Original Research Article

Production of Intracellular Carotenoid Pigment from Wild Strains of Rhodotorula

K. Shivalkar Yadav* and R. Prabha

Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bengaluru -560 024, India

*Corresponding author

ABSTRACT

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°C 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.

Keywords
Carotenoids, Intracellular Pigment, Wild strain, Rhodotorula.

Accepted: 04 April 2017
Available Online: 10 May 2017

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, Rhodospiridium, 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* RAI₃, *R.acheniorum* RC₂, *Rhodotorula* sp RA₂ and *Rhodotorula* sp RY₁ 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* RAI₃, *R.acheniorum* RC₂, *Rhodotorula* sp RA₂ and *Rhodotorula* sp RY₁ were carried out using the following flow chart (Peterson, 1953).

**Intracellular Pigment from Rhodotorula Species**

1. Collected cell pellets after centrifugation
2. Added 0.1N HCl (1:10)
3. Placed in water bath at 90°C for 10 min
4. Cooled for 10 min at 4°C
5. Centrifuged at 6000 rpm for 10 min, supernatant collected
6. 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* RAI3, *R. acheniorum* RC2, *Rhodotorula* sp RA2 and *Rhodotorula minuta* RY1 in modified malt yeast extract broth, coconut water and rice

| Type of isolate          | Source            | Viable count log<sub>10</sub> cfu/ml | Extracellular (µg/g of dry cell mass) | Viable count log<sub>10</sub> cfu/ml | Extracellular (µg/g of dry cell mass) | Viable count log<sub>10</sub> cfu/ml | Extracellular (µg/g of dry cell mass) |
|--------------------------|-------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| *Rhodotorula* sp RA2     | 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* RY1          | 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* RAI3         | 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* RC2      | 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<sub>520</sub> x volume of the acetone / Volume of the sample x 0.17
Values in the parenthesis indicates the absorbance values at A<sub>520</sub>
The yield of the pigment was calculated according to the following formula:

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

**Result and Discussion**

**Growth of phenotyped Rhodotorula species**

Phenotyped *R. minuta* RAI3, *R. acheniorum* RC2, *Rhodotorula* sp RA2 and *Rhodotorula* sp RY1 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 RAI3, R. acheniorum RC2, Rhodotorula sp RA2, R.minuta RY1 in MYEB, Coconut water and rice**

*Rhodotorula minuta* RAI3, *R. acheniorum* RC2, *Rhodotorula* sp RA2, *Rhodotorula* sp. RY1 were inoculated to broth media such as sterile MYEB, coconut water and rice as solid medium and incubated at 30°C for 9 days (Table 1). The pigment production started to visualize from 3rd day onwards and intensity was peak on 6th day and latter started to fade.

Higher production of intracellular pigment was noticed on 6th day of incubation at 30°C in all the four isolates (0.616 to 4.412 µg/g of dry cell mass). The peak production was observed in *Rhodotorula minuta* RAI3 (4.412 µg/g of dry cell mass) and the intermediary was in *Rhodotorula acheniorum* RC2 (1.678 µg/g of dry cell mass) and lowest in case of *Rhodotorula* sp RA2 (0.616µg/g of dry cell mass).

In conclusion, the intracellular pigments extracted from MYEB, coconut water and rice of *Rhodotorula minuta* RAI3 was stable both at the ambient temperature (29°C) and at 4°C up to 15 days of storage.

**References**

Dufosse, L. 2006. “Microbial production of food grade pigments”. *J. Food Techn. Biotechn.*, 44(3): 313-321.

Duran, N., Teixeira, M.F.S., de Conti, R. and Esposito, E. 2002. ”Ecological friendly pigments from fungi”. *Crit. Rev. Food Sci. Nut.*, 42(1): 53–66.

Frengova, G.I., Simova, D. and Beshkova, D.M. 2003. "Carotenoid production by lactose- negative yeasts co-cultivated with lactic acid bacteria in whey ultra filtrate”. *Zeitschrift für Naturforschung*, 58c: 562–567.

Joshi, V.K., Attri, D., Bala, A. and Bhushan, S. 2003."Microbial pigments". *Ind. J. Biotechnol.*, 2: 362-369.

Kaur, B., D. Chakraborty and H. Kaur. 2009."Production and stability analysis of yellowish pink pigments from *Rhodotorula rubra* MTCC 1446". *Int. J. Microbiol.*, 7: 1.

Latha, B.V., and Jeevaratnam, K. 2010. “purification and characterization of the pigments from *Rhodotorula glutinis* DFR-PDY isolated from natural source global”. *J. Biotechnol. Biochem.*, 5(3): 166-174.

Pattanaik, P., Roy, U. and Jain, P. 1997. “Biocolours: new generation additives for food, *Indian Food Industry*, 16(5): 21-32.

Peterson, W.J., Bell, T.A. Ettchills, J.L. and W.W.G. Sart Jr., 1953. A procedure for demonstrating the presence of carotenoid pigments in yeasts, Vol 67.

Shivalkar Yadav, K., Prabha, R. 2014. “Extraction of pigments from *Rhodotorula species* of dairy
environment”, Indian J. Sci. Technol., Vol 7(12).
Venil, C.K., and Lakshmana Perumalsamy, P. 2009. ”An insightful overview on microbial pigment: prodigiosin”. Ele. J. Biol., 5(3): 49–61.

Yadav, S., Manjunatha, K.H., Ramachandra, B., Suchitra, N., Prabha, R. 2014. “Characterization of pigment producing Rhodotorula from dairy environmental samples”. Asian J. Dairying & Foods Res., 33(1): 1-4.

**How to cite this article:**
Shivalkar Yadav, K. and Prabha, R. 2017. Production of Intracellular Carotenoid Pigment from Wild Strains of Rhodotorula. Int.J.Curr.Microbiol.App.Sci. 6(5): 679-683.
doi: [https://doi.org/10.20546/ijemas.2017.605.077](https://doi.org/10.20546/ijemas.2017.605.077)