RESEARCH ARTICLE Am. J. PharmTech Res. 2014; 4(2) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Growth Optimization for the Mass Scale Production of Carotenoids from Red Pigmented Halobacteria

Uma G[1], Ravikumar S[2]*, Gokulakrishnan R[2]

1.Noorul Islam College of Arts and Science, Department of Biotechnology, Kumaracoil, Thuckalay, Kanyakumari District, Pin – 629 180.

2.School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623 409, Ramanathapuram District, Tamil Nadu, India.

ABSTRACT

The present study was made an attempt to optimize the growth parameters of the halobacteria for the mass scale production of carotenoids. The results suggested that, the pigmented KP2 showed maximum carotenoids production at 40ºC, 20% NaCl, pH 7, 1.5% sucrose, 1% beef extract, 1.5% manganese chloride and 1.5% proline. The KT2 showed maximum carotenoids production at 30ºC, 20% NaCl, pH 7, 0.5% glucose, 1.5% beef extract, 0.5% manganese chloride and 1% proline. Moreover, the KT3 showed maximum carotenoids production at 20ºC, 20% NaCl, pH 7, 1.5% sucrose, 0.5% beef extract, 1.5% copper sulphate and 0.5% proline. The identification of individual carotenoids was done by using thin layer chromatography through standard R_f value and the results suggested that, all the pigmented halobacteria contains three different carotenoids *viz.*, bacterioruberin, mono-anhydrobacterioruberin and squalene. It is concluded from the present study that, the optimization of the microbial growth parameters of the halobacteria isolated from the saltpan of Kanyakumari district could be used for the mass scale production of pigmented carotenoids.

Keywords: Carotenoids, Halobacteria, Optimization, Pigments, Saltpan

*Corresponding Author Email: ravibiotech201321@gmail.com

Received 20 January2014, Accepted 12 February 2014

Please cite this article in press as: Ravikumar S *et al.*, Growth Optimization for the Mass Scale Production of Carotenoids from Red Pigmented Halobacteria. American Journal of PharmTech Research 2014.

INTRODUCTION

Carotenoids are natural pigments that are structurally very diverse in nature. They are typically consists of C_{40} hydrocarbon with yellow and orange pigments in bacteria, algae, plants and animals. About 750 naturally occurring carotenoids pigments have been identified [1]. Carotenoids are essential for the microorganisms with oxygenic photosynthesis due to their protective role of both depleting the energy from chlorophyll and accept the energy from reactive forms of oxygen. The red colour of many haloarchaea is mainly due to the presence of carotenoid pigments. Their function is to act as a sunscreen and to prevent the cell damage caused by UV radiation [2-3]. They are recognized to play several important physiological roles including antenna function and photo protection in photosynthetic apparatus [4] and scavenging active oxygen species [5]. Moreover, the maximum carotenoids were obtained at the optimized condition from several microbes including yeast- *Rhodotorula glutinis* [6-7]; red yeast-*Sporobolomyces pararoseus* [8]; Grape berries- *Vitis vinifera*.[9] However, studies related with the carotenoids extraction from halobacteria in Kanyakumari district is still lacking. In this connection, the present study made an attempt to extract the maximum carotenoids from the pigmented halobacteria under the optimized condition.

MATERIALS AND METHOD

Isolation of halobacteria

Sediment samples were collected from Thamaraikulam (Lat. 8°10'N and Long. 77° 26'E) and Puthalam saltpans (Lat. 8°06'N and Long. 77° 28'E) Kanyakumari district, Tamil Nadu, India. About 1g of collected sediment sample was transferred into 100 ml of sterilized 50% sea water and kept for shaking at 150 rpm for 30 min. About 100 µl of diluted sample was spreaded over the halophilic agar medium ($g.l^{-1}$) [Sodium chloride- 222; Magnesium sulphate.7H_2O- 10; Casein hydrolysate- 5; Potassium chloride-5; Disodium citrate-3; Potassium nitrate-1; Yeast extract-1; Calcium choride.6H_2O-0.2; Agar-10; pH-7.2±0.2] and incubated for 2-4 weeks at 37°C. After incubation, morphologically different colonies were re-streaked on the appropriate agar medium for further use. Morphologically different halobacterial strains isolated from Puthalam salt pan is marked as KP and the strains isolated from Thamaraikulam saltpan is marked as KT.

Extraction of Carotenoids

The carotenoids were extracted from the pigmented cells by following the method of [10]. Briefly, the dried pellets were mixed with methanol and vortexed until the methanol layer turned into red. Further, the methanol layer was mixed with hexane and distilled water (1:1) in a separating funnel and shaken well for complete recovery of carotenoids. Then the carotenoids were washed thrice by centrifugation (1000×g) for 10 minutes. After that, the polar lipids were precipitated by adding equal volume of acetone. The precipitate was discarded and the supernatant was washed and dried under rotary flash evaporator. The individual carotenoids were identified by Thin Layer Chromatography (TLC).

Quantification of carotenoids

The total carotenoids were calculated by using the following formula:

C = D.V.F / 2500

Where, C=Total carotenoids in milligram; D = Optical density at 550 nm; V=Total volume in milliliter; F=Dilution factor if any; 2500=Average extinction co-efficient for carotenoids.

Optimization of growth parameter

The isolated halobacteria were grown individually in the halophilic broth ($g.l^{-1}$) [Sodium chloride-222; Magnesium sulphate.7H_2O-10; Casein hydrolysate-5; Potassium chloride-5; Disodium citrate-3; Potassium nitrate-1; Yeast extract-1; Calcium chloride.6H_2O-0.2; Dis.H_2O-1000 ml; pH-7.2±0.2]. About 1 ml of overnight broth culture were inoculated separately in to the broth which was previously optimized with different concentrations of sodium chloride (10%, 20% and 30%), pH (5, 6, 7, 8, 9 and 10), with different temperature (20°C, 30°C and 40°C) and different concentrations (0.5%, 1% and 1.5%) of carbon sources (glucose, sucrose, maltose and mannose), nitrogen sources (beef extract, sodium nitrate and ammonium nitrate), mineral sources (copper sulphate, ferric chloride and manganese chloride) and amino acid sources (glutamine, aspartic acid and proline) were added in the halophilic broth and incubated at 37ºC for 25 days.

The individual carotenoids were identified to TLC (silica gel 60 F_{254} Merck) using the following solvent systems: The acetone-petroleum ether (20:80 v/v) for bacterioruberin and mono-anhydrobacterioruberin. The petroleum ether-diethyl ether (99.5:0.5 v/v) solvent system was used for squalene. The R_f value was calculated by using the following formula: R_f value (cm) = Distance moved by the solute (cm)/Distance moved by the solvent (cm).

RESULTS AND DISCUSSION

The microbial growth optimization is a significant task to enhance the production of industrially important products as well as other economically viable compounds. Generally, the halobacteria are slow grower in either selective or enriched medium however, it has a numerous natural products including carotenoids. Due to the slow growth rate of halobacteria, the production level of other important products derived from the halobacteria are also decreased which leads to economic loss in most of the industries. The industrial products mainly depends on the several factors *viz.*, culture medium, carbon source, mineral source, amino acid sources and some of the physical parameters.[11] The author's[12] optimized the culture media with different factors. Moreover, the halobacteria produced structurally unique diverse secondary metabolites during the log phase under the optimized condition[13]. In view of this, the present study made an attempt to optimize the growth parameters of the isolated halobacteria for the mass scale production of the carotenoids. A total of five morphologically different halobacteria was isolated. Of these, three strains *viz.*, KP2, KT2 and KT3 was produced carotenoids pigments. The present study also optimize the growth parameters of halobacteria for the mass scale production of carotenoids and the results suggested that, the isolated KT2 showed the maximum (0.000316 mg) carotenoids production at 20% NaCl whereas the KP2 showed the maximum production at 10% NaCl. But, the production level of KP2 is very low when compared with the other strains. The author's[14] reported that, the maximum carotenoids were extracted from the optimized condition at 20% NaCl. The author's[15] reported that, the concentration of NaCl below 15% induced the cell lysis. However, all the three isolates showed the minimum production at 30% NaCl (Figure 1). The KT2 showed the maximum carotenoids production at the temperature 30°C and pH 7. It could be confirmed that, the KT2 is mesophilic and neutrophilic in nature. Moreover, this optimized condition is useful for the easy maintenance and preservation and to evolve maximum amount of carotenoids. Similarly, the author's[16] optimized some physical factors for the production of β-amylase. The pH optimization reveals that, the KT3 showed the maximum (0.000412 mg) carotenoids production followed by KT2 (0.000357 mg) at pH 7 (Figure 2). The temperature optimization reveals that, the KT2 strain showed maximum (0.00024 mg) carotenoids production at 30 ºC. However, the KP2 showed maximum (0.000196 mg) carotenoids production at 40ºC (Figure 3). Moreover, all the strains showed the minimum carotenoids production at 20ºC except KT3.

The optimization of carbon source reveals that, the KT2 showed maximum (0.000238 mg) carotenoids production at 0.5% glucose. Moreover, the maximum production of carotenoids by KP2 (0.000231 mg) and KT3 (0.00018 mg) were observed at 1.5% sucrose (Figure 4). Carbon source is one of the main constituent of the cellular material. Among the several carbon sources tested, the maximum carotenoids production was obtained when glucose used as a carbon source. Similarly, the maximum carotenoids extraction was obtained from the red yeast during glucose and fructose as a carbon sources.[6] Moreover, all the halobacteria showed the maximum carotenoids production in the beef extract rather than the other nitrogen sources and this could be due to the less complexity preferred for growth[17]. The optimization of nitrogen source suggests that, all the three strains showed maximum production of carotenoids in the beef extract as a nitrogen source with the different concentrations (Figure 5). The concentration of nitrogen sources in the optimized medium might also control the regulation of the pigment metabolism in microbes. Moreover, the pigment production is influenced not only by the stimulation of nitrogen compounds but it is influenced by the catabolism of the nitrogen compound[18]. The maximum carotenoids production with different mineral source reveals that, the KT3 showed the maximum (0.000186 mg) carotenoids production at 1.5% of $CuSO_4$. Similarly, the maximum production of carotenoids was recorded by KP2 at 1.5% $MnCl_2$ and KT2 at 1.5% $FeCl_2$ (Figure 6). Most of the important microbial process can be influenced by minerals including energy generation, nutrient acquisition, cell adhesion and biofilm formation[19]. In the present study, the maximum carotenoids production was observed in KT3 when $CuSO_4$ used as a mineral source than the other mineral sources. The mineral sources are mainly involved in structural and functional role in the microbial cells.

The amino acid optimization suggests that, all the strains showed maximum carotenoid production in proline supplementation. The KP2 showed the maximum (0.00032 mg) carotenoids production at 1.5% proline. Similarly, the KT2 showed the maximum (0.00032 mg) production at 1% proline (Figure 7). Supplementation of amino acids has been found beneficial for higher production of secondary metabolites including carotenoids. Generally, plants and microbes produced proline under the stress conditions. In the present study, the pigmented strains showed the maximum carotenoids production in all the tested levels of proline. It can be concluded from the amino acid optimization results that, all the tested strains could able to produce the maximum carotenoids under the optimized amino acid level. However, excess of intracellular proline accumulation suppressed the other amino acid synthesis, cell signaling, cellular death and finally decrease the product level[20]. The individual carotenoids of the halobacteria were identified by thin layer chromatography. The results suggested that, all the halobacteria contains three different carotenoids *viz.*, bacterioruberin, mono-anhydrobacterioruberin and squalene (Table 1). There are three different carotenoids *viz.*, bacterioruberin, mono-anhydro bacterioruberin and squalene were identified from the halobacterial carotenoids. The author's [21]reported that, the bacterioruberin and its derivative mono-anhydrobacterioruberin are the major carotenoids of the halobacteria. Moreover, the bacterioruberin pigments of halobacteria and other members of the halobacteriaceae protected against high intensities of visible and UV light [22]. Moreover, the author's [10] also identified the bacterioruberin, mono-anhydro bacterioruberin and squalene from *Haloferax alexandrinus*.

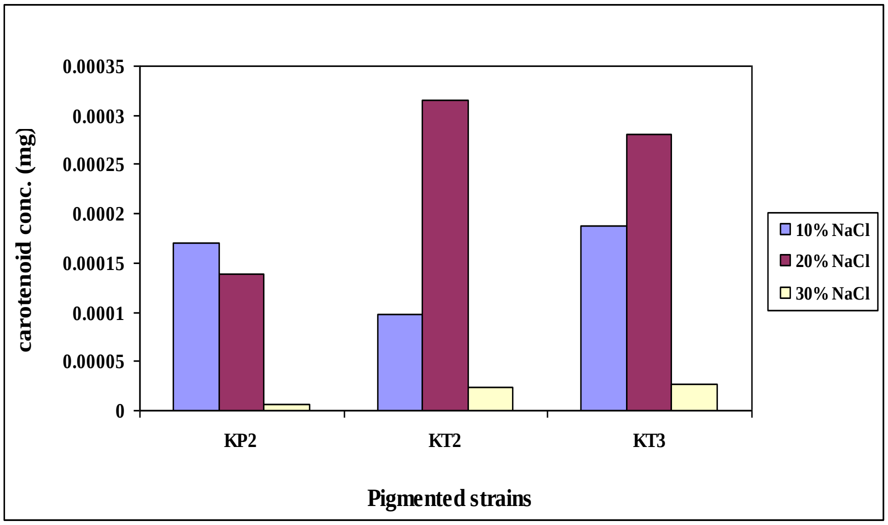

Figure 1 Production of carotenoids by the pigmented halobacteria at different salinity levels

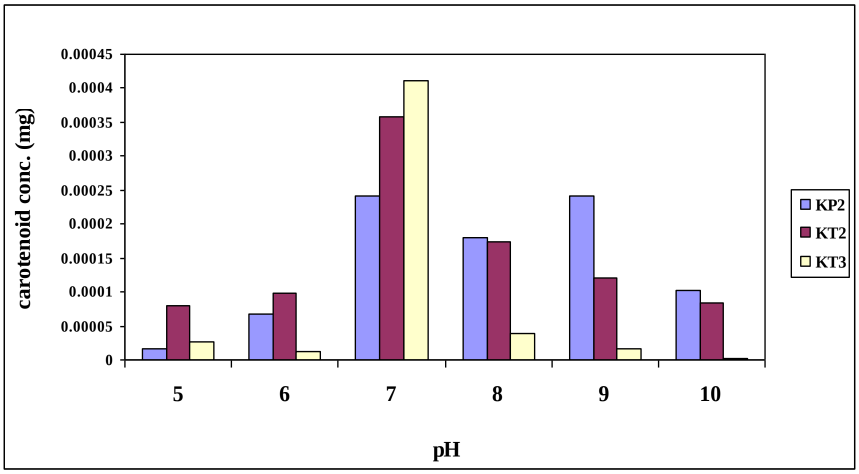

Figure 2 Production of carotenoids by the pigmented halobacteria at different pH levels

Table 1. Rf Value of the Identified Carotenoids

S.No	Carotenoids	Standard R_f Value	R_f value obtained KP2	KT2	KT3
1	Bacterioruberin	0.62	0.4	0.42	0.58
2	Mono – anhydrobacterioruberin	0.47	0.85	0.8	0.4
3	Squalene	0.60	0.64	0.64	0.67

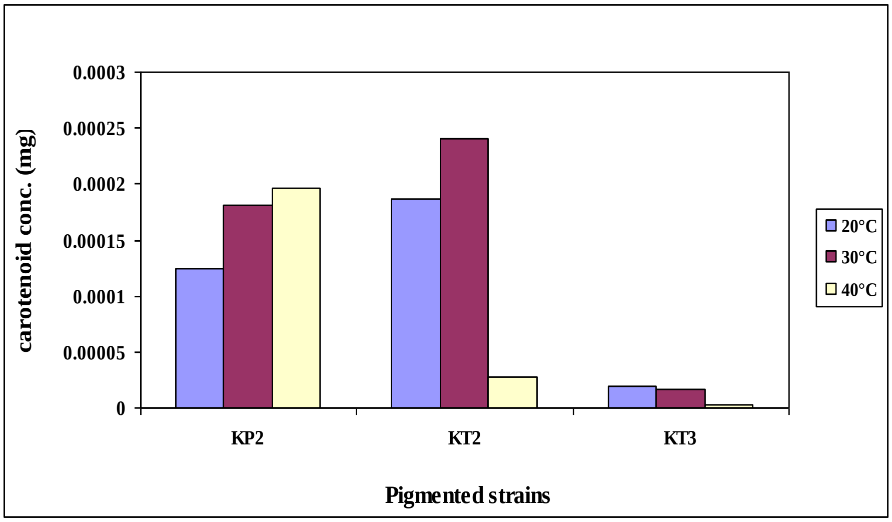

Figure 3 Production of carotenoids by the pigmented halobacteria at different temperature

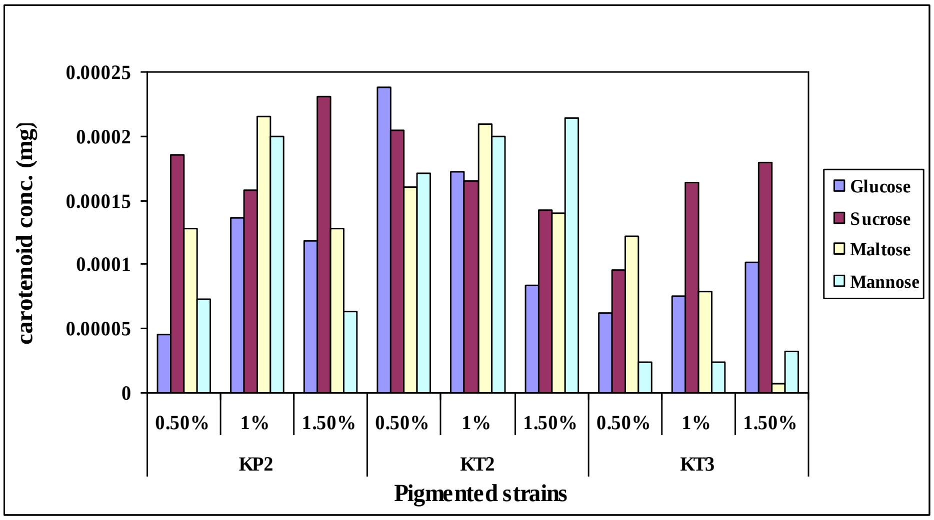

Figure 4 Production of carotenoids by the pigmented halobacteria at different carbon sources

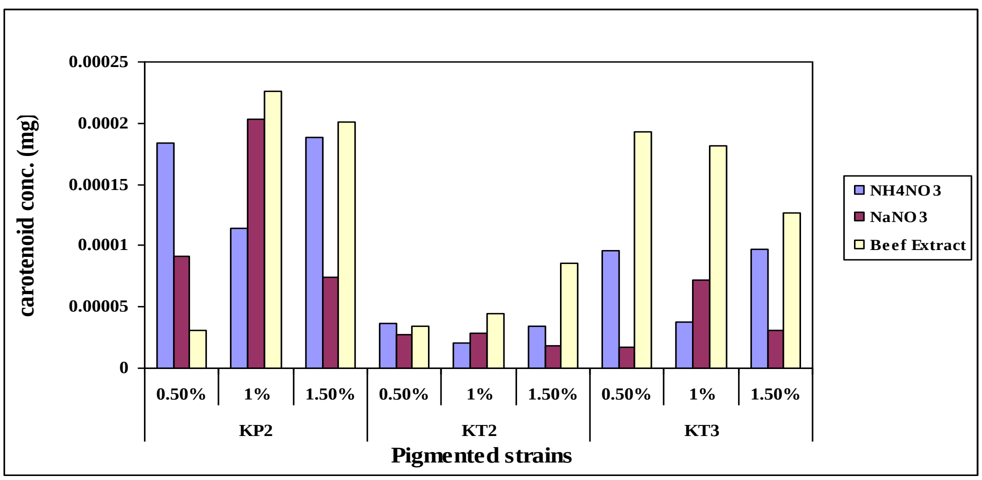

Figure 5 Production of carotenoids by the pigmented halobacteria at different nitrogen sources

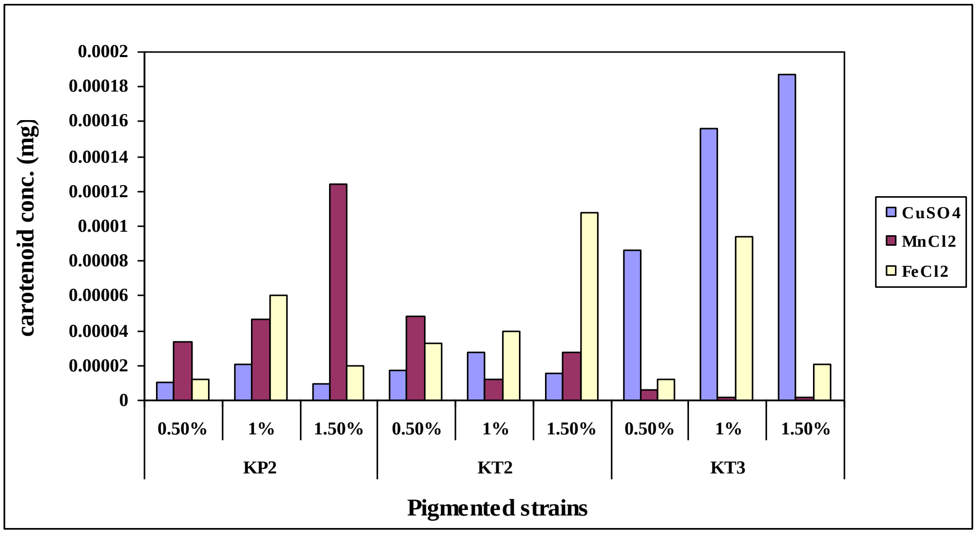

Figure 6 Production of carotenoids by the pigmented halobacteria at different mineral sources

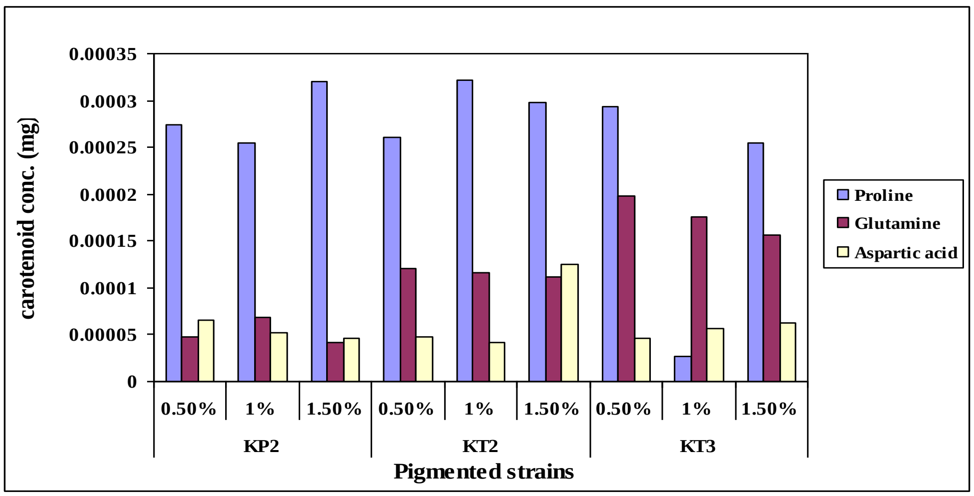

Figure 7 Production of carotenoids by the pigmented halobacteria at different amino acids sources

CONCLUSION

In conclusion, the halobacteria isolated from the saltpan sediments of Kanyakumari district could be used for the mass scale production of carotenoids under the optimized condition.

ACKNOWLEDGEMENT

The authors are greatly acknowledged to the authorities of Alagappa University for providing required facilities and also to University Grants Commission, New Delhi for financial assistance.

REFERENCES

1. Britton G, Liaaen–Jensen S, Pfander H. Carotenoids Handbook, Birkhauser Verlag AG, Basel. 2004.

2. Armstrong G. Genetics of eubacterial carotenoid biosynthesis: a colour tale. Ann Rev Microbiol 1997; 51: 629-659.
3. Margalith PZ. Production of ketocarotenoids by microalgae. Appl Microbiol Biotechnol 1999; 51: 431-438.
4. Liu Z, Yan H, Wang K, Kuang T, Zhang, J, Gui L, Anand X, Chang W. Crystal structure of spinach major light – harvesting complex at 2.72 A^0 resolution. Nature, 2004; 287–292.
5. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment I: Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest Ophthalmol Vis Sci, 1984; 25: 660–673.
6. Latha BV, Jeevaratnam K, Murali HS, Manja KS. Influence of growth factors on carotenoids pigmentation of Rhodotorula glutinis DFR-PDY from natural sources. Ind J Microbiol, 2005; 4: 353-357.
7. Bhosale P, Gadre RV. Optimization of carotenoids production from hyper producing Rhodotorula glutinis mutant 32 by a factorial approach. Let Appl Microbiol 2001; 33: 12-16.
8. Manowattana A, Seesuriyachan P, Techapun C, Chaiyaso T. Optimization of carotenoids production by red yeast sporobolomyces pararoseus TISTR 5213 using waste Glycerol as the sole carbon source. KKU Res J 2012; 17(4): 607-621.
9. Kamffer Z, Bindon KA, Oberholster A. Optimization of a method for the extraction and quantification of carotenoids and chlorophylls during ripening in grape berries (Vitis vinifera cv. Merlot). J agri food chem 2010; 58(11): 6578-6586.
10. Asker D, Awad T, Ohta Y. Lipids of Haloferex alexandrines strain TM^T: an extremely halophilic canthaxanthin producing archaeon. J Biosci Bioeng 2002a; 93(1): 37-43.
11. Weuster-Botz D. Experimental design for fermentation media development: Statistical design of global random search? J Biosci Bioeng 2000; 90(5): 473-483.
12. Kennedy M, Krouse D. Strategies for improving fermentation medium performance: a review. J Ind Microbiol Biotechnol 1999; 23(6): 456-475.
13. Jensen PR, Williams PG, OH DC, Zeigler L, Fenical W. Species-specific secondary metabolite production in marine actinomycetes of the genus Salinispora. Appl Environ Microbiol 2007; 73: 1146–1152.
14. Pathak AP, Sardar AG. Isolation and characterization of carotenoids producing halobacteria from the solar saltern of Mulund, Mumbai, India. Ind J Nat Prod Res 2012;

3(4): 483-488.

15. Asker D, Ohta Y. Production of Canthaxanthin by Haloferax alexandrinus under non-aseptic conditions and a simple, rapid method for its extraction. Appl Microbiol Biotechnol 2002b; 58: 748-750.
16. Ray RR, Jana SC, Nanda G. Optimization of physic-chemical conditions for improved production of β-amylase by Bacillus megaterium B6. Act Microbiol pol 1995; 44: 15-21.
17. Abhijit P, Ratan G, Jana SC. Optimization of Physico-chemical condition for improved production of hyperthermostable β-amylase from Bacillus subtilis DJ5. J Biochem Technol, 2012; 3(4): 370-374.
18. Certik M, Hanusova V, Breierova E, Marova I, Rapta P. Biotechnological production and properties of carotenoid pigments In: Biocatalysis and Agricultural Biotechnology, (415 p) Chapter 25, 2009; pp. 358-373. Tailor and francis group Ltd.
19. Hochella MF. Sustaining Earth: thoughts on the present and future roles in mineralogy in environmental science. Mineral Mag 2002; 66: 627–652.
20. Deuschle K, Funck D, Forlani G, Stransky H, Biehl A, Leister D, Vander Graaff E, Kunze R, Frommer WB. The role of [Delta] 1-pyrroline-5-carboxylate dehydrogenase in proline degradation. Pla Cell 2004; 16: 3413–3425.
21. Kelly M, Norgand S, Liaaen-Jensen S. Bacterial carotenoids .XXXI. C50 carotenoids 5. Carotenoids of Halobacterium salinarium especially bacterioruberin. Act Chem Scand 1970; 24: 2169-2182.
22. Dundas ID, Larsen H. The physiological role of the carotenoids pigments of Halobacterium salinarium. Arch Microbiol 1962; 44: 233-239.