



Characterization and biological activities of carotenoids produced by three haloarchaeal strains isolated from Algerian salt lakes

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Received: 15 June 2021 / Revised: 23 November 2021 / Accepted: 24 November 2021 / Published online: 4 December 2021
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Abstract

Halophilic archaea represent a promising natural source of carotenoids. However, little information is available about these archaeal metabolites and their biological effects. In the present work, carotenoids of strains *Haloferax* sp. ME16, *Halogometricum* sp. ME3 and *Haloarcula* sp. BT9, isolated from Algerian salt lakes, were produced, extracted and identified by high-performance liquid chromatography–diode array detector and liquid chromatography–mass spectrometry. Analytical results revealed a variation in the composition depending on the strain with a predominance of bacterioruberin. The evaluation of antioxidant capacity using ABTS [(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays showed that these extracts have a strong antioxidant potential, in particular those of *Haloferax* sp. ME16 which displayed antioxidant power significantly higher than that of ascorbic acid used as standard. Antibacterial activity of carotenoid extracts against four human-pathogenic strains and four fish-pathogenic strains was evaluated by agar disk diffusion method. The results showed a good antibacterial activity. These findings suggest that the C₅₀ carotenoids from the studied strains offer promising prospects for biotechnological applications.

Keywords Haloarchaea · C₅₀ carotenoids · Antioxidant · Antibacterial activity · Salt lakes · Algeria

Introduction

Carotenoids are a group of yellow, orange-red-pigmented polyisoprenoids, synthesized by plants, algae, some fungi, bacteria, and archaea. More than 1178 natural carotenoids have been properly characterized and reported in the

literature, which present huge structural diversity and physicochemical properties (Fernandes et al. 2018). They are involved in photosynthesis as accessory pigments, and function as antioxidants agents, light protectors, and cell membrane stabilizers. Their functions depend on their molecular structure such as the number of conjugated double bonds and the type of functional groups (Yatsunami et al. 2014).

Carotenoids have been proven to play important roles in human health as precursors of vitamin A, heart disease prevention agents, antitumoral molecules and enhancers of in vitro antibody production. Therefore, they are widely applied in food, medical, pharmaceutical and cosmetic industries as color additives and functional components (Vílchez et al. 2011).

Chemical synthesis is one of the production methods for carotenoids. However, the increasing consumer demand for natural ingredients, as well as the high costs, the toxic effect of some synthetic compounds and their damaging impact on the environment, have together promoted the interest for the production of carotenoids from natural sources. In this context, microorganisms are acquiring more attention for the commercial production of carotenoids than plants due

Communicated by Erko Stackebrandt.

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to their accessibility regardless of season and geographical conditions, their easy cultivation and manipulation, and their high yields (Mussagy et al. 2019).

Halophilic archaea or haloarchaea are a group of extremophilic microorganisms that require high salt concentrations for optimal growth and structural stability. They are widely distributed in saline environments such as salt lakes, solar salterns and salted foods. These microorganisms show specific metabolic pathways adapted to extreme conditions, making them a potential source of unique biomolecules with prospective biotechnological applications (Oren 2016; Torregrosa-Crespo et al. 2017). Most members of haloarchaea species are capable of producing carotenoids, and exhibit orange or red-colored colonies. The main carotenoids present in halophilic archaea are C₅₀ carotenoids, particularly bacterioruberin and its derivatives monoanhydrobacterioruberin and bisanhydrobacterioruberin (Giani et al. 2019).

The use of haloarchaea for carotenoid production might be beneficial compared to other microorganisms as the high salt requirement for their growth reduces the risk of microbial contamination, even under non-sterile conditions, reducing the energy costs. Moreover, extraction process may be simpler and faster since the exposure of the cells to low salt concentrations induces membrane lysis, and consequently avoids cost investments in terms of energy required to enable efficient cell breaking. Also, C₅₀ carotenoids biosynthesis can be easily enhanced by modifying several cultivation aspects (Giani et al. 2019; Rodrigo-Baños et al. 2021).

Despite these advantages, little is known about haloarchaeal carotenoids and few reports during the past half-century focused on these carotenoids and their biological effects have been published (Rodrigo-Baños et al. 2021).

In the present work, we report the carotenoid composition of three haloarchaea strains: *Haloferax* sp. ME16, *Halogeometricum* sp. ME3, *Haloarcula* sp. BT9, and evaluate the in vitro antioxidant and antibacterial potential of the extracted carotenoids.

Materials and methods

Microorganisms

The strains used in this study were isolated from brine samples collected at two hypersaline lakes in Algeria. Strains ME3 and ME16 were isolated from chott Melghir located in Biskra (34°11'N, 6°21'E), while strain BT9 was isolated from sebkha Bethioua located in Oran (35°42'N, 00°18'W). Modified growth medium (MGM) was used for the isolation procedure (composition per liter): 5 g peptone, 1 g yeast extract, 20 g agar, with a final total salt concentration of 23% (w/v). The stock of total salt at 30% w/v was prepared as

follows: 240 g NaCl, 30 g MgCl₂·6H₂O, 34 g MgSO₄·7H₂O, 0.5 g CaCl₂·2H₂O, and 7 g KCl; pH was adjusted to 7.5.

The isolates were identified using phenotypic tests and 16S ribosomal RNA gene sequencing as previously described elsewhere (Sahli et al. 2020). Phylogenetic analysis indicated that the three isolates corresponded to the class *Halobacteria*. Strain ME3 was affiliated with *Halogeometricum borinquense* DSM 11,551 (98.9% 16S rRNA gene sequence identity), strain M16 was closest to *Haloferax volcanii* DS2 (99.6%), while strain BT9 was most similar to *Haloarcula marismortui* ATCC 43,049 (98.3%). Sequences were submitted to NCBI database under the accession numbers MN241530, MN134045 and MN134049, respectively.

Cultivation conditions

For carotenoids production, haloarchaea strains were first cultured in 50 mL of MGM (optimum salinity and pH) at 180 rpm for seven days at optimum temperature for each strain. The precultures were then transferred into sterile 2000 mL Erlenmeyer flasks containing 500 mL of the same growth medium. Finally, the flasks were incubated in a rotary shaker at 180 rpm for 10 days at optimum temperature.

Extraction and quantification of carotenoids

The carotenoids were extracted under dim light using an adaptation of the method developed by Naziri et al (2014). Culture samples were centrifuged at 22 000×g for 30 min at 4 °C. The supernatants were separated and the harvested cells were subjected to five freezing-thaw cycles, by successive 1 min immersions in liquid nitrogen/hot water (60 °C). The pelleted cells were then resuspended in 10 mL of methanol and centrifuged at 4 °C, 10,000×g for 10 min to obtain the supernatant. Successive extractions were carried out until all visible carotenoids were dissolved in methanol. The supernatants were finally combined and evaporated to dryness in a rotary evaporator. The carotenoids were dissolved in methanol and stored at –20 °C in the dark.

Extracted carotenoids were scanned in the wavelength region of 300–700 nm using a UV–Vis spectrophotometer to determine the maximum absorption wavelength λ_{max}. The content of total carotenoids in each extract was quantified using the value obtained at λ_{max} and an absorption coefficient of 2660, according to the following expression: μg/mL = (OD_{λ_{max}}/2660) × 10⁴.

Analysis of carotenoids by HPLC–DAD and LC–MS

Aliquots of the extracted carotenoids were diluted in 1 mL of methanol containing 0.1% butylhydroxytoluene (BHT), centrifuged for 1 min at 10,000×g and then filtered through cellulose acetate filters (0.45 μm).

HPLC–DAD (high-performance liquid chromatography–diode array detector) analyses were performed on a liquid chromatograph Merck-Hitachi LaChrom Elite equipped with diode array detector (L-2455) and gradient pump (L-7100). 100 µL of each sample was injected into an RP-18 column. Separation of carotenoids was carried out at a flow rate of 1 mL/min using the following linear gradient: 0–16 min, 0–60% A; 16–30 min, 60% A; 30–35 min, 100% B. The mobile phase was composed of solvent A (ethyl acetate) and solvent B (acetonitrile/water [9:1 v/v]). The chromatograms were recorded at 450 nm.

With the purpose of elucidating the mass spectra of the different compounds, an Agilent 1100 Series LC/MS (liquid chromatography-mass spectrometry) Trap SL system was used, equipped with an electrospray ionization source operating in positive scan mode (m/z range of 300–900). Working conditions were nebulizer pressure 50 psi, drying gas flow 10 L/min, gas temperature 300 °C and capillary voltage 4,000 V psi.

Attempted identification of carotenoids was performed based on the UV–visible spectra features and characteristics of the mass spectra (protonated molecule $[M + H]^+$).

Antioxidant activity

The antioxidant capacity of carotenoids from the studied strains was evaluated by ABTS ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays.

The DPPH radical scavenging assay was performed according to the method of Hou and Cui (2018) with some modifications. Carotenoid extracts were prepared at different concentrations (40–320 µg/mL). A volume of 25 µL of each concentration was mixed with 1 mL of 100 µM DPPH (prepared with methanol). A negative control was prepared with 25 µL of absolute methanol in 1 mL of 100 µM DPPH. The mixtures were shaken vigorously and incubated at 37 °C in the dark for 5 min. After incubation, the absorbance was measured at 517 nm against methanol as a blank. Analyses were carried out in triplicate for standard and each extract. The percentage of DPPH scavenging was calculated as follows: $(\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$.

The ABTS assay was based on the method of Thaipong et al (2006) slightly modified. The $ABTS^{\cdot+}$ radical was generated by a reaction between ABTS (7 mmol L⁻¹) and potassium persulfate (2.45 mmol L⁻¹) in an aqueous solution. The two solutions were mixed and maintained in the dark at room temperature for 16 h. The solution was then diluted with methanol to obtain an absorbance of 0.7 (± 0.02) at 734 nm. 25 µL of each concentration was added to 1 mL of the $ABTS^{\cdot+}$ solution previously prepared and incubated for 5 min at 37 °C. The control was prepared by adding 25 µL of methanol rather than the sample. Analyses were performed

in triplicate. The absorbance was measured at 734 nm and the scavenging activity was calculated as follows: $(\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$.

IC50 values (concentrations providing 50% inhibition) of DPPH and ABTS scavenging activities were calculated from plotted graph of the inhibition percentage against carotenoid extracts concentration using GraphPad Prism 8.4.3 software.

Antibacterial activity

Bacterial strains

The antibacterial activity of the extracted carotenoids was tested against eight bacterial strains pathogenic to humans and to fish. Human pathogenic bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 43300 were generously supplied by the Microbiology Laboratory of the Public Hospital Establishment Hafid Boudjemaa, Constantine, Algeria. Fish pathogenic bacteria included: *Pseudomonas anguilliseptica* CECT 899, *Aeromonas salmonicida* CECT 894, *Vibrio anguillarum* CECT 522 and *Photobacterium damsela* CECT 626. They were kindly provided by the Andalusian Institute of Agriculture Research, Seville, Spain.

The tested pathogenic strains were cultured on the appropriate growth media 24 h prior to the trial. Mueller Hinton medium was used for human pathogenic bacteria, whereas fish pathogens were cultured in Tryptic Soy Agar.

Disk diffusion assay

Carotenoid extracts were dissolved in methanol to a concentration of 100 µg/mL. The antibacterial test was carried out by disc diffusion method (Suresh et al. 2015). For this purpose, bacterial suspensions of the tested pathogens were prepared, adjusted to a density of 1.0×10^8 UFC/mL and inoculated via swab streaking onto agar plates. Then, sterile 6 mm Whatman filter paper discs, containing 10 µL of each extract were placed on the agar surfaces. Gentamicin and dicentracin (fish antimicrobial peptide) were used as the positive control. Disk impregnated with methanol was used as a negative control.

The inoculated plates were incubated for 24 h at 37 °C for the human pathogenic bacteria and at 25 °C for the fish pathogenic bacteria. The antibacterial activity was evaluated by measuring the size of the inhibition zone around the discs. Experiments were carried out in triplicate.

Statistical analysis

The results are expressed as means \pm standard deviation. The data were submitted to one-way ANOVA (analysis of

variance) and the differences between means were evaluated by a Tukey's post hoc test with a significance level $p < 0.05$. Statistical analysis was performed using XLSTAT 2009.1.01 software.

Results and discussion

Characterization of carotenoids produced by the studied strains

Total carotenoids accumulated by strains: *Haloarcula* sp. BT9, *Haloferax* sp. ME16 and *Halogeometricum* sp. ME3 were extracted and attempted to be identified using HPLC/DAD and LC/MS analyses. Retention time, UV–vis wavelength maxima and the mass data obtained for the different isolated carotenoids are shown in Table 1.

Carotenoid profile of *Halogeometricum* sp. ME3

The chromatogram (Fig. 1) shows the presence of 7 major compounds. The UV–vis and mass spectra revealed that peak 1 had a $[M+H]^+$ ion at m/z 705 and absorption maxima at 464, 490, 523 nm. Peaks 2, 3, 4, 5 and 7 exhibited a $[M+H]^+$ ion at m/z 740– m/z 741 and absorption maxima at around 466, 495, 528 nm, while peak 6 showed a $[M+H]^+$ ion at m/z 738 and absorption maxima at 466, 495, 528 nm (Table 1). Comparing these results with those reported in literature (Britton 1995) shows that peak 1 corresponds to bisanhydrobacterioruberin, while peaks 2, 3, 4, 5 and 7 could be identified as bacterioruberin isomers and peak 6 was attributed to haloxanthin. Similarly, Hou and Cui (2018) have reported that the predominant carotenoids synthesized by *Halogeometricum* sp. RO1–4 and *Halogeometricum* sp. RO1–6 consisted of bacterioruberin and bisanhydrobacterioruberin.

Carotenoid profile of *Haloferax* sp. ME16

Three compounds were detected in the carotenoid extracts of this strain as shown in Fig. 1. Data obtained through HPLC–DAD and LC/MS analyses (Table 1) revealed that peaks 1 and 2 had the same molecular weight and were attempted to be identified as bacterioruberin isomers with $[M+H]^+$ at m/z 741 and absorption maxima at 465, 494, 522 nm and 468, 497, 529 nm, respectively. Peak 3 showing a $[M+H]^+$ ion at 722 and absorption maxima at 466, 494, 526 nm was attributed to monoanhydrobacterioruberin. These two carotenoids were also observed in strains *Haloferax volcanii* DS2 and *Haloferax volcanii* CGMCC 1.2150 (Hou and Cui 2018; Ronnekleiv 1995). In contrast, Fang et al (2010) and Montero-Lobato et al (2018) have reported the presence of bacterioruberin, monoanhydrobacterioruberin,

bisanhydrobacterioruberin and isopentenyldehydrorhodopin in *Haloferax mediterranei* ATCC 33500^T.

Carotenoid profile of *Haloarcula* sp. BT9

HPLC–DAD analysis revealed 8 peaks (Fig. 1) with an almost identical absorption spectra, characterized by three-fingered peaks located between 450 and 530 nm and two cis absorption maxima at lower wavelengths. These characteristics correspond to bacterioruberin and its derivatives (Britton 1995). Mass spectrometry analysis (Table 1) revealed that peaks 1, 2, 3, 4 had a $[M+H]^+$ ion between m/z 740 and 741, consistent with bacterioruberin isomers. Peaks 5, 6, 7 showed, respectively, a $[M+H]^+$ ion at m/z 682, m/z 701, m/z 720, corresponding to trisanhydrobacterioruberin, 3',4'-tetrahydrobisanhydrobacterioruberin and 3',4'-dihydromonoanhydrobacterioruberin, respectively. No assignment has been done to peak 8, although its absorption spectrum corresponds to the typical UV–vis profile of bacterioruberins. Its molecular weight does not correspond to any of the most usual bacterioruberin derivatives. It was reported that the major carotenoids synthesized by *Haloarcula japonica* JCM 7785^T were bacterioruberin and its derivatives: monoanhydrobacterioruberin, bisanhydrobacterioruberin and isopentenyldehydrorhodopin (Yatsunami et al. 2014; Yang et al. 2015).

Our results indicate a variation in the carotenoid composition depending on the strain. However, bacterioruberin was the predominant carotenoid accumulated by the three haloarchaea strains. Such dominance has also been reported in several previous studies (De La Vega et al. 2016; Flores et al. 2020; Hou and Cui 2018; Lizama et al. 2021; Montero-Lobato et al. 2018; Squillaci et al. 2017; Yatsunami et al. 2014). In fact, bacterioruberin is the main C₅₀ carotenoid component responsible for the color of the red archaea of the class *Halobacteria* (Giani et al. 2019). This carotenoid is located in the cell membrane and has a rather different molecular structure, it has a primary conjugated isoprenoid chain length of 13 C=C units with no subsidiary conjugation arising from terminal groups, which contain four –OH group functionalities (Torregrosa-Crespo et al. 2017).

Bacterioruberin presents important biological roles in halophilic archaea. It acts as cellular membrane reinforcement since it increases membrane rigidity and decreases water permeability. It also protects haloarchaeal cells from DNA damage resulting from radiography, ultraviolet radiation, and hydrogen peroxide exposure (Rodrigo-Baños et al. 2021).

Antioxidant activity

Assays using DPPH and ABTS were carried out to evaluate the antioxidant capacity of the carotenoid extracted from

Table 1 Characteristics of carotenoids produced by studied strains determined using HPLC–DAD and LC–MS techniques

Strains	Peak	Tentative identification	RT (min)	UV–Vis absorption maxima (nm)	Molecular ion (m/z) M+	Fragments profile
<i>Halo geometricum</i> sp. ME3	1	BABR	12.41	464, 490, 523	705.5	673.5, 619.4, 579.1, 540.3, 487.4, 457.6, 439.1, 394.5, 315.2
	2	BR	12.80	468, 497, 530	741.7	723.4, 683.7, 664.5, 646.0, 537.9, 484.2, 459.0, 425.2, 386.5, 346.1, 314.2
	3	BR	14.44	468, 496, 530	741.3	648.5, 593.5, 561.8, 501.4, 450.8, 406.6
	4	BR	15.84	466, 495, 528	741.2	689.4, 656.4, 632.4, 606.4, 577.4, 560.0, 523.9, 470.3, 429.1, 405.9, 384.1, 353.9
	5	BR	16.17	465, 492, 524	740.6	705.8, 688.3, 642.7, 614.4, 568.2, 527.4, 468.0, 432.1, 379.9, 313.9
	6	Haloxanthine	16.47	456, 482, 514	738.6	706.3, 687.2, 667.1, 604.4, 574.2, 526.4, 509.5, 477.3, 451.2, 411.2, 382.2, 347.6
	7	BR	16.81	466, 494, 526	740.2	723.4, 701.4, 671.1, 604.4, 570.5, 508.5, 492.4, 439.2, 421.2, 383.6
<i>Haloarcula</i> sp. BT9	1	BR	12,13	462, 490, 522	741,5	723.9, 664.5, 647.6, 606.8, 566.2, 536.8, 511.7, 475.4, 407.9, 371.4, 341.9, 312.9
	2	BR	12,61	468, 497, 530	740,5	688.4, 664.0, 646.5, 601.4, 575.3, 517.8, 441.6, 389.1, 363.3, 317.6
	3	BR	14,20	468, 497, 530	741,5	723.1, 665.4, 624.3, 577.3, 545.1, 507.5, 452.1, 428.0, 371.3, 353.3, 327.1
	4	BR	15,62	468, 497, 530	741,5	705.1, 683.3, 625.4, 606.5, 564.2, 531.6, 508.9, 458.2, 409.2, 372.0, 339.0
	5	TABR	16,31	457, 485, 517	682,2	662.9, 645.4, 604.5, 538.6, 510.5, 468.0, 424.5, 347.3, 321.1
	6	3',4'-TH-BABR	16,66	446, 473, 503	701,6	681.9, 665.2, 624.5, 603.1, 568.7, 524.3, 444.3, 399.3, 361.1
	7	3'4'-DH-MABR	16,91	455, 483, 515	720,9	691.4, 665.2, 626.9, 591.3, 550.5, 503.0, 469.8, 419.0, 358.1
	8	ND	17,26	450, 480, 510	759,3	740.2, 701.6, 654.9, 629.4, 602.6, 577.5, 550.5, 509.6, 454.4, 414.7, 390.5, 328.2
<i>Haloferax</i> sp. ME16	1	BR	12.02	464, 495, 522	741.5	723.7, 683.6, 606.3, 583.6, 559.8, 543.2, 502.3, 465.6, 420.3, 395.3, 353.3, 315.5
	2	BR	12.48	468, 497, 529	741.8	723.4, 682.4, 665.5, 624.4, 569.5, 510.9, 462.1, 410.4, 339.1
	3	MABR	14.18	465, 494, 526	722.7	643.3, 610.8, 571.6, 525.5, 482.2, 452.5, 406.2, 379.5, 361.3, 344.1

RT retention time; *BABR* bisanhydrobacterioruberin; *BR* bacterioruberin; *MABR* monoanhydrobacterioruberin; *TABR* trisanhydrobacterioruberin; *3',4'-TH-BABR* 3',4'-tetrahydrobisanhydrobacterioruberin; *3'4'-DH-MABR* 3',4'-dihydromonoanhydrobacterioruberine, *ND* not identified

studied strains. A positive response was obtained with both reagents, attesting the capacity of these carotenoids to act as antioxidant agents through the transfer of electrons to radical species. The scavenging ability was found to be dose-dependent, undergoing a significant increase with the increase of carotenoid concentration (Fig. 2). These results

are in agreement with previous reports using carotenoid extracts of halophilic archaea from various origins (Flores et al. 2020; Hegazy et al. 2020; Hou and Cui 2018; Lizama et al. 2021; Mandelli et al. 2012; Squillaci et al. 2017; Yatsunami et al. 2014).

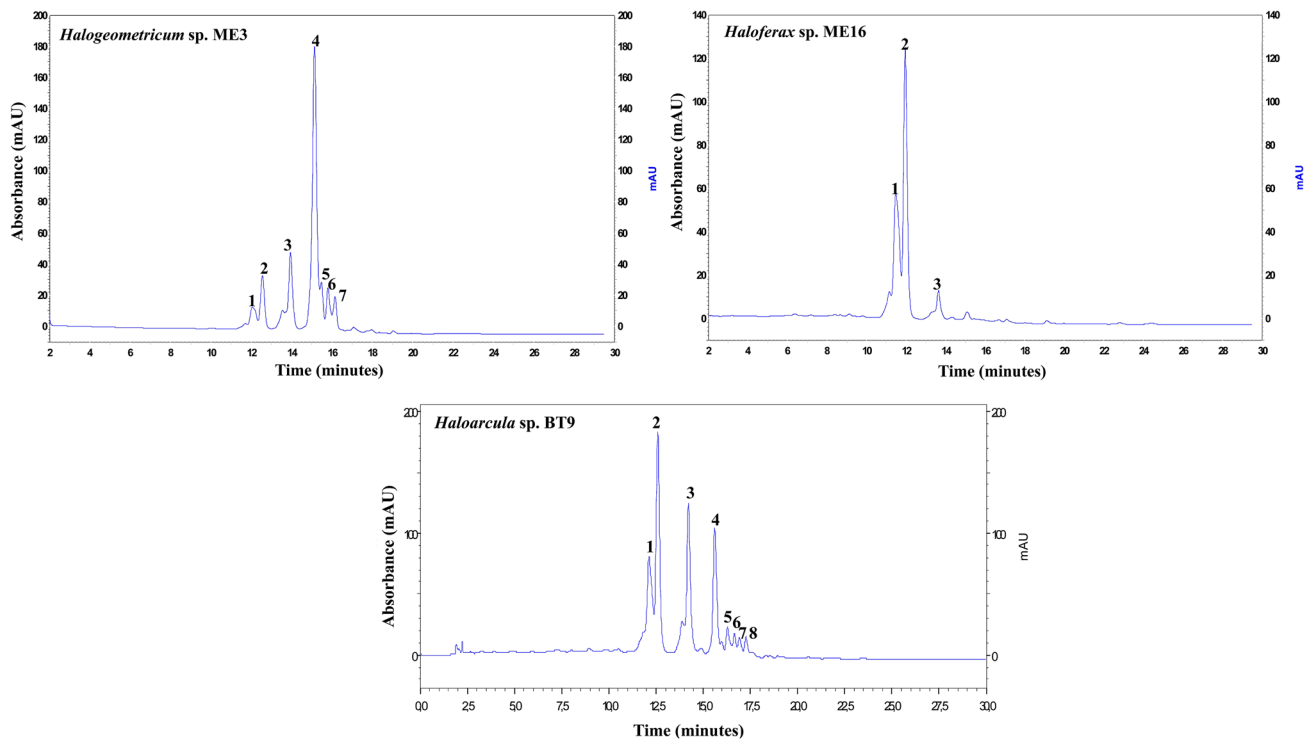


Fig. 1 HPLC elution profiles of the carotenoids obtained from studied strains

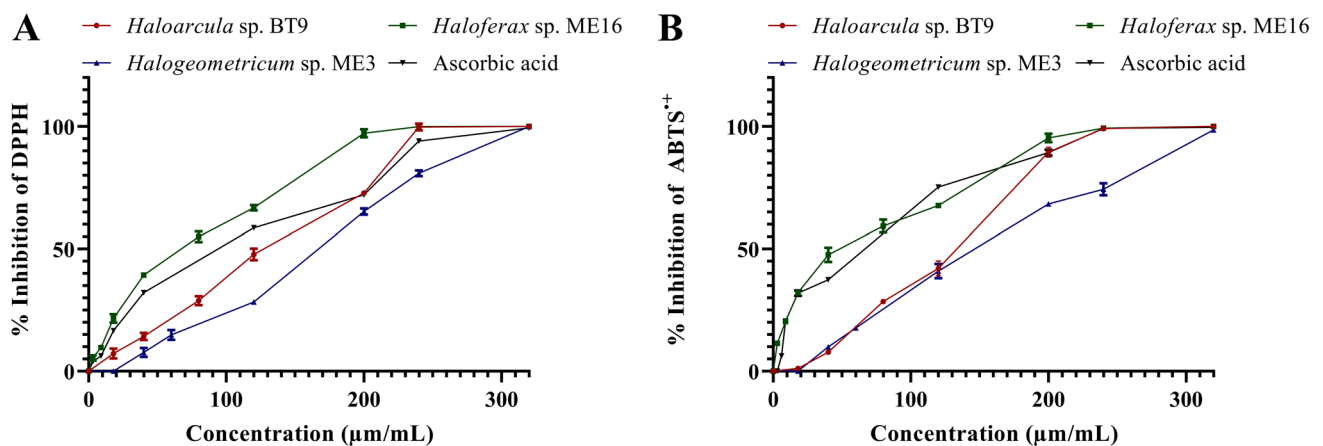


Fig. 2 Radical scavenging activity percentage of carotenoids from the studied strains against (A): DPPH and (B): ABTS + . Data are represented as means of three replicates

The IC₅₀ values given in Table 2 showed the existence of significant differences among strains ($p < 0.0001$). These differences could be explained by the difference in the qualitative composition of the extracts. The reaction between a free radical and an antioxidant compound depends on the structural conformation of the latter (Santos-Sánchez et al. 2019). The carotenoid extracts from *Haloferax* sp. ME16 exhibited the highest activity in both assays (DPPH: IC₅₀ = 56.69 µg/mL, ABTS:

IC₅₀ = 39.66 µg/mL), which was significantly higher than that of ascorbic acid used as a standard.

The obtained IC₅₀ values show that the antioxidant potential of the carotenoids extracts from the three haloarchaea strains studied is lower than that reported for other halophilic archaea. For example, IC₅₀ values for carotenoid extracts from *Haladaptatus litoreus*, *Halopelagius inordinatus*, *Halogramum rubrum*, *Haloferax volcanii*, *Haloterrigena turkmenica*, *Haloterrigena* sp. SGH1, *Haloarcula hispanica*

Table 2 IC₅₀ values of DPPH and ABTS scavenging activities of carotenoid extracts

Carotenoid extracts	IC ₅₀ (µg/mL)	
	DPPH	ABTS
<i>Haloferax</i> sp. ME16	56.69 ± 1.97 ^d	39.66 ± 1.39 ^c
<i>Halogeometricum</i> sp. ME3	170.4 ± 0.52 ^a	136.43 ± 5.08 ^a
<i>Haloarcula</i> sp. BT9	119.43 ± 0.97 ^b	120.1 ± 3.15 ^b
Ascorbic acid	69.95 ± 0.21 ^c	41.43 ± 0.83 ^c

Different letters indicate significant differences at $p < 0.0001$ according to one-way ANOVA followed by Tukey's post hoc test

HM1, *Halorubrum tebenquichense* Te Se-86 and *Haloarcula* sp. TeSe-41 varied from 1 to 10 µg/mL (Flores et al. 2020; Gómez-Villegas et al. 2020; Lizama et al. 2021; Mandelli et al. 2012; Squillaci et al. 2017). However, it is considerably greater than that of pigments produced by other microorganisms. Hu et al (2008) reported that the antioxidant capacity of carotenoids accumulated by the halophilic microalgae *Dunaliella salina* is relatively low compared to α-tocopherol with an IC₅₀ value of 8360 µg/mL. Also, Rostami et al (2016) found that carotenoids produced by the yeast *Rhodotorula glutinis* have an IC₅₀ of 555.5 µg/mL. Other researchers have reported that the IC₅₀ values recorded for the pigment extracts from the fungi *Fusarium* sp. FC1-3, *Exidia nigricans* and the bacteria *Sphingomonas paucimobilis*, *Microbacterium arborescens*, *Micrococcus roseus* were in the order of 1620, 1000, 3100, 4800 and 572 µg/mL, respectively (Jayaraman et al. 2020; Łopusiewicz 2018; Mani et al. 2015; Rostami et al. 2016).

This remarkable antioxidant capacity of haloarchaeal carotenoids may be related to their structure. The presence of a high number of hydroxyl groups positively affects the mechanism of free radical scavenging by acting as a donor of hydrogen atoms. Also, it has been suggested that the high number of conjugated double bonds in these carotenoids is

proportional to greater antioxidant capacity (Mandelli et al. 2012; Yang et al. 2015). For example, bacterioruberin contains 13 conjugated double bonds and four hydroxyl groups versus the 9 conjugated double bonds and no oxygen atoms in β-carotene (Hou and Cui 2018).

As a consequence of this extraordinary biological function, carotenoids from halophilic archaea are of great interest and could be used in food, cosmetics, and pharmaceutical industries as color additives or functional ingredients.

Antibacterial activity

Antibacterial activity of carotenoid extracts was qualitatively assayed by the agar disk diffusion method against a collection of typical fish and human pathogenic bacteria (Fig. 3). To accurately assess the antibacterial potential of our samples, the diameter taken into account was calculated by the difference between the diameter of the inhibition zones of the extracts and that of the negative control (methanol). The obtained results revealed that the carotenoid extracts from the three haloarchaea strains inhibited the growth of all the tested bacteria, excepting *Aeromonas salmonicida*, with inhibition halos up to 13 mm (Fig. 4). Zones of inhibition varied according to the targeted pathogen and the carotenoid extracts. The antibacterial activity of the carotenoid fraction extracted from *Halogeometricum* sp. ME3 against fish pathogenic bacteria was significantly higher ($p < 0.05$) than that of the other extracts. However, no significant difference was observed in the activity of the analyzed extracts against human pathogenic bacteria.

The highest inhibition zones ranging from 9 to 13 mm were obtained against *E. coli*, *K. pneumonia*, *P. aeruginosa* and *S. aureus*. However, smaller zones of inhibition were observed against *P. anguilliseptica*, *V. anguillarum* and *P. damsela*. The presence of resistance plasmids in the latter could explain this result. Indeed, these plasmids have been widely identified in a large number of fish pathogenic

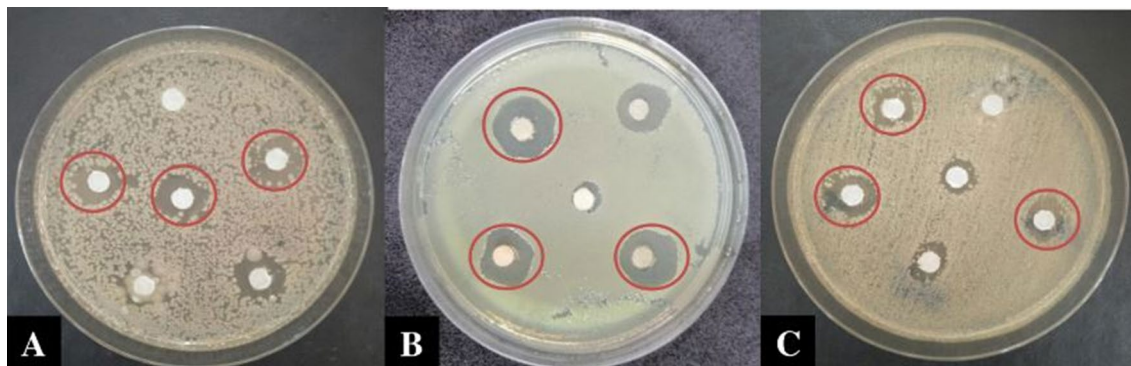


Fig. 3 Examples of antibacterial activity of carotenoid extracts. **A:** extracts of ME3 against *Vibrio anguillarum*; **(B):** extracts of BT9 against *Pseudomonas aeruginosa*; **(C):** extracts of ME16 against *Pseudomonas anguilliseptica*. The red circles delimit the zones of inhibition

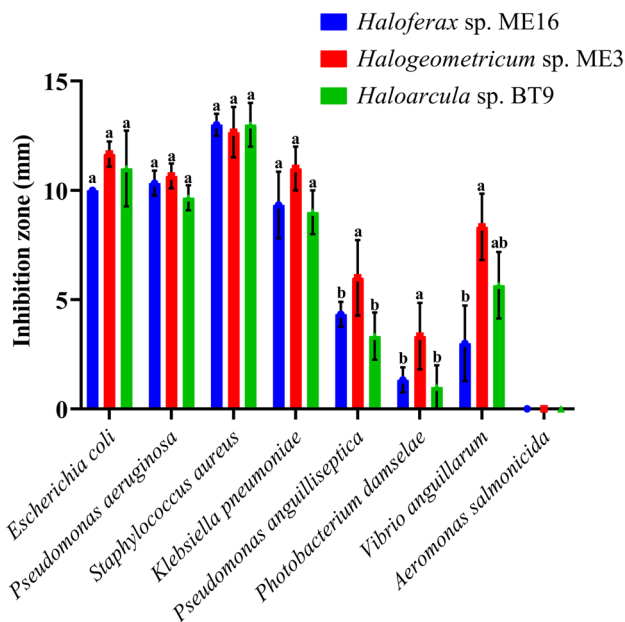


Fig. 4 Diameter of inhibition zone (mm) of carotenoid extracts from the studied strains. Columns with different superscripts differ significantly ($p < 0.05$). Data are represented as means of three replicates

species and are thought to play a significant role in the transmission of antimicrobial resistance determinants in the aquatic environment (Miller and Harbottle 2018).

It was observed that the Gram-positive bacterium *S. aureus* was more sensitive to carotenoid extracts than the other Gram-negative bacteria. This could be attributed to the differences in the cell wall structure and composition of these two groups. Lipopolysaccharide, the major component of the outer membrane of Gram-negative bacteria forms a layer that provides an effective permeability barrier against deleterious molecules such as antibiotics and antimicrobial agents (Maldonado et al. 2016).

According to the literature, the possible mechanisms of antibacterial activity of carotenoids include three basic ways: outer membrane permeability, cytoplasm leakage, and inhibition of nucleic acid formation (Karpinski and Adamczak 2019).

To sum up, our results reveal that carotenoids produced by studied haloarchaea strains exhibited a good antibacterial activity, in particular against human pathogenic bacteria, suggesting thus potential biomedical applications. Similar results were observed with pigment extracts from halotolerant and halophilic bacteria (Fariq et al. 2019; Ravikumar et al. 2016; Suresh et al. 2015). Nevertheless, except for our previous study reported recently (Sahli et al. 2020), to the best of our knowledge, there is no report on the antibacterial capacity of carotenoids produced by extremely halophilic archaea. Likewise, this study shows for the first time results of the use of carotenoids derived from *Haloferax* sp.,

Halogetometricum sp. and *Haloarcula* sp. as antibacterial compounds.

Conclusion

The present work focuses on carotenoids produced by haloarchaea strains isolated from Algerian salt lakes. Significant antioxidant and antibacterial activities were noted for these molecules, offering thus a vast potential to several applications. Further work should be carried out to encapsulate haloarchaeal carotenoids and study their stability to incorporate them in pharmaceutical, food or cosmetic products.

Funding This work was supported by the Algerian Ministry of Higher Education and Scientific Research, FEDER Programa-Andalucia 2014–2020 (UHU-1257518) and the group (VIGROB-016) Universidad de Alicante.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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