Microbiology Research Journal International
18(6): 1-8, 2017; Article no.MRJI.30967
Previously known as British Microbiology Research Journal
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Shelf Life Studies of Carotenoid Pigments Produced from *Rhodotorula minuta*

K. Shivalkar Yadav[1*] and R. Prabha[1]

[1]Department of Dairy Microbiology, Dairy Science College, Kvafsu, Hebbal, Bengaluru-560024, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author KSY managed literature searches, analyses the study, wrote the protocol and carried out laboratory experiments under the supervision of author RP. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/30967
Editor(s):
(1) Kenneth Lundstrom, Centre of Kenneth Lundstrcm Pan Therapeutics, Rue des Remparts, Switzerland.
(2) Joao Lucio Azevedo, Department of Genetics, University of São Paulo, Brazil.
(3) Lachhman Das Singla, Department of Veterinary Parasitology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, India.
(4) Alok Upadhyay, Department of Microbiology, Biochemistry & Molecular Genetics, Rutgers New Jersey Medical School, 225 Warren Street, Newark, NJ-07103, USA.
Reviewers:
(1) Juliano De Dea Lindner, Federal University of Santa Catarina (UFSC), Brazil.
(2) Clifford Nkemnaso Obi, College of Natural Scences, Michael Okpara University of Agriculture, Umudike, Nigeria.
(3) Mariya Dushkova, University of Food Technologies, Bulgaria.
(4) Fakruddin Md, Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.
(5) Murat Ozdal, Ataturk University, 25240, Erzurum, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/18006

Original Research Article

Received 12th December 2016
Accepted 23rd February 2017
Published 1st March 2017

ABSTRACT

Aim: Storage stability of carotenoid pigment (extracellular and intracellular) extracted from *Rhodotorula minuta* grown in Malt Yeast Extract Broth (MYEB), coconut water and rice was studied for a period of 15 days at ambient temperature (29℃) and refrigeration temperature i.e. 4℃ with respect to absorbance at 520 nm at 5 days interval.
Methods and Results: *Rhodotorula minuta* RAI_3 obtained from air of dairy environment was used in shelf life study of the carotenoid pigment. The yeast culture was maintained on Malt Yeast Extract Agar (MYEA) slant and working culture in Malt Yeast Extract Broth (MYEB) with incubation at 30℃ for 3-5 days. Color of pigment was stable both at ambient temperature (29℃) and refrigeration temperature (4℃) for 15 days of the study with A_{520} of 0.420, 0.140, 0.10 and 0.090 and 0.412, 0.320, 0.270 and 0.189 extracellular in MYEB, coconut water, and rice.

**Corresponding author: E-mail: shivalkaryadav@gmail.com;*

Conclusion: Storage stability for 15 days both at the ambient temperature (29℃) and the refrigeration temperature (4℃) was noticed during storage of the extra and intracellular pigment of *Rhodotorula minuta* RAI_3.

Significance and Impact of the Study: The present study aided in understanding storage stability of extra and intracellular pigments extracted from *Rhodotorula minuta* both at the ambient temperature (29℃) and the refrigeration temperature (4℃) for 15 days.

Keywords: Carotenoids; stability; extracellular; intracellular pigment; optical density.

1. INTRODUCTION

Colors are the vital constituents and probably the first characteristic properties of food observed by human senses [1]. The color of commercial products plays a vital role in attracting consumers and also represents the quality of products [2]. Nowadays, commercial markets are characterized by synthetic colorants some of which are toxic, carcinogenic causing severe damage even to vital organs [3]. This has led to development and application of eco-friendly and economical pigments from natural resources. 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 [4]. More specifically, bacteria have the potential to produce different pigments in cheap raw material supplemented to the production medium,that can radically reduce the costs of industrial production [5-7]. Moreover, the microbial pigments are superior related to stability in comparison to those derived from plants and animals [8,9]. For instance, apple pomace has been used as a cheap carbon source for production of pigment by *Micrococcus flavus* [10].

Carotenoids are the widest spread naturally occurring yellow, orange and red pigments. The abundance of carotenoids in nature is probably due to their relatively simple biosynthetic pathway, which has been demonstrated in higher plants and algae, but also in bacteria and yeasts.

As color is an important attribute to gain consumer acceptance, adding natural color to dairy products like flavoured milk, ice cream and burfi, has become a common practice in recent years. The huge international market for carotenoids has been met mainly by synthetic carotenoids with similar structures as natural carotenoids. However due to the possible toxicity of synthetic colors natural coloring alternatives have become increasingly attractive [11].

In bacteria and in yeast, carotenoids have been considered as typical secondary metabolites, playing a certain role in the survival of the fittest microorganisms. Industrially, carotenoid pigments are utilized as food colorants and feed supplements in fish and poultry [12].

Recently, carotenoids have attracted greater attention due to their beneficial effect on human health such as involvement in cancer prevention, reduction of the risk for cardiovascular diseases, macular degeneration, cataract and enhancement of immune responses [13,14]. Thus, a comprehensive screening of microbial carotenoid spectrum could help to identify novel compounds providing beneficial effects.

Microbial synthesis offers a promising approach for production of carotenoids. This explains the increasing interest in the production of microbial carotenoids as an alternative for synthetic food colorants. 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 carotenoid pigments obtained by biotechnological methods are torularhodin, -carotene, and torulene produced in various concentrations by Rhodotorula yeasts and astaxanthin from Phaffiarhodozyma or the green alga *Haematococcus pluvialis* [15].

Storage stability of carotenoids of *Rhodotorula glutinis* DFR-PDY obtained from organic solvents was studied by using two solvents such as petroleum ether (non-polar) and acetone (polar) at different temperatures i.e. 40℃, ambient temperature and 60℃ and in the presence/absence of light [16]. At 4℃ up to 90 days of storage in petroleum ether 35% stability was noticed while in the case of acetone it was only 10%. Overall stability of carotenoids

of *Rhodotorula glutinis* DFR-PDY was better at 4℃ at ambient temperature but very poor at 60℃.

In the present study the extracted pigment from *Rhodotorula minuta* was subjected to storage stability studies for a period of 15 days at ambient temperature (29℃) and refrigeration temperature i.e. 4℃.

2. MATERIALS AND METHODS

2.1 Cultures and Their Maintenance

Characterized *Rhodotorula minuta* RAI_3 obtained from the air of a dairy environment was used in a shelf life study of the pigment. The yeast culture was maintained on Malt Yeast Extract Agar (MYEA) slant and working cultures in Malt Yeast Extract Broth (MYEB) with incubation at 30℃ for 3-5 days [17].

2.2 Growth Study of Isolates in Broth and Solid Substrate

According to the reviewed literature scientists have used several media for pigment production by Rhodotorula. Based on the information the present study was carried out using MYEB as a synthetic medium, coconut water as natural liquid medium and rice as a solid substrate. Screened Rhodotorula isolates for pigment production were grown in sterile MYEB, coconut water, 50% coconut water: 50% MYEB, 50% whey: 50% coconut water, whey, Bengal gram dhal (2 part of dhal soaked in 1 part of tap water, soaked for 10 min and sterilized by autoclaving) and rice were inoculated at 1% level of pigment production. The incubation condition provided was 30℃ up to 9 days. The Direct Microscopic Count was determined every 3 days up to 9 days. The media that visually showed the presence of pigment visibly were further used for the production of pigment by selected isolates of Rhodotorula.

2.3 Production and Extraction of Pigment

R. minuta RAI_3, 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℃ for 3, 6 and 9 days, respectively [17].

2.4 Pigment Extraction Method

Extraction of extracellular and intracellular pigments from *R.minuta* RAI_3, *R.acheniorum* RC_2, *Rhodotorula* sp RA_2 and *Rhodotorula* sp RY_1 was carried out using the following flow chart [18].

Extracellular pigment from Rhodotorula Species

Inoculated Rhodotorula at 5% into 100 ml of culture media MYEB / Coconut water / Rice, incubated at 30℃ for 3 days

↓

After incubation, centrifuged at 6000 rpm for 10 min and filtered

↓

Supernatant collected as extracellular pigment

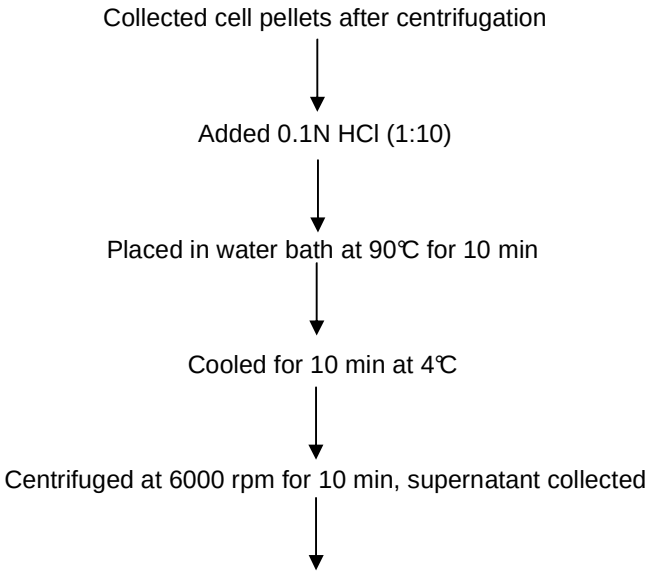
↓

Filter sterilized supernatant

↓

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

Intracellular pigment from Rhodotorula Species



Extracted pigment by using 10 ml of acetone as solvent and absorbance was measured at 520 nm
The yield of the pigment was calculated according to the following formula:

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

2.5 Shelf life of Pigment

Pigment extracted using acetone (extracellular and intracellular) was stored for a period of 15 days at ambient temperature (29℃) and refrigeration temperature i.e. 4℃ and A_{520} was measured once every 5 days to check the stability.

3. RESULTS

3.1 Storage Stability of Extra and Intracellular Pigment of *Rhodotorula minuta* RAI_3 at the Ambient Temperature

The extracellular pigment extracted from MYEB, coconut water, and rice at the ambient temperature showed retention of 69%, 70% and 82%, respectively, after 5 days of storage. After 10 days, the pigment intensity decreased to 13%, 24% and 13%, respectively and after 15 days of storage, the pigment intensity was reduced to 9% each in broth and coconut water while rice pigment showed 11% decrease. Extracellular pigment extracted from rice showed a higher retention than coconut water and MYEB. Intracellular pigment stored at ambient temperature up to 5 days, retained 97%, 68% and 48%, respectively, and later the intensity of pigment decreased to 25%, 11% and 11%, respectively. Further storage up to 15 days showed reduction in intensity of pigment by 24%, 17%, and 16%, respectively. Intracellular pigment retention was higher in MYEB extracted pigment followed by coconut water and rice (Tables 1 and 2). Significant difference was noticed among storage, media and ambient temperature statistically.

3.2 Storage Stability of Extra and Intracellular Pigment of *Rhodotorula minuta* RAI_3 at Refrigeration Temperature

The extracellular pigment extracted from MYEB, coconut water and rice stored at refrigeration temperature for 5 days showed retention of 70%, 72%, and 85%, respectively. Later after 10 days of storage, the intensity of pigment decreased to 3%, 6 and 10%. After 15 days of storage reduction in intensity of pigment was 7%, 16% and 13%. The retention of extracellular pigment was higher in rice medium followed by coconut water and MYEB (Tables 3 and 4).

Table 1. Storage stability of extracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water, and rice at ambient temperature (29℃)

Medium used for growth of pigment	Storage stability at ambient temperature 29℃							
	Control	5th		10th		15th		CD(P≥ 0.05)
	Extracellular pigment	Extracellular pigment	Percent retention	Extracellular pigment	Percent retention	Extracellular pigment	Percent retention	
MYEB	0.186 (0.243)	0.128 (0.167)	69%	0.104 (0.135)	56%	0.088 (0.115)	47%	
Coconut water	0.201 (0.262)	0.140 (0.183)	70%	0.108 (0.141)	54%	0.090 (0.117)	45%	**0.06**
Rice	0.160 (0.209)	0.132 (0.172)	82%	0.111 (0.145)	69%	0.094 (0.122)	58%	
CD (P≥0.05)				**0.08**				

Note: Acetone extracted pigment was used
Values in parenthesis indicate the quantity of pigment

Table 2. Storage stability of intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water, and rice at ambient temperature (29℃)

Medium used for growth of pigment	Storage stability at ambient temperature 29℃							
	Control	5th		10th		15th		CD(P≥ 0.05)
	Intracellular pigment	Intracellular pigment	Percent retention	Intracellular pigment	Percent retention	Intracellular pigment	Percent retention	
MYEB	0.233 (0.304)	0.226 (0.295)	97%	0.169 (0.220)	72%	0.113 (0.147)	48%	
Coconut water	0.472 (0.616)	0.320 (0.418)	68%	0.270 (0.352)	57%	0.189 (0.247)	40%	**0.06**
Rice	0.372 (0.486)	0.178 (0.232)	48%	0.138 (0.180)	37%	0.101 (0.132)	21%	
CD (P≥0.05)				**0.08**				

Note: Acetone extracted pigment was used
Values in parenthesis indicate the quantity of pigment

Table 3. Storage stability of extracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water, and rice at refrigeration temperature (4℃)

Medium used for growth of pigment	Storage stability at refrigeration temperature 4℃							CD(P≥ 0.05)
	Control	5th		10th		15th		
	Extracellular pigment	Extracellular pigment	Percent retention	Extracellular pigment	Percent retention	Extracellular pigment	Percent retention	
MYEB	0.186 (0.243)	0.131 (0.179)	70%	0.126 (0.164)	67%	0.111 (0.145)	60%	
Coconut water	0.201 (0.262)	0.144 (0.188)	72%	0.132 (0.172)	66%	0.101 (0.132)	50%	**0.07**
Rice	0.160 (0.209)	0.136 (0.177)	85%	0.120 (0.156)	75%	0.099 (0.129)	62%	
CD (P≥0.05)	**0.09**							

- *Note: Values in parenthesis indicate the quantity of pigment*

Table 4. Storage stability of intracellular pigment of *Rhodotorula minuta* RAI_3 obtained from sterile MYEB, coconut water, and rice at refrigeration temperature (4℃)

Medium used for growth of pigment	Storage stability at refrigeration temperature 4℃							CD(P≥ 0.05)
	Control	5th		10th		15th		
	Intracellular pigment	Intracellular pigment	Percent retention	Intracellular pigment	Percent retention	Intracellular pigment	Percent retention	
MYEB	0.233 (0.304)	0.211 (0.275)	90%	0.201 (0.262)	86%	0.178 (0.232)	76%	
Coconut water	0.472 (0.616)	0.331 (0.432)	70%	0.289 (0.377)	61%	0.165 (0.215)	35%	**0.07**
Rice	0.372 (0.486)	0.188 (0.245)	50%	0.159 (0.207)	33%	0.120 (0.156)	32%	
CD (P≥0.05)	**0.09**							

Note: Acetone extracted pigment was used

- *Values in parenthesis indicate the quantity of pigment*

Intracellular pigment *Rhodotorula minuta* RAI_3 extracted from MYEB, coconut water and rice stored at 4℃ showed retention of 90%, 70% and 50%, respectively, after 5 days and after 10 days of storage the intensity of pigment decreased by 4%, 9%, and 17%, respectively. Further storage up to 15 days showed a reduction in intensity of pigment by 10%, 26%, and 1%, respectively. The intracellular pigment extracted from MYEB showed better retention than coconut water and rice. A statistically significant difference was noticed among media, storage period and temperature.

4. DISCUSSION

4.1 Storage stability of extra and Intracellular Pigments of *Rhodotorula minuta* RAI_3 from Sterile MYEB, Coconut Water, and Rice

Acetone extracted pigment solution of *Rhodotorula minuta* was stored at ambient temperature (29°C) and refrigeration temperature (4℃) for 15 days with measure at A_{520}. It was shown that color was stable both at 29℃ and 4℃ up to 15 days of the study with A_{520} of 0.420, 0.140, 0.10 and 0.090 and 0.412, 0.320, 0.270 and 0.189 extracellular in MYEB, coconut water and rice.

On par with the above study, a stability study of the pigment of *Rhodotorula glutinis* DFR-PDY was carried out in different solvents at an OD of 520 nm for 3 months [16]. Stability was studied in acetone and in petroleum ether (4℃, 25℃, and 60℃) at three different temperatures, 4℃, 25℃ and 60℃ in presence of light and in the dark. The pigment showed 10 % stability and 35 % stability in acetone and petroleum ether at 4℃, respectively, compared to 25℃ and 60℃.

5. CONCLUSIONS

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pattanaik P, Roy U, Jain P. Biocolours: New generation additives for food. Indian Food Industry. 1997;16(5):21-32.
2. Shivalkar Yadav K, Prabha R. Extraction of pigments from *Rhodotorula species* of dairy environment. Indian Journal of Science and Technology. 2014;7(12): 1973–1977.
3. Duran N, Teixeira MFS, de Conti R, Esposito E. Ecological friendly pigments from fungi. Crit. Rev. Food Sci. Nut. 2002 ; 42(1):53–66.
4. Dufosse L. Microbial production of food grade pigments. J. Food Techn Biotechn. 2006;44(3):313-321.
5. Joshi VK, Attri D, Bala A, Bhushan S. Microbial pigments. Ind J Biotechnol. 2003;2:362-369.
6. Venil CK, Lakshmanaperumalsamy P. An insightful overview on microbial pigment: Prodigiosin. Ele. J. Biol. 2009;5(3):49–61.
7. Ahmad AW, Ahmad WYW, Zakaria ZK, Yosof NZ. Applications of bacterial pigments as colorant: The Malaysian perspective. New York: Springer Briefs in Molecular Science. 2012;25-44.
8. Jiang Y, Chen F, Hyde KD. Production potential of water-soluble *Monascus* red pigment by a newly isolated *Penicillium species*. J. Agr. Techn. 2005;1(1):113-126.
9. Gunasekaran S, Poorniammal R. Optimization of fermentation conditions for red pigment production from *Penicillium species* under submerged cultivation. Afr. J. Biotechn. 2008;7(12):1894-1898.
10. Deb P, Madhugiri MJ.Optimization of apple-pomace based medium for pigment production by *Micrococcus flavus*. The Bioscan. 2012;7(1):57-60.
11. Yadav S, Manjunatha KH, Ramachandra B, Suchitra N, Prabha R. Characterization of pigment producing Rhodotorula from dairy environmental samples. Asian Journal of Dairying & Foods Research. 2014;33(1):1-4.
12. Frengova GI, Simova D, Beshkova DM. Carotenoid production by lactose- negative yeasts co-cultivated with lactic acid bacteria in whey ultrafiltrate. Zeitschriftfür Naturforschung. 2003;58c:562–567.
13. Costa HL, Martelli da silva IM, Pomeroy D. Production of β-carotene by a Rhodotorula strain. Biotechnol Lett. 2005;9:373-375.

14. Iriani RM, Adilma RP Scamparini, Delia B, Rodriguez A. Selection and characterization of carotenoid-producing yeasts from Campinas region. Brazilian Journal Microbiology. 2005;40:2985-2991.
15. Ungureanu C, Ferdes M, Chirvase AA, Mocanu E. Method for Torularhodin Separation and analysis in the yeast *Rhodotorula Rubra* aerobically cultivated in lab bioreactor, Icheap-10: 10th Int. Conf. on Chemical and Process Engineering, Pts; 2011.
16. Latha BV, Jeevaratnam K. Purification and characterization of the pigments from *Rhodotorula glutinis* DFR-PDY isolated from natural source global. Journal of Biotechnology & Biochemistry. 2010;5(3): 166-174.
17. Kaur B, Chakraborty D, Kaur H. Production and stability analysis of yellowish pink pigments from *Rhodotorula rubra* MTCC 1446. Internet J.Microbiol. 2009;7:1.
18. Peterson WJ, Bell TA. Etchlls JL, Sart Jr. WWG. A procedure for demonstrating the presence of carotenoid pigments in yeasts. J Bacteriol. 1954;67.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18006