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Isolation and Identification of New Strains of Bacteria Producing Carotenoids Pigments which Isolated from a Different Sources in Basrah, Iraq

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Abstract: Carotenoids are tetraterpenoid organic pigments found in plants, bacteria, fungi, and algae, and come in a variety of colours including yellow, orange, red, and pink, fund all of these organisms, carotenoids can be complex lipids and other metabolic components. Carotenoids are used in a variety of ways, including as antioxidants, antibacterial agents, and food additives.The present study included the isolation of bacteria producing carotenoids pigments that isolated from different sources (soil, food and air) in Basrah, Iraq. Results showed, that out of 24 isolates isolated from above sources which can be produce carotenoids. Ten of those isolates were gave the highest productivity of pigments. Morphological, biochemical, and gene investigations were performed on all 10 isolates. The 16SrRNA sequence analysis of all isolates was recorded as new strains and put in the BDDJ Gene Bank, and the phylogenetic tree of the isolates was constructed using MEGA 6 software. Those strains that are the primary screening for carotenoids production.Bacterial isolates were characterized and identified based on phenotypic properties and molecular techniques. five strains, belonging to genera of *Plantibacter flavus* BKA3, *Sphingobacteruim faccium* BKA4, *Stenotrophomonas pavanii* BKA5, *Microbacteruim keltanolyticum* BKA6, *Brachybacteruim muris* BKA12, were isolated from soil and four strains, belonging to genera of *Kocuria turfanesis* BKA8, *Kocuria rosea* BKA10, *Massilia timonae* BKA11, were isolated from air, the strain *Pseudomonase cani* BkA13 and *P. fluorescens* BKA2, isolated from food. The carotenoid was extracted by methanol solvent and it's analyzed by spectrophotometric within 400-600nm and wave length maximum was found at 460nm. The results showed the isolate of *K. turfanesis* BKA8, reached a height of carotenoid content of 753.11g.gm^{-1} after 72 hours of shaking culture at 150 rpm in nutrient broth at 27˚C, pH 7.

Keywords: Bacteria, Carotenoids, Pigments.

Introduction

The colourants are classified as natural and synthetic, the synthetic are harmful to human health and the environment because they are non-biodegradable and contain toxic compounds (Devyani *et al.*, 2017). Now there is an increase in demand towards natural pigment

produced by bacteria, fungi, plant and animal (Malik *et al*., 2012). Bacteria pigments could play a key role as an additive in colourful the food (Husseiny *et al*., 2018**)** and also known to possess antibacterial (Boontosaeng *et al*., 2016**)**, antioxidant (Manimala & Murugesan, 2014) and antitumor activity (Kumar *et al*., 2015). Among various types of pigments reported from bacteria. A carotenoids group of pigments are more widely studied with respected to their application (Haddad *et al*., 2017). Most carotenoids are tetraterpenoids (C40) consisting of 8 units isoprenoids linked so that the molecule is linear and symmetrical, with the order reversed in the center (Netzer *et al*., 2010). Carotenoids are hydrophobic compounds, lipophilic, insoluble in water, and soluble in solvents such as acetone, methanol, and chloroform (Botella-Pavia & Rodríguez-Concepción, 2006). This group of fat-soluble pigments comprises more than 700 compounds responsible for the red, orange, and yellow colours, Carotenoids are mostly hydrocarbons with 40 carbon atoms and two terminal rings (Bell *et al*., 2000). In nature, there are two types of carotenoids: carotenes, such as carotenes, which are linear hydrocarbons that can be cyclized at one or both ends of the molecule (Botell-Pava & Rodríguez-Concepción, 2006), and xanthophylls, which are oxygenated derivatives of carotenes such as lutein, violaxanthin, neoxanthin, and zeaxanthin (Berman *et al*., 2015). Only 50 of the hundreds of naturally occurring carotenoids have biological function (Mezzomo & Ferreirra, 2016). *Paracoccus*, *Bacillus*, *Micrococcus*, *flavobacterium*, *Sporobolomyces*, *Blakeslea trispora*, *Rhodotorula*, and *Phaffia* are examples of microorganisms that produce carotenoids colour (Manimala & Murugesan, 2014). Carotenoids are essential because they play a role in preventing a variety of diseases caused by free radical damage, as well as acting as an antibacterial agent (Jaber *et al*., 2021). Carotenoids are utilized commercially as food colours, animal feed supplements, and, more recently, cosmetics and treatments (Yangilar & Yildiz, 2016).

The present study aimed to isolate some local bacteria producing carotenoids pigments from various sources and identified them through morphological and biochemical characteristics and gene analysis.

Materials & Methods

Collection of samples

Samples were collected from different area and locations. Soil samples were collected from the collage garden, lake, river and park in Basrah city; the samples were collected at a depth of 10cm from the surface. Food samples, Common carp *Cyprinus carpio* and cream were collected from markets; all samples were stored in cleaned polythene bags (Majeed *et al*., 2016). All the samples were immediately transported to the laboratory until further analyses.

Petri dishes containing a culture medium R2A agar (HI media-India) were exposed to the air in the laboratory near the window for 4 hours; after that, they incubated at 27 C° for 48 hours in aerobic conditions.

Isolation and identification of bacteria

Isolation and purification

From the collected samples, soil and food suspension were prepared using sterile peptone by dilution method 10^{-1}-10^{-5}(Majeed *et al*., 2016). Transport 100 µl of each dilute of samples suspension was spread by glass L-

shape on to the nutrient agar plates and the plates was incubated at 27C° for 48 hour, the pigment produced bacteria observed on 10^{-3} dilute of all samples. After that a single yellow, pink, orange and creamy colonies were Purified by transfer and sub-culture on the new nutrient agar plate for further studies.

Morphological and biochemical characterization of isolates

According to the manufactures instruction, all colonies were observed for Grams stain was carried out using kit (East tomer science-Germany). Check motility test by the hanging drop technique (Harley & Prescott, 2002). Morphological characters such as shape, colour, were further identified using biochemical tests such as catalase activity was analyzed by the generation of bubbles from 3%(v/v) H_2O_2 solution mixed with freshly grown cell according to the mothed (Benson, 2001). Oxidase activity was tested using 1% (w/v) N, N, N, N-tetra methyl-p-phenyl diamine reagent (Sigma, USA) according to the mothed of Benson (2001). Galantine hydrolysis test was by method (Macfaddin, 2000). Hemolysis test was perpetrated using the Nutrient agar medium and 5% sheep blood (Sharma & Gupta, 2014). For identification, according to Bergey's Manual of Determinative Bacteriology.

Molecular characterization of bacteria strain

DNA extraction

New bacterial strains were identified by 16SrRNA gene sequences analysis. The bacterial grown on Nutrient agar for 48 hours. Genomic DNA were extracted using the genomic DNA purification Kit (i-genomic BYE

DNA extraction mini) protocol Gram positive and used (G-spin DNA extracted Kit) protocol Gram negative bacteria according to the manufacture instruction (intron bio technology / korea). Determinates concentration extracted DNA and its pure by Nano drop spectrophotometer at 260-280nm for getting DNA concentration 1.8-2nm (Sambrook & Russel, 2001).The molecular characterization was proceeded using 16SrRNA gene sequence analysis.

Polymerase chain reaction (PCR)

16SrRNA gene sequences were amplified from genomic DNA by polymerase chain reaction (PCR) using universal primers, Forward primer 27F (5'- AGAGTTT GATCCTG GCTCAG- 3') and Reverse primer 1239R (5'- GGTTACCTT GT TA CG ACTT- 3') (Srinivasan *et al*., 2015). PCR fragment was purified using intron/PCR pre-mix Kit). PCR was carried out under the following condition in a thermal cycle using an initial denaturation at 94C for 3 min, followed by 35 circles at 94 C for 45 sec, 56C for 45 sec, 72C for 1min and final extension step at 72 C for 7 min.

Agarose gel electrophoresis of DNA

Electrophoresis has been done to determine DNA pieces after the extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel According to method Sambrook *et al.* (1989). The PCR products were separated on a 2% agarose gel electrophoresis and visualized the fragments by exposure at UV with 336 nm after ethidium bromide or Red Stain staining.

Sequencing analysis and phylogenetic tree

Sequencing of the gene was carried out by biotechnology lab, the machine is (DNA

sequencer 3730XL, Applied Biosystems) in Korea. Sequencing data were analyzed by comparison with 16SrRNA genes in the (NCBI) online at (http:// www. NCBI. nlm. nih. gov). The CLUSTAL W tool was used to build multiple alignments, and MEGA 6 software was used to construct a phylogenetic tree. The data from this study's nucleotide sequence accession numbers have been submitted to the DDBJ/Gene Bank databases.

Extraction method of carotenoid

1ml of inoculum volume was transferred to 250 ml Erlenmeyer flask containing 49 ml of nutrient broth at(pH 7), 27℃ on a rotary shaker at 150 rpm for 72 hours. Transferred the culture media to the sterile tube (50ml) and centrifuged at 6000 rpm for 10 min and the supernatant was discarded. Cell pellets were washed twice with deionized water. Followed by centrifugation at 6000 rpm for 10 min to recover the cell by discharging the supernatant again. The 25 ml of methanol (99.7% grade HPLC, GCC-UK) was added to the cell for extraction of bacterial pigment and the mixture was vortexed, extracted twice with the same solvent. The mixture of the cells and methanol was treated by ultra-sonication (59KHz, 35-40℃, 90 min, Phoenix-986, AA-U.K) and keep overnight in light protection. Pigment extracts were separated by centrifugation at 6000 rpm for 10 min and filtrated by 0.45µm filter (Boontosaenget *et al*., 2016).

Characterization of carotenoids pigment

UV-Vis spectra absorption

The pigment extracts were analyzed by scanning the absorbance wavelength region of 400-600 nm using a spectrophotometer (Apel PP-303-Japan). The solvent was the evaporated to dryness in evaporator at 40℃ and crud pigment was collected in the tub and keep light protects at -20℃. The total carotenoids were calculated by the below equations (Rodriguez-Amaya & Kimura, 2004).

ABS: absorbance

$E^{1\%}{}_{1cm}$: coefficient specific of absorption of mixtures (2500)

Sample weight (g): (Dry cell weight)

Statistical analysis

Statistical analysis using one way ANOVA. The results were obtained from a minimum of three independent experiments and averaged. Data were analyzed of variance for two factors at ($p \leq 0.05$) to estimate the differences between values of tested compounds using the SSPS program version 20 in 2019.

Results & Discussion

Isolation and purification of bacteria isolates

Twenty four bacterial isolates were isolated from different natural sources (soil, food, water and air). The isolates were selected on based on the colour of the colonies growing in solid media and were of different colours, including yellow, red, orange and creamy, which is expected to be produced by carotenoids pigments listed in table (1). After the isolation, ten isolates were screening and selected, These isolates were purified several times to obtain pure isolates,this bacterial isolates were selected for their high ability for production of carotenoids pigments were indicates in table (2). The sources of these bacteria isolates were, that five isolates were isolated from soil, three were isolated from air and two were isolated from Common carp fish.

Identification and characterization of pure of carotenoids produce bacteria

Morphological characterization

Colony characterization of the selected new strains of carotenoids producing bacteria from different sources (soil, air and Common carp fish and incubation (27℃ for 48h) was done based on its morphological characterization such as colour colony, cell shape, motility, Gram stain. The biochemical tests performed were oxidase, catalase, gelatin analysis and hemolysis. Identification of isolates obtained in pure culture were characteristics on nutrient media. Ten carotenoids producing bacteria were identified and characterized, yellow, orange, pink and creamy. These isolates were identified at genus level was done with the help of Bergey's Manual of Determinative Bacteriology, which is presented in table (2).

Table (1): Bacterial isolates of carotenoids produced.

S.NO	source of isolation	codes of isolates	No. of isolates
1	Soil	S	12
2	Water	W	1
3	Air	A	8
4	Fish	F	2
5	Cream	C	1

Table (2): Morphological and biochemical of new bacteria strains

No. isolates	Code isolates	Sources of isolates	media	Colony colour	Cell shape	motility	Gram stain	oxidase	catalase	Gelatinase	Hemolysis
1	F5	Fish	NA	Creamy	Rod	+	-	+	+	+	+
2	F23	Fish	NA	yellow	Rod	+	-	+	+	-	-
3	S6	Soil	NA	yellow	Rod	-	+	-	+	-	-
4	S7	Soil	NA	yellow	Rod	+	-	+	+	+	+
5	S10	Soil	NA	Orange	cocci	-	+	+	+	-	-
6	S11	Soil	NA	yellow	cocci	+	+	-	+	+	-
7	S19	soil	R2A	yellow	Rod	-	+	-	+	+	+
8	A14	air	R2A	yellow	cocci	-	+	-	+	-	+
9	A16	air	R2A	Pink-red	cocci	-	+	-	+	-	-
10	A17	air	R2A	Orange	Rod	+	-	-	+	+	-

Molecular identification

Ten new bacterial strain that proceeds to 16SrRNA sequencing, the results of the electrophoresis technique for genomic DNA extraction demonstrated pure and clear isolated DNA. In addition, all isolates tested using a set of universal primers,27F and 392R for the specific amplification of 16S rRNA gene sequences, yielded a single amplification of ~1 250 bp for the whole isolate (Fig 1).

In present study showed the analysis of the partial 16S rRNA genes of the ten isolates of bacteria were carotenoid production which isolated from various sources and revealed that isolates belonged to the different genera related type strains in Gene Bank are shown in fig. (1).

Tow isolates from common carp fish are coded F, Isolate (F5) and isolate (F23) presented *Pseudomonas fluorescens BKA2* that showed 99.92% sequence similarity FJ950603.1.and *P. cani BkA13* that showed 99.74% sequence similarity to MT626802.1 *P. fluorescens.* The *P. fluorescens* complex of

species is one of the most diverse groups within the *Pseudomonas* genus is belong to the Pseudomonasaceae family, members of this group have been isolated from diverse habitats, including water, soil, plant tissues (Garrido-Sanz *et al*., 2017), animals and humans (Scales *et al*., 2015).

Five isolates from the soil are coded (S), Isolates (S6) presented *Plantibacter flavus* BKA3 showed 97.18% sequence similarity to MN826584.1, other strain of this genus, *P. flavus*, was isolated from arabidopsis, lettuce, basil, and bok choy plants (Mayer *et al*., 2019). The *P. flavus*, the genus belongs to the Microbacteriaceae.

Isolate (S7) was presented *Sphingobacteruim faccium BKA4* showed 99.75% sequence similarity to FJ950587.1 of this genus, *S. faccium*, have been isolated from several types of soils and compost and, currently, several industrial applications are known colonies are smooth, convex, round with entire margins, and slightly yellowish. Catalase and oxidase reactions are present, the genus is belongs to The Family Sphingobacteriaceae (Lambiase, 2014).

Isolates (S10) presented *Stenotrophomonas pavanii BKA5*showed 99.58% sequence similarity to MN889300.1 of this genus, *S. pavanii*, was isolated from stems of a Brazilian sugar cane variety widely used in organic farming, pavanii of Pavan, named in honour of the Brazilian geneticist Crodowaldo Pavan (Ramos *et al*., 2011). The genus belongs to The Family Xanthomonodaceae.

Also Isolates (S11) presented *Microbacteruim keltanolyticum BKA6* showed 99.84% sequence similarity to MK696241.1 A total of 16 different strains of *Microbacterium* spp., was isolated from contaminated soil (Learman *et al*., 2019).

The isolate (S19) was presented *Brachybacteruim muris BKA12* showed 99.41% sequence similarity to KF87689.1, *B. muris* is a species of Gram positive, strictly aerobic, yellow-pigmented bacterium. It was first isolated from the liver of a laboratory mouse. The species was first described in 2003, and the name is derived from the Latin *muris* (mouse). The genus *Brachybacterium*, belong to the family Demabacteraceae it's isolated from soil (Singh *et al*., 2016) and liver mice (Buczolits *et al*., 2003).

Three isolates are coded (A) were isolated from air exhibited pink to yellow pigment and belonged to the family Micrococcaceae, (A14) was presented *Kocuria turfanesis BKA8* showed 99.92% sequence similarity toKY194249.1.

(A16) was presented *Kocuria rosea BKA10* showed 99.92% sequence similarity to KY194315.1, *K. rosea* belongs to genus *Kocuria* (family Micrococcaceae, suborder Micrococcineae, order Actinomycetales) that includes about 11 species of bacteria. Usually, *Kocuria* sp. are commensal organisms that colonize the oropharynx, skin and mucous membrane (Moreira *et al*., 2015). *Kocuria* sp. was isolated from soil samples (Elbendary *et al*., 2018).

The isolate (A17) was presented *Massilia timonae BKA11* showed 98.61% sequence similarity to E4373360.1; the genus *Massilia* belongs to the family Oxalobacteraceae (Betaproteobacteria) cases of *M. timonae* infection in humans, confirms the environmental nature of the species. Recently, other *Massilia* strains have been isolated from

air and drinking water (Van Craenenbroeck *et al*., 2011).

According to alignment an blast and NCBI, the results of the new strains isolated showing in fig. (1). These strains were recorded new strains due to genteic identification dependent on 16SrRNA. According to information available, after alignment with other 16SrRNA sequences in Gene Bank, they showed a high degree of similarity to references strains.The phylogentic tree shown in fig. (2) reflects the relationships of the ten selected ten different strains were isolated from soil,air and food,these new strians were rigersted in Gene Bank and were gives the accession numbers are list in table (3).

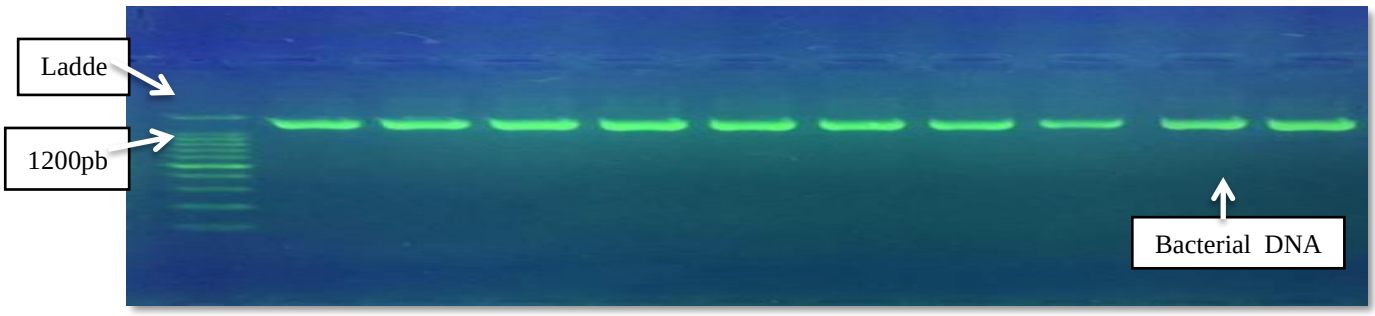

Fig. (1): Agarose gel electrophoresis of 16S rRNA gene with 100bp DNA ladder, showing a single amplification band of 1250 bp for selected isolates.

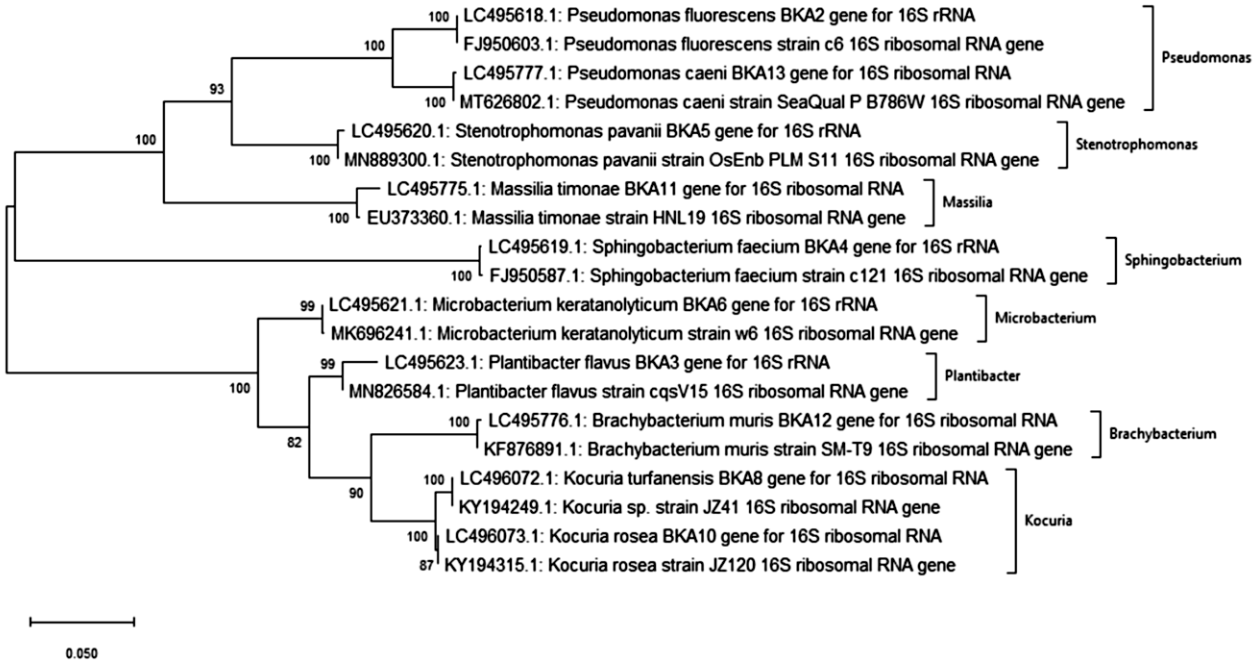

Fig. (2) Maximum-likelihood phylogenetic tree of carotenoid pigmented bacteria were obtained from the different sources .the tree was constructed using the aligned partial 16SrRNA gene sequences (602-1222bp).

Table (3): New local strains of bacteria were recorder in Gene Bank.

No. isol1ates	Code isolates	Names of a new strains	DNA	Accession NO	similarity	Accession NO of strains in Gene bank
1	F5	*P. fluorescens BKA2*	1194bp	LC495618.1	99.92	FJ950603.1
2	F23	*P. caeni BkA13*	1165bp	LC495777.1	99.74	MT626802.1
3	S6	*P. flavus BKA3*	602bp	LC495623.1	97.18	MN826584.1
4	S7	*S. faccium BKA4*	1214bp	LC495619.1	99.75	FJ950587.1
5	S10	*S. pavanii BKA5*	1186bp	LC495620.1	99.58	MN889300.1
6	S11	*M. keratanolyticum BKA6*	1214bp	LC495621.1	99.84	MK 696241.1
7	A14	*K. turfanesis BKA8*	1214bp	LC496072.1	99.92	KY194249.1
8	A16	*K. rosea BKA10*	1214bp	LC496073.1	99.92	KY194315.1
9	A17	*M. timonae BKA11*	1222bp	LC495775.1	98.61	E4373360.1
10	S19	*B. muris BKA12*	1184bp	LC495776.1	99.41	KF87689.1

Characterization of carotenoids pigment

Identification by spectrophotometric

A total of 35 samples were collected and subjected for isolation. From the 35 samples, 24 yellow, orange, pink-red and creamy pigmented colonies were isolated present in table (1).

Secondary screening (quantity) was performed by growing all isolates on the production medium of nutrient broth and in the liquid culture system (submerged fermentation method) to find the best local new strains from among ten isolates in the production of carotenoids, which included temperatures of 27 C for 72 hours and at 150 rpm, After that, the pigments of carotenoids were extracted with yellow, orange, pink by methanol absolute solvent. From ten a new strains, the methanol extracted carotenoid pigment was produced by spectrophotometer analysis by scanning the absorbance light within a wave length region of 400-600 nm (Fig. 4). The extracted pigment demonstrates the existence of region with maximum absorbance at 460 nm. Table (4), which was a typical characteristic of the absorption spectrum of carotenoid (Shatila *et al*., 2013; Varsha & Aspara, 2013).

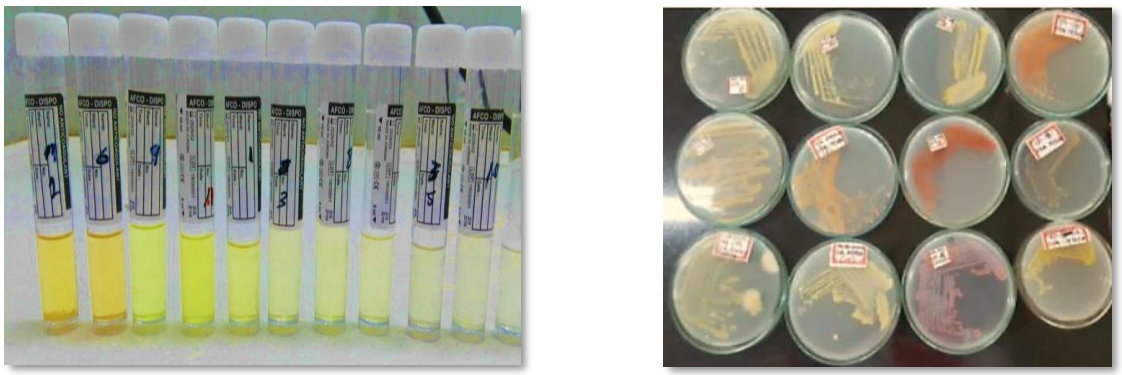

Fig. (3): Carotenoids producing bacteria and methanol extraction of carotenoids.

Carotenoid extraction for the yellow-orange pigmented isolates was carried out using methanol as a solvent. out of 24 isolates, only 10 isolates showed high amounts of carotenoid content viz., A16 > S6 > A17 > A14> S19> F5> S10> F23>S11>S7 (Table 4). From these results, isolate (A16) produced a significantly ($P < 0.05$) higher amount of carotenoids when compared to other isolates. Bhat *et al*. (2013) also suggested that carotenoid production of the organisms was mainly dependent on the environmental conditions and the composition of the culture medium and solvent. In our findings, predominant numbers of the yellow-orange pigmented colonies were obtained from the samples.

Variety in pigment production among the new bacterial strain in the current study is due to the different genetic factors (the genes responsible for the synthesis of carotenoids) for each type of bacteria as well as the influence of environmental factors (stress, UV rays, and some mutagenic factors) and the influence of optimal growth factors. The culture medium and its components necessary for the effect of bacterial growth and pigment production, and the duration of the stationary phase that the bacteria go through, since carotenoids are produced in this phase and reach their highest levels at the end of this phase (Bhat *et al*., 2013). Varsha & Arpana (2013) reported that carotenoids produced from *Planococcus maritimus* AHJ 2 absorb light in the visible region 466nm. Sasidharan *et al.* (2013) mentioned that most carotenoids absorb light maximally at wavelength with a broad shoulder at 467nm. Ramasamy & Udayasuriyan (2006) also reported the same spectral range as of absorption spectra result.

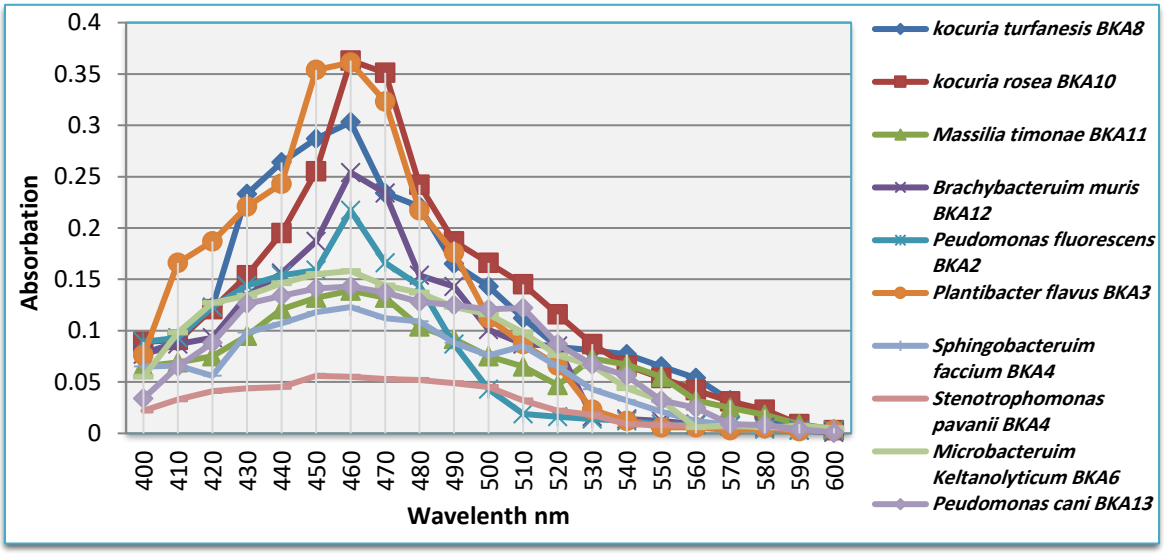

Fig. (4): Spectrum scan (λmax460nm) of the carotenoids extracted pigmen.

Table (4): Carotenoids pigment production of new strain bacteria.

isolates No:	iso1ates code	Absorption at 460nm	DCW $g.l^{-1}$	Carotenoids $\mu g.gm^{-1}$
1	F5	0.217	2.786	155.22
2	S6	0.361	2.586	279.19
3	S7	0.123	3.868	63.59
4	S10	0.055	0.872	126.14
5	S11	0.158	3.004	105.10
6	A14	0.303	3.158	192.28
7	A16	0.363	1.012	753.11
8	A17	0.139	1.088	255.51
9	S19	0.048	0.612	160.00
10	F23	0.143	2.282	125.32

Data are expressed as mean ± SD. Values given represent means of three determinations Values followed at significantly different ($p<0.05$)

The findings matched those of Boontosaenget *et al*. (2016). They looked at how different bacteria from dried seafood may produce carotenoid pigments in yellow and orange colours, and they found eight isolates with significant pigmentation levels.

Conclusion

This study deals with the isolation and identification of new strains of carotenoids producing bacteria from various sources and growth them by suitable fermentation conditions. The ten a new strains of bacteria recorded in Gene Bank as new strains which will be a new addition to list of carotenoid producing from other bacterial isolates described so far. The spectrophotometer analysis showed that the presence of carotenoids was demonstrated in ten trains. In addition, the variability among the various isolates was revealed by morphological and biochemical characterization. The obtaining various types of carotenoids pigment, ranging from yellow, orange and pink, this variety is of great importance.

Conflicts of Interest: The authors declare no conflict of interest.

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References

Bell, J. G., McEvoy, J., Tocher, D. R., & Sargent, J. R. (2000). Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. *The Journal of Nutrition, 130*, 1800-1808. https://doi.org//10.1093/jn/130.7.1800

Benson, H. J. (2001). *Microbiological Application: Laboratory Manual in General Microbiology.* 8th Edition. The McGraw – Hill, 478pp.

Berman, J., Zorrilla-López, U., Farré, G., Zhu, C., Sandmann, G., Twyman, R. M., Capell T., & Christou, P. (2015). Nutritionally important carotenoids as consumer products. *Phytochemistry Reviews, 14*, 727-743. https://doi.org/10.1007/s11101-014-9373-1

Bhat, S. V., Khan, S. S., & Amin, T. (2013). Isolation and characterization of pigment producing bacteria from various foods for their possible use as biocolours. *International Journal of Recent Scientific Research, 4*, 1605-1609. http:// www. recentscientific.com/isolation-and-haracterization-pigment-producing-bacteria-various-foods-their-possible-use-biocolour

Boontosaeng, T., Nimrat S., & Vuthiphandchai, V. (2016). Pigments production of bacteria isolated from dried seafood and capability to inhibit microbial pathogens. IOSR *Journal of Environmental Science, Toxicology and Food Technology, 10*, 30-34.

Botella-Pavía, P., & Rodríguez-Concepción, M. (2006). Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum*, *126*, 369-381.https://doi.org/10.1111/j.1399-3054.2006.00632.x

Buczolits, S., Schumann, P., Weidler, G., Radax, C., & Busse, H.-J. (2003). *Brachybacterium muris* sp. nov., isolated from the liver of a laboratory mouse strain. *International Journal of Systematic and Evolutionary Microbiology, 53*, 1955-1960. https://doi.org//10.1099/ijs.0.02728-0

Devyani, M., Shraddha, R., & Bela, N. (2017). Isolation and screening of antioxidant bacterial pigments from different ecological niche. *International Journal of Scientific & Engineering Research, 5*, 119-125.

Elbendary, A. A., Hessain, A. M., El-Hariri, M. D., Seida, A. A., Moussa, I. M., Mubarak, A. S., Kabli, S. A., Hemeg, H. A., & El Jakee, J. K. (2018). Isolation of antimicrobial producing actinobacteria from soil samples. *Saudi Journal of Biological Sciences, 25*, 44-46. https://doi.org//10.1016/j.sjbs.2017.05.003

Garrido-Sanz, D., Arrebola, E., Martínez-Granero, F., García-Méndez, S., Muriel, C., Blanco-Romero, E., Martín, M., Rivilla, R., & Redondo-Nieto, M. (2017). Classification of isolates from the *Pseudomonas fluorescens* complex into phylogenomic groups based in group-specific markers. *Frontiers in Microbiology, 8*, 413. https://doi.org//10.3389/fmicb.2017.00413

Haddad, M., S., Aghaei, S., & Zargar, M. (2017). Antimicrobial and antioxidant activity of carotenoid pigment produced by native *Rhodococcus* spp. isolated from soil. *International Journal of Molecular and Clinical Microbiology, 7*, 809-815. http://www.magiran.com/paper/1965415/?lang=en

Harley, J. P., & Prescott, L. M. (2002). *Laboratory Exercises in Microbiology*. 5th Edition. The McGraw-Hill Companies, 449 pp.

Husseiny, S. M., Abdelhafez, A. A., Ali, A. A.-A., & Sand, H. M. (2018). Optimization of β-carotene production from *Rhodotorula glutinis* ATCC 4054 growing on agro-industrial substrate using plackett–burman design. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *88*, 1637-1646. https://doi.org/10.1007/s40011-017-0908-2

Jaber, B. A., Majeed, K. R., & Al-Hashimi, A. G. (2021). Antioxidant and antibacterial activity of β-carotene pigment extracted from *paracoccus homiensis* strain bka7 isolated from Air of Basra, Iraq. *Annals of the Romanian Society for Cell Biology*, *4*, 14006-14028.

Kumar, A., Vishwakarma, H. S., Singh, J., Dwivedi, S., & Kumar, M. (2015). Microbial pigments: Production and their applications in various industries. *International Journal of Pharmaceutical, Chemical and Biological Sciences, 5*, 203-212.

Lambiase, A. (2014). *The Family Sphingobacteriaceae*. pp. 907-914. In Rosenberg, E., DeLong, E. F. Lory, S., Stackebrandt, E., & Thompson, F. (Eds.). *The Prokaryotes: Other Major Lineages of Bacteria and the Archaea*. Springer Berlin Heidelberg, Berlin, Heidelberg. 1028pp. https://doi.org/10.1007/978-3-642-38954-2_136

Learman, D. R., Ahmad, Z., Brookshier, A., Henson, M. W., Hewitt, V., Lis, A., Morrison, C., Robinson, A., Todaro, E., & Wologo, E. (2019). Comparative genomics of 16 *Microbacterium* spp. that tolerate multiple heavy metals and antibiotics. *Peer Journal*, *6*, e6258. https://doi.org/10.7717/peerj.6258

Majeed, K. R., Jaayid, T. A., & Al-Khaeun, S. N. (2016). Isolatation and identification of some types bacteria from shrimp (*Metapenaeus affinis*) and detection of

histamine producing from its. *Basrah Journal of Agricultural Sciences, 29*, 36-58. https://doi.org/10.33762/bagrs.2016.115379

Malik, K., Tokkas, J., & Goyal, S. (2012). Microbial pigments: A review. *International Journal of Microbiology Resource Technology*, *1*, 361-365.

Manimala, M., & R. Murugesan, R. (2014). *In vitro* antioxidant and antimicrobial activity of carotenoid pigment extracted from *Sporobolomyces* sp. isolated from a natural source. *Journal of Applied and Natural Science, 6*, 649-653. https://doi.org/10.31018/jans.v6i2.511

Mayer, E., de Quadros, P. D., & Fulthorpe, R. (2019). *Plantibacter flavus*, *Curtobacterium herbarum*, *Paenibacillus taichungensis*, and *Rhizobium selenitireducens* endophytes provide host-specific growth promotion of *Arabidopsis thaliana*, basil, lettuce, and bok choy plants. *Applied and Environmental Microbiology, 85*, e00383-19. https://doi.org/10.1128/AEM.00383-19

Macfaddin, J. F. (2000). *Biochemical tests for the identif ication of medical bacteria*. The Williams and Wilkins Co., 912pp.

Mezzomo, N., & Ferreira, S. R. (2016). Carotenoids functionality, sources, and processing by supercritical technology: A review. *Journal of Chemistry, 16*, 1-16. https://doi.org/10.1155/2016/3164312

Moreira, J. S., Riccetto, A. G. L., Silva, M. T. N. d., & Vilela, M. M. D. S. (2015). Endocarditis by *Kocuria rosea* in an immunocompetent child. *Brazilian Journal of Infectious Diseases, 19*, 82-84. https://doi.org//10.1016/j.bjid.2014.09.007

Netzer, R., Stafsnes, M. H., Andreassen, T., Goksøyr, A., Bruheim, P., & Brautaset, T. (2010). Biosynthetic pathway for γ-cyclic sarcinaxanthin in *Micrococcus luteus*: Heterologous expression and evidence for diverse and multiple catalytic functions of C50 carotenoid cyclases. *Journal of Bacteriology, 192*, 5688-5699. https://doi.org//10.1128/JB.00724-10

Ramasamy, A. K., & Udayasuriyan, V. (2006). Isolation and characterization of a yellow pigmented colony forming bacterium for carotenogenesis. *Biotechnology, 5*, 79-82. https://doi.org/10.3923/biotech.2006.79.82

Ramos, P. L., Van Trappen, S., Thompson, F. L., Rocha, R. C., Barbosa, H. R., De Vos, P., & Moreira-Filho, C. A. (2011). Screening for endophytic nitrogen-fixing bacteria in Brazilian sugar cane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 61*, 926-931. https://doi.org/10.1099/ijs.0.019372-0

Rodriguez-Amaya, D. B., & Kimura, M. (2004). *Harvest Plus handbook for carotenoid analysis.* International Food Policy Research Institute (IFPRI) Washington. 57pp.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1626pp. https://www.sigmaaldrich.com/IQ/en/product/sigma/m3401

Sambrook, J., & Russell, D. W. (2001). *Russell molecular cloning: A laboratory manual, III*; Cold Spring Harbor Laboratory Press: New York, 2344pp.

Sasidharan, P., Raja, R., Karthik, C., Ranandkumar, S., & Indra Arulselvi, P. (2013). Isolation and characterization of yellow pigment producing *Exiguobacterium* sp. *Journal of Biochemical Toxicology, 4*, 632-635.

Scales, B. S., Erb-Downward, J. R., LiPuma, J. J., & Huffnagle, G. B. (2015). Draft genome sequences of five *Pseudomonas fluorescens* subclade I and II strains, isolated from human respiratory samples. *Genome Announcements, 3*, e00837-15. https://doi.org/10.1128/genomeA.00837-15

Sharma, R., & Gupta, A. (2014). Differentiation of oral *Streptococcal* species by haemolysis in blood agar medium *in vitro*. *International Journal of Engineering and Advanced Technology, 4*, 143-144.

Shatila, F., Yusef, H., & Holail, H. (2013). Pigment production by *Exiguobacterium aurantiacum* FH, a novel Lebanese strain. *International Journal of Applied Research, 2*, 176-191.

Singh, H., Du, J., Yang, J.-E., Yin, C. S., Kook, M., & Yi, T.-H. (2016). *Brachybacterium horti* sp. nov. isolated from garden soil. *International Journal of*

Systematic and Evolutionary Microbiology, *66*, 189-195. https://doi.org/10.1099/ijsem.0.000696

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E. L., & Lynch, S. V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PloS one*, *10*, e.0117617. https://doi.org/10.1371/journal.pone.0117617

Van Craenenbroeck, A. H., Camps, K., Zachée, P., & Wu, K. L. (2011). *Massilia timonae* infection presenting as generalized lymphadenopathy in a man returning to Belgium from Nigeria. *Journal of Clinical Microbiology*, *49*, 2763-2765. https://doi.org/10.1128/JCM.00160-11

Varsha, C., & Arpana, J. (2013). Screening of significant nutrient parameters for pigment production from newly isolated organism *Planococcus maritimus* AHJ_2 using placket Burman design. *Journal Microbiology and Biotechnology Research*, *3*, 79-83.

Yangilar, F., & Yildiz, P. (2016). The final development related microbial pigments and the application in food industry. *Journal of Science and Technology*, *1*, 118-142.https://doi.org/10.18185/eufbed.55880

عزل وتشخيص سلالات بكتيرية جديدة منتجة لصبغات الكاروتينويدات من مصادر عزل مختلفة في البصرة، العراق

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المستخلص: الكاروتينات عبارة عن صبغات عضوية تيتراتيربينويدية (tetraterpenoid) ،توجد على نطاق واسع في الطبيعة كالنباتات والبكتريا والفطريات والطحالب ولها ألوان مختلفة مثل الأصفر والبرتقالي والأحمر والوردي ويمكن أن تكون الكاروتينات عبارة عن دهون مركبة وغيرها من عوامل الأيض(التمثيل الغذائي) في كل هذه الكائنات، الكاروتينات لها العديد من الاستخدامات كمضادات للأكسدة ومضادات للبكتيريا ومضافات غذائية. شملت هذه الدراسة عزل اربعة وعشرون عزلة نقية لها القدرة على انتاج صبغات الكاروتينويدات، من مصادر عزل مختلفة في مدينة البصرة في العراق، تم غربلة العزلات غربلة ثانوية لغرض اختيار الاكفاء في انتاجية الصبغات، تم اختيار 10عزلات نقية وتم توصيف العزلات وتشخيصها بناء على الخواص المظهرية والفحوصات الكيمو حيوية والتقنيات الجزيئية وسجلت هذه العزلات كسلالات جديدة في بنك الجينات BDDJ، خمسة سلالات تم عزلها من التربة كانت تنتمي الى الاجناس *Brachybacteruim muris* BKA12 و *Plantibacter flavus* BKA3 و *Sphingobacteruim faccium* BKA4 و *Stenotrophomonas pavanii* BKA5 و *Microbacteruim Keltanolyticum* BKA6 وثلاث سلالات تنتمي الى الاجناس *Kocuria turfanesis* BKA8 و *Kocuria rosea* BKA10 و *Massilia timonae* BKA11 تم عزلها من الهواء، وسلالتين تنتمي الى جنس *Pseudomonase cani* BkA13 و *Pseudomonas fluoresces* BKA2 عزلت من السمك. استخلصت الكاروتينويدات بواسطة مذيب الميثانول النقي وتم تحليله بواسطة مقياس الطيف الضوئي في حدود 400-600 نانومتر وتم تحديد أقصى امتصاصيه له على طول موجي 460 نانومتر. أظهرت النتائج أن عزلة *Kocuria turfanesis* BKA8 كان الاعلى في انتاج الكاروتين، اذ بلغت 753.11 ميكروغرام / غرام اثناء الغربلة الثانوية (الكمية) من خلال تنمية جميع العزلات في وسط الانتاج المرق المغذي السائل وفي نظام المزارع السائلة (تخمرات المزارع المغمورة) بالحاضنة الهزاز وبظروف تخمير ثابتة والتي شملت درجات حرارة 27م ولمدة 72 ساعة وسرعة تحريك 150 دوره/ دقيقة وبدالة حامضية 7.

الكلمات المفتاحية: بكتريا، كاروتينويدات، صبغات.