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REVIEW ARTICLE

Microbial Bacterioruberin: A Comprehensive Review

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Abstract Bacterioruberin (BR) is a fat-soluble, dipolar, reddish pigment predominantly found in halophilic archaea. BR is a rare C50 carotenoid from the xanthophyll family, and it has been extensively studied for its potent antioxidant properties, such as its ability to protect cells from oxidative stress. In addition, several studies have shown that BR-rich extracts and its derivatives exhibit significant antiviral, antidiabetic, antibacterial, and anti-inflammatory effects, making them ideal candidates for the development of novel therapeutic interventions against various diseases. Although it possesses remarkable biological properties, studies related to the regulatory aspects of biosynthesis, in vitro and in vivo studies of purified BR have been rare. However, investigations are needed to explore the potential application of BR in various industries. Additionally, optimization of the culture conditions of BR-producing haloarchaea could pave the way for their sustainable production and utilization. The current review provides comprehensive information on BR, which includes the sources of this compound and its bioproduction, extraction, stability, toxicity, and biological activities in relation to its commercial applications. This review also discusses the potential challenges and limitations associated with BR bioproduction and its utilization in various industries. In addition, this treatise highlights the need for further research to optimize production and extraction methods and explore avenues for novel applications of BR in various sectors, such as pharmaceuticals, food, and cosmetics.

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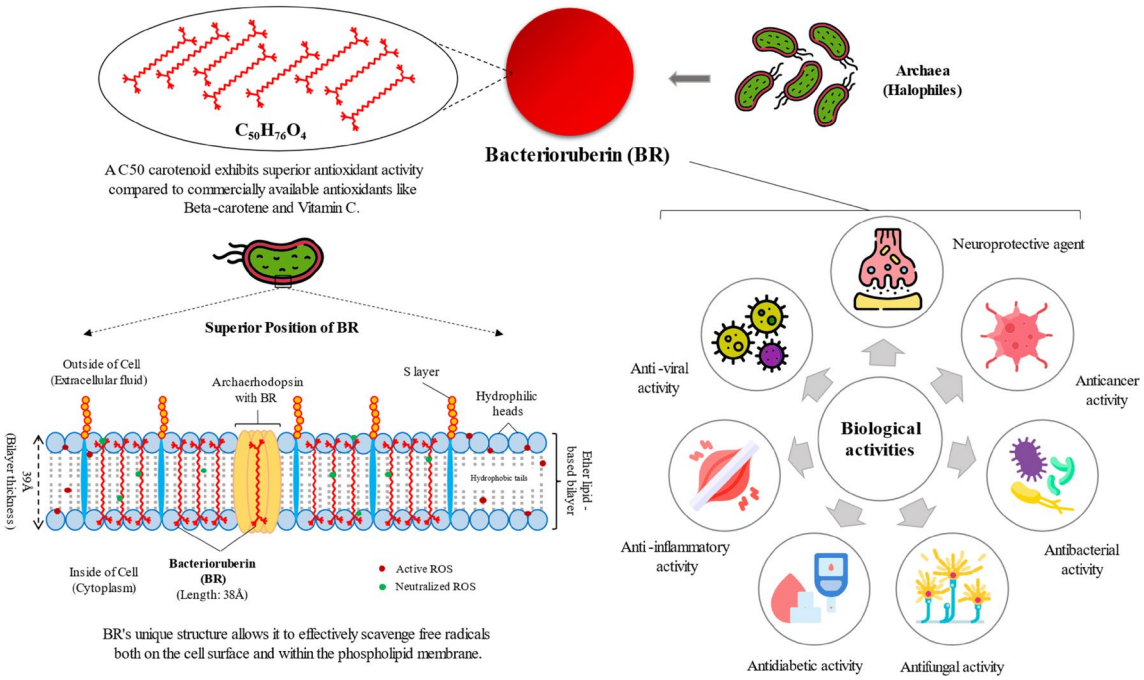
Graphical Abstract



Keywords Bacterioruberin · C50-carotenoid · Xanthophyll · Haloarchaea · Antioxidant

Introduction

Carotenoids are an important class of plant-based pigments that occur ubiquitously in nature [1]. As of now, > 1200 naturally occurring carotenoids have been identified and are distributed into two fragments: oxygenated carotenoids, known as xanthophylls and nonoxygenated carotenoids, known as carotenes. All these compounds have been isolated from bacteria (especially Cyanobacteria), eubacteria, archaebacteria, fungi (yeast), algae (both micro- and macroalgae) and higher plants [1–6]. Carotenoids are known to be involved in the reduction of free radicals, which helps to promote animal health by boosting the immune system and strengthening the endocrine system. However, since animals are unable to produce carotenoids, they need to obtain these compounds solely from their diet [5, 7]. Currently, the Carotenoids Database provides information on 1204 carotenoids, the majority of which have a C40 hydrocarbon skeleton (1121 carotenoids). However, there are fewer C30 (37 carotenoids), C35 (5 carotenoids), C45 (13 carotenoids), and C50 hydrocarbon skeletons (33 molecules), and the number of these compounds is continuing to increase as researchers discover new forms of carotenoids [6, 8]. Those carotenoids with a C45 or C50 hydrocarbon skeleton are called higher carotenoids [4]. Decaprenoxanthin, a C50 carotenoid, was first isolated from *Flavobacterium dehydrogenans* in 1966 and is the first carotenoid with more than 40 carbon atoms [1]. Since then, > 40 different kinds of higher carotenoids have been reported [4]. These higher carotenoid contents are mainly found in moderately to extremely halophilic archaea (halobacteria) [9]. Higher carotenoids are considered to be rare on the basis of their distribution among different taxa, and some of these carotenoids are C45 carotenoids, e.g., nonaflavuxanthin [10]; C50 carotenoids, e.g., bacterioruberin [11]; flavuxanthin [10, 12]; sarcinaxanthin [13, 14]; and decaprenoxanthin [12].

Bacterioruberin

Bacterioruberin (BR) is known to be a rare C50 carotenoid that is mainly found in halophilic archaea. BR is slightly or poorly soluble in water. It is a highly lipophilic molecule and can be dissolved in organic solvents and oils. It is a red–orange xanthophyll pigment responsible for the coloration observed in halophilic organisms [15, 16].

This carotenoid has 50 carbon atoms (C50) and possesses a longer system of conjugated double bonds than the C40 carotenoids often found in other organisms, such as plants, microalgae, fungi, and bacteria. Haloarchaea also contain C40 carotenoids, such as phytoene, lycopene, and beta-carotene, but in low quantities; these compounds are proposed to be the metabolic intermediates in the biosynthesis of C50 carotenoids [17]. The BR is known to serve as a highly diagnostic biomarker for halobacteria. It has been observed that some high-molecular-weight biomarkers could not be detected since they pose great challenges to analysing them by employing routinely used GC–MS techniques [18]. Carotenoids are known to play a crucial role in photosynthesis by absorbing light energy and protecting cells from harmful free radicals. On the other hand, BR not only provide antioxidant and sunlight protection activity but also aid in maintaining the structural integrity of bio-membranes when exposed to extreme salt concentrations. This unique adaptation allows halophilic archaea to survive in high-salinity environments [18]. It has been shown that C50 carotenoids, comparable to BR, are crucial for enhancing the stability of bio-membranes in psychrophiles. Due to this cellular level adaptability, these organisms are adapted to survive at extremely cold temperatures and continue to perform cellular functions normally. Furthermore, the rigidity of the biomembrane afforded by C50 carotenoids may also guard against damage caused by unfavourable freezing and thawing conditions in the environment [19, 20]. In addition, BR is known to confer resistance to gamma irradiation, intense light, and DNA damage caused by ultraviolet (UV) irradiation, radiography, and H_2O_2 exposure [21]. The potent free radical scavenging properties of BR make it a suitable candidate for use as a feed supplement in the aquaculture industry [22]. The present review offers a comprehensive overview of BR, with a focus on its diverse sources, biosynthesis, extraction methods, storage conditions, stability profiles, potential toxicity, bioproduction processes, varied biological properties, and its applications. This review provides a depth of information into every aspect of BR and sets the groundwork for understanding and harnessing the potential of this unique compound. To the best of our knowledge, this is the first review of C50-carotenoid bacterioruberin.

History of Bacterioruberin

Helena Franciska Maria Petter, a microbiologist, significantly contributed to our understanding of halophiles through her doctoral thesis on halophilic microorganisms. She carried out her research at the University of Utrecht, Utrecht, Netherlands, in the 1930s. Her thesis is titled "Over roode en andere bacterieën van gezouten visch" (On red and other bacteria of salted fish) [11, 23]. Her research focused on studying various species of halophilic prokaryotes, primarily red pigment-producing members of the *Halobacteriaceae* population, which were isolated from salted fish and Trapani salt collected from a cannery in Bergen, Norway. Her isolates included rod-shaped bacteria as well as coccoid and sarcina-shaped bacteria. Her research included descriptions of "*Bacterium trapanicum"* and *Bacterium halobium*", which are currently known as *Halobacterium trapanicum* and *Halobacterium salinarum*, respectively. In 1932, she isolated two crystalline carotenoids, α-BR and β-BR, from a bacterium named *Bacterium halobium* [24]. Helena Petter was the first to isolate and name the pink-colored carotenoid of *Halobacteriaceae* as bacterioruberin [25].

Sources of Bacterioruberin

The natural sources of BR are archaea, such as haloarchaea or halophilic archaea, and a few other extremophiles, such as psychrophiles (*Arthrobacter*, *Micrococcus*), *Azospirillum* sp., and radioresistant bacteria (*Rubrobacter*) [26]. BRs are produced predominantly by most of the members of *Halobacteriaceae* and *Haloferacaceae* (Table 1). It is abundant in halobacteria, and these bacteria are ubiquitous in salty habitats such as salt lakes and evaporating seawater pools. Thus, BR could be valuable biomarker for identifying and studying these unique microbial communities [18]. *Halobacteriaceae* are easy to distinguish by Raman spectroscopy due to the presence of distinctive carotenoid pigments (BR and its derivatives) [27–29]. Using different analytical techniques, researchers have reported the presence of BR and its derivatives in diverse microorganisms. The various microbial sources of BR and its derivatives are listed in Table 1.

Deposition of Bacterioruberin in Animals

Flamingos and pelicans glow in vibrant pink or reddish colors due to pigments that they cannot synthesize de novo. The primary source of these colours is carotenoids, which are found in food substances such as microalgae (*Dunaliella*) and small shrimp (*Artemia*) that are high in carotenoids. Recent research has indicated that microorganisms such as *Haloarchaea* inhabiting salt lakes and ponds where these birds nest may significantly contribute to the pink–reddish coloration of flamingos' feathers. Interestingly, it has been discovered that the feathers of flamingos contain live cells of *Haloarchaea* belonging to the genera *Halococcus* and *Halogeometricum*. In addition, pigment analysis of the feathers of these birds revealed the presence of BR and its derivatives [89, 90]. However, further research is needed to determine whether *Haloarchaea* may play a role in regulating

Table 1 Various microbial sources of bacterioruberin and its derivatives

Microorganism sources	References
Halorhabdus utahensis	[30]
Arthrobacter agilis GS1 *Janthinobacterium lividum* GW1	[31]
Arthrobacter agilis WB28	[32]
Salinicoccus roseus EMK96	[33]
Haloarcula sp. OS (HAE)	[34]
Natronococcus sp. TC6 *Halorubrum tebenquichense* SU10	[35]
Arthrobacter agilis NP20	[15]
Halorubrum sp. HRM-150	[36]
Arthrobacter sp. NamB2	[37]
Haloferax mediterranei	[38]
Halorubrum ruber MBLA0099	[39]
Kocuria rosea RAM1	[26]
Haloterrigena thermotolerans K15	[40]
Halogeometricum sp. ME3 *Haloarcula* sp. BT9 *Haloferax* sp. ME16	[41]
Haloarcula sp. TeSe-41 *Haloarcula* sp. ALT-23 *Halorubrum tebenquichense* Te Se-85 *Halorubrum tebenquichense* Te Se-86 *Haloarcula* sp. TeSe-89 *Haloarcula* sp. TeSe-51	[42]
Halorubrum sp.	[7]
Haloferax alexandrinus GUSF-1 (KF796625)	[43]
Haloarcula sp. M1 CA_13B53 *Halorubrum* sp. M2 Fb21 *Halolamina* sp. M3 UAH-SP14 *Halorubrum* sp. M4 SD683 *Halorubrum* sp. M5 Fb21 *Halorubrum* sp. M6 Fb21 *Halorubrum* sp. M7 Fb21 *Halorubrum* sp. M8 E302-1	[44]
Halomicrobium mukohataei DSM 12286 *Haloarcula salaria* JCM 15759 *Haloarcula japonica* JCM 7785 *Haloarcula vallismortis* ATCC 29715 *Halomicrobium mukohataei* JP 60 *Haloferax volcanii* DS2 *Halomicrobium katesii* CECT 7257	[45]
Haloterrigena sp. SGH1	[46]
Arthrobacter agilis DSM 20550 *Arthrobacter bussei* DSM 109896	[47]
Haloferax volcanii	[48]
Arthrobacter agilis 50cyt	[49]
Aquisalibacillus elongatus MB592 *Salinicoccus sesuvii* MB597 *Halomonas aquamarina* MB598	[50]
Halogeometricum rufum RO1-4 *Halogeometricum limi* RO1-6 *Haladaptatus litoreus* RO1-28 *Haloferax Haloplanus vescus* RO5-8 *Halopelagius inordinatus* RO5-2 *Halogranum rubrum* RO2-11 *Haloferax volcanii* CGMCC 1.2150	[51]
Haloferax mediterranei R4 (ATCC 33500 T)	[52]

Table 1 (continued)

Microorganism sources	References
Haloterrigena turkmenica	[53]
Halorubrum sp. SH1	[54]
Halorubrum sp. TBZ126 *Halorubrum chaoviator* Halo-G	[55]
Haloarcula japonica TR-1(JCM 7785 T)	[56]
Halobacterium sp. SP-2 *Halorubrum* sp. SP-4	[57]
Natronobacterium gregoryi DSMZ 3393	[58]
Halobacterium halobium M8	[59]
Halobacterium salinarum NRC-1 *Halobacterium salinarum* R1 *Halorubrum sodomense* *Haloarcula valismortis* *Salinibacter ruber*	[27]
Halococcus morrhuae *Halobacterium salinarium*	[60]
Halorubrum sp. SS-12	[61]
Haloferax mediterranei ATCC 33500	[62]
Halobacterium salinarium HM3 *Halobacterium salinarium* HM322 *Halobacterium salinarium* HPC1-2 *Halobacterium salinarium* AS133	[63]
Haloquadratum walsbyi	[64]
Natrinema pallidum	[28]
Haloferax alexandrinus TM	[65, 66]
Arthrobacter agilis	[20]
Curtobacterium flaccumfaciens pvar poinsettiae	[67]
Halobacterium salinarum ATCC 33170 (Formerly *Halobacterium cutirubrum* or *Halobacterium salinarum* NRC 34002)	[68]
Micrococcus roseus MTCC 678	[69]
Micrococcus roseus	[70]
Haloferax mediterranei	[71]
Haloferax volcanii DS2	[72]
Rubrobacter radiotolerans	[73]
Haloferax denitrificans comb. nov	[74]
Haloarcula hispanica *Haloferax gibbonsii*	[75]
Halobacterium denitrificans ATCC 35960	[76]
Halobacterium cutirubrum *Halobacterium halobium* *Halobacterium salinarium* *Halobacterium marismortui* *Halobacterium saccharovorum* *Halobacterium vallismortis*	[77]
Azospirillum brasilense Cd	[78]
Halobacterium marismortui	[79]
Halobacterium salinarum ATCC 33170 (Formerly *Halobacterium cutirubrum* or *Halobacterium salinarum* NRC 34002)	[80]
Arthrobacter glacialis	[81]

Table 1 (continued)

Microorganism sources	References
Halococcus morrhuae NRC 16015 (Formerly *Sarcina morrhuae,* 16015) *Halobacterium salinarium* NRC 34002 *Halobacterium cutirubrum* 54001 *Anzoebobacter morrhuae* 51001 *Halobacterizrm salinarium* PN *Halobacterium halobium* M 34014 *Sarcina litoralis* 16006 *Halobacterium halobium* 34020	[82]
Halobacterium cutirubrum NRC 34001 (Currently called *Halobacterium salinarum* ATCC 33170) *Halobacterium halobium* NRC 34020 (Currently called *Halobacterium salinarum* ATCC 43214)	[83]
Halophilic bacteria (Unknown)	[84]
Corynebacterium poinsettiae *Halobacterium salinarum*	[85]
Halophilic bacteria BOS 66	[86]
Corynebacterium poinsettiae	[87]
Halobacterium salinarum	[88]
Halobacterium salinarum (Formerly *Bacterium halobium*)	[11]

environmental factors that affect bird plumage coloration or perhaps in shielding feather microstructures from UV radiation [89]. As a result, BR is now considered to be a new pigment that needs to be investigated in relation to animal coloration in marine environments.

Biosynthesis of Bacterioruberin

Until 2015, only three biosynthetic pathways of C50 carotenoids, the ε-cyclic C50 carotenoid decaprenoxanthin in *Corynebacterium glutamicum* [12, 91, 92], the γ-cyclic C50 carotenoid sarcinaxanthin in *Micrococcus luteus* NCTC2665 [93] and the β-cyclic C50 carotenoid 2,2′-bis-(4-hydroxy-3-methybut-2-enyl)-β,β-carotene in the *Dietzia* sp. strain CQ4 [94], have been described based on their chemical structures. In 2015, Ying Yang and his coworkers at the Tokyo Institute of Technology, Yokohama, Japan, first elucidated the complete biosynthetic pathway of the C50-carotenoid-BR in *Haloarcula japonica*, an extremely halophilic archaeon. Their research showed that a gene cluster comprising three genes, *C0505*, *C0506*, and *C0507*, encodes the C50 carotenoid 2″, 3″-hydratase (CruF), a bifunctional lycopene elongase and 1,2-hydratase (LyeJ), and the carotenoid 3,4-desaturase (CrtD), respectively. In *H. japonica*, a series of chemical reactions converting lycopene into BR are catalyzed by the three carotenoid biosynthetic enzymes mentioned above [95]. The discovery of this biosynthesis pathway has provided valuable insights into the production of BR and a conceptual basis for investigating the intricacies of carotenoid biosynthesis pathways in other halophilic archaea.

The biosynthesis pathway of BR involves a series of enzymatic reactions that occur within the cell (Fig. 1). These reactions are responsible for the bioproduction of a set of precursors that eventually form BR. All carotenoids are synthesized from common precursors, namely, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are synthesized via either the well-known mevalonate (MVA) pathway or the recently discovered non-mevalonate pathway (MEP) [96]. Several enzymatic steps are involved in the initiation of carotenoid biosynthesis, including the condensation of IPP and DMAPP to produce geranyl pyrophosphate (GPP), which is catalyzed by geranyl pyrophosphate synthase (dimethylallyl transferase, or GPPS) (IspA). Subsequently, farnesyl pyrophosphate synthase (FPPS) (IspA) catalyzes the condensation of GPP with another molecule of IPP to produce farnesyl pyrophosphate (FPP). The enzyme GGPP synthase (CrtE) converts FPP into the main carotenoid precursor geranylgeranyl pyrophosphate (GGPP). The enzyme phytoene synthase (CrtB) converts GGPP, a key precursor in the biosynthesis of carotenoids, into phytoene. The first reaction specific to the carotenoid branch of isoprenoid metabolism is the formation of phytoene, a compound found in all carotenogenic organisms [97]. The enzyme phytoene desaturase (CrtI) converts phytoene to lycopene. These enzymes introduce double bonds and rearrange the carbon skeleton of phytoene to form lycopene. Lycopene is an important key molecule in global carotenogenesis since it is the precursor for several carotenogenic branches. Thus, lycopene has been unequivocally established to be a precursor for the synthesis of relevant carotenoids in nature, such as lutein and its precursors and derivatives, neurosporaxanthin,

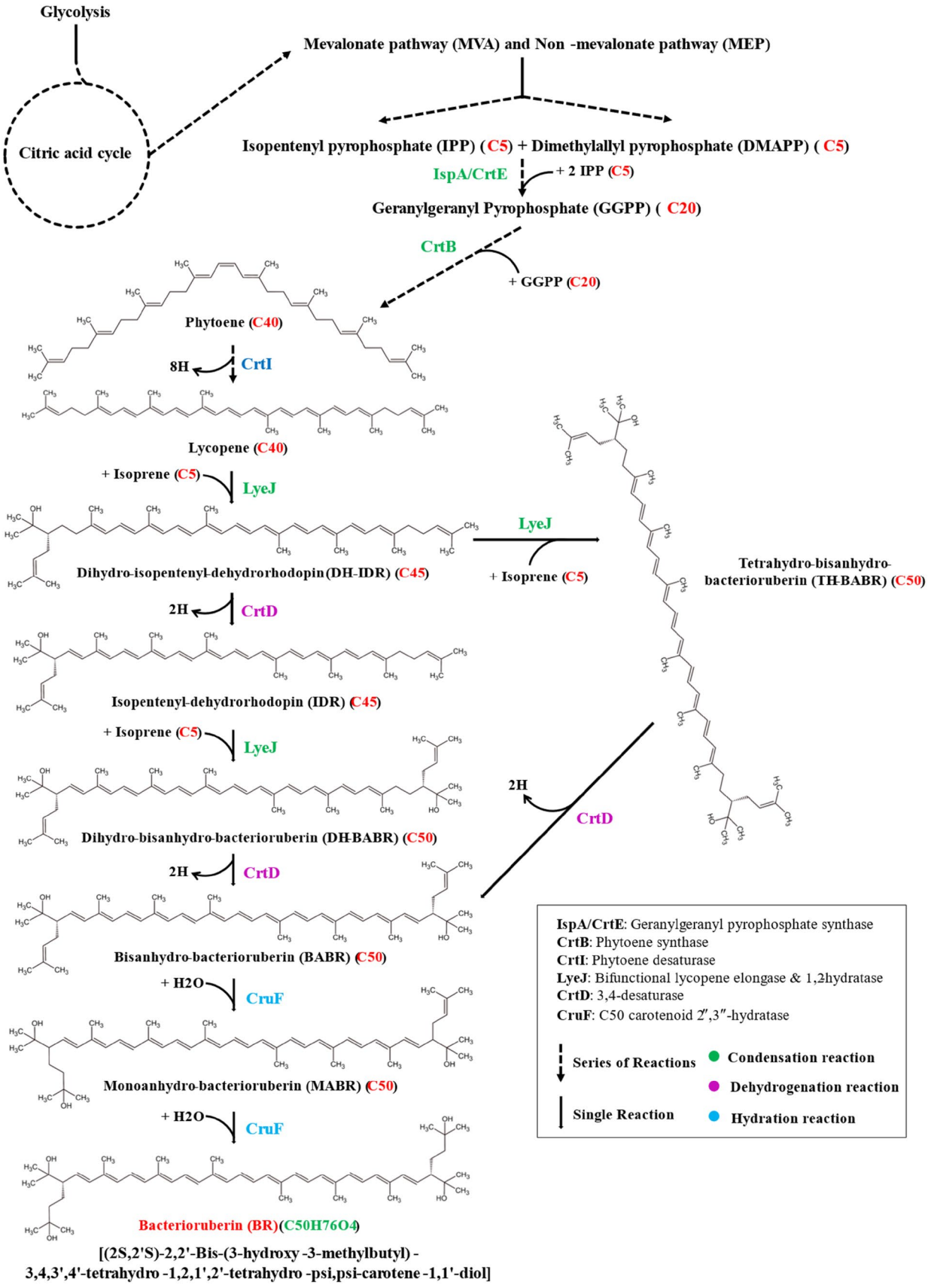
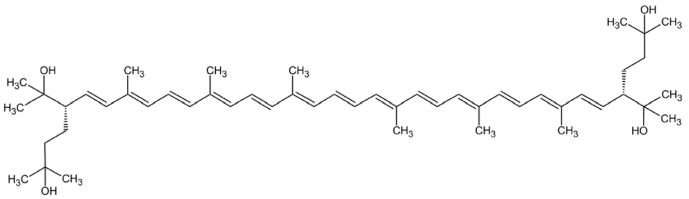
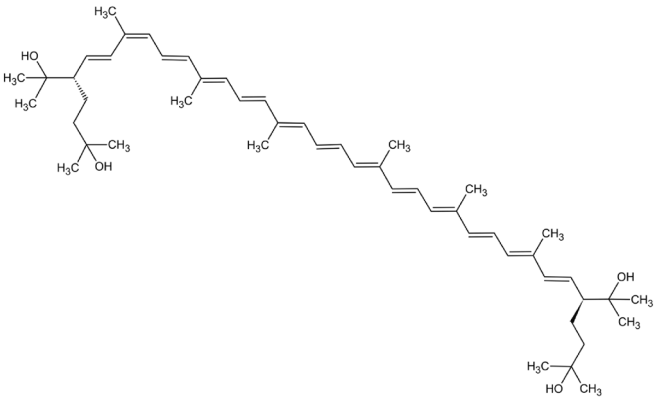
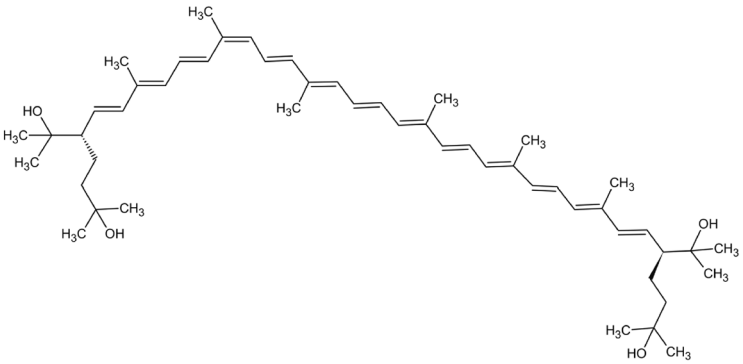
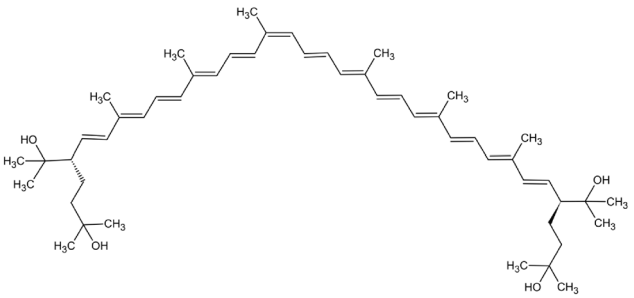


Fig. 1 Biosynthesis of Bacterioruberin

Table 2 Isomers of bacterioruberin ($C_{50}H_{76}O_4$)

Common name	IUPAC name	Chemical structure
All-trans- Bacterioruberin (Bacterioruberin)	(2S,2'S)-2,2'-Bis-(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	
5-cis- Bacterioruberin	(5Z,2S,2'S)-2,2'-Bis-(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	
9-cis- Bacterioruberin	(9Z,2S,2'S)-2,2'-Bis-(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	
13-cis- Bacterioruberin	(13Z,2S,2'S)-2,2'-Bis-(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	
15-cis- Bacterioruberin (a)	Not available	Not available
5-cis-9-cis- Bacterioruberin (b)	Not available	Not available
5-cis-26-cis- Bacterioruberin (c)	Not available	Not available
9-cis-9-cis- Bacterioruberin (d)	Not available	Not available
9-cis-26-cis- Bacterioruberin (e)	Not available	Not available

(a) to (e): The presence of these bacterioruberin isomers was reported in some research papers, but the clear or exact chemical structure and IUPAC name of the compound were not available. [(a) 15-cis- Bacterioruberin [53], (b) 5-cis-9-cis- Bacterioruberin [20, 53, 72], (c) 5-cis-26-cis- Bacterioruberin [42], (d) 9-cis-9-cis- Bacterioruberin [20, 30], (e) 9-cis-26-cis- Bacterioruberin [42]]

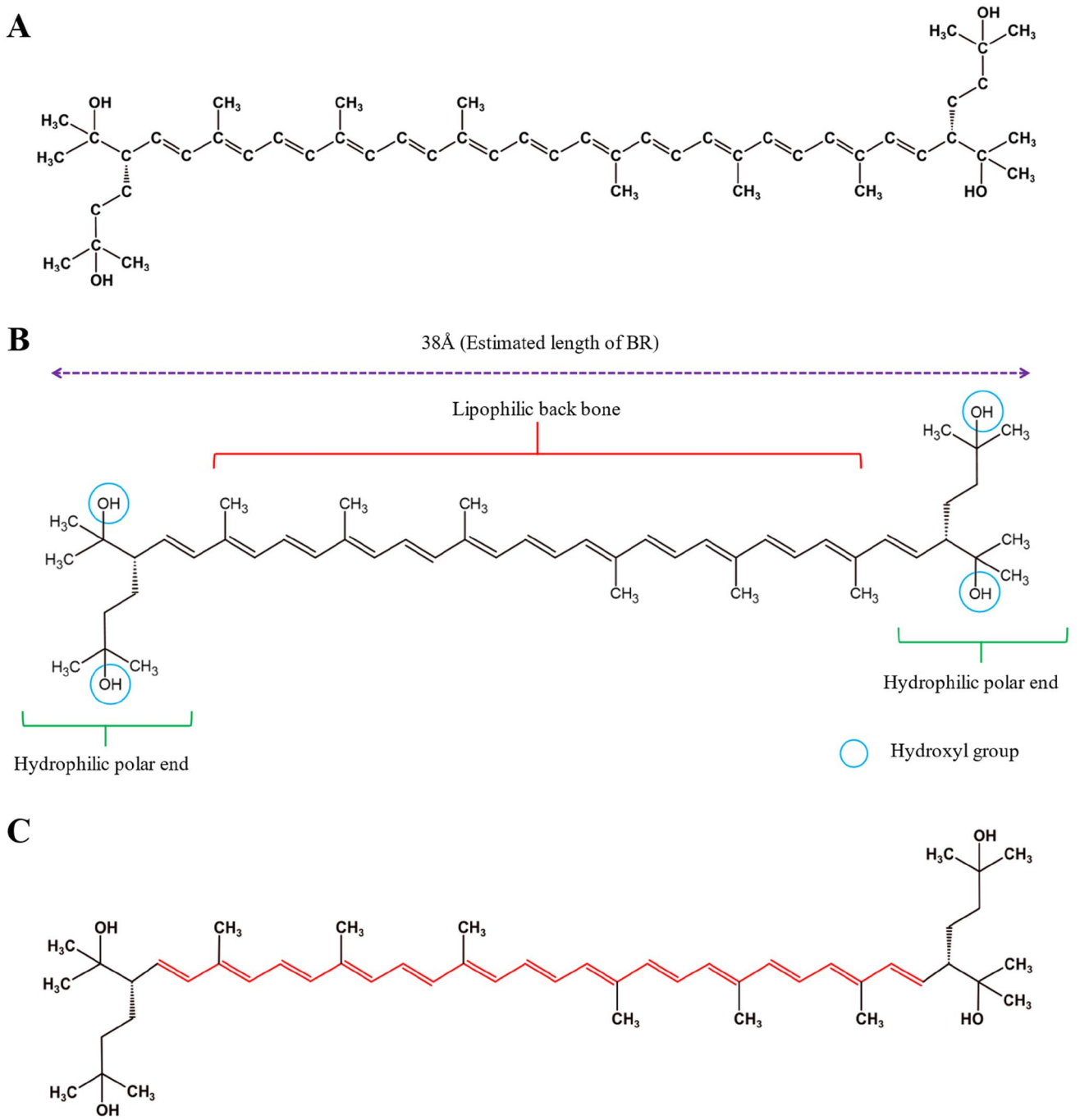


Fig. 2 **(a)** Chemical strcuture of bacterioruberin; **(b)** Structure of bacterioruberin; **(c)** Chemical structure of bacterioruberin: the thirteen conjugated carbon–carbon (C=C) double bonds (red) that together form the chromophore (aliphatic tridecaene chromophore) of the molecule are highlighted in red

and the C50 carotenoid BR [95–97]. LyeJ, CrtD, and CruF are the three key enzymes responsible for the conversion of lycopene to BR [8, 96].

The conversion of lycopene to dihydro-isopentenyl-dehydro-rhodopin (DH-IDR) is one of the crucial intermediate steps in BR biosynthesis. This reaction is catalyzed by the bifunctional lycopene elongase and 1,2-hydratase enzyme (LyeJ), which also plays a key role in converting isopentenyl-dehydro-rhodopin (IDR) to dihydro-bis-anhydro-BR (DH-BABR) and DH-IDR to tetrahydro-bis-anhydro-BR (TH-BABR). Carotenoid 3,4-desaturase (CrtD) is known to play important roles in the conversion of dihydro-isopentenyl-dehydro-rhodopin (DH-IDR) to isopentenyl-dehydro-rhodopin (IDR) and dihydro-bis-anhydro-BR (DH-BABR)

Table 3 Derivatives/metabolic intermediates of bacterioruberin

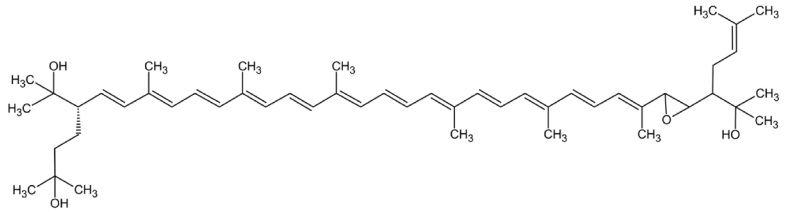
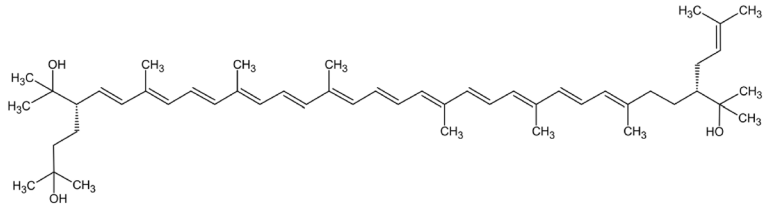
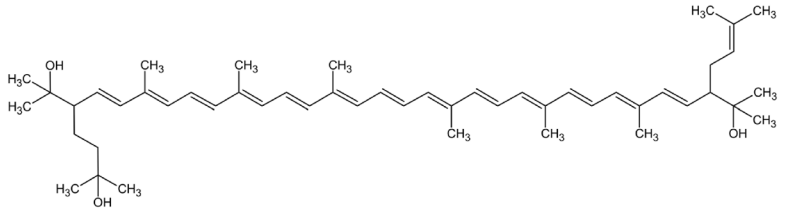
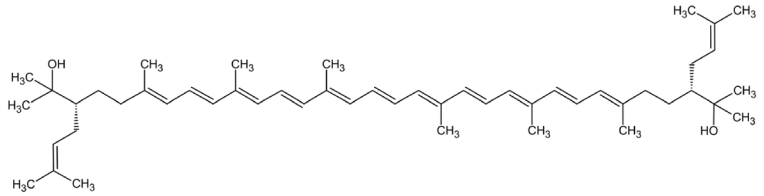
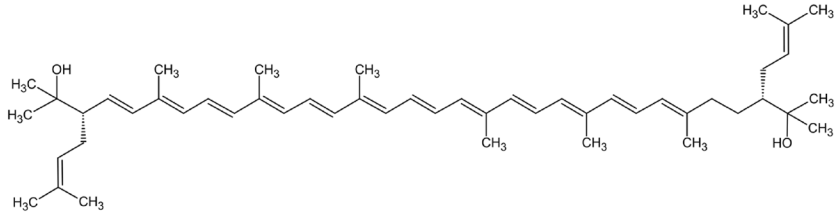
Common name	IUPAC name	Molecular formula	Molecular weight	Chemical structure
3',4'-Epoxy-monoanhydro-bacterioruberin	(2S,2'-,3'-,4'-)-3',4'-Epoxy-2-(3-hydroxy-3-methylbutyl)-2'-(3-methylbut-2-enyl)-3,4-didehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{50}H_{74}O_4$	739.092 g/mol	
Dihydro-mono-anhydro-bacterioruberin (DH-BABR)/3',4'-Dihydro-mono-anhydro-bacterioruberin	(2S,2'R)-2-(3-Hydroxy-3-methylbutyl)-2'-(3-methylbut-2-enyl)-3,4-didehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{50}H_{76}O_3$	725.108 g/mol	
Mono-anhydro-bacterioruberin (MABR)	2-(3-Hydroxy-3-methylbutyl)-2'-(3-methylbut-2-enyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{50}H_{74}O_3$	723.092 g/mol	
Tetrahydro-bis-anhydro-bacterioruberin (TH-BABR)/3,4,3',4'-Tetrahydro-bis-anhydro-bacterioruberin	(2R,2'R)-Bis-(3-methylbut-2-enyl)-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{50}H_{76}O_2$	709.108 g/mol	
Dihydro-bis-anhydro-bacterioruberin (DH-BABR)	(2S,2'S)-2,2'-Bis-(3-methylbut-2-enyl)-3,4-didehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{50}H_{74}O_2$	707.092 g/mol	

Table 3 (continued)

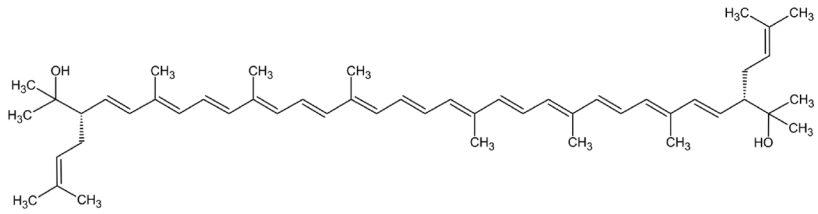
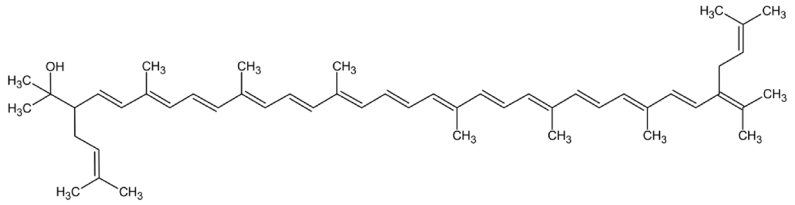
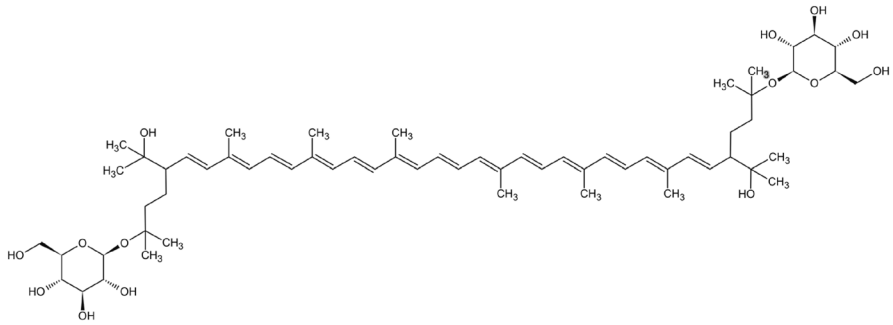
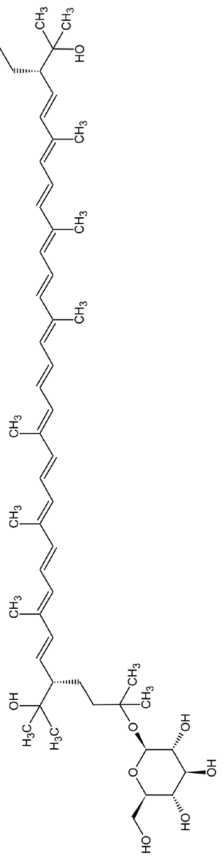
Common name	IUPAC name	Molecular formula	Molecular weight	Chemical structure
Bis-anhydro-bacterioruberin (BABR)	(2S,2'S)-2,2'-Bis-(3-methylbut-2-enyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{50}H_{72}O_2$	705.076 g/mol	
Tris-anhydro-bacterioruberin (TABR)	2,2'-Bis(3-methylbut-2-enyl)-3,4,3',4'-tetradehydro-1,2-dihydro-psi,psi-caroten-1-ol	$C_{50}H_{70}O$	687.06 g/mol	
Tetra-anhydro-bacterioruberin (TTABR) (a)	Not Available	$C_{50}H_{68}$	668.3 g/mol	Not Available
Bacterioruberin tetraglycoside (b)	Not Available	$C_{74}H_{120}O_{24}$	1,386.1 g/mol	Not Available
Bacterioruberin diglycoside	(2S,2'S)-2,2'-Bis-(3-glucosyloxy-3-methylbutyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{62}H_{96}O_{14}$	1065.388 g/mol	

Table 3 (continued)

Common name	IUPAC name	Molecular formula	Molecular weight	Chemical structure
Bacterioruberin monoglycoside	(2S,2'S)-2-(3-Glucosyloxy-3-methylbutyl)-2'-(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-psi,psi-carotene-1,1'-diol	$C_{56}H_{86}O_9$	903.248 g/mol	

Derivatives (a) and (b) were reported with molecular formula and molecular weight, but the chemical structure and IUPAC name of the compound were not available. (a) Tetra-anhydro-bacterioruberin [15, 20, 46], (b) Bacterioruberin tetraglycoside [20]

to bisanhydro-BR (BABR). In addition, it is also known to catalyze the conversion of TH-BABR to DH-BABR. This enzyme facilitates the removal of hydrogen atoms from specific positions within the molecule, leading to the formation of important carotenoids. The enzyme C50 carotenoid 2'',3''-hydratase (CruF) is responsible for the final 2 steps of BR biosynthesis. This enzyme plays a crucial role in the conversion of bis-anhydro-BR (BABR) to BR through two hydration reactions. The first step involves the hydration of bis-anhydro-BR (BABR) to form mono-anhydro-BR (MABR), followed by the second step where mono-anhydro-BR (MABR) is further hydrated to yield BR [95]. The enzymes involved in the biosynthesis of different forms of carotenoids are considered important biocatalysts because they are involved in multiple steps in their biosynthesis. It is also pertinent to mention that enzymes involved in BR biosynthesis are not exempt from their catalytic role in this bioproduction process.

The regulation of BR biosynthesis in haloarchaea is a relatively unexplored field of study. However, recent studies show that the LonB protease (membrane protease), found in the cell membrane of haloarchaea, plays a crucial role in controlling this process. One study clearly demonstrates that LonB deficiency correlates with elevated levels of BR, strongly suggesting the direct involvement of LonB in regulating BR biosynthesis [98]. Furthermore, another study reveals that LonB deficiency induces cellular over pigmentation, indicating alterations in carotenoid production, including BR [99]. Further investigation revealed that LonB protease targets phytoene synthase (PSY), a key enzyme in carotenoid biosynthesis, including BR. The rapid degradation of PSY upon LonB induction underscores the protease's role in modulating BR biosynthesis through targeted degradation of key enzymes like PSY [100]. Moreover, additional research provides compelling evidence suggesting that LonB may selectively recognize specific sequences, such as the C-terminal region of PSY, facilitating its degradation and thereby influencing BR production [101]. Collectively, these findings propose a mechanistic model wherein LonB protease finely tunes cellular BR levels by targeting key enzymes like PSY for degradation, thus intricately regulating BR biosynthesis in response to environmental stimuli.

Chemical Structure of Bacterioruberin and its Derivatives

BR is a member of the xanthophyll family because it contains not only carbon and hydrogen but also oxygen atoms (Fig. 2a). The molecular formula of BR is $C_{50}H_{76}O_4$, and the International Union of Pure and Applied Chemistry (IUPAC) name for BR is (2S,2′S)-2,2′-bis(3-hydroxy-3-methylbutyl)-3,4,3′,4′-tetradehydro-1,2,1′,2′-tetrahydro-y,y-carotene-1,1′-diol. BR was found to be a C50 carotenoid

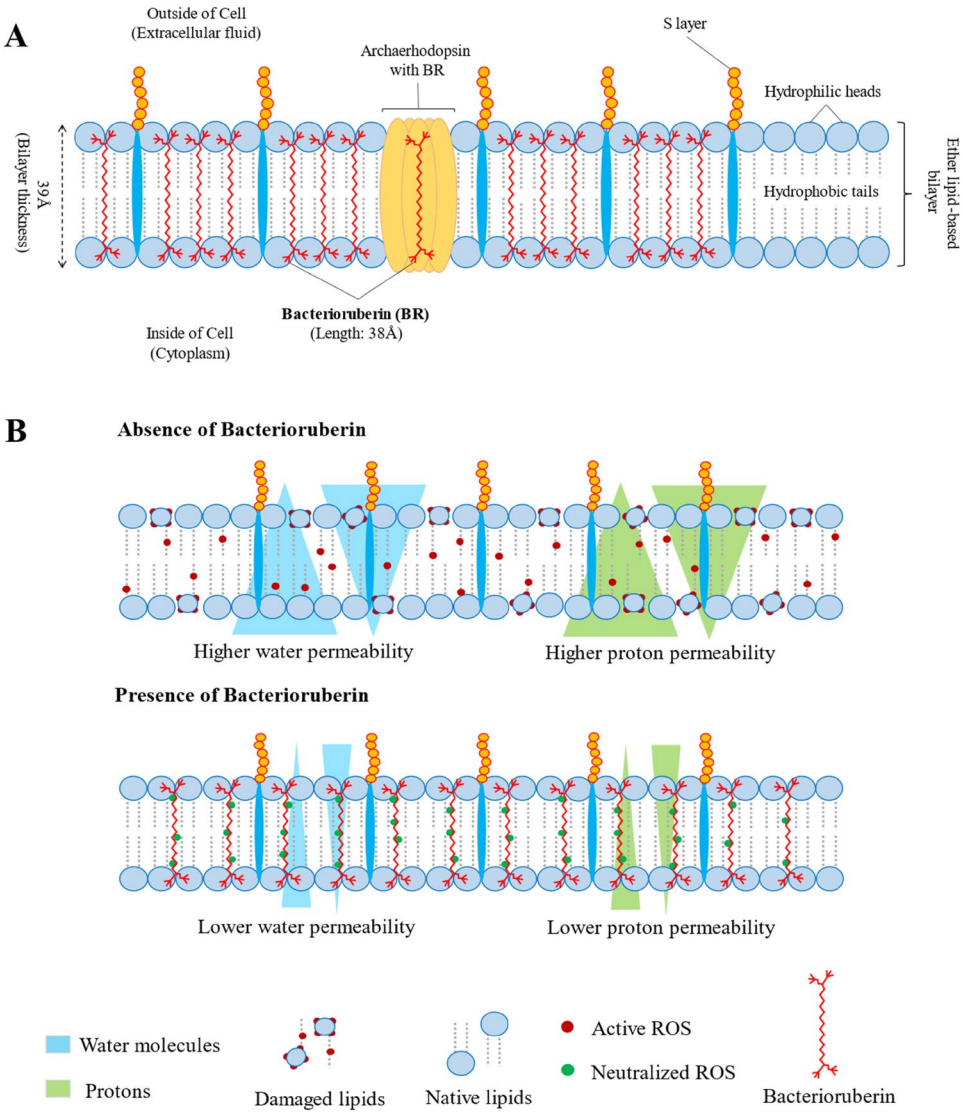


Fig. 3 **(a)** Superior position of bacterioruberin; **(b)** Biochemistry and biological roles of bacterioruberin

with a unique molecular structure compared to other carotenoids. It comprises a primary conjugated isoprenoid chain length of 13 C=C units. Moreover, it has no subsidiary conjugation arising from terminal groups, which contain only four hydroxyl groups [102]. BR is a tertiary alcohol and a tetrol (a polyhydric alcohol with four hydroxyl groups; Fig. 2b) [88]. It is a fat-soluble pigment [15, 103]. BR has a molecular weight of 741.1 g mol^{-1}. The melting point of BR is 225 °C. BR exhibits a series of geometrical isomers (Cis/E or Trans/Z). These isomers arise due to the difference in the positions of the double bonds within the carbon chain (Table 2). The variation in the double bond position gives rise to distinct spatial arrangements, resulting in different physical and chemical properties for each of the isomers. These isomers exhibit a wide range of colours, ranging from deep red to orange. All these isomers are found in natural sources (halophilic archaea). The geometric isomers of BR are all-trans-BR, 5-cis-BR, 9-cis-BR, 13-cis-BR, 15-cis-BR, 5-cis-9-cis-BR, 5-cis-26-cis-BR, 9-cis-9-cis-BR, and 9-cis-26-cis-BR [53]. In addition, different BR derivatives (Table 3) have been identified in various organisms, such as halophilic archaea and bacteria, but have not been studied for their biological activities. All these derivatives are rare and novel compounds that exhibit variations in their chemical structures and possess unique characteristics. Some of these derivatives include mono-, di-, tri-, and tetra-anhydro-BR. In addition, BR has glycosylated derivatives, including mono-, di-, and tetra-glycoside-BR. The varied chemical structures of these derivatives make them promising candidates for further research on their biological activities and therapeutic uses. However, there are no reports available suggesting that BR derivatives possess significant biological activity. Therefore, additional studies are needed to explore the potential benefits and limitations of these derivatives in different biological systems.

Biochemistry of Bacterioruberin

Conjugated systems with < 8 conjugated double bonds appear colourless to the human eye and absorb only in the UV region. With each double bond added, the excitation wavelength increases, requiring less energy to be excited, and the color we observe can range from yellow to red [104]. Therefore, the red color of BR is due to the 13 conjugated double bonds at the centre of the compound (Fig. 2c). The conjugated C = C chain of BR is found in the hydrophobic core of lipid bilayers, with glucose moieties anchored in the hydrophilic region and branched fatty acid moieties curved back into the hydrophobic region (Fig. 3a), thereby reinforcing the cell membrane of halophilic archaea [105]. Variations in the orientation of carotenoids can significantly impact membrane properties. Carotenoids like zeaxanthin, with two polar end groups spanning the membrane, can act as structural "rivets," enhancing membrane rigidity and mechanical strength. Building on this, it was proposed that BR, with its four hydroxyl substituents, could act as a similar "rivet" in haloarchaeal cell membranes. Despite their similar lengths, BR integrates more effectively into lipid vesicles than zeaxanthin or decapreno-zeaxanthin. Therefore, the integration of BR into the haloarchaeal lipid vesicles has some effect on membrane fluidity, acts as a barrier to water, allows permeability to oxygen and other molecules, and increases the rigidity of bilayers (Fig. 3b); thus, strains can survive in hypersaline or low-temperature conditions [62, 106, 107]. In addition, BR has been proven to present greater antioxidant activity than other commercially available carotenoids, such as beta-carotene, ascorbic acid, butylated hydroxytoluene (BHT), lycopene, astaxanthin, alpha-tocopherol, and trolox (a water-soluble derivative of vitamin E) [33, 46, 53, 56], because it can traverse the cell membrane from the inside to the outside (Fig. 3a). Molecular dynamics simulations revealed that the thickness of the archaeal tetraether monolayer is 39 Å. The length of the BR is 38 Å, which indicates that the BR can connect both leaflets of the phospholipid bilayer in specific regions of the cell membrane and easily interact with transmembrane proteins [108]. BRs are not occurred free in cells but appear to be rather firmly bound to proteins. When the cells are lysed by exposing them to low salt concentrations, an almost clear red solution is obtained from which the pigment cannot be extracted by nonpolar solvents. This also applied to cell extracts made through sonic disintegration. When such cell extracts are heated, the pigment remains attached to the precipitated protein. However, the pigment-protein complex splits when the protein is precipitated by polar organic solvents [109]. Microbial rhodopsins (MR) are a class of photoreceptors found in halophilic archaea and bacteria. They are retinal-binding proteins that share a seven-transmembrane structure and a light-sensitive retinal molecule (a primary chromophore) that is covalently bound to a lysine residue on helix G through a protonated Schiff base linkage [110]. MR is also an integral membrane protein that provides light-dependent ion transport, which captures and utilizes sunlight for the synthesis of ATP [111] and sensory functions in halophilic cells. Additionally, it has been found to play a role in an array of biological processes, such as phototaxis. Certain proteins contain not only the retinal chromophore but also a noncovalently bound pigmented carotenoid molecule as the second chromophore to enable their function (e.g., BR, Fig. 3a) [110]. The C50 carotenoid "bacterioruberin" was identified as a second chromophore in some MRs and is thought to protect against photobleaching. Research via crystallographic studies demonstrated that BR is tightly aligned in the crevices between the adjacent protein subunits within the trimer of archaerhodopsins [111–113], cruxrhodopsins [114], deltarhodopsins [115], and halorhodopsins [103, 116].

Bioproduction of Bacterioruberin and its Current Status of Commercialization

Only a few studies have attempted to enhance the bioproduction of BR in halobacteria. Several recent studies on the production of BR from haloarchaea have reported that the production of this rare C50 carotenoid may be readily enhanced by modifying culture conditions, such as pH, oxygen availability, salt concentrations, light incidence, and temperature [117]. Additionally, the biosynthesis of BR has been shown to be induced by the presence of different compounds, such as aniline [118]. One finding suggested that the response surface methodology (RSM) approach is highly useful for determining the optimal conditions of cell culture, such as temperature, pH, and salinity, for large-scale production of BR by haloarchaea [52]. On the other hand, Noby et al. reported that cheese whey-based medium has been proven to be a potent and nutritious supplement for producing BR from *Arthrobacter agilis* NP20. This newly developed, cost-effective medium highlights the great potential for large-scale bioproduction of BR. Furthermore, the results of the study suggested that the use of this rare C50 carotenoid in the food, cosmetics, and pharmaceutical industries could be achieved through the low-cost production of BR from whey-based media [15]. In addition, a few other researchers have worked on the optimization of culture conditions to augment the BR yield and biomass of halobacteria, with a focus on commercial applications [54]. For instance, the yield of

BR from *H. volanii* increased 1.7-fold under low-salt conditions but decreased cell growth under osmotic stress [119]. To address this, a 2-step cultivation of *H. mediterranei* was tested in a 20-L jar fermenter, in which the biomass was first produced under optimal growth conditions and subsequently transferred to a hypoosmotic medium optimized for BR production. This process increased production 6.4-fold in fermented broth [62]. However, this process increases the number of cultivation steps and work needed for production. Furthermore, single-step cultivation of *H. mediterranei* at relatively low salt concentrations under optimized conditions was shown to increase both the BR yield and biomass concentration. At a salt concentration of 230 g/L, this species yielded 125 mg/L total carotenoids and a maximum cell density of 7.7×109 cells/mL. This remarkable increase in productivity represents the highest production ever reported for a wild-type strain. This increase corresponds to a 4.4-fold increase in yield and a 20% increase in biomass [120]. Additionally, hyperpigmented mutants, known as HVLON3, exhibited even more impressive performance, producing BR at an astonishing rate of 3.14 mg/g CDM, which is approximately 15 times higher than that of the wild type (0.2 mg/g CDM). This unprecedented achievement resulted in the highest yield ever observed for haloarchaea [98, 121].

As mentioned before, only a few studies on haloarchaea carotenoid accumulation support the idea that these microorganisms might be good carotenoid producers, specifically for BR and other C50 carotenoid pigments. All these studies reported that accelerating the rapid growth of halophilic archaea requires high salt concentrations (from 20 to 25% w/v) in the culture medium. However, it has been observed that promoting higher level of carotenoid accumulation in halophilic bacteria requires a relatively lower concentration of NaCl, which is < 16% w/v. It was also observed that a lower salt concentration of NaCl leads to slower growth rates or even cell lysis. Therefore, carotenoid accumulation and the growth of halophilic archaea are often inversely related. In addition to the salinity of the culture medium, other physicochemical factors, such as pH and temperature, have been shown to significantly affect the accumulation of carotenoids and the growth rate of halophilic microorganisms [102].

In the current scenario, the only commercially available products from archaea were extracted from halophilic archaea: C50-BR and C30-Squalene (two nonpolar archaeolipids), Bacteriorhodopsin (a membrane-bound protein), and Di- or tetraether lipids. These products do not qualify for “Biotechnological Readiness Level 3” (BTRL 3), which means that even though they are commercialized, none of them are manufactured on an industrial scale. Instead, their demand is fulfilled by selling very small quantities of these products at very expensive prices [22]. Currently, only two companies have commercialized cosmetic products in micro quantities that contain BR as an active ingredient or a BR-rich extract. It is predicted that more companies may enter the market in the near future.

Storage, Stability and Toxicity of Bacterioruberin

The stability of BR under various environmental conditions is not well understood. Investigating the effects of temperature, pH, and light exposure on the stability of BR could provide valuable insights into its potential applications. Mongkol Yachai’s PhD thesis titled "Carotenoid Production by Halophilic Archaea and Its Applications" [63] indicated that BR extracted from *H. salinarum* HM3 exhibited good stability under high-temperature conditions and light exposure in soybean oil and surimi paste (a paste made from bigeye snapper fish). No color bleeding was observed in white surimi gel containing BR under steaming. During refrigerated storage, the color of the surimi gels supplemented with BR slightly changed. Moreover, BR is known to prevent lipid oxidation in surimi gels during storage and does not alter the taste or texture of the gel. BR was found to be stable at 70–90 °C in soybean oil, with 78%-87% retention of the BR content [63]. In addition, this study further investigated the acute oral toxicity of BR from *H. salinarum* HM3 in Wistar rats (50 rats). During the course of the study, fifty rats were divided into 5 groups (5 from each sex) and fed BR in soybean oil at different dosages (0, 125, 250, 500, 1000 mg/kg/day). At 24 h intervals, the Wistar rats were carefully examined for symptoms of toxicity. Both male and female rats fed BR exhibited a slight decrease in body weight gain during the first week. In the second week, no significant differences were observed in male rats, but female rats showed decreases in body weight gain at 1000 mg/kg/day BR. No deaths were observed in any group during the 14-day study, and all surviving rats did not exhibit any major pathological lesions [63]. Therefore, the stability and nontoxicity of BR at high temperatures make it a valuable ingredient for food, pharmaceutical, and nutraceutical applications. Microbial pigments are widely used in textile and paper printing as a long-term alternative to synthetic dyes [122]. According to this, BR’s vibrant red color and stability make it an excellent candidate for the future development of environmentally friendly and safe natural colorants for various industries. Additionally, the growing consumer demand for clean label products has further fuelled interest in natural pigments like BR. As a result, it is inferred that BR can be effectively used in the production of heat-resistant items, food, and textile coloring agents.

Extraction and Analysis of Bacterioruberin

BR is present in the claret membrane of haloarchaeal cells, is slightly soluble in water and is a highly lipophilic molecule

Table 4 Some of the extraction methods, yield, and analysis of bacterioruberin

Organism	Extraction method	Solvents used for extraction	Yield	Analysis and characterization	References
Arthrobacter agilis NP20	Solvent extraction (Cell bleaching/Cell disruption/ Homogenization/Lyophilization/ Freeze–thaw followed by centrifugation)	Methanol	5.13 mg/L	UV–visible spectroscopy, FTIR, HPLC–DAD and HPLC-APCI-MS	[15]
Halorhabdus utahensis		Methanol (containing 0.5% butylhydroxytoluene)	-	RP-HPLC and UV–visible spectroscopy	[30]
Halobacterium salinarium HM3		Ethanol	-	Mass spectroscopy and HPLC	[63]
Haloferax alexandrinus GUSF-1 (KF796625)		Acetone/petroleum ether, Methanol/ chloroform mixture	2 µg/g	HPLC–DAD-ESI–MS(QTOF): UHPLC and Nano-HPLC, TLC, FTIR and UV–visible Spectroscopy	[43]
Haloferax volcanii HVLON3		Acetone/methanol mixture, Acetone	3.14 mg/g	HPLC and GC–MS	[121]
Halopelagius inordinatus RO5-2, *Halogranum rubrum* RO2-11		Acetone	82%	HPLC–MS/MS	[51]
Haloarcula japonica		Acetone/methanol mixture	68.10%	HPLC, UV–visible, NMR, CD, and Mass spectroscopy	[56]
Halorubrum sp. HRM-150		Methanol	84.12%	UV–visible, TLC, and HPLC–MS	[124]
Haloterrigena sp. SGH1		Methanol	50%	HPLC, UHPLC, UV–visible, and Raman Spectroscopy	[46]
Haloterrigena turkmenica		Methanol	74.5 µg/g	RP-HPLC, UV–visible, and Mass spectroscopy	[53]
Halorubrum sp. TBZ126		Acetone/methanol mixture	-	UV–visible spectroscopy, TLC and LC–MS	[55]
Haloferax mediterranei ATCC 33500	Solvent Induced Protein precipitation followed by polishing method	Ethanol and Aqueous solution of Tween 20 (surfactant)	0.37 ± 0.01 mg/g	UHPLC–MS	[125]
Haloferax mediterranei ATCC 33500	"One-pot" recovery of BR using hydrophobic eutectic solvents (HES) and a counter-solvent (Protein-Induced Precipitation)	HES: menthol & levulinic acid mixture, counter-solvent: water	2.13 mg mL^{-1}	H-NMR, FTIR-Attenuated total reflectance (ATR) spectroscopy	[126]

Table 5 Bioavailability of bacterioruberin (according to Lipinski's rule)

	An active compound/drug should posses	Bacterioruberin
Molecular weight (MW)	<450 g mol^{-1}	741.1 g mol^{-1}
Topological polar surface areA (PSA)	<90 Å^2	80.9 Å^2
Hydrogen bond donor count (HBD)	<7	4
Hydrogen bond acceptor count (HBA)	<4	4
XLOGP3-AA (log P value)	≤ 5	13.7

that can be dissolved in organic solvents. Extracting BR pigments involves several steps, including cell lysis, centrifugation, solvent extraction, and chromatography. Cell bleaching by cell lysis involves breaking the haloarchaeal cell membrane through sonication [95, 123], freeze–thawing [53], lyophilization [46], or homogenization [124]. Once the haloarchaea cells have been lysed, centrifugation is used to separate the cell components from the BR-containing supernatant. The supernatant was subsequently subjected to solvent extraction, in which a suitable solvent was used to dissolve and extract BR pigments from other cellular components. Methanol [53, 124], acetone [51], or a methanol/acetone mixture [55, 56, 121] are the most widely used solvents for extracting BR. Chromatography techniques, such as liquid chromatography–mass spectrometry (LC–MS), thin-layer chromatography, gas chromatography–mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC), are being extensively employed to analyse, quantify, purify, and separate BR from other cellular components. BR is characterized by analysing their molecular structure and chemical properties through various techniques. These methods include UV–visible Spectroscopy [15], Ultrahigh-Performance Liquid Chromatography (UHPLC) [125], and Mass Spectrometry (MS) [63] to determine the absorption spectrum (430–530 nm), retention time, and molecular weight (741.1 g mol^{-1}), respectively. Additionally, the functional groups present in BR and its chemical structure were determined by employing Fourier Transform Infrared Spectroscopy (FTIR) [43], Raman Spectroscopy [46], and Nuclear Magnetic Resonance (NMR) spectroscopy [56]. These techniques provide information about the composition and chemical properties of BR. Some of the extraction methods and analysis techniques for BR are listed in Table 4. Most of the studies listed in Table 4 extracted the total carotenoids from archaea and estimated the percentage of BR as a function of total carotenoid content (TCC). It could be inferred that only a few studies have extracted and purified BR to homogeneity.

Bioavailability of Bacterioruberin

The term "bioavailability" describes how much carotenoids are absorbed through circulation and made available for both physiological processes and storage in the human body. Factors such as digestion, absorption, movement, and storage influence carotenoid availability. Sometimes, crystallization of carotenoids can decrease their bioavailability, with only five percent being absorbed in the intestine. However, several investigations have shown that thermal treatment increases carotenoid availability by disrupting cell walls and loosening bonds [5]. The ability of BR to be absorbed and utilized by the human body is referred to as its bioavailability. The potential health benefits of BR, including its antioxidant activity and several other biological properties, have been studied. Understanding its bioavailability is important for determining its effectiveness as a therapeutic agent or nutritional supplement. Due to its chemical lability, poor water solubility, and low bioavailability, the application potential of this compound has significantly decreased, especially for therapeutic uses [127]. Additionally, factors such as dosage, formulation, and individual variations may also influence bioavailability. Until now, there has been no clear evidence to suggest that its bioavailability is limited. However, further research is needed to fully understand how BR is metabolized and distributed in the body. It can be inferred that Lipinski's rule of five methodology might be useful in identifying bioavailability and pharmacokinetic drug properties by employing computational methodologies. According to Lipinski's rule, an active compound or drug (orally active) with good permeability has the following criteria: molecular weight (MW) <450 g mol^{-1}, log P ≤ 5, hydrogen bond acceptor (HBA) <4, hydrogen bond donor (HBD) <7, and polar surface (PSA) <90 Å^2 [128]. According to Lipinski's rule and based on the information available from both the PubChem and carotenoid databases [6], BR has various drug properties, as listed in Table 5, which show almost reasonable drug likeness criteria.

Table 6 Biological properties of bacterioruberin-rich carotenoid pigments and bacterioruberin

Source	Biological activities	References
BR rich carotenoid pigments		
Halorubrum sp. HRM-150 (CGMCC 17350)	Antioxidant activity	[124]
Arthrobacter agilis NP20	Antioxidant activity	[15]
Haloarcula sp. OS (HAE)	Anti-inflammatory activity and Intracellular ROS Assessment	[34]
Haloferax mediterranei R-4 (ATCC33500)	Antiproliferative activity	[133]
Haloarcula sp. A15	Cytotoxicity (induces apoptosis in breast cancer cells) and anticancer activity	[134, 135]
Natronococcus sp. TC6 *Halorubrum tebenquichense* SU10	Antioxidant and the matrix metallopeptidase 9 (MMP-9) inhibition activities	[35]
Halorhabdus utahensis	Hyaluronidase inhibition assay and Antioxidant activity	[30]
Kocuria rosea RAM1	Antibacterial, anti-inflammatory, antioxidant, cytotoxicity, wound healing, anticancer, antidiabetic, and antiviral activities	[26]
Haloferax mediterranei R-4	Antioxidant, Antiglycemic (α-glucosidase, α-amylase), and Antilipidemic (lipase) activities	[38]
Haloterrigena thermotolerans K15	Antioxidant activity	[40]
Haloferax sp. ME16, *Halogeometricum* sp. ME3, *Haloarcula* sp. BT9	Antioxidant, and antibacterial activities	[41]
Halorubrum tebenquichense Te Se-85, *Halorubrum tebenquichense* Te Se-86, *Haloarcula* sp. ALT-23, *Haloarcula* sp. TeSe-41, *Haloarcula* sp. TeSe-51, *Haloarcula* sp. TeSe-89	Antioxidant and cholinesterase enzymes inhibitory activities	[42]
Haloferax alexandrinus GUSF-1 (KF796625)	Antioxidant activity	[43]
Halorubrum sp. BS2	Antioxidant, and antibacterial activities	[136]
Natrialba sp. M6	Anticancer and antiviral activities	[132]
Haloferax volcanii HVLON3	Antioxidant activity and enhance sperm cells viability	[121]
Halomonas aquamarine, Aquisalibacillus elongatus, Salinicoccus sesuvii	Antioxidant, antifungal, and antibacterial activities	[50]
Halogeometricum rufum RO1-4, *Halogeometricum limi* RO1-6, *Haladaptatus litoreus* RO1-28, *Haloferax Haloplanus vescus* RO5-8, *Halopelagius inordinatus* RO5-2, *Halogranum rubrum* RO2-11, *Haloferax volcanii* CGMCC 1.2150	Antioxidant, anti-haemolytic, and anticancer activities	[51]
Haloterrigena turkmenica DSM-5511	Antioxidant activity	[53]
Halobacterium halobium M8	Antiproliferative and antioxidant activities	[59]
Halococcus morrhuae, Halobacterium salinarium	Antioxidant activity	[60]
BR rich cell suspension/halo-archaea extracts		
Halobacterium salinarum ET 1001	Antioxidant activity	[137]
Haloarcula hispanica HM1, *Halobacterium salinarum* HM2	Antimicrobial, neuroprotective (Acetylcholinesterase), antidiabetic (α-glucosidase, α-amylase), antioxidant, anti-inflammatory (Cyclooxygenase 2) and anti-algal activities	[17]
Halobacterium salinarum ATCC 33170 (Formerly *Halobacterium cutirubrum* or *Halobacterium salinarum* NRC 34002)	Resistant to DNA damaging agents (H_2O_2, mitomycin-C), Photoprotective (Gamma-irradiation), and Radio protective (UV) activities	[68]
Bacterioruberin (pure)		
Haloterrigena sp. SGH1	Cytotoxicity and antioxidant activity	[46]
Haloarcula japonica TR-1 (JCM 7785 ^{T})	Antioxidant activity	[56]
Halobacterium salinarum HM3	Antioxidant activity	[63]
Rubrobacter radiotolerans	Antioxidant activity	[131]
Bacterioruberin loaded nanoparticles/nanovesicles		
Halorubrum tebenquichense	Anti-proliferative, anti-inflammatory, antioxidant, anti-psoriatic and anti-*Staphylococcus aureus* activities	[138]
Halorubrum tebenquichense	Antioxidant, cytotoxicity, anti-haemolytic and anti-inflammatory activities	[127]
Halorubrum tebenquichense	Anti-inflammatory and antioxidant activities	[139]

Biological Properties of Bacterioruberin

The carotenoids produced by halophilic archaea exhibit a stronger antioxidant capacity than the carotenoids produced by other microbes (whether they are extremophilic or not). BR, a fat-soluble, bright red carotenoid pigment produced by halophilic archaea, has potent and superior antioxidant activity [15]. According to antioxidant studies on carotenoids so far, the capacity for oxygen-reactive species (ROS) scavenging depends on the concentration of carotenoids. This means that higher concentrations of carotenoids generally result in greater antioxidant activity. Therefore, increasing the concentration of carotenoids in a system can potentially enhance its overall antioxidant capacity. Carotenoids with longer carbon chains and more pairs of conjugated double bonds tend to have greater antioxidant capacities [56, 129]. Additionally, the functional groups and their positions within the carotenoid molecule [129, 130], as well as oxygen-containing substituents [56], can also affect its antioxidant activity. BR contains 13 carbon double bonds, which are more than the nine carbon double bonds of beta-carotene. Therefore, BR is a more effective radical scavenger than beta-carotene is [56, 131]. Furthermore, studies have shown that increasing the concentration of BR can increase its overall antioxidant activity, making it an effective natural antioxidant. On the other hand, carotenoid extracts from halophilic archaea, which are rich in BR, exhibit antimicrobial, anti-haemolytic [51], anticancer [26], and antiviral activities [132]; enhance sperm cell viability during freezing and thawing [121]; and inhibit cholinesterase [42]. The potential of BR extract to repair UV-induced damage to human DNA strands has led to research into its potential for preventing skin cancer [68]. The various biological properties of the BR and BR-rich carotenoid extracts are listed in Table 6. Most of the studies so far have used BR-rich total carotenoid pigment extracts and evaluated the biological properties of total carotenoid pigments. However, further investigations are needed to determine the individual contributions of BR and its potential applications in various fields. Currently, there is limited research on the specific effects of BR. Most related studies have evaluated its anti-inflammatory and antioxidant effects (Table 6). Therefore, there is still a lack of comprehensive understanding of its other potential benefits. Further research is needed to explore the potential therapeutic applications of BR beyond its antioxidant and anti-inflammatory properties. Investigating its impact on immune function, cellular signalling pathways, and disease prevention could provide valuable insights into its biological properties. Additionally, studying the safety profile and potential side effects of BR is crucial for its future use in clinical settings.

Current Applications of Bacterioruberin

Animal Uses

Aquaculture industry: BR is considered a potential feed additive in aquaculture. Metazoans that thrive in salt get their food from halophilic archaea. For instance, *Artemia* may thrive by consuming nutrients from a mono-diet that is based on halophilic archaea [140]. In one study, researchers reported that the feed containing *Haloferax volcani* improved *Artemia nauplii* biomass production and antioxidant content, with BR being the major contributor [141]. In another study, Wei et al. applied carotenoids containing Archaea *Halorubrum* to aquaculture for the first time. The *Halorubrum* strain used in their study is a high-BR-producing halobacteria. The *Halorubrum* strain was fermented in a culture medium, and the cells were fed to *Litopenaeus vanammei* post-larvae through *A. nauplii* enrichment. The results showed that *Halorubrum*-enriched *A. nauplii* improved *L. vanamme* survival and growth. The study discovered that dietary supplementation with *Halorubrum* had a beneficial impact on *L. vanammei*'s ability to tolerate osmotic stress and ammonia stress. This could be linked to the antioxidative capacity of BR, which exists in the archaea. The results suggest that the red halophilic archaea *Halorubrum* could be a useful feed supplement in shrimp larviculture [7].

Human Uses

Cosmetic industry: HALOTEK, a Germany-based company, has recently launched a product called Halorubin, which is a skincare product made from a natural haloarchaeal ingredient, BR [142]. Another company, ADEKA, a Japanese company, uses halorubin (a BR-rich source) as an active ingredient in more than 15 COSMOS-approved cosmetic products. Some of the ADEKA products that have halorubin as an active ingredient are Pure Serum Retinal, Pure Serum Retinal Jellified, Perfect Eye Contour, Regenerating Retinal Face Cream, Transparent Pectin Gel Lotion, Crystal Make-Up Remover, The Oléo Cleansing Balm, Clear Night Scalp Serum, Natural Concrete Perfume, Crystalline Hand Gel Care, Silky Body Spray, Purified Anti-Acne, Blue Light Power Mask, and Refresh Transparent Cleansing Oil [143]. These halorubin-based products are found to provide several healthcare benefits for the skin. Its unique formula combines the power of BR with a variety of advanced skincare solutions. These products offer a comprehensive barrier against environmental stressors due to their ability to protect against ultraviolet and the consequent DNA damage. Furthermore, its radical scavenging activity helps to combat signs of aging and promote skin rejuvenation, making it an ideal choice for

those looking for effective anti-pollution, sun care, and regenerative face cosmetics [143].

While BR currently finds applications in aquaculture and cosmetics, its potential biological properties suggest it may have future applications in other industries, including food and pharmaceuticals. Research and development in this area may uncover additional uses for BR in the future.

Limitations and Conclusion

According to the current literature, BR has various biological properties, including protection against DNA damage; antioxidant, antimicrobial, anti-inflammatory, and anticancer activity; and use as a natural food colorant. Because of these properties, there will be increased demand for BR in the near future. However, the effects of BR on human nutrition, metabolism, and intracellular targets have not yet been unequivocally reported. Until now, BR has not been used in preclinical trials due to constraints related to limited biological sources, optimized cell culture conditions, and a lack of understanding of its potential therapeutic applications. Its unique structure and biological properties and recent advancements in research have sparked interest in exploring the therapeutic applications of BR, opening doors for future clinical research. Accordingly, it is being emphasized that intense research is needed to fully understand the benefits and limitations of BR in preclinical settings before progressing to clinical trials. Furthermore, there is a scarcity of research on the potential health benefits of pure BR. Many of the biological properties discussed in the literature are based on BR-rich extracts. Thus, the existing lacuna in research findings and the consequent speculation related to the projected biological properties of pure BR need to be substantiated with appropriate methodologies and research studies. Hence, there is a need for extensive research to evaluate the possible biological properties of BR. Additionally, it is important to investigate the potential interactions of BR with other compounds commonly found in environmental samples to better understand its stability and potential applications. Further, it is crucial to explore the effect of different storage conditions, such as temperature and light exposure, on BR's longevity, which will provide valuable insights for its practical use in various industries. Overall, a comprehensive understanding of BR's stability and toxicity profile is crucial for its successful integration into biotechnological and biomedical applications. Currently, there is no synthetic source of BR available; it is predominantly present in natural resources such as halophilic archaea. Despite the fact that haloarchaea species have the innate ability to produce BR, especially under optimized culture conditions, the production of BR in a laboratory setting and on large scales remains a challenge, making it difficult to investigate its potential applications and benefits. Only a few studies have been conducted on the optimization of culture conditions for haloarchaea to produce BR under in vitro conditions. Accordingly, further investigations are needed to fully understand and maximize its potential for commercial use. There is growing interest in optimizing culture conditions for high production of BR as well as in searching for alternative production methods due to the limitations in the in vitro production of BR from haloarchaea. Researchers are actively investigating synthetic and biotechnological approaches for increasing the production of BR that involve the utilization of genetic engineering methodologies to augment BR biosynthesis in halobacteria and other hosts. One promising approach involves harnessing the power of CRISPR-Cas9 technology to directly manipulate the genes responsible for BR biosynthesis. This innovative technique holds great potential for significantly boosting BR yields. Moreover, advancements in metabolic engineering offer another avenue for enhancing the metabolic pathways involved in BR bioproduction, thereby leading to increased yields. Furthermore, it is important to explore the feasibility of genetically modifying plants or microbes to serve as a sustainable and cost-effective source of BR production. By employing these cutting-edge approaches, it is hoped that the benefits of BR can be fully assessed and that the demand for BR in sectors such as pharmaceuticals, cosmetics, and food production can be effectively met in the near future. Furthermore, further investigations are needed to understand the intricacies of BR biosynthesis and the regulatory mechanisms of the BR biosynthesis pathway. By delving deeper into BR biosynthesis and its regulation, researchers can uncover the intricate mechanisms underlying BR production and elucidate the factors that influence its production. This knowledge could ultimately pave the way for enhanced production strategies and the utilization of BR in various industries. Additionally, the need for the development of cost-effective and scalable production methods for BR is crucial for its widespread commercialization.

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Author contributions MP performed data collection and analysis and wrote the draft. SR designed and revised the manuscript. All authors have read and approved the manuscript.

Data Availability Statement All the data and materials provided in the manuscript are obtained from included references and available upon request.

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Conflict of interest No Conflict of Interest.

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