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Strain Improvement for the Increased Production of Beta-carotene by *Rhodotorula minuta* (3359)

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Carotenoids are chemical compounds, synthesized by plants, fungi, algae bacteria and yeast. *Rhodotorula minuta* (3359) is a yeast species (pink red in colour), producing various carotenoids such as beta-carotene, astaxanthin, torularhodine, and torulene. Beta-carotene acts as an anti-oxidant as well as increases pigmentation and colouration of organisms. To increase production of beta-carotene form *Rhodotorula minuta*, strain improvement program was carried out. To improve beta-carotene production physical and chemical mutagens are used, which block the biosynthesis of other carotenoids and so the golden colonies were formed. These colonies are selected for media optimisation to increase in production of beta-carotene from mutant *Rhodotorula minuta*.

Key Words: Carotenoid, Mutation, Torularhodine, *Rhodotorula minuta*, Beta-Carotene and UV Radiation.

Beta-carotene (β-carotene), derived from the Latin name for carrot, belongs to a family of carotenes or Carotenoids. Carotene gives their rich colours yellow or orange to fruits and vegetables. Some microorganisms produce β–carotene which help to protect themselves from damaging to the cells caused by harmful radiations. β-carotene is the most pronounced naturally occurring pigment. It is of great scientific and commercial interest because of their distribution in nature and varied application. β–carotene is considered to be an important antioxidant, anti-carcinogenic, neutraceutical and more commonly as food colorant (Ye *et al*., 2006). Like all other carotenoids, β–carotene is an antioxidant and appears to protect the body from damaging molecules called free radicals. It has been seen that dietary intake of β-carotene may reduce the risk of two types of chronic illness, heart diseases and cancer. Epidemiological events and experimental results suggest that dietary carotenoids inhibit onset of many diseases mainly initiated by free radicals. The possible benefits of theses pigments in human health have led to their large scale use in foods, cosmetics and neutraceuticals. The choice of microorganisms also has a great influence on production of β–carotene. Yeasts are more convenient than algae and fungi for large-scale production due to their unicellular nature and comparatively higher growth rate. *Phaffia rhodozyma*, *Asporogenous* yeast, *Rhodotorula*, is known to produce characteristic carotenoids viz. torulene, torularhodine and β–carotene in various proportions (Schroeder *et. al*., 1995). Previously, a few researchers have attempted mutation on

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Table 1.Represents physical change of *R. minuta* during the screening of mutant producing higher amount of β-carotene.

Properties	Parent strain	Mutant 1	Mutant 2	Mutant 3
Colour	Pink.	White.	Light orange.	Golden yellow.
Main carotenoid	Torulene.	Torularhodine and torulene.	Torularhodine and torulene.	Beta-carotene.

Table 2.Shows variation of carbon source with difference in production of β-carotene from *R. minuta*.

Carbon source	Dry cell mass (g/l)	Beta-carotene (mg/l)
Glucose	10.1	1.9
Sucrose	9.6	0.8
Candy sugar	13.1	2.8
Fructose	7.8	0.06
Maltose	3.8	0.02

Use of different carbon sources shows difference in dry cell mass with variation in production of β-carotene as shown in table 2. Candy sugar was giving highest production of β-carotene with highest dry cell mass production.

Table 3. Shows variation of nitrogen source with effect of variation in dry cell mass with difference in production of β-carotene.

Nitrogen source	Dry cell mass (g/l)	Beta-Carotene (mg/l)
Peptone	7.5	1.5
Soya peptone	12.5	2.8
Tryptone	10.5	0.9
Yeast extract	9.1	0.28
Urea	2.9	0.004

Different nitrogen sources were checked for increase in production of β-carotene from mutant *R. minuta* with the effect of dry cell mass. Soya peptone showed highest yield of dry cell mass with highest production of b-carotene as shown in Table 3.

Table 4. Shows the effect of Tracer salts on mutant *R. minuta* for the production of β-carotene with increase in dry cell mass.

Tracer salts	Dry cell mass (g/l)	Beta-Carotene (mg/l)
Cacl,	11.3	2.9
CuSO„	8.4	0.8
FeSO„	10.2	1.8
MgSO„	6.4	0.04
MnSO„	7.2	0.6

Different tracer salts show difference in dry cell mass with variation in production of β-carotene as shown in table 4. Calcium chloride (Cacl,) was giving highest production of β-carotene with highest dry cell mass production.

Rhodotorula species to increase yield of carotenoids in general. However, such mutation was focussed on increase in torulene and torularhodine. In order to get an increase content of β-carotene from hyper producing mutant, present study focused on media optimization of *R. minuta* which blocked the biochemical pathways of torularhodin and torulene synthesis.

MATERIALS AND METHODS

Strain used for the strain improvement program was *R. minuta* 3359, wild type strain obtained from NCIM (National Collection of Industrial Microorganisms). Two different media were used, one for growth (M1) of *R. minuta* containing glucose, meat extract, yeast extract, peptone, K_2HPO_4, $KH_2PO_4.7H_2O$, agar and other media for production (M_2) of β–carotene from *R. minuta* containing glucose, yeast extract, peptone, $CaCl_2$, $MgSO_4.7H_2O$ and micro minerals. The parent culture of *R. minuta* 3359 was provided by NCIM on nutrient agar medium which was activated by inoculating culture in to the growth medium i.e., M1 medium for its optimum growth. Then from M1 media parent culture of *R. minuta* 3359 was incubated in M2 media for β–carotene production for 14 hours; one at 28°C in normal incubator (non-shaker incubator) and other was kept on shaking incubator (200 rpm) at 28°C. Media was optimized for growth for pH, agitation, temperature and exposed to light.

Mutations

Formaldehyde as a chemical mutagen (also called as induced mutations) was used to improve *R. minuta* strain for increase on production of β-carotene. UV radiation as physical mutagen was used to improve *R. minuta* strain for increase in production of β-carotene. Screenings of mutant was done for a new mutant strain with increase in production of β-carotenoid from *R. minuta*.

Media optimization was carried for carbon source, nitrogen source and tracer salts for increase in production of β-carotene from *R. minuta*.

Sample analysis

The samples were regularly analyzed by checking various parameters. 1 ml of culture was taken for centrifugation; supernatant was used to check various parameters such as OD at 660 nm for growth, DNSA at 540 nm for sugar utilization, pigment extraction at 420 nm. The remaining cell mass as a pellet was washed with 1 ml of phosphate buffer saline, centrifuge for 10 minutes. To the pellet 1ml of saline was added and was poured in a petri plate. Oven dried, then weighted in pre-weighted petri-plate to calculate dry cell mass for growth.

RESULTS

Comparative growth of *R. minuta* was measured between shaker and non-shaker incubator; it was found that the growth of *R. minuta* on shaker incubator was more superior to non-shaker incubator. It is observed that in chemically induced mutation no colour mutants were observed which were producing higher concentration of β–carotene than parent strain but mutation was seen in the form of change in the colony characters and morphology of the cells. When culture was subjected to physical mutation by UV radiation was given at different time intervals ranging from 10 seconds to 300 seconds. The mutant colonies were white in colour, some light orange and some was golden yellow in colour. The colony was accordingly named as mutant 1, mutant 2, and mutant 3 respectively. All the three mutants of *R. minuta* were checked for producing higher concentration of β-carotene. To observe the mutation UV exposure was given at different time intervals ranging from 10 seconds to 300 seconds and a kill curve was plotted for the minimum number of survivals. For further mutation this time was taken as a standard time and plates were exposed at that particular standard time till golden yellow coloured colonies were obtained. The mutation was also seen in the form of change in the colony characters and morphology of the cells as shown in Table 1.

The mutant 3 obtained by mutagenesis was golden yellow in colour, producing higher concentration of β–carotene from 1.6 to 2.8 mg/l by optimising the carbon source, nitrogen source and tracer salts. There is increase in production of β–carotene with increase growth taking OD at 660 nm, decrease in sugar by taking OD of DNSA at 540 nm, increase in pigment β–carotene by taking OD at 420nm and increase in dry cell mass.

DISCUSSION

Rhodotorula minuta (3359) is industrially important strain used for the production of torularhodin. *R. minuta* (3359) was studied for the increase in production of β–carotene to several folds by proceeding with strain improvement programme having two-step approach, consisting of media optimization and mutagenesis. Shaker type of incubator showed increase in production of β–carotene with maximum growth of mutant *R. minuta*, as it provides more oxygen for growth and production of β–carotene from of *R. minuta*. A selected component was systematically optimized with increase in volumetric production and cellular accumulation of β–carotene as compared to the initial basal medium in the shake flask. Similar type of study was carried out by Anderson *et. al*, (2001) and observed increase in production of β–χαροτενε using shaker flasks by plus and minus unmated strains of *Choanephoraceae*. Metabolism in *P. rhodozyma* associated with astaxanthin biosynthesis is extremely oxygen-demanding. UV radiations as a physical mutagen produce mutant *R. minuta* with golden yellow colonies having increased production of β–carotene. Biosynthesis of the commercial carotenoids canthaxanthin and astaxanthin from *Agrobacterium aurantiacum* was carried out on Alanine-scaning mutagenesis (Ye *et al*., 2006). *R. minuta* was further studied for the effect of different media components for carotenoid production by optimization of other culture conditions like carbon source, nitrogen source and tracer salts. Similar type of optimization of media of molasses by *Blakeslea trispora* for the increase in production of β–carotene was carried out by Goksungur *et al.,* (2002). Anderson *et al.,* (2001) studied the effect of various nitrogen sources such as oats, wheat, barley, corn, rice and rye on β–carotene production by mated strains of *B trispora* and found that the maximum pigment concentration varied between 36.4 and 177.4 mg dm^{-3}.

CONCLUSION

There are very few sources for the production of β–carotene from other microbial sources like algae and fungi which requires rigorous maintenance of process conditions. The physical mutagen was advantageous for increase in production of β–carotene from wild strain of *R. minuta* (3359). Role of carbon, nitrogen and tracer salts was clearly observed in β–carotene production. More oxygen is required for the growth of mutant *R. minuta* as sample on shaker incubator showed higher growth β–carotene helps to enhance pigmentation, reduce damage caused by reactive oxygen species or phototoxic molecules, to prevent or treat cancer or cardio vascular disease, to provide a vitamin A supplement, to enhance lactation, and to increase fertility. Therefore strain improvement programme is a two-step approach consisting of media optimization and mutations can improve carotenoid production from microorganisms by several folds. The study shows that the isolation of mutant *Rhodotorula minuta* by mutagenesis can be used as an industrially important source for the production of carotenoids.

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