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Production of Intracellular Carotenoid Pigment from Wild Strains of *Rhodotorula*

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A B S T R A C T

Microorganisms produce certain commercially important secondary metabolites like antibiotics, carotenoid pigments, toxins and so on, some of which are of commercial importance. Carotenoid is a group of pigment and its production is a natural phenomenon in case of certain microorganisms. Yeast is a unicellular eukaryotic organism occurring in soil, air, feed and fodder of dairy farm environment. Among yeast, *Rhodotorula* sp. produces both extra and intracellular carotenoid pigments. The pigment producing 11 isolates of Rhodotorula were obtained from air (3), apple (4), can milk (2) and yoghurt (2). Phenotyped *Rhodotorula minuta* RAI3; *Rhodotorula acheniorum* RC2, *Rhodotorula* sp RA2, *Rhodotorula minuta* RY1 were selected for pigment production. Sterile synthetic media Malt Yeast Extract Agar (MYEB), coconut water as liquid media and rice as solid substrate medium were used for intracellular pigment production from *Rhodotorula minuta* RAI3; *Rhodotorula acheniorum* RC2, Rhodotorula sp RA2, *Rhodotorula minuta* RY1. Coconut water and rice media showed maximum production of intracellular pigment at 30^0C for 6 days. Among the 4 isolates, *Rhodotorula minuta* RAI3 showed maximum intracellular pigment production of 4.412 µg/g of dry cell mass respectively. The present study was taken to the production and characterization of pigments from wild strains of phenotyped *Rhodotorula* sp.

Introduction

Colours are the vital constituents and probably the first characteristic properties of food observed by human senses (Pattanaik *et al*., 1997). The colour of commercial products plays a vital role in attracting consumers and also represents the quality of products (Shivalkar Yadav and Prabha, 2014). Nowadays, commercial markets are characterized by synthetic colourants, some of which are toxic, carcinogenic causing severe damage even to vital organs (Duran, 2002). This has led to development and application of eco-friendly and economical pigments from natural resources even in dairy products like flavoured milk, ice cream and burfi. The various sources of natural pigments are microbes, insects and plants. Microbes have immense potential to produce various pigments like carotenoids, monascins, violacien and flavins on industrial wastes like apple pomace, sugar baggasse and others, which can radically reduce the costs of industrial production (Dufosse, 2006; Joshi *et al*., 2003; Venil and Lakshmanaperumalsamy, 2009).

Carotenoids are the widest spread naturally occurring yellow, orange and red pigments due to their relatively simple biosynthetic pathway not only in higher plants and algae, but also in bacteria and yeasts.

The huge international market for carotenoids has been met mainly by synthetic carotenoids and however due to the possible toxicity natural carotenoids have become increasingly attract (Yadav *et al*., 2014). Industrially, carotenoid pigments are utilized as food colourants and feed supplements in fish and poultry (Frengova, 2003)**.**

Several algae (Dunaliella, Dictyococcus, and Haematococcus), bacteria (many species of eubacteria in addition to halobacteria in archaebacteria), some filamentous fungi (belonging to lower fungi and Ascomycetes), yeasts (*Cryptococcus, Phaffia, Rhodosporidium, Rhodotorula, Sporidiobolus,* and *Sporobolomyces*) are reported to produce carotenoid**.**

The major yeast based carotenoid pigments obtained by biotechnological methods are torularhodin, -carotene and torulene produced by Rhodotorula species (Latha and Jeevaratnam, 2010)**.**

Materials and Methods

Cultures and their maintenance

Characterized *Rhodotorula* species obtained from air sample, can milk, fodder (spoilt fruits) and yoghurt samples were maintained on Malt Yeast Extract Agar (MYEA) slant and working cultures in Malt Yeast Extract Broth (MYEB) with incubation at 30°C for 3-5 days (Kaur *et al*., 2009).

Production and extraction of pigment

R.minuta RAI_3, *R.acheniorum* RC_2, *Rhodotorula* sp RA_2 and *Rhodotorula* sp RY_1 were inoculated to broth media such as sterile MYEB as a semi-synthetic medium, coconut water as the natural medium and rice as the natural solid medium and incubated at 30°C for 3, 6 and 9 days, respectively (Kaur *et al*., 2009).

Pigment extraction method

Extraction of intracellular pigments from *R.minuta* RAI_3, *R.acheniorum* RC_2, *Rhodotorula* sp RA_2 and *Rhodotorula* sp RY_1 were carried out using the following flow chart (Peterson, 1953).

Intracellular Pigment from Rhodotorula Species

Collected cell pellets after centrifugation

↓

Added 0.1N HCl (1:10)

↓

Placed in water bath at 90°C for 10 min

↓

Cooled for 10 min at 4°C

↓

Centrifuged at 6000 rpm for 10 min, supernatant collected

↓

Extracted Pigment by using 10 ml of acetone as solvent and absorbance was measured at 520 nm

Table.1 Production of intracellular carotenoid pigment from *R. minuta* RAI_3, *R. acheniorum* RC_2, *Rhodotorula* sp RA_2 and *Rhodotorula minuta* RY_1 in modified malt yeast extract broth, coconut water and rice

Type of isolate	Source	Viable count log_{10} cfu/ml	Extracellular (µg/g of dry cell mass)	Viable count log_{10} cfu/ml	Extracellular (µg/g of dry cell mass)	Viable count log_{10} cfu/ml	Extracellular (µg/g of dry cell mass)
Rhodotorula sp RA_2	MYEB	7.03	1.520 (0.103)	7.13	1.770 (0.124)	7.11	1.485 (0.094)
	Coconut water	7.03	1.090 (0.191)	7.06	2.160 (0.378)	7.05	2.120 (0.371)
	Rice	7.02	0.588 (0.268)	7.04	0.616 (0.310)	7.03	0.537 (0.260)
R.minuta RY_1	MYEB	7.09	0.405 (0.068)	7.12	0.651 (0.128)	7.11	0.451 (0.121)
	Coconut water	7.09	0.405 (0.071)	7.13	2.588 (0.496)	7.11	2.422 (0.424)
	Rice	7.04	0.388 (0.070)	7.09	0.700 (0.114)	7.07	0.691 (0.079)
R.minuta RAI_3	MYEB	7.10	1.897 (0.233)	7.15	3.200 (0.705)	7.14	3.057 (0.676)
	Coconut water	7.09	3.840 (0.672)	7.18	4.412 (0.873)	7.16	4.274 (0.748)
	Rice	7.10	1.331 (0.332)	7.17	3.990 (0.560)	7.16	3.862 (0.535)
R.acheniorum RC_2	MYEB	7.08	0.445 (0.082)	7.14	1.051 (0.153)	7.11	0.920 (0.142)
	Coconut water	7.12	0.405 (0.071)	7.18	1.091 (0.191)	7.14	1.062 (0.186)
	Rice	7.08	0.468 (0.078)	7.13	0.841 (0.184)	7.10	0.811 (0.161)

Note: For viable count MYEA as used with pH of 6.0 and incubated at 30°C/3-5 days
Carotenoid yield (µg/g of dry cell mass) = A_{520} x volume of the acetone / Volume of the sample x 0.17
Values in the parenthesis indicates the absorbance values at A_{520}

The yield of the pigment was calculated according to the following formula:

$$\text{Carotenoid yield } (\mu g/g \text{ of dry cell mass}) = \frac{A_{520}\,(\text{Absorption at } 520nm) \times \text{volume of acetone}}{\text{Volume of the sample} \times 0.17}$$

Result and Discussion

Growth of phenotyped *Rhodotorula* species

Phenotyped *R.minuta* RAI_3, *R.acheniorum* RC_2, *Rhodotorula* sp RA_2 and *Rhodotorula* sp RY_1 were isolated from air sample, can milk, fodder (spoilt fruits) and yoghurt samples sources. The *Rhodotorula* species when grown in MYEB, rice and coconut water showed maximum production of intracellular carotenoid pigment.

Production and extraction of intracellular carotenoid pigment from *Rhodotorula minuta* RAI_3, *R.acheniorum* RC_2, *Rhodotorula* sp RA_2, *R.minuta* RY_1 in MYEB, Coconut water and rice

Rhodotorula minuta RAI_3, *R.acheniorum* RC_2, *Rhodotorula* sp RA_2, *Rhodotorula* sp. RY_1 were inoculated to broth media such as sterile MYEB, coconut water and rice as solid medium and incubated at 30^0C for 9 days (Table 1). The pigment production started to visualize from 3^{rd} day onwards and intensity was peak on 6^{th} day and latter started to fade.

Higher production of intracellular pigment was noticed on 6^{th} day of incubation at 30^0C in all the four isolates (0.616 to 4.412 µg/g of dry cell mass).The peak production was observed in *Rhodotorula minuta* RAI_3 (4.412 µg/g of dry cell mass) and the intermediary was in *Rhodotorula acheniorum* RC_2 (1.678 µg/g of dry cell mass) and lowest in case of *Rhodotorula* sp RA_2 (0.616µg/g of dry cell mass).

In conclusion, the intracellular pigments extracted from MYEB, coconut water and rice of *Rhodotorula minuta* RAI_3 was stable both at the ambient temperature (29°C) and at 4°C up to 15 days of storage.

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