IJRM
INTERNATIONAL JOURNAL OF RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
Vol.:1, Issue:2

Screening for Potential Carotogenic Producing Bacteria from Soils of Arid Region of Rajasthan

IJRM
INTERNATIONAL JOURNAL OF RESEARCH METHODOLOGY
An Official Publication of Human Journals

Richa Jain, Nitish Sharma, Heeralal Choudhary, Ankit Chokiwal and Abhijeet Singh*

Department of Biosciences, Manipal University, Jaipur, India-303007.

HUMAN JOURNALS

www.ijrm.humanjournals.com

Keywords: carotenoids, microorganisms, staining, spectrophotometer

ABSTRACT

The present study is aimed to screen and isolate microorganisms from the arid zone having the potential to produce carotenoids. To achieve the goal three soil samples were collected from different areas under different environmental and climatic conditions such as garden, industrial and sanganer. In total nine pigmented colonies were isolated by serial dilution and quadrant streak method. These colonies were further screened for the production of carotenoids. Among these isolated bacteria, four cocci (circular) and five are rod shaped. These were further characterized by gram staining, which revealed that 5 are Gram positive and 4 are Gram negative. All nine bacteria were tested for production of carotenoids using UV spectrophotometer. It revealed that out of nine, three bacteria (S1B, G2D and S2E) have the potential of producing carotenoids.

INTRODUCTION

Carotenoids are a group of bioactive compounds and are responsible for bright yellow, orange, red pigments of various plants, microorganisms and animals and are widely distributed in nature [1]. These pigments have an important function to act as protective agents against oxidative damage [2]. Recently carotenoids have attracted greater attention due to the beneficial role on human health [3]. Interest in carotenoids has been increased considerably, due in part to the growing evidence of benefits to human health and also to the growth of certain areas of agriculture, especially aquaculture and poultry industry [4]. The utility of carotenoids as anticancer agents [5], and as singlet oxygen or free radical scavengers, as immune response stimulants [6] as coloring agents for cooking sausage, soft drinks, baked goods and as additive to cosmetics [7,8] are well known to us. Carotenoids are natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. There are several dozen carotenoids in the foods that all of us eat, and most of these carotenoids have antioxidant activity. Beta-carotene produced for carotenoid production has been best studied, since in most countries it is the most common carotenoid in fruits and vegetables. However, in the U.S., lycopene from tomatoes now is consumed in approximately the same amount as beta-carotene. Antioxidants (including carotenoids) have been studied for their ability to prevent chronic disease.

Microbial synthesis offers a promising method for production of carotenoids. This explains the increasing interest in production of microbial carotenoids as an alternative for synthetic food colorants [4]. Several algae (Dunaliella, Dictyococcus and Haematococcus), bacteria (many species of eubacteria in addition to halobacteria in archaebacteria), some filamentous fungi (belong to lower fungi and Ascomycetes), yeasts (Cryptococcus, Phaffia, Rhodosporidium, Rhodotorula, Sporidiobolus, and Sporobolomyces) are reported to produce carotenoids [4, 9-13]. Microorganisms produce various pigments like carotenoids, melanin's, flavins, monascins, violacein and indigo [15]. It also protects "life style –related" diseases such as cardiovascular disease and age related macular degeneration due to their antioxidant activity and pro-vitamin A function [16]. They are used as colorants in the food industry to pigment salmon, trout and poultry flesh (or) to identify the color of egg yolk [17]. Engineering of microbial pathway enzyme can produce high amount of carotenoids in an industrial process. The present study was

aimed at isolation of bacterial isolates from different environment source, capable of producing carotenoids of possible commercial importance. Bacterial isolates were classified and identified through morphological and Biochemical characteristics. Carotenoid production was confirmed by UV Spectrophotometer.

MATERIALS AND METHODS

Materials

Soil sample collection

Soil samples were collected from various locations with different environmental condition of Jaipur district like Industrial (I1 and I2), Sanganer (S1 and S2), and Garage Soil (G1 and G2). Samples were collected by scraping of the soil surface with the sterile spatula and about 10 g of soil were obtained from a depth of 2-5 cm.

Isolation of colored pigmented bacteria

Bacteria present in the soil was isolated by serial dilution and spread plated on LB (Luria Bertani) medium (g^{-1}peptone-10, NaCl- 10, yeast extract-5 and pH 6.8 ± 0.2) and incubated overnight at 37°C. Basic biochemical test like gram's staining was done.

Carotenoids production analysis

The bacterial isolates were grown in LB broth in a rotary shaker at 120rpm at 37ºC. After 3-5 days, cells were harvested by centrifugation (Remi C-24 plus) at 8000rpm for 10 min, and 4ºC. The pellet was washed with distilled water and pigments were extracted from the pellet with acetone as a solvent at 60ºC for 20 min or until all visible pigments were extracted. Further centrifugation, the colored supernatant was separated and filtered using the syringe filter of 0.45µm (Millipore) for the examination of carotenoids under the UV-Visible spectrophotometer ranging from 350-700nm with acetone as a blank.

RESULTS AND DISCUSSION

Soil samples were collected from different environmental conditions such as Industrial, Garage, and Sanganer locations of Jaipur district. A total of 3 soil samples was collected and subjected to isolation. The selection of bacterial colony by their pigment due to carotenoids producing bacteria is showing yellow to orange and red to brown color colonies[18]. These bacteria were isolated and further identified based on their colony morphology (Table no1). Such kind of bacteria maintained in LB media (Fig 1). Hong Zhang and Qing-ping (2015)[19] were isolated *Rhodopseudomonas faecalis* PSB-B from Fenhe River in China has the ability to produce carotenoids. Bhat and Marar (2015)[20] were reported the orange color pigment from *Salinicoccus sp*. M KJ997975. Preliminary morphological observations revealed that the colonies were circular, rod shaped, and varied colors. Furthermore, gram staining was performed (Fig.2).

Table No. 1. Colony morphology

S. no	Sample Location	Name of bacteria	Gram Staining	Color	Elevation	Margin	Form
1	Garage soil	G1A	+ve	Dark yellow	Convex	Entire	Rod
2	Sanganer soil	S1B	-ve	Light Brown	Convex	Entire	Circular
3	Sanganer soil	S1C	+ve	Orange	Convex	Entire	Rod
4	Garage soil	G2D	-ve	Pale Yellow	Convex	Entire	Circular
5	Sanganer soil	S2E	-ve	Yellow	Convex	Entire	Circular
6	Sanganer soil	S2F	+ve	Yellow	Convex	Entire	Rod
7	Industrial soil	I1G	+ve	Dark Yellow	Convex	Entire	Rod
8	Industrial soil	I1H	+ve	Cream	Convex	Entire	Rod
9	Industrial soil	I2I	-ve	Yellow	Convex	Entire	Circular

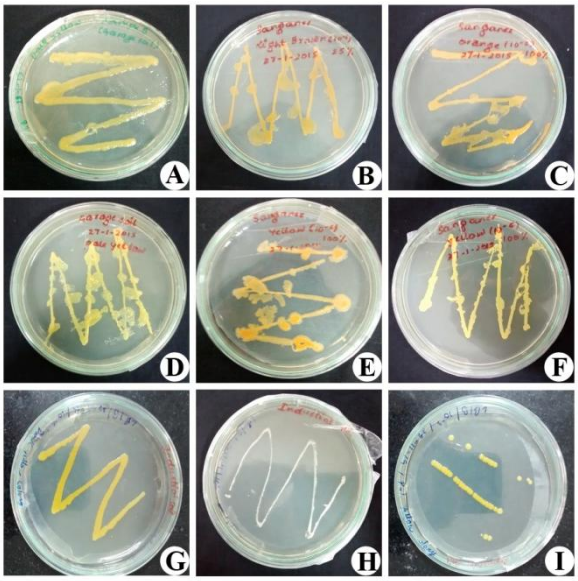

Fig. 1. Pure Isolated Bacteria (A) G1A (B) S1B (C) S1C (D) G2D (E) S2E (F) S2F (G) I1G(H) I1H (I) I2I

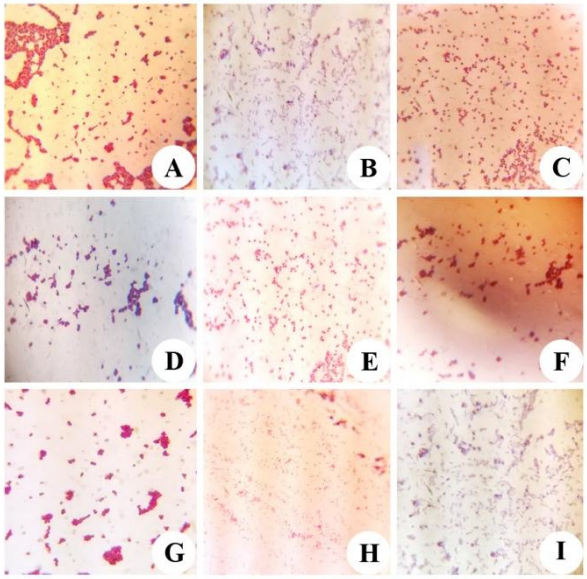

Fig. 2. Gram staining (A) G1A (B) S1B (C) S1C (D) G2D (E) S2E (F) S2F (G) I1G(H) I1H (I) I2I

The carotenoids are lipophilic in nature and soluble in polar organic solvent such as acetone and methanol is widely used. All the bacteria stain carotenoids were extracted by acetone as a

solvent. Among the nine bacteria stains, S1B, G2D and S2E are showing the best result for carotenoids production, maximum peak in the UV spectrum analysis 447, 456 and 449nm (Fig.3). Most of the carotenoids are showing peak between 400-500nm ranges[21]. Such result observed by the Ramasamy and udayasuriyan (2006)[22] were found standard beta carotenoids showing peak at 450nm. These bacteria showing predominately beta carotenoids producing bacteria.

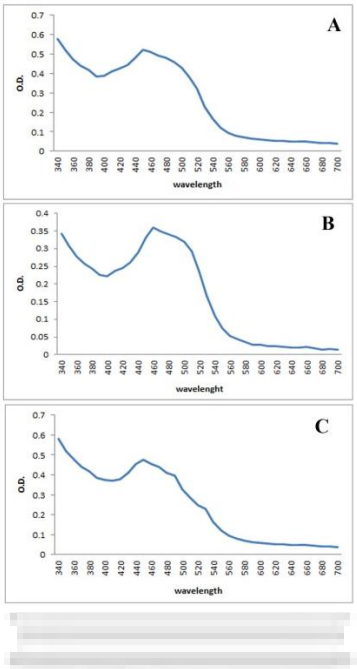

Fig. 3. UV spectrophotometer analysis showing graph A, B and C for bacteria stain S1B, G2D and S2E receptivity

CONCLUSION

In the present study, bacteria were isolated from soil of arid region by the serial dilution and quadrant streak method. Their distinctive pigmented colonies were found is due to carotenoid, which is selected for further testing. Based on the morphological and biochemical properties the isolated bacterium was identified as Rod and cocci shape, and Gram positive and Gram negative. The organism was mass multiplied on LB medium and pigment was extracted. The UV-Visible absorption of the sample was measured and found to be maximum of 447, 456, 449nm, by the bacteria stain S1B, G2D and S2E receptivity which indicate the presence of carotenoid pigment. The maximum absorption peak was showing near about beta carotenoids is 450nm. Further studies are required to analyze the carotenoid sample.

ACKNOWLEDGEMENT

The authors are grateful to the Hon'ble President, Dean, and Director, Faculty of science for providing all the necessary facilities and encouragement.

REFERENCES

1. Wilhelm S, Helmut S.Lycopene: A biologically important carotenoid forhumans? ArchBiochemBiophys. 1996; 336: 1-9.
2. Goodwin TW, Britton G.Distribution and analysis of carotenoids in plant pigments.Acad Press London United Kingdom. 1988; 61-132.
3. Scolnik PA, Bartley GE. Nucleotide sequence of a putative geranylgeranylPyrophosphate synthase from Arabidopsis. Plant Physiol.1995; 104: 1469-1470.
4. Johnson EA and Schroeder WA,Microbial Carote-noids, In: A. Fiecher, Ed., Advances Biochemical Engi-neering Biotechnology. Springer-Verlage, Heidelberg. 1995; 53: 119-178.
5. Hennekens CH.*β*-Carotene Supplementation and Can-cer Prevention.Nutrition. 1997; 13: 697-699.
6. Rapta P, Polovka M, Zalibera M, Breierova E, Zit-nanova I, Marova I and Certik M.Scavenging and Anti-oxidant Properties of Compounds Synthesized by Caro-tenogenic Yeasts Stressed by Heavy Metals, EPR Spin Trapping Study.Biophysical Chemistry.2005; 116: 1-9.
7. Bauernfeind JC. Carotenoids, Colorants and Vitamin A Precursors, Technological and Nutritional Applica-tions. Academic Press.1981; 938.
8. Borowitzka MA.Micro-Algae as Sources of Fine Chemicals.Microbiol Sci.1986; 3: 372-375.
9. Ninet L and Renaut J,Carotenoids, In: PapplerHJand Perimen D. Eds., Microbial Technology, 2nd Edition, Academic Press, New York. 1979; 1: 529-544.
10. Goodwin TW.Fungi, In: Goodwin TW. Ed., The Biochemistry of the Carotenoids, Plants, 2nd Edition, Vol. 1, Chapman and Hall, London. 1980; 257-290.
11. Oren A.Review: A Hundred Years of *Dunaliella*Research. Saline Systems.2005; 1(2): 1-14.
12. Aksu Z and Eren AT. Production of Carotenoids by Isolated Yeast of *Rhodotorulaglutinis*.BiochemEngJ.2007; 35(2): 107-113.
13. Dufossé L, Microbial Pigments, Encyclopedia of Microbiology, 3rd Edition. 2009; 457-471.
14. Sasidharan P, Raja R, Karthik C, Sharma RK, IndraArulselvi P. Isolation and characterization of yellow pigment producing *Exiguobacteriumsps.* J Biochem Tech.2013; 4 (4): 632-635.
15. Dufosse L. Pigments. Microbial, Encyclopedia Microbiol.2009; 4: 457-471.
16. Young AJ, Lowe GM. Antioxidant and prooxidant properties ofCarotenoids. Arch BiochemBiophys.2001; 385: 20-27.
17. Johnson EA, Schroeder WA.Microbial carotenoids. AdvBiochEngBiotech.1995; 11: 297-326.
18. Khoo H, Prasad KN, Kong KW, Jiang Y and Ismail A. Carotenoids and Their Isomers: Color Pigments in Fruits and Vegetables. Molecules. 2011; 16: 1710-1738.
19. Zhang H and Qing-ping HU.Isolation, identification and physiological characteristics ofhigh carotenoids yield *rhodopseudomonasfaecalis*PSB-B. Int J Recent Sci Res. 2015; 6(5): 3893-3899.
20. Bhat MR and Marar T. Media Optimization, Extraction and Partial Characterization of an Orange Pigment from *Salinicoccus sp.* MKJ 997975.Int J Life SciBiotechnolPharma Res. 2015; 4(2): 85-89.
21. ShlaF and Hyolai Y. Pigment productin by *Exiguobacteriumantc.* FH, a novel Labanes strain. Int. J. Cur. Micobl. Ap. Sci. 2013; 2: 76-91.
22. RamasamyAK and Udayasuriyan V.Isolation and charterization of a yellow pigmented colony forming bacterium for carotenogenesis. Biotechnol.2006; 5 (1); 79-82.