and ergosterol were quantified using external standards of lycopene (ψ,ψ-carotene, concentration range 10–100 μg/mL), β-carotene (β,β-carotene, concentration range 10–100 μg/mL), ergosterol (concentration range 0.1–1 mg/mL), all from Sigma-Aldrich, St. Louis, MO, USA. Individual carotenoids were analysed by on-line RP-HPLC/PDA/ESI-MS (Finnigan Surveyor PDA detector, Finnigan LCQ Advantage Max MS detector, Thermo Finnigan, USA) using column type, mobile phase and chromatography conditions as described above. Analysis was performed at the flow rate of 0.5 mL/min and mass spectra were analyzed by ESI ionization in negative mode.

Based on total peak area of HPLC chromatograms, approximate yield of total carotenoids was expressed as 1.5-fold value of β-carotene concentration.

Statistical analysis

The results of stress experiments carried out in Erlenmeyer flasks were expressed as means±S.D. of three measurements. Results were analysed by the Student's *t*-test with a use of Statistica for Windows v. 5.0 (Statsoft, Tulsa, OK, USA).

Results and Discussion

According to the previous results of a comparative stress study obtained using several carotenogenic yeasts (*9*), especially strains of *Rhodotorula glutinis* were shown as good producers of carotenoids and/or enriched biomass in optimal conditions as well as under exogenous stress. Thus, in this work, the influence of exogenous stress on biomass and pigment production in red yeast strain CCY 20-2-26 of *Rhodotorula glutinis* was studied in detail. A typical HPLC chromatogram of carotenoids present in yeast cell extract (80-hour cultivation in the production medium; see Materials and Methods) is presented in Fig. 1. The main pigment produced by the analyzed stationary cells was identified as β,β-carotene; further pigments were identified as torularhodin alcohol, torularhodin, torulene and lycopene (*14*). Quantitative analysis was done for β-carotene and lycopene only.

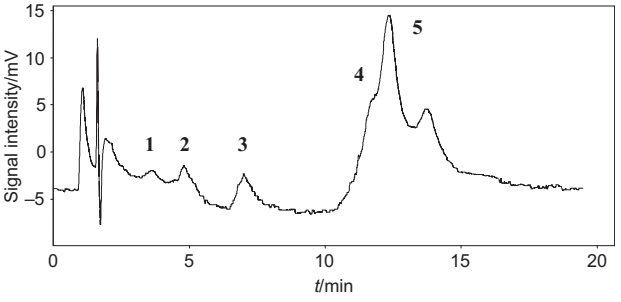

Fig. 1. A typical HPLC chromatogram of carotenoids present in the *Rhodotorula glutinis* extract after 80-hour cultivation in production medium. Peak **1** (3.81 min) – torularhodin alcohol (A_{max}=419, 452 and 496 nm; *m/z*=552.9, peak **2** (4.96 min) – torularhodin (A_{max}=425, 453 and 486 nm; *m/z*=565.97), peak **3** (7.25 min) – torulene (A_{max}=453, 489 and 518 nm; *m/z*=534.38), peak **4** (12.79 min) – lycopene (A_{max}=438, 469 and 518 nm; *m/z*=536.63) and peak **5** – β,β-carotene (A_{max}=425, 450 and 483 nm; *m/z*=536.46)

Growth and production of metabolites by R. glutinis strain CCY 20-2-26 under optimal conditions

The growth curve of *Rhodotorula glutinis* CCY 20-2-26 exhibited typical two-phase character with prolonged stationary phase, probably due to the ability of the yeast cells to utilize lipid storages formed during growth as additional energy source. The production of carotenoids during growth fluctuated and some local maxima and minima were observed. The major carotenoid produced by *R. glutinis* cells is β-carotene. The maximum of its production was reached in stationary phase after 80 h of cultivation (Fig. 2a).

Ergosterol was observed partly as the additional parameter of biomass quality and also to monitor the competition of two specialized branches of isoprenoid pathway, which is used for the biosynthesis of both carotenoids and sterols. The production of ergosterol was very similar to the production of β-carotene (Fig. 2b), even if these metabolites were formed in competitive branches of isoprenoid metabolic pathway (*3*). According to these results, growth of *R. glutinis* cells during stress experiments was carried out for 80 h. Two parallel growth experiments were monitored, so that statistical parameters could not be evaluated. Samples in both experiments were taken at the same time. Differences in β-carotene as well as ergosterol production in parallel samples varied from 10 to 15 %.

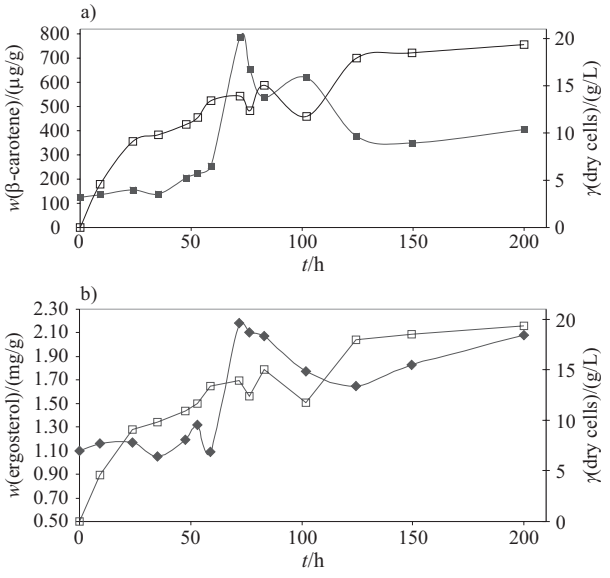

Fig. 2. Course of production of β-carotene (a) and ergosterol (b) during growth of *Rhodotorula glutinis*. (□) growth of *R. glutinis* cells, (■) β-carotene production and (◆) ergosterol production

Practically simultaneous oscillation in carotenoid and ergosterol production under optimal conditions (see Fig. 2) could be caused by the role of both metabolites in *R. glutinis* stress response. Carotenoids act as antioxidants and may prevent cells or cell membranes against negative effects of increased oxidative stress. Ergosterol is an integral component of yeast cell membranes, which are very sensitive to external stress. Recently, it has been found that the major changes in intact cells of red yeast *Rhodotorula minuta* irradiated by UV-B were interpreted as combination of changes observed in the cell wall and membrane, while the changes observed in the membrane preparations were attributed to ergosterol (*4*,*12*).

Influence of exogenous stress factors on the growth and metabolism of R. glutinis

Physical stress

UV irradiation was studied mainly for strain description. We suppose that the acquisition of the ability to produce carotenoids may be an important property of the studied strain *R. glutinis* CCY 20-2-26 to protect cells against oxidative damage caused by UV irradiation and, thus, to promote its wider distribution in the natural world.

In our work short-term UV irradiation of the production medium led to minimal changes in biomass production (Table 1). The production of carotenoids in *R. glutinis* cells was stimulated in all samples of exponentially growing cells when compared with control cultivation. In stationary phase, the production of carotenoids was induced only by 35-minute irradiation. Ergosterol production exhibited very similar changes as β-carotene production both under temperature and UV stress. It should be mentioned that during the study of UV stress, only one series of experiments was done. Nevertheless, our results are in good agreement with recent findings of the effect of weak white light irradiation on carotenoid production by a mutant of *R. glutinis* (*11*).

Chemical stress

In this part of the work, the influence of osmotic (salt) stress, oxidative (peroxide) stress and combined effects of these stress factors on the morphology, growth and production of metabolites by *R. glutinis* CCY 20-2-26 cells were studied.

The first part of the experiments was focused on studying the influence of intensive stress on yeast cells. Except for 10 % NaCl, no significant changes in biomass production were observed. The addition of peroxide and salt led to a significant increase in carotenoid as well as ergosterol production (Table 2a), above all in stationary phase.

In further experiments, the effects of mild stress on the metabolism of *R. glutinis* were studied, including pre-conditioning of cells by several stress factors (see Materials and Methods). The results obtained in this part of the work are presented in Table 2b. Both oxidative and salt stress stimulated the production of carotenoids in logarithmic as well as in stationary phases. The highest yield of β-carotene per dry mass of cells (2.9 mg/g), more than 3-fold higher when compared with

Table 1. Production of biomass and metabolites by *R. glutinis* cells irradiated with UV light

t(irradiation)/min	γ(dry cells)/(g/L)	*w*(lycopene)/(μg/g)	*w*(β-carotene)/(μg/g)	*w*(ergosterol)/(μg/g)
0	15.36	6.85	58.04	391.78
5	16.26	3.44	38.64	293.06
10	13.50	0.12	3.67	83.97
20	16.36	0.69	5.96	152.97
35	16.24	7.89	73.86	311.36
55	15.03	0.55	5.65	59.77

Cultivation time was 80 h; during the study of UV stress, one series of experiments was done; no data for statistical analysis were obtained

Table 2. Influence of chemical stress factors on the growth and metabolism of *R. glutinis* cells
a) Chemical stress induced by high concentration of stress factors

Stress factor	γ(dry cells)	w(lycopene)	w(β-carotene)	w(ergosterol)
	g/L	μg/g	μg/g	mg/g
Control	11.12±1.86	201.67±37.64	880.35±145.62	1.35±0.45
10 % NaCl	6.81±1.12	266.46±58.82	1749.29±312.65	5.44±0.98
10 mmol/L H_2O_2	10.53±2.10	385.74±49.86	2619.28±387.48	6.61±1.16

b) Combination of stress factors in inoculum II and production medium

Stress factor	γ(dry cells)	w(lycopene)	w(β-carotene)	w(ergosterol)
	g/L	μg/g	μg/g	mg/g
Control	11.12±1.16	201.67±39.15	880.35±166.85	1.35±0.28
2 % NaCl/inoculum II	10.88±1.56	208.96±58.18	1467.20±325.65	0.71±0.11
2 mmol/L H_2O_2/inoculum II	12.01±1.64	434.32±62.25	1946.39±365.27	0.99±0.12
2 % NaCl+5 mmol/L H_2O_2	10.31±1.27	614.16±127.16	2924.29±369.56	3.70±0.15
2 mmol/L H_2O_2+5 % NaCl	10.33±1.47	329.75±90.82	2112.62±289.42	4.98±0.68

Three parallel experiments were carried out; the results are expressed as means±S.D.

control, was obtained in an 80-hour culture preincubated with 2 % of salt and then cultivated with 5 mmol/L peroxide in the production medium. Similar increase in ergosterol production was observed under the same conditions, the ergosterol yield was about 3.7 mg/g. About 2.5-fold increase in the production of β-carotene and 3.7-fold increase in ergosterol were obtained in stationary culture preincubated with 2 mmol/L peroxide and grown in the medium with 5 % salt. However, only preincubation of *R. glutinis* cells with 2 % salt or 2 mmol/L peroxide exhibited substantially lower increase of carotenoid production and decrease in ergosterol formation.

Lycopene production formed only a small part of total carotenoid yield. Changes of its formation during stress experiments exhibited very similar course to β-carotene changes (see Table 2). According to the experimental data, it seems that during 80-hour cultivation of *R. glutinis* cells carotenogenic biosynthetic pathway is not finished because of the presence of lycopene (biosynthetic precursor of β-carotene) and relatively low amount of torulene and torularhodin (final oxidized products of carotenoid pathway in *Rhodotorula* cells) found in yeast cell extract.

During their lifespan, yeast cells may be exposed to numerous chemical stresses, which result from many natural activites as well as industrial processes. The exposure of *R. glutinis* CCY 20-2-26 cells to most of the tested stress combinations resulted in higher production of carotenoids as well as ergosterol. Oxidative and salt stresses were described to stimulate the production of carotenoids also in other microbial strains. For example, extremely halophilic bacteria produced 2.06 mg of total carotenoids per g of dry mass, and *R. glutinis* mutants cultivated in seawater medium produced 2-fold higher yield of β-carotene (86 mg/L) as compared to the medium prepared in distilled water (*12*).

Cultivation of R. glutinis under stress conditions in a laboratory fermentor

In the next series of experiments, a fed-batch cultivation of *R. glutinis* CCY 20-2-26 cells was carried out in a 2-litre laboratory fermentor. The production of yeast biomass in the laboratory fermentor was in most types of cultivation more than 30 g/L (about 3-times higher yield than in Erlenmeyer flasks, Tables 3a and b). The balance of cultivation in the fermentor under optimum conditions is as follows: about 37.14 g/L of biomass containing about 19.22 mg/L of the sum of lycopene+β-carotene (approx. 26.30 mg of total carotenoids) and 7.79 mg/L of ergosterol were obtained (Table 3b). The production of β-carotene was induced by most types of stress combinations except for the combination of peroxide in inoculation and production medium and simple preincubation with salt. High total yield of β-carotene was obtained after preincubation of cells with 2 mmol/L peroxide (22.69 mg/L, lycopene+β-carotene 23.34 mg/L and total carotenoids 34.03 mg/L), and also in these preincubated cells grown in the presence of 5 % NaCl (691.60 μg/g and 19.60 mg/L). The production of ergosterol under combined peroxide/salt stress was about 70 mg/L. The highest total yield of β-carotene was obtained using combined salt/salt stress (36.07 mg/L, lycopene+β-carotene 35.88 mg/L and total carotenoid yield 54.11 mg/L); this cultivation was accompanied also with relatively high biomass production (50.85 mg/L).

The aim of the preliminary experiments carried out in laboratory fermentor was to obtain basic information about potential biotechnological use of the tested *R. glutinis* strain for the industrial production of β-carotene-enriched biomass. The results of the first cultivation of *R. glutinis* are very promising. The biomass yield (30–37 g/L) produced in minimal cultivation medium was similar to the maximal biomass yield obtained in fed-batch cultivation of *Phaffia rhodozyma* (36 g/L), which is widely used as an industrial producer of astaxanthin (*8*). The maximal production of total carotenoids by *P. rhodozyma* mutant strain was 40 mg/L, which is also similar to the yields obtained in *R. glutinis* CCY 20-2-26 cells grown under controlled stress.

For yeast biotechnology, it is vitally important to understand cellular stress physiology in order to alleviate as far as possible the detrimental influences of the

Table 3. The results of pilot stress experiments carried out in laboratory fermentor
a) The yield of biomass and metabolites

Stress factor	γ(dry cells)	w(β-carotene)	w(lycopene)	w(ergosterol)
	g/L	μg/g	μg/g	mg/g
Control	37.14	482.76	34.65	0.21
2 % NaCl/inoculum II	66.84	156.34	9.15	0.26
2 mmol/L H_2O_2/inoculum II	52.70	430.55	12.45	0.18
2 mmol/L H_2O_2+5 mmol/L H_2O_2	39.05	134.96	7.15	0.04
2 % NaCl+5 mmol/L H_2O_2	30.24	534.66	19.22	2.76
2 mmol/L H_2O_2+5 % NaCl	28.34	691.60	36.15	2.49
2 % NaCl+5 % NaCl	50.85	709.42	16.21	0.25

b) The yield of industrially significant metabolites

Stress factor	γ(β-carotene)	γ(ergosterol)	γ(β-carotene+lycopene)	γ(total carotenoids*)
	mg/L	mg/L	mg/L	mg/L
Control	17.93	7.79	19.22	26.30
2 % NaCl/inoculum II	10.45	17.37	11.06	15.67
2 mmol/L H_2O_2/inoculum II	22.69	9.49	23.34	34.03
2 mmol/L H_2O_2+5 mmol/L H_2O_2	5.27	1.56	5.55	7.90
2 % NaCl+5 mmol/L H_2O_2	10.12	83.46	11.16	15.12
2 mmol/L H_2O_2+2 % NaCl	19.60	70.57	20.62	29.40
2 % NaCl+5 % NaCl	36.07	10.21	35.88	54.11

*approximate values of total carotenoids exhibited a sum of concentrations of all pigments including lycopene and β-carotene (based on total peak area, calculated as 1.5-fold value of β-carotene yield)

changes in the growth environment. Further study is needed to obtain more detailed information about complex metabolic role of carotenoids in red yeasts.

Conclusions

Rhodotorula glutinis CCY 20-2-26 strain could be a suitable candidate for biotechnological applications in the area of carotenoid-rich biomass production. Preliminary cultivation in a 2-litre laboratory fermentor after preincubation with stress factors in well-ballanced experiments led to the yield of about 40–50 g per litre of biomass enriched with 20–40 mg of β-carotene+lycopene (approx. 30–50 mg of total carotenoids per litre) and about 70 mg of ergosterol per litre. Therefore, this strain takes advantage of the utilization of the whole biomass (complete nutrition source), which is efficiently enriched with carotenoids (provitamin A, antioxidants) and also ergosterol (provitamin D). Such a product could serve as an additional natural source of significant nutrition factors in feed and food industry.

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