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Application of bacterioruberin from *Arthrobacter* sp. isolated from Xinjiang desert to extend the shelf-life of fruits during postharvest storage

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ABSTRACT

Post-harvest losses and rapid fruit ripening at room temperature are major challenges in preserving fruit quality. This study aimed to reduce such losses by applying a red carotenoid pigment, bacterioruberin extracted from an *Arthrobacter* sp. The carotenoid was characterized as bacterioruberin and its derivative tetra anhydrous bacterioruberin (λmax 490 nm), and an *m/z* value of 675 and 742 $(M+ 1H)^{+1}$. The annotated LIPID MAP demonstrated the presence of over 360 isoprenoids annotated transcripts. The compound exhibited significant antioxidant activity, with an IC_{50} of 22 μg/mL, iron chelation and antibacterial activities indicating its potential as a natural preservative. When applied to grapes, peaches, and apricots, bacterioruberin (2 %) effectively prevented spoilage for six days at room temperature. Statistical analysis using one-way ANOVA revealed a significant correlation *($p = 0.05$)* between treated and control groups in subjective quality attributes. Computational investigation with phospholipase D and VQ22 motif protein further supported the preservative potential of bacterioruberin.

1. Introduction

China is familiar as the as cradle of orchard due to its consideration as centers of origin for fruits (Yan et al., 2016). An estimate showing over more than 300 fruit species throughout the country for economic and commercial purposes. Government of China is investing a lot and paid a great at tension for indigenous fruit production that has recorded a rapid development and contributed to its economy. Xinjiang province is famous for fruits production and one of the advantageous sectors (Ming & Yun-wei, 1986). In 2023, Xinjiang exported over 200,000 tons of fruits which is estimated as 150 % increase compared to 2022. The export value is estimated as $ 0.28 billion represent almost 80 % increase from the previous year. Like other fruits apricot, grapes and peach undergo a prompt and rapid ripening at room temperature due to ethylene production and accelerated respiration. The fruits become vulnerable to mechanical damage, rot, spoilage hindering the long-distance shipping. Although low temperature storage is considered to be effective, the chilling injury in short term storages the freeze thaw cycle compromising the quality and nutritional value of fruits (Chaves & Zaritzky, 2018). The major chilling injury symptoms include peel browning and pulp softening. Moreover, maintaining the cold chain cycle for local market shipping is not cost effective due to electric consumption and other utility charges. The freeze thaw cycle also results in accumulation of the reactive oxygen species, toxic aldehyde and ketones and ultimately senescence. Softening is a complex process which is always associated with fruit cell wall components degradation, a decline in covalently bound and insoluble pectin, and an increase in hydrophilic pectin (Zerpa-Catanho et al., 2017).

The wide use of antibiotics and other chemical preservatives in the food and feed industry has contributed to the emergence of drug-

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resistant super-bugs. Moreover, food spoilage issues and use chemical preservatives are considered unsafe to eukaryotic cells. These issues have prompted the researchers to discover and identify new antimicrobials and bio-preservatives. In this contrast, the natural compounds have attracted the researchers, food and pharma sector as they are considered as value added compounds and safe to the eukaryotic cells (Asfour, 2018; Hakimi Alni et al., 2020).

In an era, where the consumers demand for more safe, nutritious, chemical additive free, ready to eat and long shelf life food, the market is evolving quickly due to emerging technologies (Gokoglu, 2019). Nowadays carotenoids are promising alternative to the petroleum-based food additives due to its diverse color, antioxidant, anti-cancerous and antibacterial potentials. Carotenoids are a group of compounds that give colors to microbes, plants and animals. In desert microbes' carotenoids are present to protect the cells from high UV radiation, protein oxidation and other cellular damages in extreme environments. Due to its high biotechnological applications in cosmetic, food and pharmaceutical industries microbial carotenoids have fascinated the courtesy of many researchers. Microbial carotenoids are preferred over other sources because of its seasonal independency, standardization, commercial viability, not-toxicity, dual solubility nature and provitamin A activity. The consumption of carotenoid as food supplement is also highly associated with prevention several non-infectious diseases due to its high antioxidant and anti-inflammatory activities (Griffiths et al., 2016). The global market for carotenoid is highly driven by consumer's preferences for more safe and natural compounds and is expected to reach $ 2.45 billion by 2034.

Among carotenoids the naturally occurring bacterioruberin (BR), C50 is found in microbe's in particular inhabiting in extreme environments. This compound possesses extraordinary antioxidant capabilities and maintain the microbial membrane fluidity during the stress and with a large potential application in food and feed industry. In the future carotenoid market, the BR can be a key player as an antioxidant and food preservative. BR is dominantly found in halobacterium or archaea inhabiting in the extreme environment. This bright pigment is formed from the isoprenoid with conjugated double carbon units along with four OH groups thereby making this slightly soluble in water (Jain & Sirisha, 2020). This compound along with other carotenoids have been reported to play an adaptive role during stress in harsh conditions like salinity, drought, desiccation and high UV radiation. The glycosylated and other derivatives of BR which are specifically produced by *Arthrobacter* sp. maintain the membrane fluidity in psychrophilic conditions, reducing the freeze thaw effect. The BR also increases the membrane rigidity to resist the harsh conditions and prolong its survival (Chen et al., 2023). Glycosylation increases bioavailability, photo-stability, and biological activities thereby making it a suitable candidate for food preservation and to inhibit food spoilage (Cyboran-Mikołajczyk et al., 2018). The quality of BR produced by *Arthrobacter* sp. makes it a considerable source as compared to the carotenoids produced by haloarchaea group.

In this context, our current research focuses on the isolation of an *Arthrobacter* sp. from Xinjiang province and extraction of the C50 carotenoid and its derivatives. The carotenoid was evaluated for its antioxidant and iron chelating activities followed by antimicrobial activities against food borne pathogens. The bacterioruberin was also investigated for its applications on the fresh fruit surface to evaluate its potential in extending the fresh fruit shelf life. Furthermore, the bioinformatic analysis was carried out to study the binding affinity and interaction of BR with phospholipase D and VQ motif containing protein (JVA1/VQ22) which involve in responses to biotic and abiotic stresses.

2. Material and methods

2.1. Reagents, chemicals and equipment's

All the Microbiological media were purchased from Solaribio life sciences China. The chemicals like Ferrozine and DPPH were purchased from AOBOX Biotechnologies China. The HPLC used was from Waterse2695 separation module, for LC/MS and Lipidomic we used Water Acquity I Class PLUS ultra-high performance liquid tandem Waters Xevo G2-XS QT (Beijing Biomarker Technologies China) in positive mode. The ultrahigh centrifuge used was Beckman Coulter Avanti J-26S XP at XIEG, IKA RV10 Rotary Evaporator with HB10 Bath, Costar ref. 3599 96 well culture plates.

2.2. Sampling

The soils samples were collected from the Rhizosphere of Apocynum Pictum from Karamay Region (Latitude and longitude coordinates are: 43.825592, 87.616852) Urumqi at the Xinjiang Provence. The samples were collected in a sterile sample bottles and transported to Xinjiang Institute of Ecology and Geography Urumqi China. All the samples were stored at 4 °C before processing it further for screening.

2.3. Isolation

For isolation the soil samples were serially diluted in 10 mL of 1 % saline (dilution factor 10^{-1}–10^{-5}). R2A agar medium (Reasoner's 2A agar) was prepared and marked with their respective dilution factors. An aliquot of 100 μL of the sample was transferred to respective plates and spread uniformly on the surface of the agar medium followed by incubation at 25 °C for 3 days. The plates were observed for any possible growth followed by picking only the colored colonies which were further purified by sub-culturing technique. The purified colonies were also stored at −20 °C in 15 % glycerol.

2.4. Microscopic and biochemical characterization

The selected red to pink color strain was examined microscopically using Gram stain and morphologically on the R2A agar medium. Catalase, amylase and protease tests were performed using standard protocol assays.

2.5. DNA extraction, amplification and sequencing

For DNA extraction an overnight grown culture in LB was used following the manufacturer protocol (QIA amp DNA Mini Kit no. 51306; Qiagen). The DNA was quantified through nano-drop and amplified for 16S rRNA by Polymerase Chain Reaction (PCR). The conserved bacteria specific primers 1492R′ (5′-CTACGGCTACCTTGTTACGA-3′), 27F′ (5′AGAGTTTGATCCTGGCTCAG-3′) were used (Lane, 1991). The PCR was performed according to the manufacturer's recommendations (Promega). PCR thermal condition optimized at 95 °C (denaturation) for 5 min and 35 cycles which includes for 95 °C for 30s, 55 °C (polymerization) for 30 s, 72 °C annealing for 2 min, and a final extension cycle at 72 °C for 7 min. The amplicons, forward and reverse were sequenced at Sangon Biotech (China). The computed sequence of nucleotide was evaluated using BLAST n tool accessible through NCBI. Phylogeny was constructed using MEGA X software to determine the taxonomic relationship of the isolate.

2.6. Carotenoids extraction and quantification

For the extraction of carotenoid 20 mL of the fresh inoculum was prepared in R2A broth supplemented with $MgCl_2$, and NaCl, as catalytic and stress activators. 5 % of this inoculum was transferred to 500 mL R2A broth previously sterilized in 1 L flask. Fermentation was carried out at 25 °C for 96 h. Following the standard protocols the cells were harvested through ultrahigh centrifugation at 4 °C for 10 min at 14,000 rpm using Beckman Coulter Avanti J-26S XP series centrifuges. The cell pallets (6 g by dry weight) were incubated at −80 °C for 30 min followed by rapid transfer to extraction mixture acetoine: methanol: water in a

ratio 7:2:1. Cells were washed in extraction mixture using sonicator probe at a frequency of 20 kHz for 5 s repeating the step three time to ensure the complete lysis of the cell. The extract was again centrifuged at 4 °C for 10 min at 14,000 rpm for removing cell debris and other suspended particles and collecting the colored supernatant.

2.7. Quantification of total carotenoids and thin layer chromatography

The extracted pigment was scanned in a wavelength region of 200-800 nm using Agilent spectrophotometer 8453. The extract was resuspended in methanol and total carotenoid concentration was determined at the obtained maximum wavelength (λ_{max} 490) through applying an average extension coefficient (Liaaen-Jensen & Jensen, 1971). For carotenoid confirmation thin layer chromatography TLC was used. The acetone extract was placed on silica gel plate (GF254) with a mobile phase in chloroform-methanol 7:3. The plate was visualized at 354 nm and spots were identified by visibility and spraying with a solution of ceramic sulfate. Ceramic sulfate solution is widely used to coat the TLC plates and visualize the mixture of compounds under UV.

2.8. Reversed-phase high-performance liquid chromatography

Reverse phase high performance liquid chromatography (RP-HPLC) on a Waters e 2690 Alliance system (Waters Corp., Milford, MA) was used to analyze the carotenoids. The sample was washed twice with ethanol to precipitate any protein. Hypersil OCS C18 (pore size 5 µm, 4.6 × 250 mm) column was used. To degas, the HPLC grade solvents were sonicated. The sample prepared in methanol was filtered through a 0.2-µm filter prior to the HPLC analysis. The column was maintained at a constant temperature of 25 °C using a standard column thermostat. A mixture containing acetonitrile, methanol and isopropanol in a ration (40,50,10) was used. The apparatus was run in isocratic conditions with a flow rate of 1 mL/min with a total run time of 10 min. (Saito et al., 2004). The fractions eluted were monitored with a Waters 996 photodiode array detector (Waters Corp.), building the chromatogram at 490 nm.

2.9. LCMS-MS and lipidomic analysis

The most widely produced carotenoids and other metabolites were detected for metabolic pathway analysis through metabolomics. The analysis platform used is the Waters Acquit I Class PLUS ultra-high-performance liquid chromatography coupled with the Waters Xevo G2- XS QTOF high-resolution mass spectrometer. Sample analysis was performed according to the corresponding parameters. The raw data collected using MassLynx V4.2 was processed using Progenesis QI software for peak extraction, peak alignment, and other data processing operations. Identification is performed using the Progenesis QI and mass softwares with the online database. The lipidomic was also conducted to analyze the different expressions and annotation analysis. A 10-µL sample dissolved in LC–MS grade methanol was injected onto a column (C18; internal diameter 5 µm, 250 × 4 mm; Bischoff, Germany) using UV/Vis photodiode array (PDA) detector and auto sampler). The mobile phase comprised methanol (solvent A) and acetonitrile (solvent B) with 0.1 % (*v*/v) formic acid, used in a gradient mode for B [0.0/30; 25/100; 35/100; 45/30 (min/%)] at a flow rate of 0.8 mL/min (Inbaraj et al., 2008). The mass spectrometer was used in positive ion mode to detect *m/z* transitions $[M + H]^{+1}$.

2.10. Bioassays

2.10.1. Antioxidant assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant potential of (Singh, 2005). Different concentration of the compound (5–20 µg/mL) was transferred to 96-well microtiter plate, followed by raising the volume uniformly up to 200 µL using previously prepared DPPH in methanol. The reaction mixture was incubated for 30 min at 37 °C in a shaking incubator. Absorbance was measured at 517 nm on the UV–visible spectrometer using methanol as the blank and ascorbic acid as a positive control. IC_{50} value (half maximal inhibitory concentration) for the tested as well as the ascorbic acid was calculated by plotting the data concentration vs inhibition. The following formula was used to calculate the free radical scavenging activity:

$$\%scavenging = control–test/control \times 100$$

2.10.2. Iron chelation assay

The ferrous ion chelation activity was determined by using ferrozine through calorimetric assay (Liang et al., 2004). 50 µL of 2 mM Ferrous chloride was mixed with different concentrations of compound (10–40 µg/µL) in a 96 well microtiter plate and the reaction was initiated by adding 200 µL of 5 mM ferrozine previously prepped in sodium acetate buffer (2 mM). The reactants were mixed properly and incubated for 40 min at room temperature. The absorbance was recorded at 562 nm by UV–Vis spectrometer while using EDTA (10–30 µg/µL) as positive control. Chelation activity of bioactive extract was calculated by formula:

$$\%chelation = control–test/control \times 100$$

2.10.3. Evaluation of antimicrobial activity

The antimicrobial efficacy of bacterioruberin was investigated using the dilution method with 96 well plates (Cetin-Karaca & Newman, 2015). The isolated BR was dissolved in 20 % DMSO. Four foodborne pathogens, *S. aureus*, *B. cereus*, *E. coli*, and *Salmonella typhi*, were prepared to 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL) in Mueller–Hinton Broth. 100 µL of the aliquots were transferred to the 96 well plates. Different concentration ranges form 10–40 µg of the BR was used as test compound against each strain. Negative control was run in parallel using DMSO, sterile media (blank) and strains without addition of the bacterioruberin. The plate was incubated at 37 °C for 48 h measuring the turbidity after each 24 h to evaluate the minimum inhibitory concentration and EC_{50} value.

2.11. Application of bacterioruberin spray on fresh fruits to extend shelf life

A 2 % suspension of bacterioruberin was prepared using a sodium citrate and sodium bicarbonate solution (10 mL). Fresh peaches, grapes, and apricots were purchased locally and washed with warm water to remove dirt. Four groups of fruits were prepared:

1. Environmental control with no treatment.
2. Fruits are treated with the sodium citrate and sodium bicarbonate solution.
3. Fruits sprayed with the 2 % bacterioruberin suspension.
4. Fruits treated with commercially available ectoine from BioSynth China.

All groups were placed in clean plastic containers covered with cloth gauze to prevent cross- contamination. The fruits were stored at room temperature for 7 days and monitored for spoilage, odor, cellular damage, dryness, and freshness.

2.12. Organoleptic properties

To broaden the scope of the study and application of the bacterioruberin, on day six the sensory attributes and consumer overall impression of the control and tested fruit-groups were evaluated. A 5-point hedonic scale evaluation test by 10 independent researchers at the Xinjiang Institute Chinese academy of sciences, was conducted. Data collected through evaluation forms for fruit spoilage, sensory attributes, freshness was processed for descriptive analysis and ANOVA.

Independent researchers at XIEG conducted organoleptic evaluations.

2.13. Docking studies with phospholipase D and VQ motif protein JVA1/VQ22

Phospholipase D and VQ motif protein play a significant role in plant response to biotic and abiotic environment. The structural details of Phospholipase-D (PDB ID:7e0m), and JAV1 motif (AlphaFold ID: AF-Q9LIE6-FI), were obtained from RCSB Protein Data Bank and AlphaFold, respectively. The crystallographic water molecules as well as Hetero Atom (HETATM) from all the selected target enzymes were removed. Using the Graphical Interface program AutoDock Tools (ADT) (Handoko et al., 2012). The pdbqt files for the targeted enzyme and protein and ligand bacterioruberin was prepared for the intermediary steps of molecular docking. For the grid box properties in the configuration file AutoDock/Vina was employed along with the ligand information. The targeted enzyme and protein selected includes phospholipase D and VQ22 motif protein with a significant role in biotic and abiotic stresses. During the procedure both the tested compound and targeted enzyme/protein were considered as rigid. 3D protonation of the targeted enzyme and protein was conducted subsequently followed by energy minimization on the structures using MOE algorithm. The results of less than 1.0 Å in a root-mean square deviation (RMSD) were clustered to represent the results with most favorable free binding energy. The binding efficacy and affinity of the bacterioruberin to the pockets of target proteins with lowest energy was aligned with target structures for further analysis.

3. Results

3.1. Isolation

A total of 5 different strains with potential to produce colors were isolated on R2A medium as shown in the table S1. The appearance of higher proportion-colored colonies on the R2A agar medium was observed. Based on the dense and prominent color PKT-29 was selected for further studies.

3.2. Morphology and biochemical characterization

The strain PKT-29 showed Gram-positive reaction in microscopy with coccoid or in form of short rods, catalase-positive. On the R2A medium agar plates, PKT-29 colonies appeared round, smooth, dry with bright red color with noticeable color darkness after storage at 4 °C.

3.3. Molecular characterization

The 16S rRNA sequence assembled and subjected to BLAST in NCBI database demonstrated 99 % similarity to *Arthrobacter agilis*. The phylogeny constructed and the strain sequence submitted to NCBI for accession number PQ313135. Strain *Arthrobacter* PKT-29 (PQ313135) clustered into class *Actinomycetia*, order *Micrococcales* and genus *Arthrobacter* group among the sequences obtained from NCBI (Fig. 1).

3.4. Extraction and thin layer chromatography

The red color carotenoid extracted in acetone:methanol:water (7,2,1) showed λ_{max} at 490 nm. The extract was dried in rotary evaporator and weighed. 22 mg of the carotenoid compound was extracted from 200 mg of the dried cell biomass. The carotenoid passed through the Solid Phase Extraction (SPE) cartridge using water, methanol and finally dichloromethane. Two distinct spots appeared on the TLC plates with blue color. The Rf values calculated were 5.2 and 8.2. Finally, 8 mg/mL of the stock was prepared in extra pure methanol for further analysis.

3.5. HPLC

The carotenoids were extracted from the cells of *Arthrobacter* sp. PKT-29 and analyzed by HPLC. The carotenoid was washed thrice with ethyl acetate and acetone to remove any other impurities or bounded protein and filtered subsequently subjected to RP-HPLC. Seven distinct peaks were observed with a retention time of 1.0, 1.8, 2.0, 2.5, 3.9, 5.8 and 6.2 mins, respectively; these were collected separately and labelled F1-F7, as shown in Fig. S2. An aliquot of 5 mg of pure compound was extracted from *Arthrobacter* sp. with a purity of >80 %.

3.6. LCMS lipid profile

The top 20 metabolites from *Arthrobacter* sp. annotated in lipid maps database are shown in the figure along with heatmap. The results demonstrated abundance of fatty acids and conjugates along with isoprenoids which include tetra terpenes with C40 and C50 as shown in Fig. 2. The number at the tail of each line represents the number of metabolites identified which represent higher production of the metabolites with isoprenoid rings. A heatmap for the relative variation of metabolites is shown where the color-coding from red to green indicates abundances from high to low, respectively. The spectra demonstrated an abundance of carotenoids which falls in *m/z* from 568 to 600. The

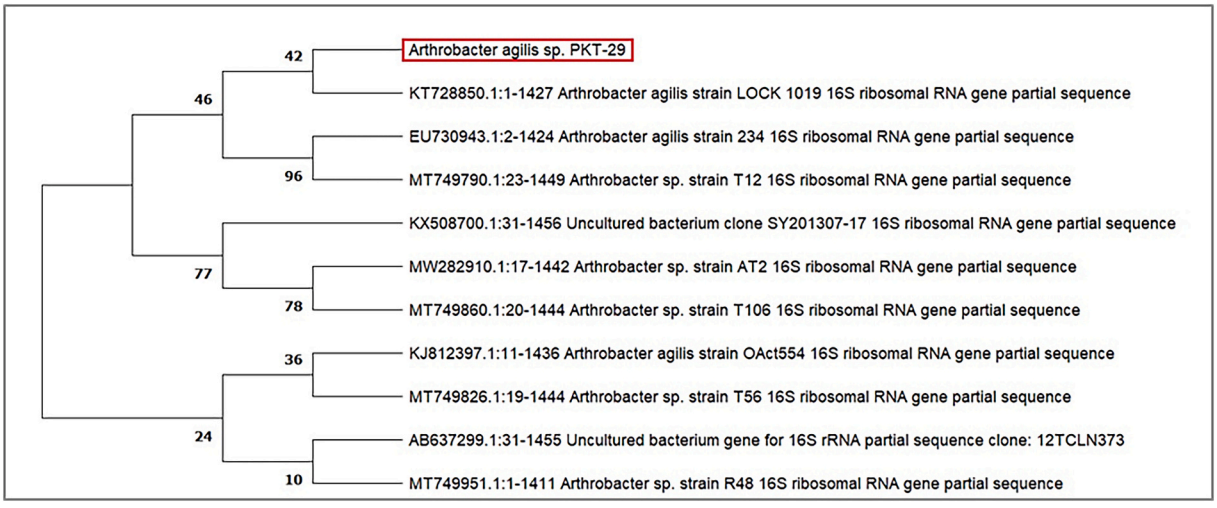

Fig. 1. Phylogenetic tree based on 16S rRNA analysis with neighbor joining, showing the position of isolate PKT-29 to *Arthrobacter* sp. The number at nodes represent the % bootstrap values based on 100 replications. The tree is constructed to a scale with branch lengths restrained in the number of switches per site.

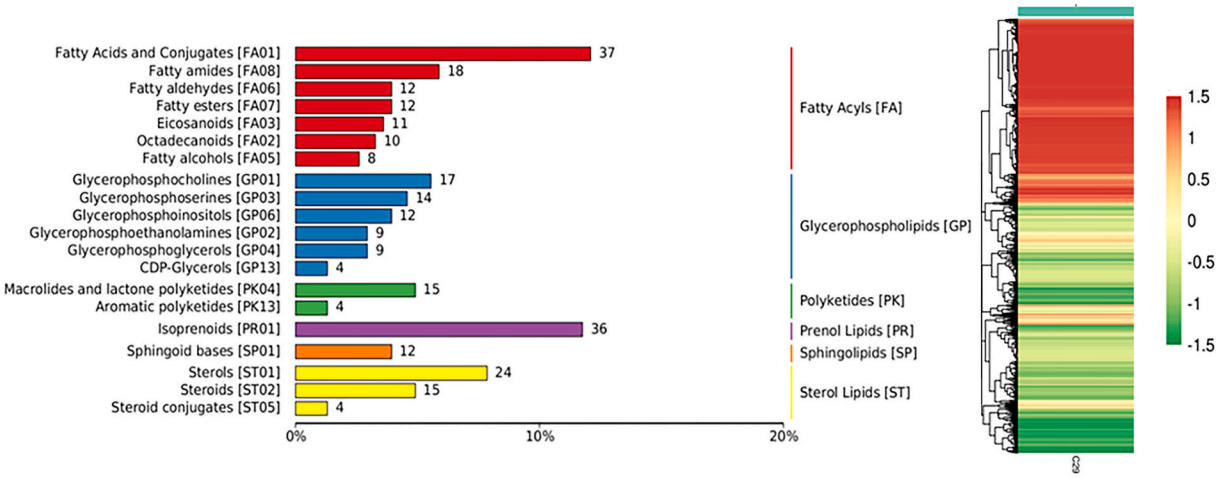

Fig. 2. The KEGG, and LIPID MAPS annotations of the entire metabolites identified from *Arthrobacter* sp. The colors in the figures represent different pathways or metabolites.

presence of two distinct peaks with m/z $^{+1}$, 675 ($C_{50}H_{72}O_2$) and 742 ($C_{50}H_{76}O_4$) also confirmed the presence of bacterioruberin at its derivative tetra anhydrous bacterioruberin Fig. 3.

3.7. Bioassays

3.7.1. DPPH

The concentration dependent 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the bacterioruberin was confirmed by changing the color of DPPH from violet to transparent. The highest scavenging activity of 67 % was achieved at 40 µg/mL in comparison to the standard ascorbic acid which demonstrated 76 % at the same concentration (Fig. 4 A). However, the IC_{50} calculated by linear regression analysis using formula $y = ax \pm b$, was 22 µg/mL to that of ascorbic acid 26 µg/mL. The dose response curve was plotted between the % scavenging (*y-axis*) and concentration of the compound used (*x-axis*) as shown in Fig. 4A.

3.7.2. Iron chelation activity

The iron chelation potential of BR was observed by fading the dark color of ferrozine. The compound demonstrated a significant chelation (82 %) with an IC_{50} value of 27 µg/mL. The standard EDTA revealed 92 % chelation activity as shown (Fig. 4 B). The IC_{50} was calculated by plotting the data between % chelation and concentration of the compound by using the formula $y = ax \pm b$. where the R^2 value was 0.92.

3.7.3. Minimum inhibitory concentration against food borne pathogens

The carotenoid was tested against four foodborne pathogens, *E. coli, Salmonella typhi, Bacillus cerus,* and *S. aureus*. Concentrations inhibiting 50 % of bacterial growth (EC_{50}) are reported in Fig. 5. In general, the carotenoid was most active against *S. aureus* with an EC_{50} calculated 18.35 ± 2 µg/mL followed by *Bacillus cerus, E. coli and S. typhi*. In details the carotenoid was statistically (one-way ANOVA Bonferroni post-test analysis) more effective on Gram positive followed by Gram Negative with an EC_{50} value ranges from 33.16 ± 2 µg/mL 95.06 ± 2 µg/mL. The dose response curve plotted against the % inhibition and concentration to reach its half-maximal effective response showed results in a concentration dependent manner Fig. 5. The obtained primary data were analyzed mainly by using ANOVA followed by Tukey's post-hoc multiple comparison test. The analysis revealed significant variations

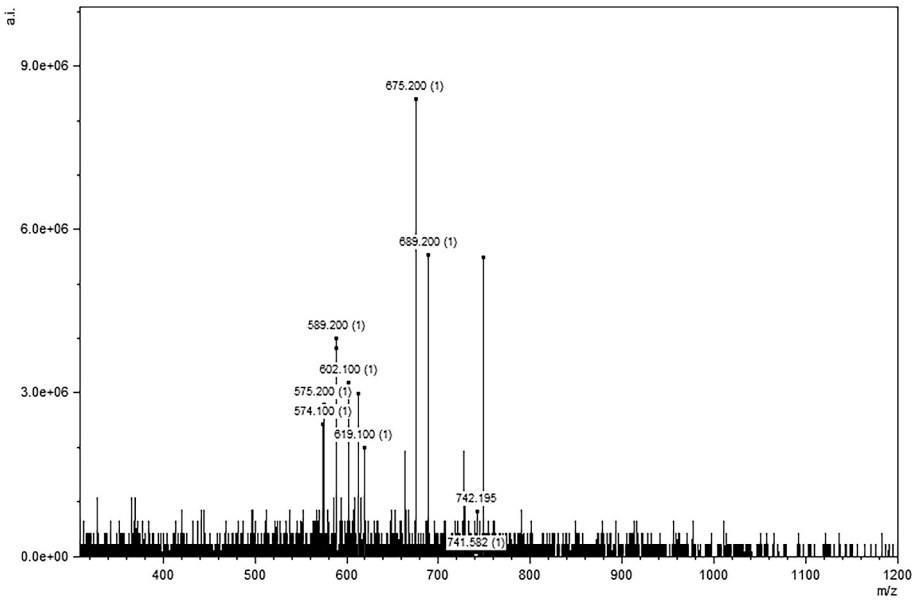

Fig. 3. Showing the LC/MS $m/z + 1$ of the identified compounds demonstrated the presence of tetra anhydrous bacterioruberin 675-1, and bacterioruberin 742-1.

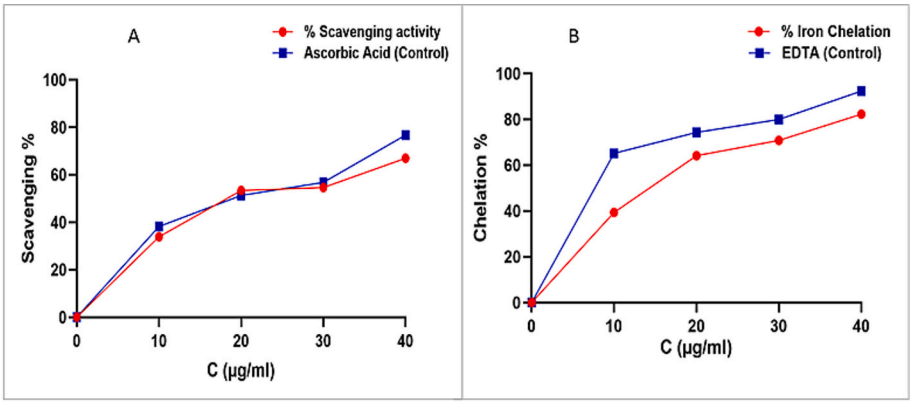

Fig. 4. A) % scavenging activity of BR from strain *Arthrobacter* strain PKT-29. B) iron chelating ability of BR in comparison to positive control EDTA. The test compound exhibited % scavenging and inhibition in a dose dependent manner. The compound concentration causing 50 % inhibition of the desired activity (IC_{50} was obtained by linear regression analysis. B) Where R^2 value was calculated as 0.96.

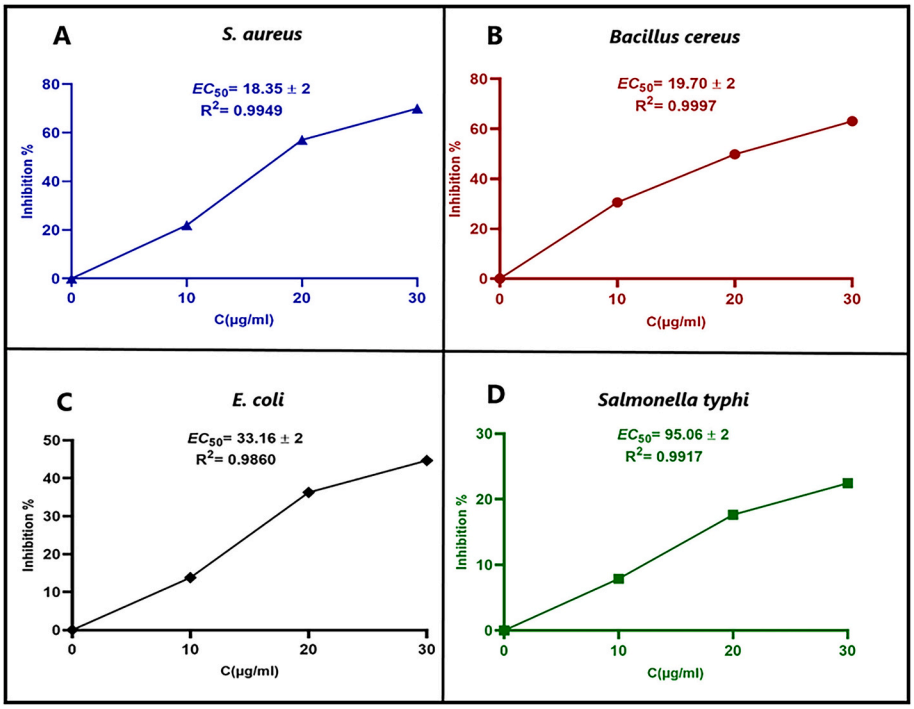

Fig. 5. EC_{50} calculation of the bacterioruberin on (A) *S. aureus* (B*) Bacillus cereus* (C) *E. coli* (D) *Salmonella typhi.* The EC_{50} was calculated by plotting the dose response curve and 5 inhibition of the respective strains. R^2 value shows the regression value to determine the proportion of variance.

between the four groups (*S. aureus*, *B. cereus*, *E. coli,* and *S. typhi* as indicated by a *p*-value of less than 0.05, as stated in Table S2.

3.8. Application of bacterioruberin spray on fresh fruits to extend shelf life

All fruit groups, including peaches, grapes, and apricot, were evaluated every 24 h for 7 days. Group D, which received a carotenoid spray, remained fresh without signs of spoilage, while the control groups spoiled within 3 days. The carotenoid-treated fruits maintained their water content, surface membrane integrity, and organoleptic properties (such as odor, texture, and appearance) for 6 days at room temperature. In contrast, untreated fruits and those treated with sodium citrate or bicarbonate water experienced water loss, membrane damage, and fungal spoilage. The results clearly demonstrate that carotenoid spray significantly extends the shelf life of post-harvest fresh fruits, even at room temperature. While untreated fruits and those sprayed with carbonated water alone showed spoilage, the carotenoid-treated fruits preserved their quality by resisting biotic and abiotic stress, reducing damage, and preventing over ripening. This suggests the carotenoid treatment promotes a healthier physiological state in fruits, likely due to a decreased respiration rate and improved turgidity as shown in fig. S2.

3.9. Organoleptic properties

Mean acceptance of sensory attributes of the 4 samples varies from 2.33 to 3.28. The best obtained range of sensory attributes with no spoilage, fruit freshness, integrated membranes was the sample treated with 2 % carotenoid followed by fruits treated with 2 % ectoine. The most liked group D (treated with 2 % bacterioruberin) has a significant *p* value indicating the high-quality attributes of the post-harvest fruits even after 6 days of storage at room temperature. The one-way ANOVA (Tukey) multiple compression test revealed a high good sensory acceptance (Table 1) with extended shelf life and overall high general

Table 1
ANOVA (Tukey) multiple comparisons for evaluation of control and Tested Samples.*

Pair	Comparison of Attributes	Mean	STD	Std. Error	Group (A) Environmental Control		Group (B) Aqua Water Control	
					(P values)		*(P values)*	
Pair 1	Aroma	2.42	1.217	0.192	GC	$p < 0.001$	GC	0.064
					GD	$p < 0.001$	GD	$p < 0.001$
Pair 2	Appearance	3.10	1.297	0.205	GC	$p < 0.005$	GC	0.090
					GD	$p < 0.001$	GD	$p < 0.001$
Pair 3	Texture	2.28	1.320	0.209	GC	$p < 0.001$	GC	0.014
					GD	$p < 0.001$	GD	$p < 0.001$
Pair 4	Spoilage	2.33	2.030	0.321	GC	$p < 0.001$	GC	$p < 0.001$
					GD	$p < 0.001$	GD	$p < 0.001$
Pair 5	Necrotic Spots	3.28	1.339	0.212	GC	$p < 0.001$	GC	0.006
					GD	$p < 0.001$	GD	$p < 0.001$
Pair 6	Membrane Damage	3.03	1.687	0.267	GC	$p < 0.001$	GC	$p < 0.001$
					GD	$p < 0.001$	GD	$p < 0.001$
Pair 7	General Impression	2.05	1.867	0.295	GC	$p < 0.001$	GC	$p < 0.001$
					GD	$p < 0.001$	GD	$p < 0.001$

* GA (Group A, Environmental Control), GB (Group B, Aqua Water Control), GC (Group C, Tested Group Ectoine), GD (Group D, Tested Group Carotenoid). For each biological replicate, three technical replicates were performed to minimize experimental variability and ensure reproducibility.

impression of the bacterioruberin treated samples. Our results indicated the high anti-food spoilage properties of the carotenoid thus suggesting a good commercial potential for the bacterioruberin.

3.10. Docking studies

Docking assays were performed on phospholipase D and VQ motif protein JVA1/VQ22, an important player in many physiological processes like response to biotic and abiotic stresses. The bacterioruberin demonstrated a high binding affinity towards both the targets JVA1/VQ22 (−9.4 kcal/mol) and phospholipase D (−8.5 kcal/mol). Phospholipase D is the key enzyme in membrane degradation. The inhibition of this enzymes leads to better preservation of the fruits and can extend the shelf life by slowing down the senescence. Bacterioruberin upon interaction with phospholipase D demonstrated high hydrophobic interactions with residues of catalytic pockets as shown in Fig. 6 A & B. Furthermore, the interactions with VQ motif protein revealed both the hydrophobic and hydrophilic interactions due to H-bonding as shown in 7B. The carotenoid fragments as shown in fig. S3 have an interaction

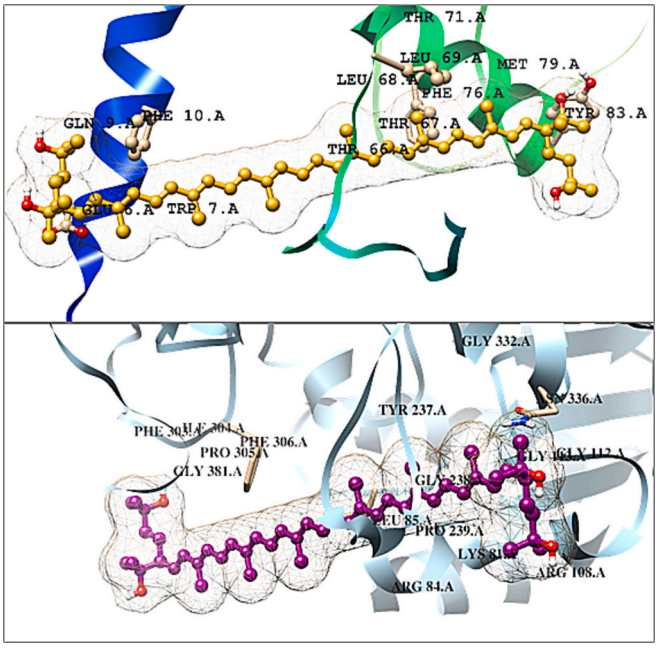

Fig. 6. Three-Dimensional Representation of Best Docked Poses: A) Phospholipase D-. Bacterioruberin, B) JVA1/VQ22-Bacterioruberin.

that favor possible inhibitory activity given by a bound consistently fitting into the enzyme pocket and near to the catalytic site and showing a possible effect on the enzyme catalysis and VQ motif proteins.

4. Discussion

The significance of fruits in a balanced diet in a daily routine cannot be underscored. A wide range of determinants of desire quality attributes like nutritional value, color, shelf life, texture and other processing qualities have been reported by many researchers (Singh et al., 2011). However, the use of chemical preservatives, antioxidants in fruits, has been a subject of controversy due adverse health consequences on animal and human health (Alabdaly et al., 2021; Salehi et al., 2018). In the light of growing demand for natural food colorant, preservative and antioxidants which are environmentally friendly, biocompatible carotenoids have gained a great interest in biotechnology and food industry (Mussagy et al., 2019). Microbes that live in extreme environments are good producers of coloring compounds contributing to its survival, desiccation and UV resistance. These carotenoids also have the ability to maintain membrane fluidity in stress, preventing oxidative damage, protein and lipid peroxidation. *Arthrobacter* genera is ubiquitous in nature and is considered one of the is one of the known producers of bacterioruberin and their tetra anhydrous derivatives. Among the *Arthrobacter* the *Arthrobacter agilis* is strong producer of bacterioruberin which appears red in color on the surface of agar medium. This coloring compound along with its derivatives are secreted in a temperature dependent manner in several cold loving bacteria to enhance its membrane plasticity thereby preventing the cells from freeze-thawing cycles. This also helps microbes in regulating the osmotic shock and cold shock proteins to prolong its survival (Flegler & Lipski, 2022; Fong et al., 2001). Due to its vast biological activities, and other advantages for human health carotenoids are nowadays widely used as food colorants, dietary supplements and for aesthetic applications (Ashokkumar et al., 2023; Guleria et al., 2017; Gupta et al., 2022). BR is C50 isoprenoid with 13 conjugated double bonds with four OH groups; highly antioxidant and anti-inflammatory compound compared to other carotenoids (Jehlička & Oren, 2013; Yatsunami et al., 2014). They have a higher activity in reducing the damage of H2O2 exposure, UV and gamma irradiation. This compound is also found in the membranes of halophilic archaea acting as photoprotection system in high salinity and long-lasting UV exposure (Saito et al., 1997; Singh & Gabani, 2011).

In our current investigations the carotenoid characterized as bacterioruberin and its derivative tetra-anhydrous bacterioruberin demonstrated strong antioxidant and iron chelating activities suggesting its preservative nature to extend the shelf life of post-harvest fresh fruits. BR is highly lipophilic in nature, but 4 terminal hydrophilic groups are

present which determines its slight solubility in water. These characteristics make the extracted BR an excellent candidate for the Nanocomposite, and opportunity to design a most compatible preservative with control release and high bioavailability (Palanisamy & Ramalingam, 2024). The presence of conjugated double bond structure and high resonance makes this an excellent candidate to quench the superoxide's, stabilize the membranes and inhibit the Lipoxygenases and phospholipases. Its iron chelation ability also contributes to a unique characteristic to prevent the Fenton pathway which is a key contributor in oxidation and membrane damages during fruit spoilage. The iron chelation potential of the BR demonstrated that no free iron is available to initiate Fenton pathway thereby inhibiting the protein and lipid oxidation during fruit storages (Hwang et al., 2024). Due to its high antioxidant potential this bacterioruberin and bacterioruberin-rich microbial extracts have attracted the biotech, food and pharma sector to be used in many industrial products (Giani et al., 2024). However, the bacterioruberin significance as a preservative in fresh fruits, vegetables and other items, which are vulnerable to oxidation and may produce toxic unstable compounds, has not been studied. The EC_{50} for BR against several foods borne pathogen was also calculated indicating as strong antibacterial potential specifically against the Gram-positive bacteria. The analysis revealed significant variations between the four groups (*S. aureus, B. cereus, S. typhi* and *E. coli* as indicated by a *p*-value of less than 0.05, as stated in Table. Due to its conjugated and resonance structure these compounds like to fits in transmembrane proteins like porins and to the outer membrane of bacterial cell wall. This phenomenon leads to forming strong polymer bonds to destroy the porins and reducing permeability of bacterial membranes thereby inhibit bacterial growth (Evans & Cowan, 2016). When applied to the fruits as 2 % suspension, demonstrated anti-spoilage activity which indicates its antifungal nature. This also suggests the anti-biofilm nature of the compound and could be used as quorum sensing inhibitors.

Moreover, these compounds, noteworthy for their colors ranging from yellow to green and orange to red, serve as potent natural colorant to contribute to the most important attributes of food both for aesthetic value and for quality (Pereira da Costa & Campos Miranda-Filho, 2020). Furthermore, these carotenoids play a vital role in shielding the membrane damage, dryness and water leakage to prolong the shelf life, thereby ensuring that the nutritional value of fruits remains intact (Hrebień-Filisińska, 2021). The bioinformatics data demonstrated a strong binding interaction of the BR to phospholipase D and VQ22. The expression of phospholipase D is highly linked to membrane damage and membrane catabolism. Any compound with potential to inhibit this enzyme has been observed to enhance the shelf life and quality of food products (Padmanabhan & Paliyath, 2020). Moreover, these compounds also give a characteristic color and keep the membrane intact, maintain fluidity and prevent any significant loss of aroma and freshen of the fruits. It is likely the induction of several transcription factors, enzymes like, β-1,3-glucanase and chitinase in response to BR application might confer protection to fruit from postharvest fungal infections.

Studies suggested that silencing the VQ motif protein VQ22 enhances resistance to necrotrophic fungi whereas transgenic plants expressing VQ22 were highly susceptible to biotic and abiotic stresses (Hu et al., 2013). This indicate that VQ22 functions as a negative regulator of JA-mediated plant defense. Our study revealed a strong binding mechanism of BR with VQ22 through hydrophobic interactions making it a suitable candidate for anti-food spoilage to extend the shelf life of post-harvest fruits studied among the 4 groups.

5. Conclusions

Bacterioruberin extracted from *Arthrobacter* sp. isolated from the Xinjiang desert offers a viable and novel approach to extending the shelf life of fruits in postharvest storage. Its notable antimicrobial properties not only combat spoilage but also preserve the nutritional integrity and overall quality of the fruits. Utilizing this organic substance in postharvest procedures can improve food sustainability, paving the way for more environmentally friendly methods in the food industry. Continued exploration of bacterioruberin's potential could significantly impact food preservation strategies and contribute to better food security.

6. Limitations

Bacterioruberin offers a wide range of industrial applications, but the sanitation step-in large-scale production is considered one of the costs and risk factor from microbes. If there are any contaminations in the process the whole production is compromised. These compounds are usually present in membranes along with other carotenoproteins which make the purification process difficult. Most of the color compounds are sensitive to light and other environmental factors and could easily degrade. Moreover, during the lab investigation sometime variation in the results may be observed which may require independent feedback for evaluation of sensory attributes through crowdsourcing.

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Ethical Permission

Ethical permission was not required.

Consent

All panelists involved in this sensory research provided informed consent prior to participation, affirming their voluntary involvement and understanding of the study's objectives.

CRediT authorship contribution statement

Wasim Sajjad: Writing – original draft, Visualization, Software, Methodology, Investigation, Conceptualization. **Murad Muhammad:** Resources, Formal analysis, Data curation. **Sayed Muhammad Ata Ullah Shah Bukhari:** Visualization, Formal analysis, Data curation. **Sumra Wajid Abbasi:** Software, Resources, Formal analysis, Data curation. **Osama Abdalla Abdelshafy Mohamad:** Validation, Resources, Methodology, Formal analysis, Data curation. **Yong-Hong Liu:** Writing – original draft, Visualization, Project administration, Formal analysis. **Wen-Jun Li:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The article and Supplementary material contain the original contributions made during the study; for additional information, contact the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2024.100239.

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