Journal of Scientific and Innovative Research 2013; 2 (6): 1044-1051
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2013; 2(6): 1044-1051

Received: 04-10-2013
Accepted: 31-12-2013

Varsha M.Chaudhari
Department of Microbiology, PSGVPM's SIPArt's, GBPatel Science, STSKVS Commerce College, Nandurbar, Maharashtra 425409, India

Correspondence:
Varsha M.Chaudhari
Department of Microbiology, PSGVPM's SIPArt's, GBPatel Science, STSKVS Commerce College, Nandurbar, Maharashtra 425409, India
Tel: +919850184264
E-mail:
mail.varsharaj_294@rediffmail.com

Optimization of the extraction parameters for the production of biopigment from the new isolate of distillery effluent

*Varsha M.Chaudhari**

Abstract

The present study has been carried out to investigate the effects of single factors such as temperature, extraction time, nature of solvent, and concentration of solvent on the contents of biopigments from the newly isolated organism *Planococcus maritimus* AHJ_2 isolated from distillery spent wash. On this basis, an L16 orthogonal multiple factors design of experiment was performed to determine the optimal conditions for the extraction of biopigments. The amount of pigments extracted reached its maximum value when extracted at 80ºC for 10 min by using 80% methanol with 2 times of extraction. Spectrophotometric, TLC and FTIR performed for the optimized extracts proved the presence of carotenoids.

Keywords: Carotenoids, Orthogonal experiments, Single-factor experiments, *Planococcus maritimus* AHJ_2.

Introduction

Pigments are colorants which have been widely used in food, cloth, painting, cosmetics, pharmaceuticals and plastics.[1] The currently used colorants are almost exclusively made from nonrenewable resources such as fossil oil. The production of the synthetic colorants is economically efficient and technically advanced with colors covering the whole color spectrum. However, synthetic colorants are facing the following challenges: dependence on non-renewable oil resources and sustainability of current operation, environmental toxicity, and human health concerns of some synthetic dyes.[2] In the food, cosmetics and pharmaceutical industries, due to the serious environment and safety problems caused by many artificial synthetic pigments research has focused on processes for the production of safe and natural pigments from natural resources. Thus, searching renewable and environmentally friendly resources for production of colorants is an urgent need.[3]

Biological pigments are natural and a better substitute to chemical dyes used in the industries and laboratories.[4] Nature produces many biocolorants from various resources including plants, animals, and microorganisms, which are possible alternatives to synthetic dyes and pigments currently employed.[5] One promising method is to produce biopigments by microbes because of their fast growth rate and the feasibility of bioprocess development.[6] Microorganisms produce a large variety of stable pigments such as carotenoids, flavonoids, quinones, and rubramines, and the fermentation has higher yields in pigments and lower residues compared to the use of plants & animals.[7]

Thus, biosynthesis of dyes and pigments via fermentation processes has attracted more attention in recent years.[8] Among the natural pigments produced by microbes reported so far, most research has focused on yellow and red pigment production, such as monascue produced by Meniscus sp.[9], carotenoid from Phaffia rhodozyma[10], Micrococcus roseus[11], Brevibacterium linens[12] and Bradyrhizobium sp. [13], and xanthomonadin from *Xanthomonas campestris* pv. [14]

Numbers of methods are available for extraction of pigments from various sources. Pigment extractions from microbial sources are generally carried out employing organic solvents. A wide range of polar and non-polar solvents either individually, sequentially or in combination have been employed for optimal extraction of pigments from algae, fungi and bacteria with and without pretreatment to biomass. Ultrasonic and hydrochloric acid assisted extractions, as well as supercritical CO_2 extraction have also been attempted for carotenoids extraction from microbial source. These studies revealed that the method of extraction and solvent to be employed depends on the nature of pigment as well as biomass produced by the typical microbial source. But there is a need to develop a suitable extraction method with maximum pigment yield from microbial sources, which can be applied on a commercial scale. Hence, present studies were conducted to determine the optimum conditions to extract pigment from *Planococcus maritimus* AHJ_2 in order to explore a proper process to utilize the pigment in the food as well as in pharmaceutical industry.

Materials and methods

All chemicals and culture media used for the growth of pigment producing bacteria were purchased from Hi-Media laboratories (Bombay, India). Double glass distilled water was used for the experimentation. Solvents used for the extraction of pigments were of HPLC grade and purchased from S.D Fine chemicals (Bombay, India).

Instruments

The instruments used in this study were UV/Visible spectrophotometer (UV Mini 1240 Shimadzu, Japan), commercial heavy duty shaker (REMI), cyclomixer and water bath (REMI).

Collection of distillery spent wash

Distillery spent wash from Shri Satpuda Tapi Sahakari sugar factory and distillery section, Purushottamnagar, Shahada was collected in sterile 500 ml Erlenmeyer flasks. Analysis of the sample was carried out in the research laboratory of Department of Microbiology, PSGVPM'S 'ASC' College, Shahada.

Microorganism

Culture of *Planococcus maritimus* AHJ_2 was isolated on Luria- Bertani agar from distillery spent wash of Shri Satpuda Tapi Sahakari sugar factory and distillery section. Isolate was purified and identified from Institute of Microbial Technology (IMTECH), Chandigarh, India. It was maintained on slants of Luria- Bertani Agar (LB) medium at 40^0 C and sub cultured after every 30 days.

Preparation of inoculum

Inoculum of *Planococcus maritimus* AHJ_2 was prepared from single colony of actively growing culture in sterile saline. 100 µl of suspension was inoculated in 500 ml Erlenmeyer flask containing 100 ml sterile Luria Bertani broth (casein enzyme hydrolysate $10gl^{-1}$; yeast extract, $5gl^{-1}$; NaCl $10gl^{-1}$; pH 7±0.5) Flask was incubated on rotary shaker at 120 rpm for 48 h. 100 µl of inoculum from this flask was used for further study.

Extraction of biopigment

The main factor that affects the extraction of pigments is the nature of the solvent, temperature, extraction time and amount of solvents. In the present study optimum extraction conditions were determined by orthogonal design of experiments i.e., three levels and four different parameters. R value (Range analysis) was carried out to investigate the effect of each factor in the extraction process of biopigments. Further as the carotenoids are thermolabile and sensitive to temperature; the extraction method was modified to obtain maximum yield.

Spectrophotometric analysis

Spectrophotometric analysis and estimation of the pigment was carried out at 466 nm by UV –VIS spectrophotometer.

Experimental design

Basically, experimental design used in the present study comprised of single-factor experimental design, which was used for screening of extraction process and orthogonal design that was as a multiple factors for the optimization of extraction process.[15]

Single factor experiment

Preliminary experiment was carried out to determine the effect of different variables on the extraction of biopigments.

1. Solvent Type

Extractions of biopigment using various different types of solvents like acetone, methanol, ethanol, dichloromethane and dimethyl sulphoxide (DMSO) was carried out. Each solvent was used at fixed condition of temperature and a fixed extraction time of 10 min. The best solvent type was chosen based on the highest value of total pigment content express as a dry weight in mg /100 g cell mass.

2. Solvent concentration

The solvent concentration was investigated using the best solvent with concentration ranging from 60% to 100% (v/v). Various concentrations of solvent were prepared in the double distilled water ranges from 60% to 100% (v/v). The best solvent concentration was selected based on the highest value of total pigment present as a dry weight in mg/100 g cell mass.

3. Extraction Temperature

In order to determine the effect of extraction temperature, extraction was executed by using the best solvent composition at fixed time, under various temperatures, which were 70, 80, 90 and 100^0C. The extraction procedures were repeated. The best extraction temperature was chosen based on the highest value of pigment (mg/ 100 g cell mass).

4. Extraction Time

The impact of extraction time on the yield of biopigment was varied from 5, 10, 15, 20, min. Extraction was accomplished by applying the best solvent composition at optimum temperature. The extraction procedures were repeated. The best extraction time was chosen according to the highest value of pigment (mg / 100 g cell mass).

Multiple factor design

Development and Optimization of pigment extraction using L16 orthogonal design

The orthogonal experiment was designed based on the single factor experiment. The parameters and the orthogonal design of the experiment for the extraction of biopigment were given in the Table 1 and 2. The results were made in the form of range analysis. The results were shown in Table 3 & 4.

Factors for the extraction of biopigment

Table 1: Orthogonal Experiment of the extraction

	A	**B**	**C**
Levels	**Temperature (^{0} C)**	**Extraction Time (min)**	**Solvent (%)**
1	60	5	60
2	70	10	70
3	80	15	80
4	90	20	90

Table 2: L16 Orthogonal design of experiment

No.	**A**	**B**	**C**
1	1	1	2
2	1	2	3
3	1	3	4
4	1	4	1
5	2	1	2

6	2	2	3
7	2	3	4
8	2	4	1
9	3	1	2
10	3	2	3
11	3	3	4
12	3	4	1
13	4	1	2
14	4	2	3
15	4	3	4
16	4	4	1

Results and discussions

Effect of different solvent type on the extraction of biopigment

The result in Figure 1 revealed that the maximum amount of pigment content was observed when extracted in methanol. Considering one of the aims of this work is to propose a suitable solvent for extracting the pigment. Among various solvents like acetone, ethanol, methanol, dimethyl sulphoxide (DMSO), dichloromethane (DCM) etc., methanol and ethanol were selected as a right choice because it is environmentally benign and relatively safe to human health. Methanol was found to be more effective solvent for the extraction; it interacts with the pigments probably through non-covalent interactions and promotes a rapid diffusion of the pigment into the solution.

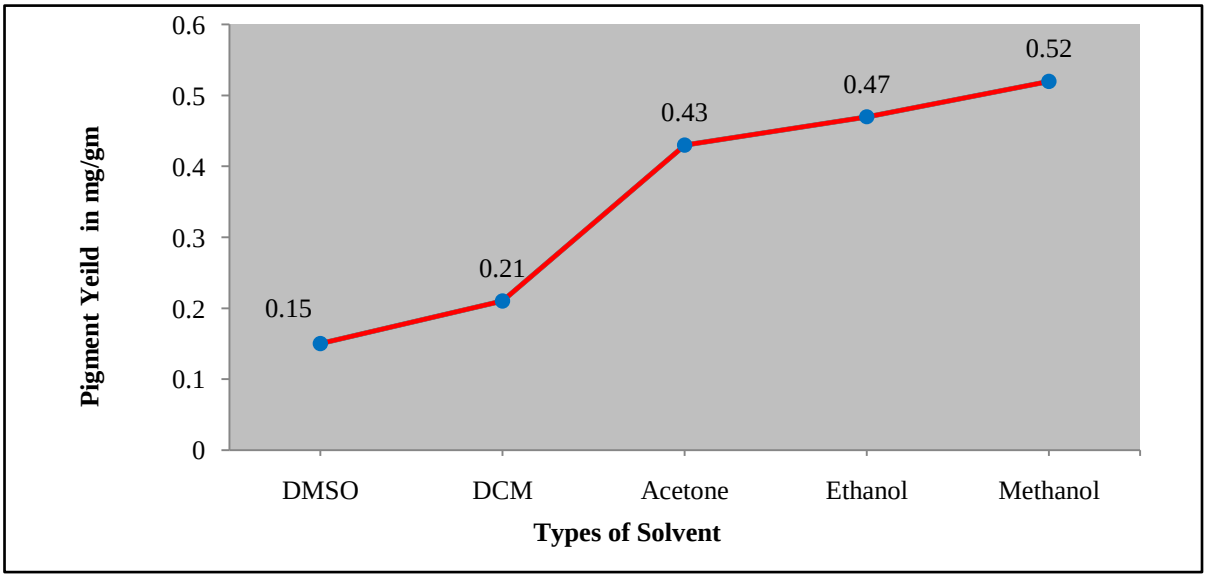

Figure 1: Effect of different solvent type on the extraction of pigment

Effect of concentration of solvent on the extraction of biopigment

It observed from the Figure 2 that the content of pigment extracts, increases with the concentration of methanol i.e., 60% to 100%. Various concentration of methanol prepared in distilled water used exhibited different effect in the content of pigment. Change in the fluid polarity had diverse effect on the solubility enhancement of the pigment but optimal methanol concentration for extraction of pigment was found to be 80 %.

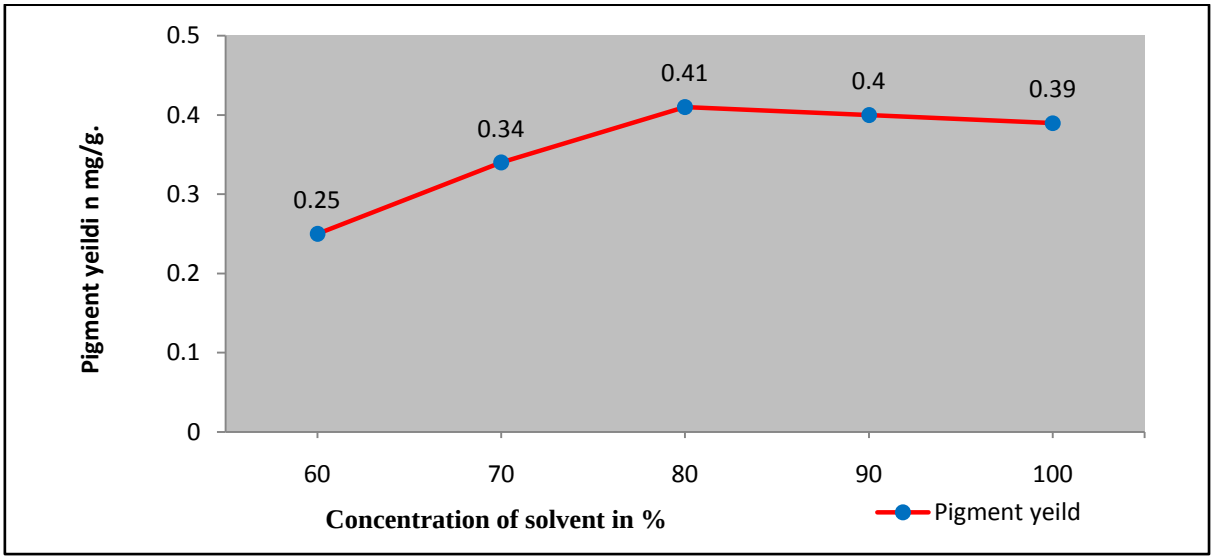

Figure 2: Effect of the methanol concentration on the extraction of biopigment

Effect of extraction time on the content of biopigment

The results of the Figure 3 showed that the contents of pigment extracted for 10 min reached maxima and prolonged time for extraction may not yield an increased content.

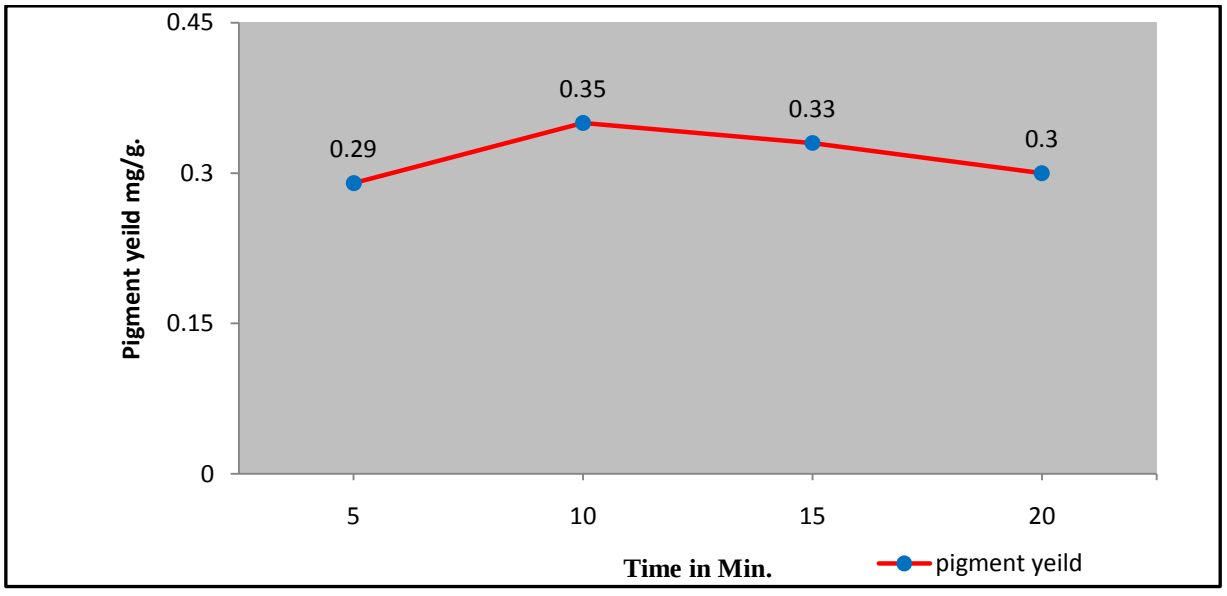

Figure 3: Effect of extraction time on the production of biopigment

Effect of temperature on the extraction of biopigment

Figure 4 showed the contents of pigment tended to increase gradually with a rise in the temperature range from 70ºC to 100ºC. The contents of pigments gradually increased with a rise in the temperature in a range of 70ºC to 100ºC with a 10ºC temperature interval. It may be probable that at higher temperature causes the diffusion of pigment more quickly from cell to extracting agent. Temperature's effect on extraction is dual. On one hand, higher temperature can accelerate the solvent flow and thus increase the increase the extraction of pigment.

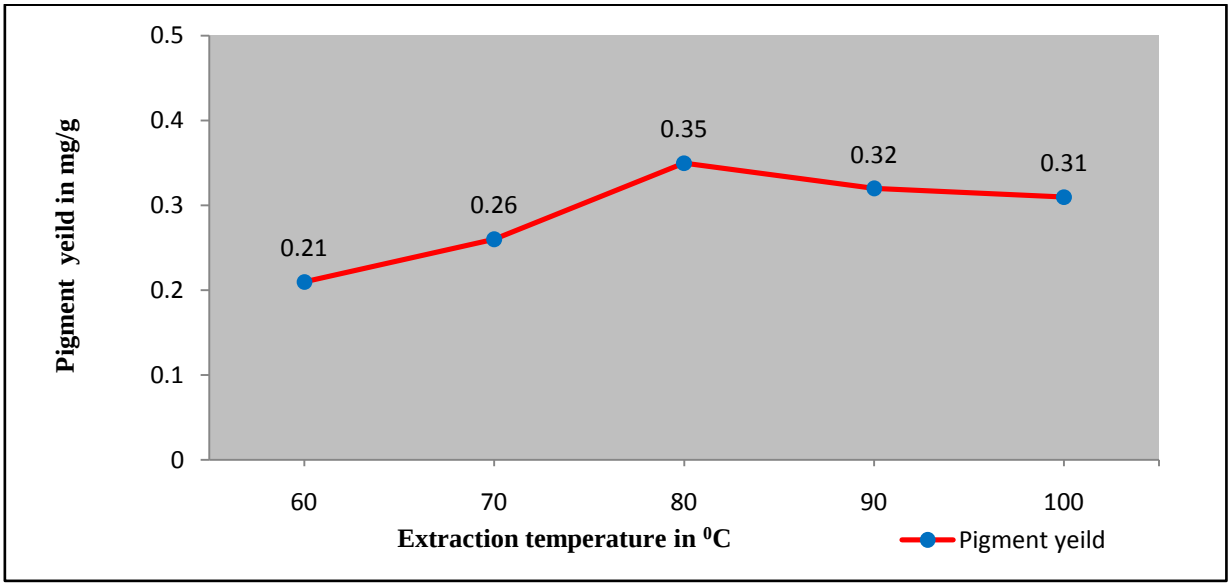

Figure 4: Effect of extraction temperature on the production of biopigment

Multiple factor experiment

Optimization of biopigment extractions using L16 orthogonal design

The parameters and the orthogonal design of experiment for the extraction of pigments were given in the Table 3. The results were made in the form of range analysis. The order of the effect of factors on pigment extraction was A>C>B. The concentration and nature of the solvent had the greatest effect on the extraction procedure. An equivalent effect was observed in the temperature. The other factors such as time of extraction and number of extractions did not play a vital role in extracting the pigment to a higher yield. The optimum extraction conditions obtained from the range analysis were A3B2C3. It means that 80°C, 10 min of extraction duration, and 80% methanol concentration.

Table 3: Yield of biopigment and range analysis

No.	A	B	C	Pigment Yield mg\g
1	1	1	2	1.92
2	1	2	3	2.54
3	1	3	4	3.10
4	1	4	1	2.64
5	2	1	2	3.84
6	2	2	3	4.57
7	2	3	4	3.21
8	2	4	1	2.95
9	3	1	2	3.05
10	3	2	3	5.04
11	3	3	4	3.74
12	3	4	1	3.01
13	4	1	2	2.36
14	4	2	3	1.92
15	4	3	4	2.60
16	4	4	1	1.90

K1	2.55	2.79	2.63	
K2	3.64	3.51	2.8	
K3	3.71	3.16	3.52	
K4	2.19	2.62	3.16	
k_1	0.63	0.69	0.65	
k_2	0.91	0.87	0.69	
k_3	0.92	0.79	0.88	
k_4	0.54	0.65	0.79	
R	0.38	0.22	0.23	

TLC and Spectrophotometric results

The results of TLC and Spectrophotometric revealed the presence of carotenoids by absorption at 466 nm by spectrophotometer UV mini Shimadzu.

Conclusion

In conclusion, the extraction conditions for biopigments were optimized to find that the extraction temperature 80ºC, 10min of extraction duration, 80% methanol and 2 times of extraction were the optimal conditions. Moreover, temperature was found to be a significant factor that affects the extraction procedure. The Spectrophotometric, TLC and HPLC results of the optimized extracts were found to contained carotenoids compounds.

Acknowledgements

The authors are greatly thankful for the financial support by UGC WRO Pune, India, for carrying out the above research work.

References

1. Yuan Lu., Liyan Wang., Yuan Xue., Chong Zhiang., Xin-Hui Xing., Kai Lou. *Et al*. Production of violet pigment by a newly isolated psychrotrophic bacterium from a glacier in Xinjiang, China. Bioche. Engi. Journal. 2009; 43: 135–141.

2. Farzaneh Alihosseini, Kou-San Ju, Jozsef Lango, Bruce D., Hammock Gang Sun., Antibacterial Colorants: Characterization of Prodiginines and Their Applications on Textile Materials. Biotechnol. Prog. 2008; 24: 742-747.

3. Cho Y.J., Park J.P., Hwang H.J., Kim S.W., Choi J.W., Yun J.W., Production of red pigment by submerged culture of *Paecilomyces sinclairii*. Lett. Appl. Microbiol. 2002; 3:195–202.

4. Palanichamy V., Aachhari Hundet., Bhaskar Mitra., Reddy N., Optimization of cultivation parameters for growth and pigment Production by streptomyces spp. isolated from marine sediment and rhizosphere soil. Int.J.of Plant, Animal and Envir.Sci. 2011; 1(3):158-170.

5. Mapari S. A. S., Nielsen K.F., Larsen T.O., Frisvad J.C., Meyer A.S., Thrane U., Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. Curr. Opin. Biotechno. 2005; 16 (2): 109-238.

6. Zhao, D.H., Lu L., Qin H.L., Studies on the properties of a microbial blue pigment. Food Ferment. Ind. 1998; 5: 21–24.

7. Dura´n, N., Teixeira M.F.S., R.De. Conti. Esposito E., Ecological-friendly pigments from fungi. Crit. ReV. Food Sci. Nutr.42, 2002; 1:53-66.

8. Hobson D. K., Wales D.S., Green colorants. J. Soc. Dyers Colour. 1998; 114: 42-44.

9. Yongsmith B., Krairak S., Chaisrisook C., Fermentation of yellow pigments by cassava starch utilizing Monascus spp., Biotechnol. Sustainable Util. Biol. Resour.Trop. 1998; 12: 235–244.

10. Vazquez M., Santos V., Parajo J.C., Effect of the carbon source on the carotenoids profiles of *Phaffia rhodozyma* strains. J Ind. Microbiol. Biotechnol. 1997; 19: 263–268.

11. Chattopadhyay M.K., Jagannadham M.V., Vairamani M., Shivaji S., Carotenoid pigments of an Antarctic psychrotrophic bacterium *Micrococcus roseus*: temperature dependent biosynthesis, structure and interaction with synthetic membranes. Biochem. Biophys. Res. Commun. 1997; 239: 85–90.

12. Guyomarc'h F., Binet A., Dufossé L.,Production of carotenoids by *Brevibacterium linens*: variation among strains, kinetic aspects and HPLC profiles. J. Ind. Microbiol. Biotechnol. 2000; 24: 64–70.

13. Lorquin J. F., Molouba B.L., Dreyfus, Identification of the carotenoid pigment canthaxanthin from photosynthetic Bradyrhizobium strains. Appl. Environ. Microbiol. 1997; 3: 1151–1154.

14. Poplawsky A.R., Urban S.C., Chun W., Biological role of Xanthomonadin pigments in *Xanthomonas campestris* pv. Campestris. Appl. Environ. Microbiol. 2000; 9: 5123–5127.

15. Bohua W., Liping L., Lei L., Weiping C., Optimization of β-carotene production by a newly isolated *Serratia marcescens* strain. Electronic Journal of Biotech.2012; 6:1-8.