BIOTECHINTELLECT, 2026, 3(1) e20 (1-11)
https://jbiotechintel.com/index.php/biotechintel

Research Article

eISSN: 3115-7920

OPEN ACCESS

Screening of Pigment Producer from Native Halophilic Archaea

Milad Behroozi[1], Maryam Jalili Tabaii [*1], Soheila Abbasi[1], Giti Emtiazi[1], Ramona Massoud[2*]

[1] Department of Biology, SR.C., Islamic Azad University, Tehran, Iran

[2] Microorganisms Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran

[3] Department of Pathogenic Microorganisms, Faculty of Basic Sciences and Advanced Technologies, University of Science and Culture, Tehran, Iran

Article history:
Received 06 July 2026
Revised 10 Sep 2026
Accepted 24 Sep 2026
Published online 25 Sep 2026

Keywords: Pigments, haloarchaea, carotenoid, antioxidant, antibacterial

How to cite this article:
Behroozi M, Jalili Tabaii M, Abbasi S, Emtiazi G, Massoud R. Screening of pigment producer from native halophilic archaea. BiotechIntellect, 2026; 3(1): e20, 1-11.
https://doi.org/10.66224/BiotechIntellect.3.1.58

*corresponding author's
Maryam Jalili Tabaii
Email: maryjtabaii@gmail.com

© 2026 the authors. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License.

ABSTRACT

Background and Objective: Halophilic archaea have a high potential to discover new compounds with high biotechnological potential, one of these compounds is carotenoids.

Materials and Methods: A collection of 23 native highly halophilic archaea isolates were isolated from distinct saline habitats in different regions of Iran. Among the examined isolates, the isolates that had produced colored colonies within a week were further investigated by absorption spectrophotometric method in order to extract and measure the amount of pigment produced with different solvents of methanol, acetone, hexane, petroleum ether and ethanol. Also, the microscopic evaluation of morphological changes in melanoma cells exposed to carotenoid pigment was studied.

Results and Conclusion: According to the results obtained from the screening of the strains, the best methanol solvent and the best absorbance 0.239 at the wavelength of 498 belonged to the *Haloferax Mediterranei* ASFM403 strain. The light absorption data of the pigment shows the structural similarity of this carotenoid with bacterioruberin. The information obtained shows that bacterioruberin is the dominant carotenoid. It has a toxic effect on melanoma cancer cells in concentrations of 10 micrograms/liter. The cells lost their original morphology and underwent morphological changes at a pigment concentration of 10 µg/mL and 1000 µg/mL.

What is "already known":	• Halophilic archaea are promising sources of bioactive compounds, including carotenoids with potential biotechnological applications. • Carotenoids from microbial sources have attracted interest for their potential anticancer and cell-protective effects. • Carotenoids produced by halophilic archaea, particularly bacterioruberin, are known to possess antioxidant and antimicrobial properties.
What this article adds:	• This study identified *Haloferax mediterranei* ASFM403 as a promising native halophilic archaeal strain for the production of carotenoids, with bacterioruberin identified as the dominant pigment. • The findings demonstrated selective biological effects of the extracted carotenoid, including cytotoxicity toward melanoma cells at 10 and 1000 µg/L.

1. INTRODUCTION

Archaea is a good source of producing pigments such as carotenoids with considerable antioxidant and anti-inflammatory properties [1-5]. Halophiles are exposed to severe oxidative stress and therefore produce carotenoids that are highly active in neutralizing reactive oxygen species (ROS) [6]. Various carotenoids are produced by archaea. Carotenoids are generally mainly of the bacterioruberin and canthaxanthin types, with small amounts of 3-hydroxychinenone and beta-carotene [7,8], or zeaxanthin [9]. Archaea also produce thermostable enzymes [10]. Because they can grow under harsh conditions of pH, salinity, and high temperature, the risk of contamination is reduced, and even non-sterile media can be used for archaeal growth, substantially reducing cultivation costs. One advantage of haloarchaea for C50 carotenoid production is that biosynthesis can be readily increased by replacing a highly saline growth medium (20–25% w/v) with a low-salt medium (<16% w/v), which results in rapid bacterioruberin accumulation [11].

Therefore, easy carotenoid production by haloarchaea should preferably be carried out through a two-stage process consisting of biomass production under high-salt conditions (stage one), followed by rapid carotenoid biosynthesis and increased accumulation under low-salt conditions (stage two) [10, 11].

After production, pigment extraction is an important step and may sometimes be associated with difficulties such as disruption of the rigid cell wall. However, for carotenoid extraction from haloarchaeal species, reducing the salt concentration of the medium can cause cell lysis, making cell disruption easier [8]. Karthikeyan et al. reported the optimum growth temperature of haloarchaea in Natrinema to be 42 °C [11]. Similar reports have been published by Chaudhary et al. [12] and Enache et al. [13]. The studies indicate the possibility of discovering new compounds with high industrial potential [14]. Up to know only limited attention has been paid to halophilic micro-organisms, particularly haloarchaea with a high capacity for carotenoid production. However, research has shown that some haloarchaeal species are capable of producing carotenoids at high concentrations and on an industrial scale [15].

Considering the advantages of archaea for industrial pigment production, this study was designed to improve archaeal cultivation conditions. The aim of this study was to isolate archaea and optimize the growth medium for pigment production for industrial applications.

2. MATERIALS AND METHODS

2.1. Preparation of McFarland Standards

The McFarland method is based on the precipitation reaction resulting from the combination of barium chloride and sulfuric acid and is used as an approximation of the turbidity of a bacterial suspension. These standards are used when equal concentrations of bacterial strains are required for inoculation. The method for preparing these standards is shown in Table 1.

Table 1. Preparation of McFarland standards

McFarland standards	0.5	1	2	3	4
10% barium chloride (mL)	0.05	0.1	0.2	0.3	0.4
10% sulfuric acid (mL)	9.59	9.9	9.8	9.7	9.6
Cell density ($\times 10^8$ CFU/mL)	1.5	3.0	6.0	9.0	12.0
Optical density (OD)	0.145	0.260	0.457	0.585	0.673

2-2. Sampling, Isolation, and Maintenance of Haloarchaeal Strains

2-2-1. Sampling

The samples were collected from six different regions in autumn 2023 as follows:

1) Salt Mountain, Kuhrang County, Chaharmahal and Bakhtiari Province (samples of salt, soil, and water from three different locations) (Fig. 1a);

2) the Kuhrang salt spring, which emerges from the ground and forms salt pools along its course (local

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

residents used its salt as edible salt): samples of salt, soil, and water were collected from three different locations. The soil along the flow of the salt-spring water was red (Fig. 1b);

3) Alikuh salt spring, Ardal County, Chaharmahal and Bakhtiari Province, where the spring flows through a valley between two mountains. The spring bed was marshy and soil-colored, while the stream bed was black and muddy (Fig. 1c); samples of salt, soil, and water were collected from three different locations;

4) Howz Sultan Salt Lake, Qom: a 30-cm-deep pit was dug, revealing three layers from top to bottom that were brown, green, and pink. Three salt samples were collected from each pit, with sampling performed from three pits in total (Fig. 1d);

5) Maharloo Lake, Shiraz, which is particularly pink in summer (samples were collected from three different locations of water, soil, and salt) (Fig. 1e);

6) the Varzaneh desert area and Khara Salt Lake, 110 km from Isfahan (soil samples from three different locations). Samples of salt, water, and soil were also collected from three different locations in Khara village, south of Gavkhouni Wetland, and Khara Salt Lake. The lake had dried up, and only a small amount of water remained because of rainfall (Fig. 1f).

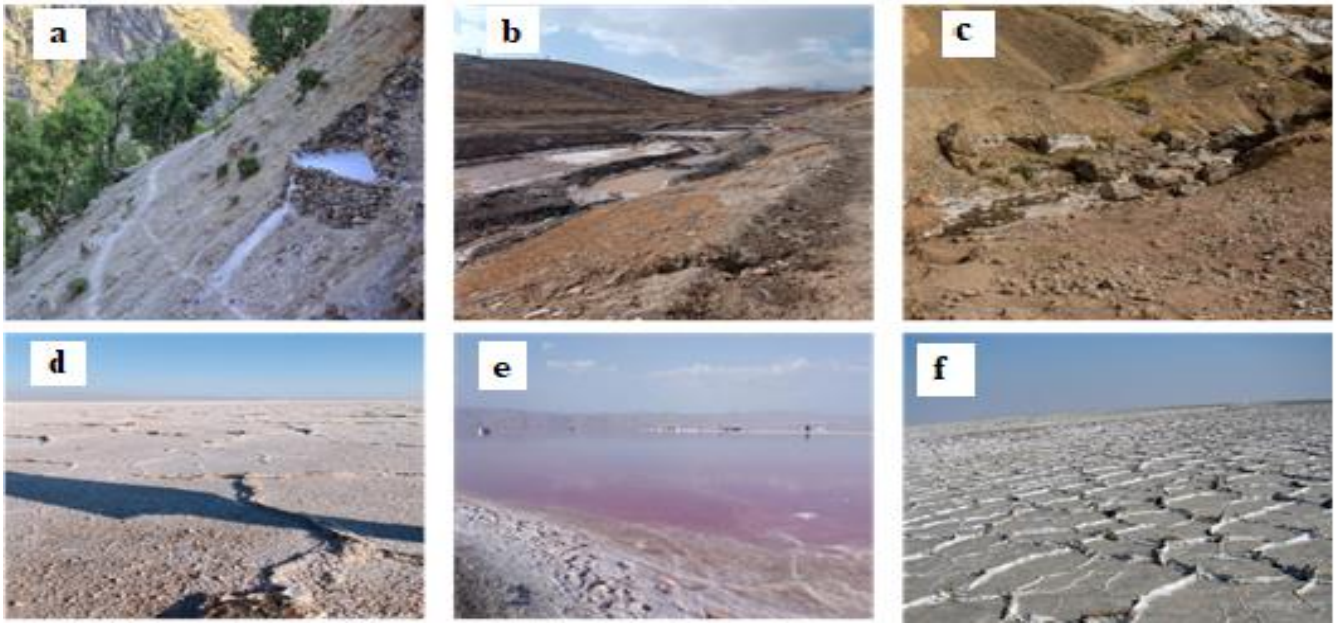


Figure 1. Sampling sites for isolation of Archaeal strains (a-f). Kuhrang salt mountain (a); Kuhrang salt spring (b); Alikuh salt spring (c); Howz Sultan salt lake Qom (d); Maharloo lake Shiraz (e); Varzaneh &Khara salt lake Isfahan (f).

2-2-2. Preparation of archaeal culture media

Archaea broth medium with varying NaCl concentrations was used for the isolation and cultivation of halophilic microorganisms and archaea. The culture composition was as following: glucose 5(g/L), yeast extract 1(g/L), peptone 1(g/L), sodium chloride 125 (g/L), magnesium chloride 6 (g/L), calcium chloride 0.013 (g/L), and potassium sulfate 5 (g/L). After adjusting the pH to 6.8 –7, the media were autoclaved for 20 min at 120°C, and solid medium was prepared by adding 2.2% agar.

2-2-3. Isolation of Archaea

900 mL of liquid Archaea broth medium was prepared. 300 mL of water was poured in a 1000 mL Erlenmeyer flask and put on a shaker with a magnetic stirrer, and the other materials were added sequentially while being mixed. After dissolution, 100 mL of the culture medium was dispensed into 250 mL Erlenmeyer flasks and autoclaved at 120 °C for 20 min.

Then, 0.18 g chloramphenicol was added to 9 mL of sterile distilled water and vortexed. One milliliter of this antibiotic solution was then added to each Erlenmeyer flask containing sterile Archaea broth

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

(final concentration, 0.02 g/L). To isolate pigment-producing halophilic archaea, 1 g of each collected sample (soil, water, and salt) was separately added to each flask. The flasks were incubated in a shaking incubator at 30 °C for 3 weeks. The cultures were then plated on solid Archaea agar medium. The resulting colonies were examined for morphology, size, and pigment production, and colonies with different characteristics and pigment production were purified by successive subculturing on the same medium. To confirm archaeal isolation in this study and to screen archaeal strains among halophilic strains isolated in previous studies, after preparing solid Archaea agar medium and culturing the strains of interest on separate plates, anisomycin powder was placed on an empty sterile plate and paper disks were impregnated with the antibiotic. The disks were then transferred with sterile forceps and placed on the media inoculated with the strains of interest. The plates were incubated at 30 °C for 7 days, after which the formation of growth-inhibition zones was examined. Formation of an inhibition zone around the disks confirmed the archaeal nature of the isolate because archaea are sensitive to anisomycin and resistant to chloramphenicol.

2-2-4. Maintenance of isolates in refrigerator/ freezer

Isolate colonies were transferred with a sterile loop from the selective medium to plates containing sterile Archaea agar. After 48 h of incubation and adequate growth, the cultures were transferred to Archaea agar slants and incubated at 30 °C. After sufficient growth, the slants were stored in a refrigerator. A loopful of a pure colony was inoculated into a suitable liquid medium such as Archaea broth and incubated at 30 °C and 120 rpm. After 48 h, 0.5 mL of the dense culture was mixed with 0.5 mL of 50% glycerol, vortexed, and stored in appropriate microtubes in a −80 °C freezer.

2-3. Primary Screening of Carotenoid-Producing Archaeal and Halophilic Isolates

After sampling, isolation, and purification, the best archaeal colony/colonies among the purified indigenous isolates obtained in this study and indigenous isolates obtained in previous studies were selected for subsequent experiments if they produced pigmented colonies after one week of incubation. The remaining isolates were discarded.

2-4. Carotenoid Production

A fresh culture of the screened isolates was prepared on Archaea agar. A turbidity equivalent to 0.5 McFarland was prepared in 12.5% saline, and 10 mL was inoculated into Archaea broth. The inoculated flasks were incubated at 30 °C and 120 rpm for 7 days. The samples were collected at different times to determine cell dry weight (CDW) and evaluate carotenoid production. Archaea broth without chloramphenicol was used as the liquid carotenoid-production medium in Erlenmeyer flasks and for determining cell dry weight and the amount of extracted carotenoid.

2-5. Carotenoid Measurement Method

Five pigmented archaeal isolates were used. For carotenoid measurement, the isolates were cultured in broth and incubated in a shaking incubator at 120 rpm for 48–72 h at 30 °C. After 72 h, the cultures were centrifuged at 3500 rpm for 15 min and washed twice with sterile 12.5% saline, centrifuging for 5 min each time. Then, 5 mL of methanol, acetone, hexane, or petroleum ether was added per gram of cell biomass to the pellet and incubated in a 62 °C water bath for 20 min. The extract was filtered through filter paper, and the resulting liquid was measured at wavelengths of 400–700 nm using a spectrophotometer. Dry carotenoid powder was obtained by drying the resulting solution in an oven at 70 °C.

2-5-1. Determination of cell dry weight

The culture medium of this strain contains a high amount of salt; therefore, determining the net archaeal biomass requires estimating the amount of salt precipitate that sediments together with the actual biomass during centrifugation. Thus, 100 mL of archaeal culture medium and 100 mL of archaeon-free culture medium containing all the same medium components were centrifuged in pre-weighed Falcon tubes. After removing the supernatant, the tubes were weighed again. The exact archaeal biomass was calculated by subtracting the weight of the salt precipitate obtained from the archaeon-free medium from the weight of the precipitate obtained from the

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

archaeon-containing medium. To determine archaeal dry weight, the Falcon tube containing the biomass was placed in an oven at 70 °C for 72 h. After this period, the weight of the resulting dry precipitate was measured.

2-6. Selection of the Suitable Carotenoid-Producing Strain

Among the five pigmented archaeal isolates, one showed the highest carotenoid-production potential. It had been isolated from water of Lake Urmia in previous research and identified *Haloferax Mediterranei* ASFM403, accession number PQ327049. This strain was selected for subsequent production experiments and analyses. To confirm the strain characteristics, colony morphology, cell morphology, physiological, and biochemical tests of *Haloferax Mediterranei* ASFM403 were performed under optimal growth conditions on solid medium after aerobic incubation at 30 °C for 7 days. Cell morphology and bacterial motility were observed by light microscopy during the logarithmic growth phase in liquid culture.

2-6-1. Staining of halophilic archaea

Several attempts have been made to stain halophilic bacteria. Because these organisms grow in culture media containing more than 12% sodium chloride, halophiles are highly sensitive to changes in osmotic pressure and may lyse after immersion in salt-free water. Therefore, smears for microscopic examination should be prepared in 20% saline, and after proper preparation, a desalination step should be performed before staining. In this study, an effective technique for staining halophilic isolates, with a minor modification of previously used techniques, was applied. The procedure was as follows.

First, the organisms were suspended in sterile 20% saline and mixed thoroughly to homogenize the culture, which is usually viscous. A large loopful was spread as a thin layer on a clean glass slide. After air-drying, the smear was fixed and then immersed in 2% acetic acid for 5 min for desalination. It was then removed and dried without washing. This is important because washed slides have produced poor results. The smear was covered with 0.25% aqueous crystal violet solution for 3 min. Safranin can also be used as a counterstain.

2-6-2. Physiological and Biochemical Tests

Catalase, oxidase, and indole formation tests were performed according to modified standard methods. Acid production from different sugars (glucose, lactose, mannitol, sucrose, xylose, fructose, arabinose, rhamnose, and sorbitol) was tested using 1% sterilized sugar solutions without sodium citrate, supplemented with 0.001% phenol red and containing sodium chloride.

2-6-3. Microscopic evaluation of morphological changes in melanoma cells exposed to carotenoid pigment

Microscopic studies were also performed to investigate morphological changes. For this purpose, the carotenoid obtained from *Haloferax mediterranei* ASFM403 was used at concentrations of 10 and 1000 µg/mL. If the carotenoid has an adverse effect on the cells, the cells undergo morphological changes prior to lysis, including shrinkage of cells in suspension and rounding of adherent cells.

3. RESULTS AND DISCUSSION

The results were evaluated after two weeks of surface cultivation of the samples on plates. During the isolation stage, a total of 70 inoculated Erlenmeyer flasks and 102 plates prepared by surface plating from the flasks were examined. Many showed no growth, and only 23 developed colonies, of which 4 were pigment-producing colonies. Secondary screening based on pigment production was then performed on these 5 strains, and the results are presented below. Ultimately, *Haloferax Mediterranei* ASFM403 was selected as the best strain.

3.1. Secondary Screening of Isolates Based on Pigment Extraction Using Different Solvents and Measurement of Pigment Level by Optical Absorbance

The results of pigment extraction using five solvents; methanol, ethanol, acetone, petroleum ether, and hexane and assessment by optical absorbance at wavelengths of 400, 500, 600, and 700 nm for five archaeal strains are presented in Table 2. According to

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

the results, among the 5 isolates examined with methanol, strains 1 and 5 showed the highest pigment levels with this solvent at 400 nm (OD400 = 0.182 for strain 1 and 0.247 for strain 5) and 500 nm (OD500 = 0.239 for strain 1 and 0.199 for strain 5).

According to the pigment-extraction results for the 5 pigment-producing strains using ethanol, strains 1 and 4 showed the highest amounts of extracted pigment with ethanol at 400 nm (OD400 = 0.182 for strain 1 and 0.247 for strain 4) and 500 nm (OD500 = 0.150 for strain 1 and 0.118 for strain 4).

According to the pigment-extraction results for the 5 pigmented strains using acetone, strain 1 showed the highest pigment extraction with acetone at 400 and 500 nm. With petroleum ether, strain 1 also showed the highest pigment extraction at 400 and 500 nm. Similarly, with hexane, strain 1 showed the highest pigment extraction at 400 and 500 nm.

Among all solvents used for pigment extraction, methanol and acetone produced the best results. Methanol was considered the best extraction solvent due to its better performance (Table 2). Strain 1 also achieved the highest pigment extraction compared with the other isolates using the different solvents and at 400 and 500 nm. This strain (*Haloferax Mediterranei* ASFM403) was therefore selected as the best. Then, the solution was dried at 70 °C in an oven to obtain dry carotenoid powder.

Table 2. Pigment extraction using 5 solvents: methanol, ethanol, acetone, petroleum ether, and hexane

Sample	Solvent	Wavelength 400 (nm)	Wavelength 500 (nm)	Wavelength 600 (nm)	Wavelength 700 (nm)
1	Methanol	0.182	**0.239**	0.038	0.017
2		0.114	0.057	0.023	0.045
3		0.051	0.058	0.018	0.028
4		0.051	0.039	0.008	0.039
5		0.247	0.199	0.058	0.052
1	Ethanol	0.183	0.150	0.115	0.108
2		0.068	0.048	0.012	0.030
3		0.048	0.058	0.034	0.049
4		0.159	0.118	0.133	0.074
5		0.066	0.060	0.037	0.039
1	Acetone	0.196	0.231	0.037	0.042
2		0.034	0.040	0.003	0.016
3		0.044	0.016	0.038	0.023
4		0.036	0.019	0	0.005
5		0.089	0.046	0.019	0.068
1	Petroleum ether	0.123	0.095	0.045	0.034
2		0.054	0.030	0.019	0.018
3		0.046	0.025	0.014	0.017
4		0.050	0.034	0.020	0.015
5		0.031	0.018	0.017	0.011
1	Hexane	0.108	0.143	0	0.045
2		0.061	0.056	0	0.049
3		0.043	0.045	0	0.043
4		0.028	0.013	0	0.010
5		0.102	0.068	0.025	0.032

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

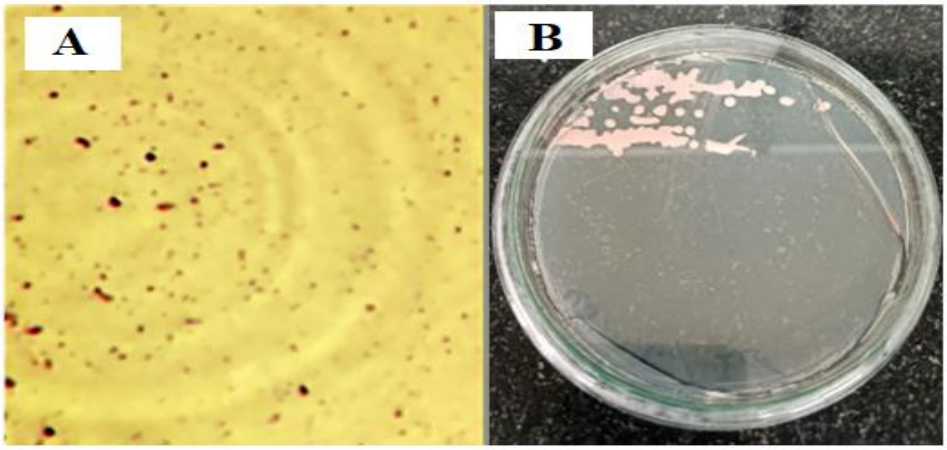


Figure 2. (A) Morphology of Haloferax Mediterranei ASFM 403 after Archaeal-specific staining under a light microscope, (B) Colony morphology on Archaea agar medium.

3-2. Characteristics of Haloferax mediterranei ASFM403

The physical and chemical characteristics of the strain were examined to confirm maintenance of the characteristics of *Haloferax Mediterranei* ASFM403. Macroscopic and microscopic examination of *Haloferax Mediterranei* ASFM403 on Archaea agar showed that: The colonies were pink, approximately 4 mm in diameter, circular, smooth, and mucoid, while the cells were triangular in microscopic morphology, Gram-negative, and motile (Fig. 2).

Physiological and biochemical analyses were performed using liquid or solid Archaea medium at 30 °C. The results showed that this halophilic archaeon grows anaerobically with nitrate. The Results of biochemical sugar tests for *Haloferax Mediterranei* ASFM403. Glucose, fructose, xylose, arabinose, rhamnose, and nitrate tests were positive. It can utilize individual carbon substrates such as glucose, fructose, xylose, arabinose, and rhamnose (Fig. 3).

Catalase and oxidase tests were negative. Tests for gelatin hydrolysis, casein hydrolysis, indole, and H2S were also negative (Table 3).

3-3. Relation of Pigment Production and Growth Phase in Haloferax Mediterranei ASFM403

As shown in Fig. 4, growth and pigment production by the producing strain *Haloferax Mediterranei* ASFM403 were monitored for up to 240 h. The correlation between the growth curve and pigment production showed that the maximum pigment production occurred during the logarithmic growth phase. In the pigment optimization analysis and assessment of the time factor, the highest pigment level (0.184) was obtained on day 7. On day 7, *Haloferax Mediterranei* ASFM403 also showed its highest growth (0.642), after which growth remained constant. The strain that produced the highest amount of carotenoid in this study belonged to *Haloferax Mediterranei* and produced pink colonies. Members of this group possess C50 carotenoids of the bacterioruberin group.

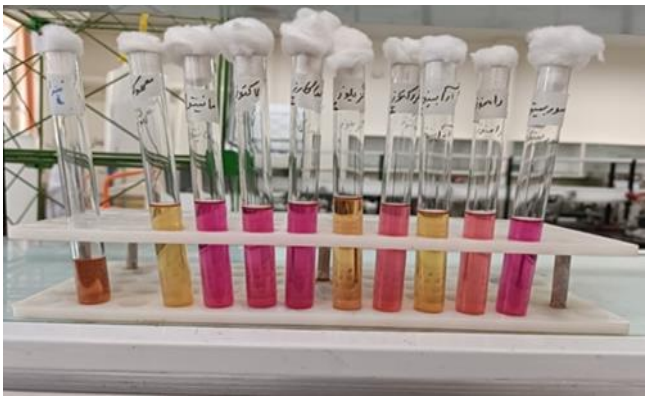


Figure 3. Biochemical sugar tests for *Haloferax Mediterranei* ASFM403. Glucose, fructose, xylose, arabinose, rhamnose, and nitrate.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

Table 3. Morphological and physiological characteristics of *Haloferax Mediterranei* ASFM403

Morphological characteristics	
Colony morphology	Pink,round regular edges
Form	Triangular
Gram stain	Negative
Spore	Negative
Motility	Positive
Physiological characteristics	
Anaerobic growth	Positive
Acid from glucose	Positive
Gas from glucose	Positive
Hydrolysis of fructose	Positive
Hydrolysis of arabinose	Positive
Hydrolysis of rhamnose	Positive
Hydrolysis of xylose	Positive
Hydrolysis of sucrose	Positive
Hydrolysis of gelatin	Negative
Utilization of citrate	Positive
Hydrolysis of Casein	Negative
Indol formation	Negative
H2S	Negative
Metyl Red test	Positive
VP-test	Negative
Urease	Negative
Nitrate reduction	Positive

Production of pigments ranging from red to orange is common among microorganisms inhabiting salt lakes and brines [16]. The absorption range of the pigments is 300–700 nm, with maximum optical absorption around 489 nm. The role of these pigments in protecting these microorganisms against ultraviolet radiation from sunlight in their natural habitats has been demonstrated [17].

3.4. Determination of Carotenoid Content

To determine the total carotenoid content, the principal carotenoid absorbance in methanol was considered. The approximate carotenoid content per gram of archaeal dry weight was calculated and found to be approximately 950 µg/g of archaeal biomass. This indicates that the carotenoid pigment production level of this archaeon is relatively favorable compared with some known carotenoid-producing microorganisms, including *Haloarcula* (830 µg/g) [16] and the radiation-resistant bacterium *Rubrobacter radiotolerans* (722 µg/g) [18]. Accordingly, this organism can be considered a valuable carotenoid producer. The correlation between the growth curve and pigment production showed that maximum pigment production occurred during the logarithmic growth phase, although pigment production continued throughout all growth phases.

3.5. Microscopic Evaluation of Morphological Changes in Melanoma Cells Exposed to Carotenoid Pigment

Images of the cells after exposure to the obtained carotenoid sample and the control sample after 48 h are shown in Fig. 5. The images show that the cells lost their original morphology and underwent morphological changes at a pigment concentration of 10 µg/mL and 1000 µg/mL.

4. CONCLUSION

In this study, carotenoid production by the extremophilic strain *Haloferax Mediterranei* ASFM403 was investigated. Using the cell dry-weight determination method and methanol extraction, the strain was identified as having high potential for carotenoid production. The optical absorption data indicated structural similarity between this carotenoid and bacterioruberin.

5. DECLARATIONS

5.1. Acknowledgements

The authors would like to thank all individuals and institutions who supported this study. The authors also appreciate the constructive comments and suggestions provided by the reviewers and the Editor, which significantly improved the quality of this manuscript.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

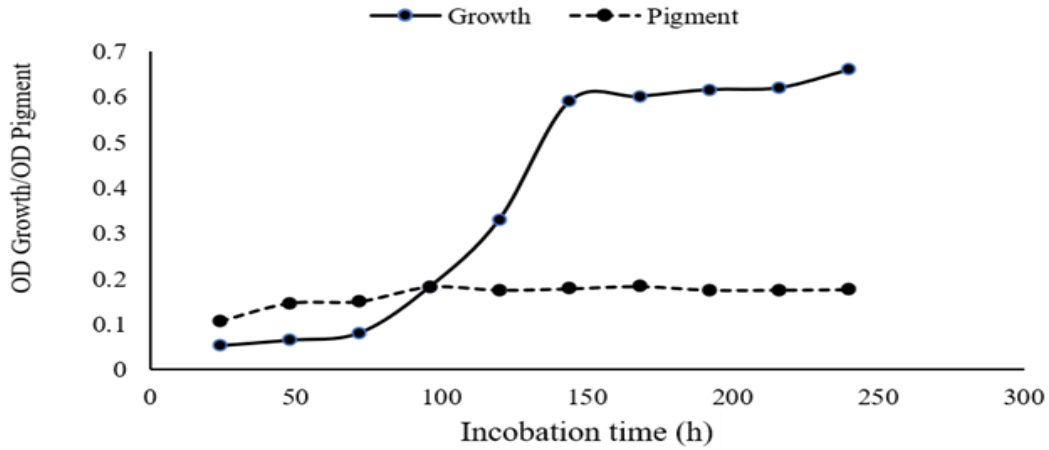


Figure 4. Relationship between the growth curve and the amount of pigment produced in the culture medium by *Haloferax Mediterranei* ASFM403.

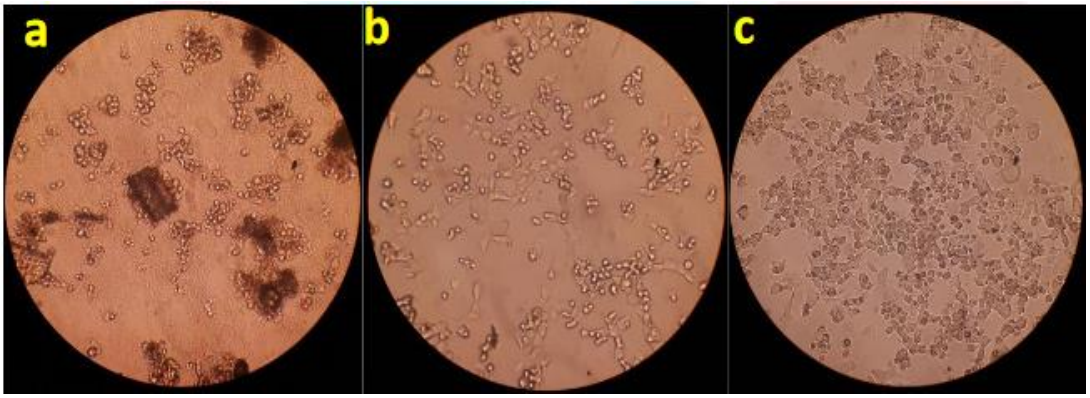


Figure 5. cells exposure to the obtained carotenoid sample and the control sample after 48 h (a). Concentration of 10 µg/mL(b); concentration of 1000 µg/mL(c).

5. 2. Conflict of Interest

The authors declare no conflict of interest.

5.3. Authors Contributions

Formal analysis, graphic investigation and writing original draft preparation Milad Behroozi. Writing review and editing, Giti Emtiazi, Maryam Jalili Tabaii, Soheila Abbasi and Ramona Massoud.

5.4. Using Artificial Intelligent chatbots

AI chatbots used for graphic abstract.

6. REFERENCES

1. Milani, A., M. Basirnejad, S. Shahbazi and A. Bolhassani, Carotenoids: biochemistry, pharmacology and treatment. *British Journal of Pharmacology*, 2017. **174**(11): p. 1290-1324. https://doi.org/10.1111/bph.13625

1. Mortensen, A., L.H. Skibsted and T.G. Truscott, The interaction of dietary carotenoids with radical species. *Archives of Biochemistry and Biophysics*, 2001. **385**(1): p. 13-19. https://doi.org/10.1006/abbi.2000.2172

2. Bayir, H., Reactive oxygen species. *Critical Care Medicine*, 2005. **33**(12 Suppl): p. S498-S501.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

https://doi.org/10.1097/01.ccm.0000186787.64500.12

3. Jomova, K. and M. Valko, Health protective effects of carotenoids and their interactions with other biological antioxidants. *European Journal of Medicinal Chemistry*, 2013. 70: p. 102-110. https://doi.org/10.1016/j.ejmech.2013.09.054

4. Koklesova, L., A. Liskova, M. Samec, C. Buhrmann, S.M. Samuel, E. Varghese, M. Ashrafizadeh, M. Najafi, M. Shakibaei, D. Büsselberg, F.A. Giordano, O. Golubnitschaja and P. Kubatka, Carotenoids in cancer apoptosis—The road from bench to bedside and back. *Cancers*, 2020. **12**(9): p. 2425. https://doi.org/10.3390/cancers12092425

5. Oren, A., The microbiology of red brines. *Advances in Applied Microbiology*, 2020. 113: p. 57-110. https://doi.org/10.1016/bs.aambs.2020.07.003

6. Massoud, R. and K. Khosravi-Darani, A review on the impacts of process variables on microbial production of carotenoid pigments. In: *Food Biosynthesis*, 2017. p. 183-211. https://doi.org/10.1016/B978-0-12-811372-1.00006-3

7. Vílchez, C., E. Forján, M. Cuaresma, F. Bédmar, I. Garbayo and J.M. Vega, Marine carotenoids: biological functions and commercial applications. *Marine Drugs*, 2011. **9**(3): p. 319-333. https://doi.org/10.3390/md9030319

8. Rønnekleiv, M., Bacterial carotenoids 53. C50-carotenoids 23; carotenoids of *Haloferax volcanii* versus other halophilic bacteria. *Biochemical Systematics and Ecology*, 1995. **23**(6): p. 627-634. https://doi.org/10.1016/0305-1978(95)00047-X

9. Nicolaus, B., V.S. Moriello, L. Lama, A. Poli and A. Gambacorta, Polysaccharides from extremophilic microorganisms. *Origins of Life and Evolution of the Biosphere*, 2004. **34**(1-2): p. 159-169. https://doi.org/10.1023/B:ORIG.0000009837.37412.D3

10. Karthikeyan, P., S.G. Bhat and M. Chandra-sekaran, Halocin SH10 production by an extreme haloarchaeon *Natrinema* sp. BTSH10 isolated from salt pans of South India. *Saudi Journal of Biological Sciences*, 2013. **20**(2): p. 205-212. https://doi.org/10.1016/j.sjbs.2013.02.002

11. Chaudhary, M., B. Bajpai, K. Suthar and J. Saxena, Characterization of a halotolerant archaeon *Haloferax larsenii* HA8 isolated from solar saltern for halocin production. *Chiang Mai Journal of Science*, 2018. **45**(6): p. 2244-2255.

13. Enache, M., T. Itoh, M. Kamekura, G. Teodosiu and L. Dumitru, *Haloferax prahovense* sp. nov., an extremely halophilic archaeon isolated from a Romanian salt lake. *International Journal of Systematic and Evolutionary Microbiology*, 2007. **57**(2): p. 393-397. https://doi.org/10.1099/ijs.0.64674-0

14. Kumar, V. and S.K. Tiwari, Halocin diversity among Halophilic Archaea and their applications. In: *Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Volume 1. Microbial Diversity in Normal & Extreme Environments*, 2019. p. 497-532. https://doi.org/10.1007/978-981-13-8315-1_16

15. Grivard, A., I. Goubet, L.M.d.S. Duarte Filho, V. Thiéry, S. Chevalier, R.G. de Oliveira-Junior, N. El Aouad, J.R.G.d.S. Almeida, P. Sitarek, L.J. Quintans-Junior, R. Grougnet, H. Agogué and L. Picot, Archaea carotenoids: natural pigments with unexplored innovative potential. *Marine Drugs*, 2022. **20**(8): p. 524. https://doi.org/10.3390/md20080524

16. Oren, A., Molecular ecology of extremely halophilic Archaea and Bacteria. *FEMS Microbiology Ecology*, 2002. **39**(1): p. 1-7. https://doi.org/10.1111/j.1574-6941.2002.tb00900.x

17. Asgarani, E., H. Funamizu, T. Saito, H. Terato, Y. Ohyama, O. Yamamoto and H. Ide, Mechanisms of DNA protection in *Halobacterium salinarium*, an extremely halophilic bacterium. *Microbiological Research*, 1999. **154**(2): p. 185-190. https://doi.org/10.1016/S0944-5013(99)80013-5

18. Saito, T., H. Terato and O. Yamamoto, Pigments of *Rubrobacter radiotolerans*. *Archives of Microbiology*, 1994. **162**(6): p. 414-421. https://doi.org/10.1007/BF00282106

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/