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Extraction of extracellular and intracellular carotenoid pigment from wild strains of *Rhodotorula*: A comparative study

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Abstract
Carotenoids are natural pigments produced by a wide range of microorganisms (i.e., fungi, yeasts, bacteria, and microalgae), lichens, and plants. These pigments are responsible for creating colors, such as yellow, orange, red, and pink in plants, fish, and shellfish in aquaculture and microorganisms. Carotenoids have numerous applications in food, feed, medical, pharmaceutical, chemical and cosmetic industries. Nowadays, a lot of pigments is chemically synthesized, however, their application in food, pharmaceutical, and cosmetic industries is limited due to their toxicity. Carotenoids are commercially produced by using microorganisms that have high efficiency and are also easy to work with. Many research have been carried out on carotenoids biosynthesis by microorganisms, such as bacteria (e.g., Dietzia), algae (e.g., Haematococcus), fungus (e.g., Phycomyces), and yeast (e.g., *Rhodotorula*). 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* RAI_3; *Rhodotorula acheniorum* RC_2, *Rhodotorula* sp RA_2, *Rhodotorula minuta* RY_1 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 extracellular and intracellular pigment production from *Rhodotorula minuta* RAI_3; *Rhodotorula acheniorum* RC_2, *Rhodotorula* sp RA_2, *Rhodotorula minuta* RY1. Coconut water and rice media showed production of extracellular and intracellular pigment at 30^0C for 6 days. Among the 4 isolates, *Rhodotorula minuta* RAI_3 showed maximum intracellular pigment production of 4.412 µg/g of dry cell mass compared to extracellular1.20 µg/g of dry mass respectively.

Keywords: carotenoid, extracellular pigment, wild strain, *Rhodotorula*

1. Introduction

Coloured pigments used in the food, feed and cosmetic industries are obtained by synthetic or natural way [1]. The first synthetic pigment “mauve” (aniline purple) identified by William Henry Perkin in 1856, considered as the beginning of the modern colour industry. The colour of commercial products plays a vital role in attracting consumers and also represents the quality of products [2]. This led to the replacement of natural pigments by synthetic ones mainly due to their lower cost, higher stability, and ease of large-scale production possibilities [3]. However, the consumers’ increasing interest in human safety and environmental conservation has led to the request for natural sources of pigments.

The carotenoid pigment group has antioxidant and provitamin a activity [4]. New potential sources of natural pigments are thus highly desired. Plants are also predominant source of natural pigments, but their use is limited by the irregularity of harvests, land regulations and their labour [5-6].

Pigments can also be produced by some filamentous fungi, which are gaining interest as a promising fermentation-derived natural pigment alternative [7-8].

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 [9].

These microorganisms can produce variety of pigments such as carotenoids, melanins, flavones, quinines, prodigiosin, and monascins [10]. Many yeasts like Rhodotorula (pink), *Yarrowia lipolytica* (brown), *Cryptococcus* sp. (red) and *Phaffia rhodozyma* (carotenoids) are good source of microbial pigments. The pigment production by molds of Monascus group especially *Monascus purpureus* and *Monascus anka* for use as a good color is well known. The algae which produce pigments are Chlorococcum, Chlamydomonas, Chlorella, Hematococcusi and Sporangium. Another alga namely, *Dunaliella salina* belonging to class chlorophylaceae occur in marine environment and produces β-carotene which can be used as food colorant [11]. The major yeast based carotenoid pigments obtained by biotechnological methods are torularhodin, beta carotene and torulene produced by Rhodotorula species [12]. An attempt has been made in this study to compare the yield of extracellular and intracellular pigment from *Rhodotorula minuta* RAI_3 in semi - synthetic and natural media.

2 Materials and Methods

2.1 Cultures and their maintenance

Characterized *Rhodotorula* species maintained in the Department of dairy Microbiology, Dairy Science College, KVAFSU, Bengaluru were used in the study. Yeast cultures 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 [13].

2.2 Production and extraction of pigment

Rhodotroula 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 [13].

2.3 Pigment extraction method

Extraction of extracellular and intracelluar 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 [14].

Extracellular pigment from rhodotorula species

Inoculated Rhodotorula into 100 ml of culture media MYEB / Coconut water / Rice

↓

Centrifuged at 6000 rpm for 10 min and filtered

↓

Separated the supernatant for Extracellular Pigment

↓

Filter sterilized the supernatant

↓

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

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

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

Intracellular pigments from the selected isolates of rhodotorula

Collected all the cell pellets

↓

Added 0.1N HCl (1:10)

↓

Placed in water bath for 90 °C for 10 min

↓

Cooled in ice water for 10 min

↓

Centrifuged at 6000 rpm for 10 min

↓

Collected the Supernatant

↓

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

The yield of the pigment was calculate according to the following formula

$$\text{Carotenoid yield (ug/g of dry cell mass)} = \frac{A_{520} \text{ x volume of the acetone}}{\text{Volume of the sample x 0.17}}$$

3. Result and Discussion

3.1 Growth of phenotyped *Rhodotorula* species

The *Rhodotorula* species when grown in MYEB, rice and coconut water produced extracellular and intracellular carotenoid pigments.

3.2 Comparison of pigment production of extracellular and intracellular of *Rhodotorula minuta* RAI_3, *Rhodotorula acheniorum* RC_2, *Rhodotorula* sp RA_2, and *Rhodotorula minuta* RY_1, in modified malt yeast extract broth, coconut water and rice

In this study it was observed that pigment production in Rhodotorula isolates was more intracellular rather than extracellular. Out of synthetic media MYEB; coconut water (natural medium) and rice as solid medium, all the four isolates of Rhodotorula showed maximum pigment production both extra and intracellular in coconut water.

Rhodotorula sp. RA_2 showed extracellular pigment production of 0.280 to 0.890 µg/g and intracellular of 0.537 to 2.160 µg/g of dry cell mass while *Rhodotorula minuta* RY_1 had pigment production ranged from 0.348 to 1.259 µg/g and intracellular of 0.388 to 2.588 µg/g of dry cell mass. Extracellular pigment production of 0.691 to 2.877 µg/g and 0.862 to 1.678 µg/g of dry cell mass and intracellular pigment production of 1.331 to 4.412 µg/g and 0.405 to 1.091 µg/g of dry cell mass were exhibited by *Rhodotorula minuta* RAI_3 and *Rhodotorula acheniorum* RC_2 respectively. Statistically significant difference did not exist among extra and intracellular pigment production with respect to types of media used in the present study. Yield wise difference was noticed between the extracellular and intracellular pigment production significantly.

Among extracellular and intracellular pigment, intracellular pigment production was more among all the 4 isolates such as *Rhodotorula minuta* (RAI_3) and *Rhodotorula acheniorum* (RC_2), *Rhodotorula* sp (RA_2), and *Rhodotorula minuta* (RY_1) among MYEB, coconut water and rice mediaCoconut water, a natural medium showed maximum extracellular and intracellular pigment production on 6th day with absorbance at 520 nm of 0.210 (1.200 µg/g of dry mass) and 0.873(4.412

µg/g of dry mass) in *Rhodotorula minuta* RAI_3 when compared to MYEB and rice media.

Table 1: Comparison of pigment production of extracellular and intracellular of R*hodotorula* spp in various media

Type of isolate	Source	3rd day		6th day		9th day	
		Extracellular (µg/g of dry cell mass)	Intracellular (µg/g of dry cell mass)	Extracellular (µg/g of dry cell mass)	Intracellular (µg/g of dry cell mass)	Extracellular (µg/g of dry cell mass)	Intracellular (µg/g of dry cell mass)
Rhodotorula sp RA_2	MYEB	0.702	1.520	0.890	1.770	0.765	1.485
	Coconut water	0.388	1.090	0.514	2.160	0.451	2.120
	Rice	0.280	0.588	0.388	0.616	0.367	0.537
R. minuta RY_1	MYEB	0.817	0.405	1.259	0.651	1.137	0.451
	Coconut water	0.348	0.405	0.640	2.588	0.548	2.422
	Rice	0.822	0.388	1.080	0.700	1.040	0.691
R. minuta RAI_3	MYEB	0.914	1.897	1.325	3.200	1.287	3.057
	Coconut water	0.691	3.840	1.200	4.412	0.965	4.274
	Rice	1.062	1.331	2.877	3.990	2.544	3.862
R .acheniorum RC_2	MYEB	0.971	0.445	1.354	1.051	1.277	0.920
	Coconut water	0.862	0.405	1.678	1.091	1.342	1.062
	Rice	1.062	0.468	1.548	0.841	1.222	0.811

5. Conclusion

The study carriedout on the carotenoid pigment extracted both extracellular and intracellular and it performance.

Extraction of extracellular and intracellular carotenoid pigment from wild strains of *Rhodotorula* showed maximum extracellular and intracellular pigment production on 6^{th} day with absorbance at 520 nm of 0.210 (1.200 µg/g of dry mass) and 0.873(4.412 µg/g of dry mass) in coconut water *Rhodotorula minuta* RAI_3.

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)

6. References

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