## Original Research Article

# Pigment production by *Exiguobacterium aurantiacum* FH, a novel Lebanese strain

**Fatima Shatila\*, Hoda Yusef and Hanafy Holail**

Beirut Arab University, Faculty of Science, Department of Biological and Environmental Sciences, Debbieh-Lebanon
*\*Corresponding author*

### A B S T R A C T

**Keywords**

Microbial pigments, *Exiguo-bacterium aurantiacum* FH,

An orange pigmented strain of *Exiguobacterium aurantiacum* FH was isolated from air. The analysis of pigment produced revealed the presence of carotenoids. Both carotenes and xanthophylls were detected in the methanolic extract of carotenoids. The carotenoids produced were characterized by considerable stability and demonstrated antifungal activity against *Fusarium* sp., *Penicilium* sp., and *Alternatria* sp.. By optimization of the factors affecting carotenoid production, cellular pigment content (534.51 µg pigment /g dry weight) was recorded when *E. aurantiacum* FH was cultivated at 30 °C under shaken condition (150 rpm) in 250 Erlenmeyer flasks containing 40 ml LB medium (pH 7), for three days in light.

## Introduction

Health issues and environmental concerns due to unmonitored utilization of synthetic colorants revived interest in natural dyes as they are safer, healthier, biodegradable, and exhibit higher compatibility with the environment (Ahmad et al, 2012). Natural pigments can be obtained from animals, plants, algae, and microorganisms (Mortensen, 2006). However, few of them are available in sufficient quantities for commercial use as food colorants which necessitate continuous investigation for new resources (Aberoumand, 2011). Microorganisms produce wide variety of pigments including carotenoids, flavins, monascins, chlorophyll, quinines, prodigioson, violacien (Dufosse, 2006);

thus they represent a potential source for natural pigments of commerical interest in several industries as food, feed, cosmetics, pharmaceuticals and neutraceuticals (Varsha and Arpana, 2013). In addition to their natural character, microbial pigments are safe to use, moreover, they maintain controllable and predictable yields, and unlike plant pigments, they don't exhibit seasonal and geographical variations (Joshi *et al.*, 2003; Mohanasrinivasan *et al.*, 2013).

The current study aimed to characterize the orange pigment produced by a bacterial isolate, *Exiguobacterium aurantiacum* FH, and investigate the

cultural and environmental conditions that lead to maximum pigment production. The pigmented extract was also assessed for its stability in light, and in various pHs (5, 7 & 9) and temperatures (25, 50, 75 & 100ºC). It was also screened for potential antimicrobial activity.

## Materials and Methods

### Microorganisms

The experimental strain, an orange pigment producing bacterium, was isolated from air in Dibbieh-Lebanon. Bacterial and fungal plant pathogens were kindly provided by the Microbiology lab, faculty of Science, BAU.

### Characterization and identification of the pigmented bacterial strain

The morphological features of the pigmented bacterial strain grown on nutrient agar were described. The bacterial cells were Gram stained; the Gram reaction was confirmed by KOH test.

Molecular characterization was proceeded using 16S RNA sequence analysis. Single colony of the selected bacterial isolate was suspended in 100 ml of 33 mM Tris HCL buffer (pH 8.0). The bacterial suspension was incubated for 10 min at 95 °C and centrifuged to precipitate cell debris. The supernatant was transferred to a fresh tube and used as total genomic DNA samples. The bacterial 16S rDNA was amplified from the total genomic DNA using universal eubacteria specific primers, designated to amplify 1500 bp fragment of the 16S rDNA regions. The forward primer was: 27F (5'AGAGTTTGATCMTGGCTCAG 3') and the reverse primer was: 1492R (5'TACGGYTACCTTGTTACGACTT3')

which yielded a product of approximately 1500 bp. The PCR reaction conditions included 35 cycles denaturation at 94 °C for 1 min in addition one cycle of extension at 72 °C for 10 min. The PCR product was purified using QIA quick PCR purification reagents (Qiagen) according to the kit manual. The purified product was directly sequenced. DNA sequences were obtained using an ABI PRISM 377 DNA Sequencer and ABI PRISM Big Dye Terminator Cycle Sequencing (Perkin Elmer). The PCR product was sequenced using the same PCR primers. Blast program (www.ncbi.nlm.nih.gov/BLAST) was used to access the DNA similarities and multiple sequence alignment and molecular phylogeny were performed using Bio Edit software. The phylogenetic tree was displayed using the TREE VIEW program.

### Media

Media used in this study were prepared using distilled water, adjusted to initial pH $7 \pm 0.2$ and sterilized by autoclaving at 121 ºC for 20 minutes at pressure 15 lb/inch$^2$. The solid media were prepared by adding 15 grams agar/ liter, ingredients are given in g/l, Nutrient broth, NB (Baskar *et al.*, 2010): Beef extract, 3; peptone, 5; sodium chloride, 5..
Luria Bertani broth, LB (Hardjito *et at.*, 2002): Yeast extract, 5; peptone, 10; sodium chloride, 5.
Synthetic medium: glucose, 5; $(NH_4)_3PO_4$, 1; NaCl, 5; MgSO4, 0.2; $KH_2PO_4$, 1.

Mueller Hinton, MH: Beef extract, 2; digest of casein, 17.5; starch, 1.5; agar, 17. Sabouraud dextrose agar, SDA: Enzymatic digest of casein, 5; enzymatic digest of animal tissue, 5; dextrose, 14; agar, 15. pH $5.6 \pm 0.2$.

**Inocula preparation, cultivation and growth determination**

Seed cultures were prepared by inoculating 100 ml Erlynmeyer flask containing 30 ml nutrient broth by a loopfull from a single colony and shaken at 150 rpm at 30 °C till bacterial growth reached OD $_{550\ nm}$ = 1. Two percent inoculum size from the seed culture was introduced into 250 ml Erlenmeyer flasks containing 50 ml of sterile growth medium (pH adjusted to 7). Inoculated flasks were incubated in shaking incubator (150 rpm) at 30 °C for three days in light (unless otherwise indicated).

The culture medium was centrifuged at 6000 rpm for 15 minutes to separate cells from the culture medium. The harvested cells were washed twice and centrifuged. The harvested wet cell pellets were used for pigment extraction and for growth determination after drying at 60 °C till constant weight was obtained.

**Pigment extraction**

Different solvents (acetone, absolute methanol, methanol, ethanol, petroleum ether & ethyl ether) were investigated for their ability to extract the intracellular water insoluble pigment. Pigment extraction from the harvested cells was done in dim light. The wet cell pellets were suspended with the most potent solvent (absolute methanol), subjected to vigorous vortexing for 1 minute, followed by a resting period for 10 minutes then centrifugation for 15 minutes at 6000 rpm. The process was repeated till the cell pellets became colorless (Khanafari *et al.*, 2010; Ibrahim, 2008).

**Pigment identification**

**Spectrophotometric analysis**

The absorption spectrum of pigment extract was measured within visible a wavelength region 400 - 690 nm.

**Chemical identification**

The bacterial extract was chemically tested to detect the presence of carotenoids. 1 g dry weight of harvested cells was extracted with 10 ml chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and few drops of 85 % sulfuric acid were added. A blue color appeared at the interface indicated the presence of carotenoids (Mrak *et al.*, 1949; Karrer & Jucker, 1950; Ajayi *et al.*, 2011).

**Pigment separation**

Methanolic extracts of pigment was concentrated using a rotary evaporater, then two ml were transferred to 5 ml petroleum ether and partitioned with equal volume of 90% methanol. After vigorous shaking in a separatory funnel, the hypophase and epiphase obtained were further analyzed using TLC.

Silica gel coated on plastic sheets were used as stationary phase. Few drops from each phase were spotted on the base line of the TLC plate and then the plate was placed inside presaturated TLC chamber containing a mobile phase (methanol: benzene: ethyl acetate 5: 70: 25) (Forgacs & Caserhati, 2002). The chromatogram was analyzed visually for banding patterns and the spots were marked. Relative Rf values were calculated using the following formula:

Rf=

$$\frac{\text{Distance traveled by the substance}}{\text{Distance traveled by the solvent}}$$

**Stability assessment of the pigmented extract**

Stability of the methanolic extracted pigment was tested after incubation in different temperatures (25, 50, 75 & 100 °C) for five hours, various pH (5, 7 & 9), as well as in light and dark for 24 hours. The absorbance of extracted pigment was measured at wavelength 450 nm and the loss of color was calculated according to the following equation:

$$\text{Color loss (\%)} = \frac{OD\,i - OD\,t}{OD\,i} \times 100 \; ,$$

Where ODi = Initial OD; & ODt= OD at time (t)

**Antimicrobial effect of the pigmented extract**

The antimicrobial effect of the extract was tested against several bacterial pathogens (*E coli, Klebsiella* sp*., Staphycococcus aureus* & *Erwinia* sp.) as well as against five plant fungal pathogens (*Penicillium* sp*., Alternaria* sp*., Botrytes* sp*., Fusarium* sp., & *Sclerotium* sp.).

Sensitivity testing using disc diffusion method was carried on Mueller Hinton medium for bacterial antibiograms, and on sabouraud dextrose agar for fungal antibiograms. Sterile discs were soaked with the filter- sterilized pigmented extract, and added to the petri dishes on which microbial isolates were plated. The control disks were prepared by saturating sterile discs with absolute methanol (Amanullah *et al*., 2011). The plates were incubated for 24 hours at 30°C and 37°C

for fungal and bacterial pathogens respectively. After incubation period, the plates were checked for inhibition zones formation. The presence of inhibition zone around the discs soaked with pigmented extract but not around the control discs indicated the presence of antimicrobial activity.

**Carotenoids determination**

Total carotenoid content of bacterial cells was determined according to the following equation:

$$\text{Carotenoid }_{\mu g/g} = \frac{A \times V(ml) \times 10^4}{E1\% \times W(g)} \; ;$$

A= absorbance at 450 nm;
V= volume of extract;
W= dry weight of bacterial cells;
$10^4$ = conversion factor to obtain concentration in units of µg/g (Rodriguiz-Amaya & Kimura, 2004; Rivera & Canela, 2012).

**Optimization of culture conditions**

The effect of several factors on growth and pigment production by the isolated bacterium was investigated, these included: effect of culture media (Luria Bertani broth, nutrient broth and a synthetic medium), effect of static and shaken conditions (100 & 150 rpm), effect of incubation period (1, 2, 3, 4, 5 & 6 days), effect of incubation temperature (25, 30 & 37 °C), effect of pH of culture medium (4, 5, 6, 7, 8, 9 & 10), effect of culture volume (30, 40, 50 & 60 ml/ 250 ml Erlynmeyer flask), effect of inoculum size percentage (1, 2, 4, 6 & 8 %), also the effect of light & dark incubation was also investigated. Statistical analysis using one way ANOVA and student's T-test were performed to detect significance of factors affecting pigment production.

## Results and Discussion

### Isolation, phenotypic and molecular characterization of the bacterial isolate

An orange pigmented bacterium isolated from air and purified was described on nutrient agar plates. The colonies of the isolate were 2 mm in diameter with a regular circular form, entire margin, glistening surface, viscous texture, and convex elevation. Gram staining followed by microscopic examination and confirmed by KOH test, revealed that the bacterial isolate was Gram positive cocci arranged in pairs. The molecular characterization of the isolate revealed 97% similarity to *Exiguobacterium aurantiacum* and was referred to as *Exiguobacterium aurantiacum* FH (Figure. 1). The sequence was submitted to GenBank and it is available under the accession number KC699817.

### The effect of different solvents on pigment extraction

*Exiguobacterium aurantiacum* FH was cultivated in nutrient broth medium for 3 days. Then the colored cell pellets were separated by centrifugation from the culture medium. Polar and non polar solvents (used individually or in mixture) were investigated for their ability to extract the non water soluble orange pigment from the wet cell pellets. The pellets were totally decolorized when absolute methanol was utilized.

Suhnel et al. (2009) mentioned that the chemical composition of the pigment extracted directly influences the choice of organic solvent and yield. Carotenoids are lipophilic and soluble in organic solvents, such as chloroform, hexane, acetone, petroleum ether, etc. (Belitz *et al*., 2004;

Lee & Schwartz, 2012). Britton (1985) and Fikselova et al. (2008) recommended water miscible organic solvents, such as ethanol, methanol and acetone, to extract carotenoids. In accordance with the present findings, methanol was used for carotenoids extraction from several bacterial species; *Kocuria polaris* (Reddy *et al*., 2003), a marine *Micrococcus* sp. (Ibrahim, 2008), and *Rhodobacter sphaeroides* WL-APD911 (Wu & Liu, 2011).

### Pigment identification

### Spectrophotometric identification of the methanolic extract

The methanolic extract of *E. aurantiacum* FH, analyzed spectrophotometrically by scanning the absorbance within a wave length region 400-690 nm, demonstrated the presence of a shoulder peak with maximum absorbance at $\lambda = 463$ (Figure. 2) which was a typical pattern of absorption spectrum of a carotenoid. Liakopoulou-Kyriakides and Kyriakidis (2002) mentioned that carotenoids absorb light in the visible region between 400 and 500 nm; and the obtained absorption spectra can be used for the identification of carotenoids. Bridoux (2008) mentioned that most carotenoids absorb light maximally at three wavelengths thus resulting in a three peak spectra.

Carotenoids that contain polyene system of 10 or 11 double bonds show a three banded absorption spectrum, in which the band of maximum intensity is located at an intermediate distance from the other two bands, thus form broad bands having fine details of a shoulder (Fieser, 1950).

**Figure.1** Phylogenetic relationships among representative experimental isolate and the most related bacteria based on 16S rRNA sequences
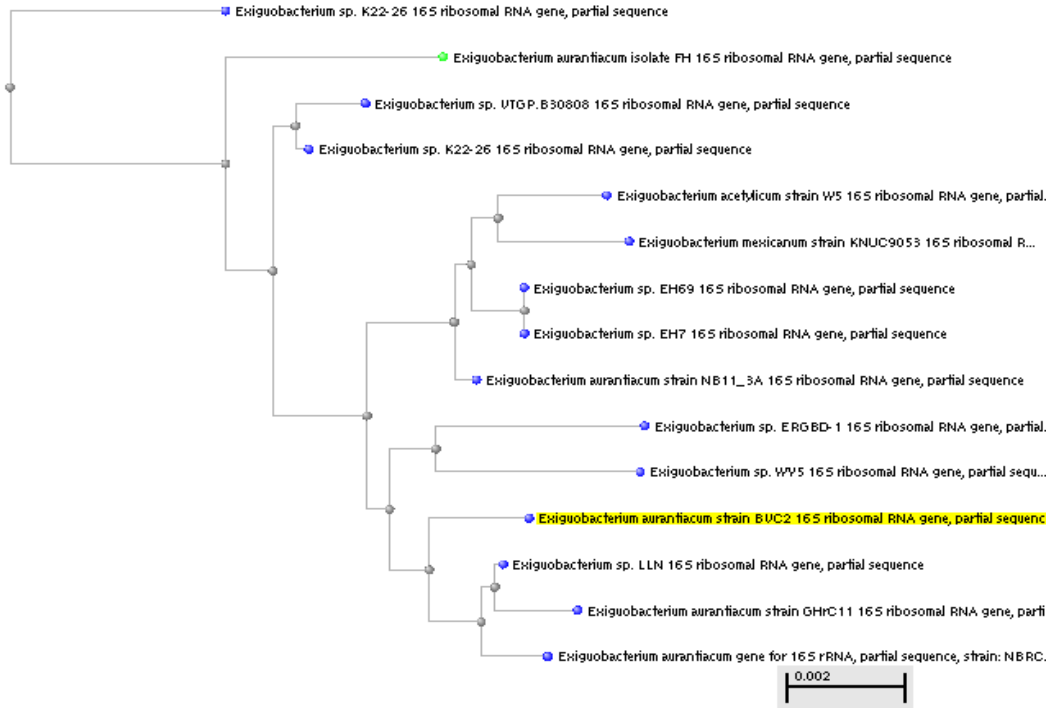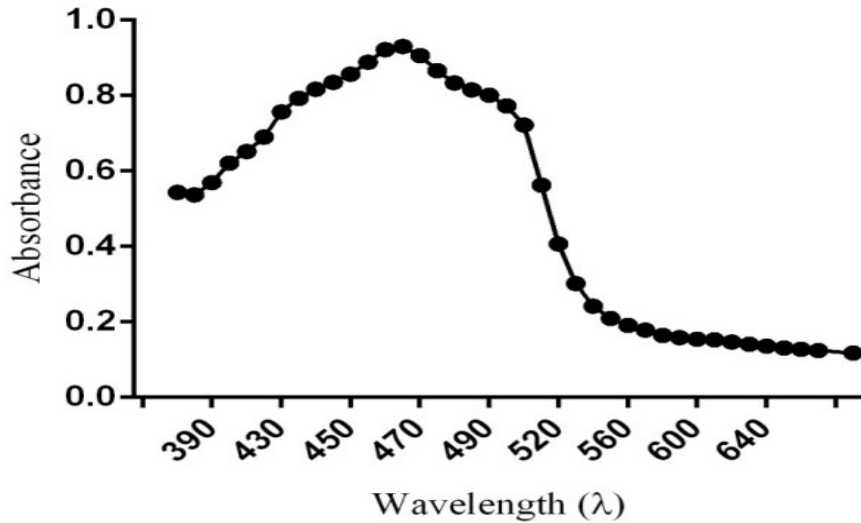


**Figure.2** Visible spectra of the extracted pigment

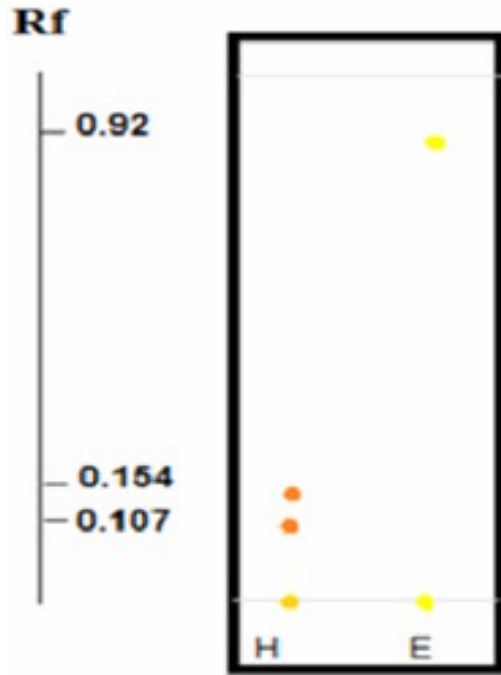**Figure.3** Diagram of TLC chromatogram showing; (H) hypophasic and (E) epiphasic carotenoids
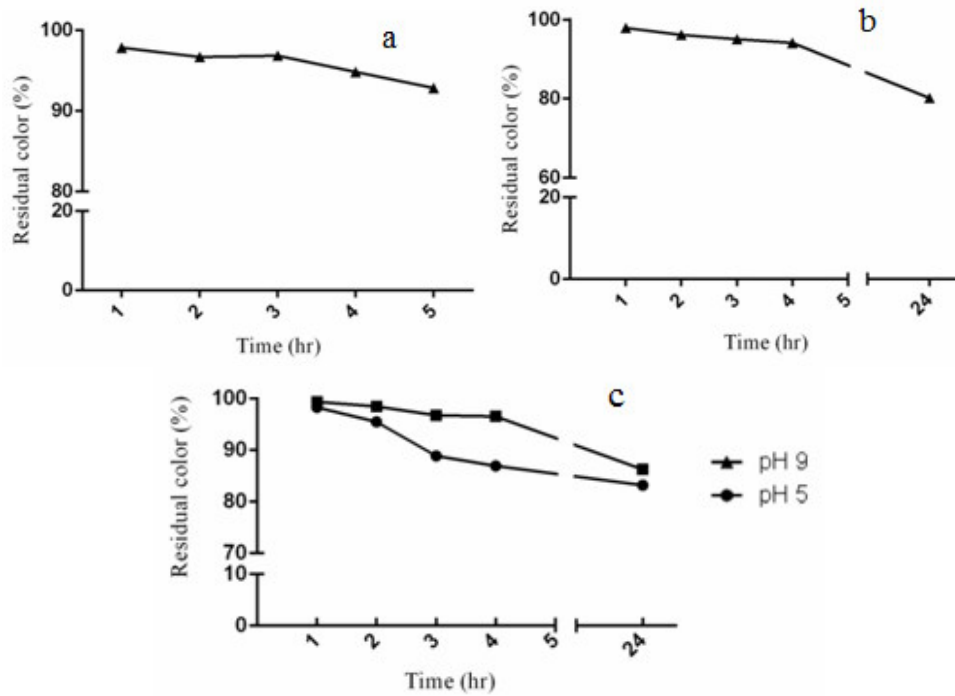


**Figure.4** Carotenoids stability in light (a); at temperature 100 °C (b) and at pH 5 & pH **9 (c)**

**Chemical identification of the extracted pigment**

Mrak et al (1949), Karrer & Jucker (1950), and Ajayi et al (2011) mentioned that the appearance of blue or violet ring at the interface between pigment extract and concentrated sulfuric acid indicates the presence of polyene pigments and it is confirmatory for the presence of carotenoids. Similarly, the chloroform extract of *Exiguobacterium aurantiacum* FH, exhibited dark blue coloration upon the addition of concentrated sulfuric acid which revealed the presence of carotenoids. The production of carotenoids by *Exiguobacterium acetylicum* and Exiguobacterium sp., and *Exiguobacterium aurantiacum* was previously reported by Kim et al. (2007), Asker et al (2012) & Sasidharan et al (2013) respectively.

**Carotenoids separation into carotenes and xanthophylls**

The addition of concentrated carotenoids, extracted from *Exiguobacterium aurantiacum* FH into equal volumes of petroleum ether and 90% methanol allowed their separation into upper petroleum ether layer (epiphase) which contained the carotenes, and lower 90% methanol layer (hypophase) which contained the xanthophylls. Vallentyne (1956) elaborated that the reason for such classification is based on the structural basis of carotenes and xanthophylls, the former doesn't contain any hydroxyl group while the latter contains two hydroxyl groups. Carotenoids contained two or more hydroxyl groups occupy the hypophasic layer and those without hydroxyl group occupy the epiphase (Karrer & Jucker, 1950; Vallentyne, 1956;

Fogg & Belcher, 1961). The epiphasic and hypophasic layers, analyzed by Thin Layer Chromatography (TLC), revealed the presence of at least one carotene and 2 dihydroxylated xanthophylls (Figure. 3). The separation of the pigmented extract under investigation was based on the number of their functional (-OH) group. Mishra and Singh (2010) mentioned that carotenes aren't retained and they migrate with the solvent front, while monohydroxylated compounds migrate to an intermediate distance while dihydroxylated compounds remain close to the baseline of the chromatography sheet.

**Antimicrobial effect of extracted carotenoids**

The work was extended to evaluate the antimicrobial effect of the carotenoids produced by *E. aurantiacum* FH. The methanolic extract demonstrated varying fungicidal effect against *Fusarium* sp., *Penicilium* sp., and *Alternatria* sp. with inhibition zones equivalent to 10 mm, 11 mm and 10 mm respectively. Tao et al. (2010) mentioned that carotenoids extracted from *Shatian pumello* had antifungal activity against *Saccharomyces cerevisiae* and *Rhizopus oryza*. Ma et al. (2004) also mentioned that the ether soluble pigment extracted from citrus peel had strong antifungal activity against *Penicillium* sp..

**Stability of carotenoids extract**

One of the main concerns of this study is to examine the stability of the produced carotenoids. The methanolic extract of *E. aurantiacum* FH maintained residual color equivalent to 80.21 % after exposure to light for 24 hours. A good stability was

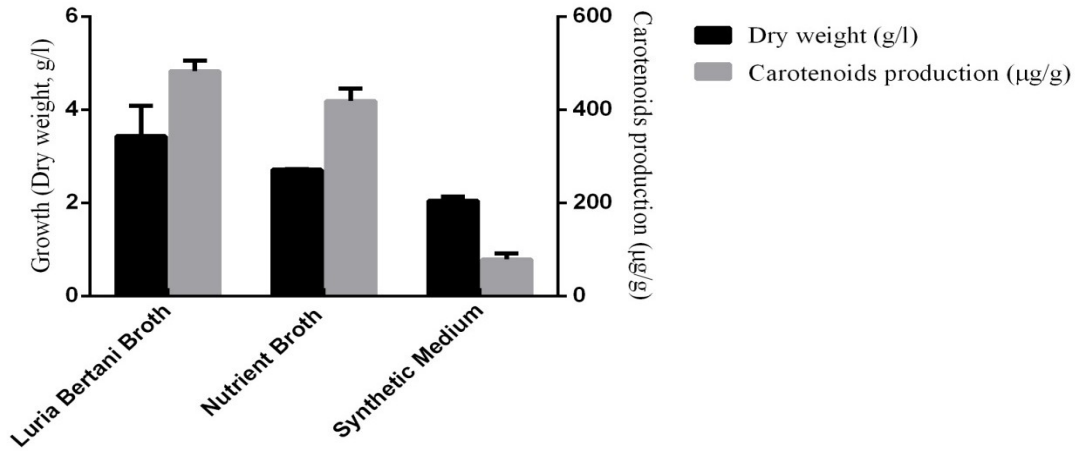**Figure.5** Effect of culture media on growth and carotenoids production



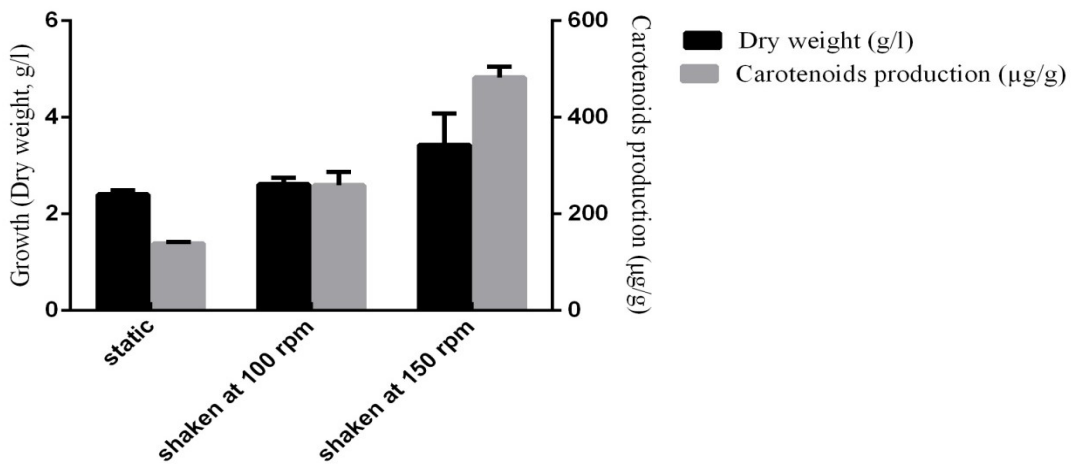**Figure.6** Effect of incubation conditions on growth and carotenoids production



**Figure.7** Effect of incubation time on growth and carotenoids production
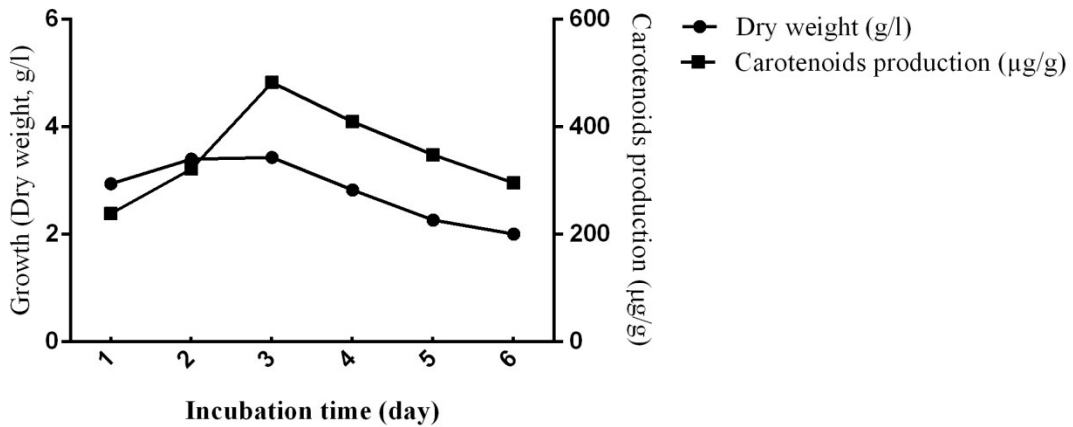
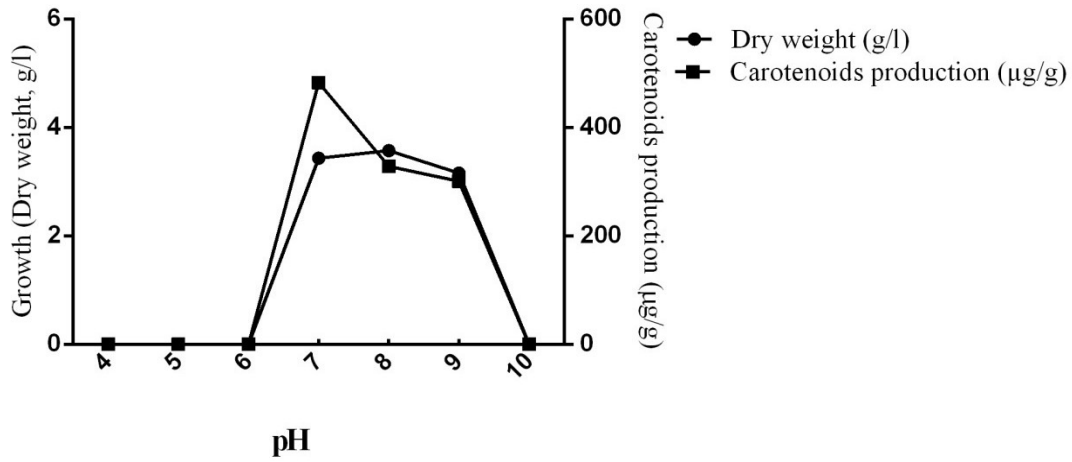**Figure.8** Effect of pH of culture medium on growth and carotenoids production



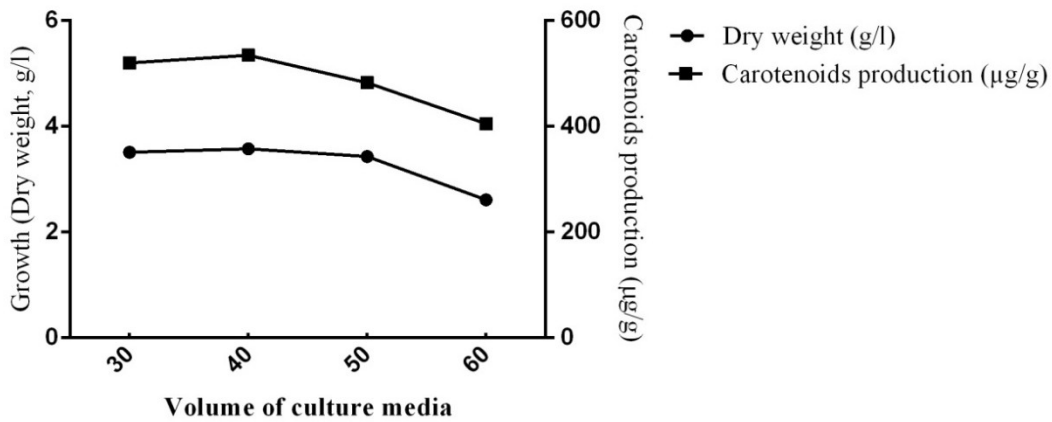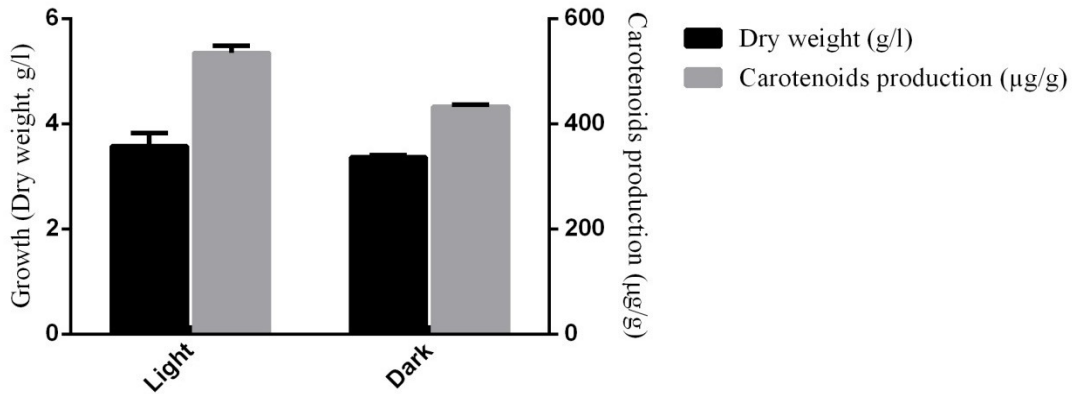**Figure.9** Effect of volume of culture media on growth and pigment production



**Figure.10** Effect of light and dark incubation on growth and pigment production

obtained after exposure to a temperature 100 °C for 5 hours with only 7.04 % color loss; whereas residual color equivalent to 83.24 and 86.27 % were recorded after 24 hours exposure to pH 5 & 9 respectively (Figure. 4). Kaur et al. (2009) monitored the stability of pigments extracted from the yeast *Monascus purpureus* MTCC 410, they found that the ethanolic extracts retained good stability at temperatures below 70 °C within a pH range from 6-8, while residual color equivalent to 35 % was recorded after 48 hours of exposure to direct sun light. The loss of color is due to the presence of polyene chain which renders carotenoids unstable; therefore, susceptible to oxidation and geometrical isomerization from trans to cis form which is enhanced in presence of light, heat and acidic medium (Dutta *et al*., 2005).

**Optimization of growth and carotenoids production**

Several factors affecting the growth and carotenoids production by *E. aurantiacum* FH were investigated. Growth and pigment production were higher when the experimental strain was grown in complex media, such as Luria Bertani medium (3.43 g/l; 482.82 µg/g**)** and nutrient broth (2.68 g/l, 418.99 µg/g), than when grown in synthetic medium (2.05 g/l, 78.96 µg/g) (Figure. 5). The microbial growth and the accumulation of carotenoids in cells requires complex media (Schaechter, 2009). Bhosale & Gadre (2001) found that presence of complex organic nitrogen sources (peptone, soy peptone, soy bean meal and urea) in culture medium resulted in higher accumulation of carotenoids by a mutant of *Rhodotorula glutinis* as compared with inorganic sources (ammonium sulphate and ammonium chloride). Previous studies reported that microbial pigments produced by *Monascus* sp, and *Rhodototula* sp.,

*Sarcina* sp., *Cryptococcus* sp., *Monascus purpureus*, *Phaffia rhodozyma*, and *Bacillus* sp. required a production medium that is too complex (Sandhu & Joshi, 1997; Joshi *et al*., 2003; Attri & Joshi, 2005; Joshi *et al*., 2011; El Banna *et al*., 2012). These observations may be attributed to the protective role of carotenoids in microorganisms against peroxides which may be produced after exposure of a complex growth medium to light in presence of air (Mackey, 2000).

*Exiguobacterium aurantiacum* FH exhibited an increase in growth as well as in carotenoids production as the incubation conditions changed from static (2.41 g/l, 138.68 µg/g) to shaken conditions at different agitation rates (100 rpm and 150 rpm) with growth and carotenoids production equivalent to 2.62 g/l, 259.77 µg/g and 3.43 g/l, 482.82 µg/g respectively (Figure. 6); The data revealed that maximum growth and carotenoid yields were obtained shaken cultures at 150 rpm. This could be attributed to the aeration which was beneficial to the growth and performance of microbial cells by improving the transfer of substrates and oxygen (Ibrahim, 2008; Valduga *et al*., 2009). Juneius et al. (2012) reported that maximum production of secondary metabolites by *Rhodobacter sphaeroides* MSB 57 was obtained when agitation rate was set to 150 rpm. Shaken conditions were found to be favorable for pigment production by other bacterial sp. such as *Flavobacterium* sp. (Masetto *et al*., 2001).

Similar findings were also reported for yeasts (*Rhodorula* sp. and *Phaffia* sp.) as they exhibit maximal growth and pigment production when incubated within an agitation range of 180-900 rpm (Frengova & Beshkova, 2009). Moreover, Maniyom & Markx (2012) demonstrated that an increase in agitation rate from 400 rpm to

1200 rpm increased pigment production by *Monascus* sp.

The time course of carotenoids production by *Exiguobacterium aurantiacum* FH is displayed in Figure. 7. Maximum growth and carotenoids production by *Exiguobacterium aurantiacum* FH were reached after three days, extending incubation period beyond the optimum incubation time resulted in decline in both growth and pigment production. Microbial pigments, as secondary metabolites, are produced during stationary or late log phase by a variety of microorganisms (Kaur *et al*., 2009). *Exiguobacterium aurantiacum* FH reached stationary phase after 48 hours which coincided withwith the results recorded by Mohanty & Mukherji (2008) for another strain of *E. aurantiacum*. Also, the study of Attri & Joshi (2006) showed that maximum growth and pigmentation of *Chromobacter* sp. were recorded after 2 days. Rea et al. (2010) explained the delay in secondary metabolites production by the utilization of carbon mainly for primary metabolism.

The estimation of growth and carotenoids production by *Exiguobacterium aurantiacum* FH incubated at different incubation temperature (25, 30 & 37 °C) revealed that maximum growth and pigment production were obtained at temperature 30°C, lower results were obtained at 25 and 37ºC (Data not shown). Temperature is one of the most important environmental factors affecting the growth of microorganisms and it causes changes in many biosynthetic pathways, such as carotenoid biosynthesis (Khodaiyan *et al*., 2007). Pigment production is related to the specificity of each microorganism and its optimum temperature and incubation conditions. Similarly to the results obtained,

*Exiguobacterium* sp. PMA grew maximally when incubation temperature was set to 30 °C (Arora *et al*., 2012), while maximum pigment production at 30 °C was recorded by *Serratia* sp. and *Micrcococcus* sp. (Hardijito *et al*., 2002 and Ibrahim, 2008).

The effect of pH of culture medium on growth and carotenoids production by *E. aurantiacum* FH was also investigated. It was noticed that the bacterial isolate exhibited growth and carotenoids production within pH range 7 to 10, however, maximum carotenoids production equivalent to 482.82 µg/g was obtained at pH 7, while maximum growth equivalent to 3.57 g/l was recorded at pH 8 (Figure. 8). Gee et al (1980) reported that the growth of *Exiguobacterium aurantiacum* occurs at pH above 6.5. Also, *Exiguobacterium oxidotoleranse* exhibited growth within pH range 7-10 (Yumoto *et al*., 2004) and maximum growth for a psychrotrophic *Exiguobacterium* sp. was obtained at pH 8 (Thankamani & Lipin Dev, 2011). No growth or minimal growth would occur at pH 4 and below unless the bacterial isolate belongs to acid tolerant bacteria (Joshi *et al*., 2011). In accordance to the data obtained, optimum growth and pigment production at pH 7 were recorded for *Serratia marcescens*, *Halorubrum sodomense* ATCC 33755, *Halobacterium* sp. TM (Asker & Ohta, 1999; Hardijito *et al*., 2002; Yusef *et al*., 2004; Khanafari *et al*., 2010).

The effect of different volume of culture medium was also studied; maximum growth and pigment production were obtained when *E. aurantiacum* FH was incubated in 250 ml flasks containing 40 ml of culture medium (Figure. 9). It was reported that the variation in the medium volume controlled the growth and

carotenoid production by *Agrobacterium auranticum* (Yokoyama & Miki, 1995) and that any increase in the volume of culture medium beyond the optimum volume caused a decline in both growth and pigment production maybe ascribed to the decreases the amount of dissolved oxygen leading to a decline in growth and pigment production by strict aerobic isolates (Goswami *et al*., 2010).

The influence of inoculum size was tested. It was noticed that 2% inoculum size was conducive to maximum carotenoids production by *E. aurantiacum* FH, while maximum growth was obtained when flasks were fortified with 6% inoculum size (Data not shown). Hamdi et al. (1997) found that the optimum pigment production by *Monascus purpureus* was recorded when 2 % inoculum was added to culture medium. Ji et al. (2012) and Babitha et al. (2007) mentioned that high inocula sizes may increase biomass but decreases pigment production, the inhibition of pigment formation was due to the lack of some substances in culture medium which were consumed by the high bacterial biomass.

To elucidate the effect of light on carotenoids production, *E. aurantiacum* FH was incubated under the previously mentioned optimized conditions in light and dark. Growth and carotenoids production were equivalent to 3.57 g/l, 534.51 µg/g respectively in light. These yields were higher than that obtained in dark incubation (3.36 g dry weight/l, 432.52 µg carotenoids/g dry weight) (Figure. 10) which was analogous to what was mentioned that carotenogenesis is a photoregulated process, light stimulates carotenogenesis in some moulds (*Phycomyces* sp., *Neurospora crasa*, and *Aspergillus*) and yeast (*Xanthophyllomyces dendrorhous*) (Meyer & Du Preez, 1994; Vázquez, 2001), while this process is inhibited in other species such as *Trichophyton mentagrophytes* and *Blakeslea trispora*. These findings indicate that microorganisms are not affected by light in the same way (Vázquez, 2001). Light alters the metabolic activities of microorganisms (Baker, 1938; Linden *et al*., 1997), and induces carotenogenesis, by the production of photooxidized metabolite which enhances carotenogenic enzyme or inactive the repressors of carotenogenesis (Batra 1967).

Statistical analysis using one way ANOVA revealed that all factors had statistically significant effect on carotenoid production by E. *aurantiacum* FH except the volume of culture medium with $p < 0.05$.

*Exiguobacterium aurantiacum* FH, a novel Lebanese strain that produced both carotenes & xanthophylls, exhibited high carotenoid production (534.51 µg/ g dry weight cells) which makes it worth investigation for pigment production on industrial scale.

## References

Aberoumand, A. 2011. A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. World J. Dairy.Food Sci. 6: 71-78.

Ahmad, W.A., Ahmad, W.Y.W., Zakaria, Z.A., and Yusof, N.Z. 2012. Application of Bacterial Pigments as Colorants: The Malaysian Perspective. Springer, New York.

Ajayi, I.A., Ajibade, O., and Oderinde, R.A. 2011. Preliminary phytochemical analysis of some plant seeds. Res. J. Chem. Sci.1: 58-62.

Amanullah, S., Chinnakonda, H., Kumar, V.A., and Khatheeja, S. 2011. Antimicrobial activity of *Triphala* against bacterial isolates from HIV infected patients. J. Microbiol. 4: S9-S17.

Arora, P.K., Sharma, A., Mehta, R., Shenoy, B.D., Srivastava, A., and Singh, V.P. 2012.

Metabolism of 4-chloro-2-nitrophenol in a Gram positive bacterium, *Exiguobacterium* sp. PMA. Microbial Cell Fact. 11: 150-160.

Asker, D., Awad, T.S., Beppu, T., and Ueda, K. 2012. Isolation, characterization, and diversity of novel radiotolerant carotenoid-producing bacteria. .ology. 892: 21-60.

Asker, D., and Ohta, Y. 1999. Production of canthaxanthin by extremely halophilic bacteria. J. Biosci. Bioengineer. 88: 617-621.

Attri, D., and Joshi, V.K. 2005. Optimization of apple pomace based medium and fermentation conditions for pigment production by *Micrococcus* species. J.Sci. Industrial Res. 64: 598-601.

Attri, D., and Joshi, V.K. 2006. Optimization of apple pomace based medium and fermentation condition for pigment production by *Chromobacter* species. J.Food Sci. Technol. 43: 515-520.

Baker, J.A. 1938. Light as a factor in the production of pigment by certain bacteria. J. Bacteriol. 35: 625-631.

Batra, P.P. 1967. Mechanism of photoinduced carotenoid synthesis. The J. Biol. Chem. 242: 5630-5635.

Babitha, S., Soccol, C.R., and Pandey, A. 2007. Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed. Biores. Technol. 98, 1554-1560.

Baskar, V., Madhanraj, P., Kanimozhi, K., Panneervelvam, A. 2010. Characterization of carotenoids from selected strains of Streptomyces sp.. Ann. Biol. Res. 1: 194-200.

Belitz, H.D., Grosch, W., and Schieberle, P. 2004. Food Chemistry. 3rd ed., Springer, Verlag, Berlin.

Bhosale, P., and Gadre, R.V. 2001. Production of beta carotene by a mutant of *Rhodotorula glutinis*. Appl.Microbiol. Biotechnol. 55: 423-427.

Bridoux, M.C. 2008. Algal biomarkers and their metabolites in the lower food web of the great lakes, analyzed by HPLC-PDA/FL, LC-MS and GC- MS. A thesis submitted in partial fulfillment of the requirements of a PHD degree, Albany State University of New York. Proquest, USA.

Britton, G. 1985. General carotenoid methods. Methods in Enzymology: 111, 113-149.

Dufosse, L. 2006. Microbial production of food grade pigments. Food Technol. Biotechnol.44, 313-321.

Dutta, D., Chaudhuri, U.R., Chakraborty, R. 2005. Structure, health benefits and antioxidants property and processing and storage of carotenoids. African. J. Biotechnol. 4:, 1510-1520.

El-Banna, A.A., Abd, El Razek, A., and El-Mahdy, A.R. 2012. Some factors affecting the production of carotenoids by *Rhodotorula glutinis* var. *glutinis*. Food and Nutrition Sciences. 3,64-71.

Fikselova, M., Silhar, S., Marecek, J., and Francakova, H. 2008. Extraction of carrot (*Daucus carota L.*) carotenes under different conditions. Czech. J. Food .26: 268-274.

Fieser, L.F. 1950. Absorption spectra of carotenoids, structure, of vitamin A2. The J. Organic Chem. 15: 930-943.

Fogg, G.E., and Belcher, J.H. 1961. Pigments from the bottom deposits of an English lake. New Phytol. 60, 129-142.

Forgacs, E., and Caserhati, T. 2002. Thin-layer chromatography of natural pigments: New advances. J. Liquid .Related .25: 1521-1541.

Frengova, G.I., and Beshkova, D.M. 2009. Carotenoids from *Rhodotorula* and *Phaffia*: yeasts of biotechnological importance. J.Indus. Microbiol. Biotechnol. 36:163-180.

Gee, J.M., Lund, B.M., Metcalf, G., and Peel, J.L. 1980. Properties of a new group of alkalophilic bacteria. J. General Microbiol. 117, 9-17.

Goswami, G., Chaudhuri, S., and Dutta, D. 2010. Effect of pH and temperature on pigment production from an isolated bacterium. Chemical Engineer. Trans.. 20, 127-132.

Hamdi, M., Blanc, P.J., Loret, M.O., and Goma, G. 1997. A new process for red pigment production by submerged culture of *Monascus purpureus*. Bioprocess Engineer. 17, 75-79.

Hardijito, L., Huq, A., and Colwell, R.R. 2002. The Influence of environmental conditions on the production of pigment by *Serratia marcescens*. Biotechnol. Bioprocess Engineer . 7: 100-104.

Ibrahim, A.S.S. 2008. Production of carotenoids by a newly isolated marine *Micrococcus* sp. Biotechnol. 7, 469-474.

Ji, H., Jiang, D., and Cao, L. 2012. Optimization of fermentation parameters on T-DNA inserted *Monascus pyrpureus* mutant MT24 with high pigment production capacity. Res. J. Biotechnol. 7: 9-14.

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

Joshi, V.K., Attri, D., and Rana, N.S. 2011. Optimization of apple pomace based medium and fermentation conditions for pigment production by *Sarcina* sp.. *Indian* J. Natural Product. Res. 2: 421-427.

Juneius, C.E.R., Selvin, J., Sabrathnan, B., Asha,

D., Pandian, V., and Sathiyanaranan, G. 2012. *Rhodobacter sphaeroides* MSB 57 - A natural source of 2-piperidinone, 1-(3, 4, 5, 6-tetrahydro-2-pyridinyl): A novel inhibitor for reverse transcriptase of HIV. Central European J. Experi. Biol. 1: 36-44.

Karrer, P., and Jucker, E. 1950. Carotenoids. Elsevier Publishing Company Inc., New York, Amsterdam, London, Brussels.

Kaur, B., Chakraborty, D., and Kaur, H. 2009. Production and evaluation of physicochemical properties of red pigment from *Monascus purpureus* MTCC 410. The Internet. J. Microbiol..7. DOI:10.558/d4a.

Khanafari, A., Khavarinejad, D., and Mashinchian, A. 2010. Solar salt lake as natural environmental source for extraction halophilic pigments. Iranian. J. Microbiol. 2: 103-109.

Khodaiyan, F., Razavi, S.H., Djomeh , Z.E., and Mousavi, S.M.A. 2007. Optimization of canthaxanthin production by *Dietzia natronolimnaea* HS-1 using response surface methodology. Pakistan. J. Biol. Sci. 10: 2544-2552.

Kim, D., Song, H., Lim, S., Yun, H., and Chung, J. 2007. Effects of gamma irradiation on the radiation- resistant bacteria and polyphenol oxidase activity in fresh kale juice. Radiation Phys. Chem.76: 1213-1217.

Lee, J.H., and Schwartz, S.J. 2012. Analysis of carotenoids and chlorphylls in foods. In: Methods of Analysis of Food Components and Additives. 2nd ed., Ötleş S (Ed.), CRC press, Boca Raton, USA, pp. 231-252.

Liakopoulou-Kyriakides, M., and Kyriakidis, D.A. 2002. *Croccus sativus*-biological active constituents. In: Studies in Natural Products Chemistry: Bioactive Natural Products.Vol.26, part C, Ur-Rahman A (Ed.), Elseiver. Amesterdam, pp: 293-312.

Linden H, Ballario P & Macino G (1997). Blue light regulation in *Neurospora crassa*. Fungal Genetics and Biology. 22: 141-150.

Ma, Q.Y., Chen, C.T., Jing, X.Y., and Yuan, W.X. 2004. Studies on extraction of hesperidin and other active components from Citrus peels and their antimicrobial effects. Food Sci. 25: 112-115.

Mackey, B.M. 2000. Injured bacteria. In: Microbiological Safety and Quality of Food, Lund B, and Baired- Parker TC, Warwick Gould G. Aspen Publishers Inc. USA, pp: 315-335.

Maniyom, S., and Markx, G.H. 2012. Biomass and pigment production by *Monascus* during miniaturized submerged culture on Adlay.

World Acad. Sci. Engineer. Technol. 68: 1786-1791.

Masetto, A., Flores-Cotera, L.B., Diaz, C., Langly, E., and Sanchez, S. 2001. Application of a complete factorial design for the production of zeaxanthin by *Flavobacterium* sp.. J. Biosci. Bioengineer. 92: 55-58.

Meyer, P.S., and Du Preez, J.C. 1994. Effect of culture conditions on astaxanthin production by a mutant of *Phaffia rhodozyma* in batch and chemostat culture. Appl. Microbiol. Biotechnol. 40: 780-785.

Mishra, P., and Singh, N.K. 2010. Spectrophotometric and TLC based characterization of Kernel carotenoids in short duration maize. Maydica. 55: 95-100.

Mohanasrinivasan, V., Sriram Kalyan, P., Ipsita, N., Subathradevi, C., Selvarajan, E., Suganthi, V., and Jemimah Naine, S. 2013. Fermentative production of extracellular pigment from *Streptomyces coelicolor* MSIS1. Res. J. Biotechnol. 8: 31-41.

Mohanty, G., and Mukherji, S. 2008. Enhancement of NAPL bioavailability by induction of cell-surface hydrophobicity in *Exiguobacterium aurantiacum* and *Hurkholderia cepacia*. Indian J. Biotechnol. 7: 295-306.

Mortensen, A. 2006. Carotenoids and other pigments as natural colorants. Pure. Appl. Chem. 78: 1477-1491.

Mrak EM, Phaff HJ & Mackinney G.1949. A simple test for carotenoid pigments in yeasts. J. Bacteriol. 57: 409-411.

Rea, G., Antonacci, A., Lambreva, M., Margonelli, A., Ambrosi, C., and Giardi, M.T. 2010. The nutra–snacks project: Basic research and biotechnological programs on nutraceutics. In: Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors. GIARDI, M.T., Rea, G., Berra, B. (Eds.), Springer Science and Landes Bioscience, USA, pp. 1-17.

Reddy, G.S.N., Prakash, J.S.S., Prabahar, V., Matsumoto, G.I., Stackerbrandt, E., and Shivaji, S. 2003. *Kocuria polaris* sp. nov., an orange-pigmented psychrophilic bacterium isolated from an Antarctic cayanobacterial mat sample. Inter. J. System. Evol. Microbiol. 53: 183-187.

Rivera, S., and Canela, R. 2012. Influence of sample processing on the analysis of carotenoids in maize. Molecules. 17: 11255-11268.

Rodriguez-Amaya, D.B., and Kimura, M. 2004. HarvestPlus Handbook for Carotenoid Analysis. HarvestPlus Technical Monograph

Series2; IFPRI: Washington, DC, USA, and CIAT: Cali, Colombia.

Sashidharan, P., Raja, R., Karthik, C., Ranandakumar, S., Indra Arulselvi, P. 2013. Isolation and characterization of yellow pigment producing Exiguobacterium sps. Journal of Biochemical Technology. 4,632-635.

Sandhu, D.K., JOSHI, V.K. 1997. Development of apple pomace based medium, optimizing pigment production by *Rhodotorula* and its characterization. Adv. Food Sci. (CMTL). 19: 31-34.

Schaechter, M. 2009. Encyclopedia of Microbiology. Vol.1, 3$^{rd}$ ed., Academic Elsevier Inc., UK.

Suhnel, S., Lagreze, F., Ferreira, J.F., Campertini, L.H., and Maraschin, M. 2009. Carotenoid extraction from the gonad of the scallop *Nodipecten nodosus* (Linnaeus, 1758) (Bivalvia: Pectinidae). Brazilian. J. Biol. 69: 209-215.

Tao, N., Gao, Y., Liu, Y., Ge, F. 2010. Carotenoids from the peel of Shatian pummelo (*Citrus grandis* Osbeck) and its antimicrobial activity. American-Eurasian J.Agri. Environ. Sci. 7: 110-115.

Thankamani, V., and LipinDev, M.S. 2011. *Bacillus* isolates VTGP. A-D 30808 *Alcaligenes* sp., *Exiguobacterium* sp., B. *pumilus* and B. *fusiformis* producing extracellular alkaline protease, amylases and cellulases- a preliminary report. Res. Biotechnol. 2:20-30.

Vallentyne, J.R. 1956. Epiphasic carotenoids in post-glacial lake sediments. Limnol. Oceanogra. 1: 252-262.

Valduga, E., Tatsch, P.O., and Tiggemann, L., Zeni, J., Colet, R., Cansian, J.M., Treichel, H., and Luccio, M. 2009. Evaluation of the conditions of carotenoids production in a synthetic medium by *Sporidiobolus salmonicolor* (CBS 2636) in a bioreactor-Original article. Inter. J. Food . Technol. 44:2445-2451.

Varsha, C., and Arpana, J. 2013. Screening of significant nutrient parameters for pigment production from newly isolated organism *Planococcus maritimus AHJ_2* using Plackett-Burman design. J. Microbiol. Biotechnol. Res. 3: 79-83.

Vázquez, M. 2001. Effect of light on carotenoid profiles of *Xanthophyllomyces dendrorhous* strains (formerly *Phaffia rhodozyma*). Food Technol. Biotechnol. 39: 123-128.

Wu, W.T., and Liu, W.S. 2011. Anti-inflammatory property of biomaterial carotenoids production by *Rhodobacter sphaeroides* WL-APD911. Adv. Materials Res.. 287-290: 2028-2031.

Yokoyama, A., and Miki, W. 1995. Composition and presumed biosynthetic pathway of carotenoids in the astaxanthin-producing bacterium *Agrobacterium aurantiacum*. FEMS Microbiol. Lett. 128: 139-144.

Yumoto, I., Hishinuma-Narisawa, M., Hirota, K., Shingyo, T., Takebe, F., Nodasaka, Y., Matsuyama, H., and Hara, I. 2004. *Exiguobacterium oxidotolerans* sp. nov., a novel alkaliphile exhibiting high catalase activity. Inter. J. System. Evol. Microbiol. 54: 2013-2017.

Yusef, H.H., Ghozlan, H., Sabry, S., and Khalil, R. 2004. Isolation and characterization of an extremely halophilic archeon, *Halobacterium salinarum* SHR from a solar saltern in Egypt. Fresenius Environ. Bull. 13: 494-500.