Chapter 4

Biocompounds from Haloarchaea and Their Uses in Biotechnology

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Abstract

New advances in the understanding of haloarchaea physiology, metabolism, biochemistry, and molecular biology show that these kinds of microorganisms produce several compounds in response to the extreme conditions of their ecosystems. Thus, the complete metabolic and genetic machineries are fully adapted to nutrient starvation, high sun radiation, and high ionic strength. Due to these adaptations, some of the primary and secondary metabolites produced by haloarchaea are of high interest in terms of potential biotechnological uses. The principal goal of the chapter is to present a review about the main characteristics of these biocompounds and their potential uses in biomedicine, pharmacy, and industry.

Keywords: biotechnology, carotenoids, enzymes, haloarchaea, natural pigments, polyhydroxyalkanoates

1. Introduction

Hypersaline environments such as hypersaline lakes, soils, springs, solar salterns, and rock salt deposits are widely distributed around the world. Organisms inhabiting these ecosystems are characterized by their high salt tolerance/requirements [1]. The organisms living under these conditions are usually termed "halotolerants/halophiles/halophilic organisms." The term "halophilic" means "salt-loving." The most widely used definitions distinguish different categories:

(i) Extreme halophiles: growing best in media containing 2.5–5.2 M salt;

(ii) Borderline extreme halophiles: growing best in media containing 1.5–4.0 M salt;

(iii) Moderate halophiles: growing best in media containing 0.5–2.5 M salt; and

(iv) Halotolerant microorganisms: salt is not an absolute requirement for their growth, but they can grow even in the presence of very high salt concentrations (considered extremely halotolerant if the growth range extends above 2.5 M salt).

These kinds of definitions have proved valuable in the classification of microorganisms based on their relationship to salt [2–5].

Halophilic microorganisms can be found in all three domains of life (Archaea, Bacteria, and Eukarya). However, those that require high salt concentrations for optimal growth (2–4 M NaCl) are mainly archaea grouped into the families *Halobacteriaceae* and *Haloferacaceae*, phylum Euryarchaeota [6]. They constitute the main populations in highly salty environments like marshes or salty ponds, where NaCl is obtained for human consumption [7].

Halophilic archaea (haloarchaea) are mostly aerobic, although some species can grow anaerobically using nitrate as the final electron acceptor (denitrification) [8]. Most of the species are generally red-pigmented. To be alive under these extreme conditions (low water availability, high sun radiation, nutrient starvation, and high ionic strength), halophilic microbes show different metabolic adaptations. Some of the main adaptations are:

(i) Cells accumulate high KCl intracellular concentrations or some osmolytes such as 2-sulfotrehalose to deal with high ionic strength [9]. This "salt-in" strategy is mainly used by haloarchaea and it requires the adaptation of the intracellular enzymatic machinery, as proteins should maintain their proper conformation and activity at near-saturating salt concentrations [1, 3, 4].

(ii) Amino acidic residues predominate on halophilic proteins' surface. Thus, proteins become stable and active within cytoplasms containing high KCl concentrations. Consequently, the proteome of such microorganisms is highly acidic, and most proteins denature when they are suspended in low salt concentration [1, 3, 4].

(iii) Cellular bilayers have different composition and structure [10].

(iv) Genomes from halophilic microorganisms contain significant amount of salt resistance genes [11].

Due to these adaptations, some of the primary and secondary metabolites produced by haloarchaea are of high interest in biotechnology. Thus, several biocompounds such as enzymes [12], carotenoids [13], PHAs/PHBs [14], and halocins (bacteriocin-like peptides) [15] have focused the attention of many researchers around the world. Many of the studies on these subjects were published between the 1990s and the first decade of the current century. However, large-scale industrial applications from archaeal cultures are yet to come. Several technical difficulties must be addressed in the near future to make possible efficient large-scale biotechnological applications using archaea. In that sense, aspects related to fermenter corrosion, for instance, should be properly analyzed.

This work summarizes what has been described so far about biocompounds produced by haloarchaea (mainly enzymes, pigments, and bioplastics), their production at large scale, as well as the potential uses of these biocompounds in biotechnology, biomedicine, pharmacy, and industry.

2. Carotenoids

2.1. Carotenoids: definition, classification, and metabolism

Natural pigments are widespread in all organisms. They provide attractive colors and play basic biological roles in the development of organisms [16]. Among natural pigments, carotenoids are of high interest due to their biotechnological applications and their potential beneficial effects on human health [17–19]. These compounds are the second most abundant naturally occurring pigments in nature ranging from colorless to yellow, orange, and red [20, 21]. The production of these pigments has been described in plants and some microorganisms such as algae, cyanobacteria, yeast, and fungi [22, 23]. From a chemical point of view, carotenoids are hydrophobic compounds, which essentially consist of a C_{40} hydrocarbon backbone in the case of carotenes (i.e., they contain 40 carbon atoms in eight isoprene residues), often modified by various oxygen-containing functional groups to produce cyclic or acyclicxanthophylls. Thus, all carotenoids are characterized by the following common features: long-conjugated chain of double bond and a near-bilateral symmetry around the central double bond [24]. This chain may be terminated by cyclic groups (rings), and it can be complemented with oxygen-containing functional groups [25].

Carotenoids can be classified into different groups using different criteria. Considering the chemical structure and the oxygen presence, two types can be distinguished: carotenes or carotenoid hydrocarbons, composed of carbon and hydrogen only; and xanthophylls or oxygenated carotenoids, which are oxygenated and may contain epoxy, carbonyl, hydroxyl, methoxy, or carboxylic acid functional groups [26]. Lycopene and β-carotene are examples of carotene carotenoids and lutein, canthaxanthin, zeaxanthin, violaxanthin, capsorubin and astaxanthin are xanthopyll carotenoids [27].

These natural pigments are derived from the general isoprenoid biosynthetic pathway, along with a variety of other important natural substances such as steroids and gibberellic acid. In this pathway, mevalonic acid is the starting product which is further transformed into a phosphorylated isoprene upon phosphorylation; this isoprene subsequently polymerizes. During polymerization, the number and position of the double bonds are fixed. The conversion of two molecules of geranylgeranyl pyrophosphate (GGPP) to phytoene, a compound common to all C_{40} carotenogenic organisms, constitutes the first reaction unique to the carotenoid branch of isoprenoid metabolism. From this step, slightly different reactions can be found in different organisms [13].

The synthesis and degradation of carotenes and xanthophylls, the regulation of carotenogenesis, as well as the role of these compounds, have been very well described in plants [20] and mammals [28]. Animals are not able to synthesize carotenoids *de novo*, and consequently, they are acquired through diet. In most of the organisms, carotenoids show powerful antioxidant properties, which directly emerge from their molecular structure [13].

2.2. Carotenoids produced by haloarchaea

Bibliography about carotenoids of extremophile microorganisms is scarce as compared to all information available from other organisms [29]. Nevertheless, it has been demonstrated that most members of the families *Haloferacaceae* and *Halobacteriaceae* can synthesize C_{50} carotenoids, including bacterioruberin (as the most abundant C_{50} in most of the analyzed haloarchaeal species) and its precursors (2-isopentenyl-3,4-dehydrorhodopin (IDR), bisanhydrobacterioruberin (BABR), and monoanhydrobacterioruberin (MABR)) [30, 31]. Bacterioruberin has a rather different molecular structure. It has a primary conjugated isoprenoid chain length of 13 C═C units with no subsidiary conjugation arising from terminal groups, which contain four ─OH group functionalities only (**Figure 1**) [32].

Several other derivatives have been found in minor amounts: 3,4-dehidromonoanhydrobacterioruberin, haloxanthin (which is a derivative of the previous one containing a peroxide end group), and 3,4-epoxymonoanhydrobacterioruberin, identified in *Haloferax volcanii* [33]. Carotenoids such as phytoene, lycopene, and β-carotene are also produced by these species but at lower concentration. Usually, these carotenoids are in the cell membranes, and they provide color to the colonies when haloarchaea cells grow on solid media or sustain the red color shown by salted coastal ponds (mainly in summer).

Thanks to bacterioruberin and its derivatives, haloarchaeal cells are protected against UV sun radiations. They are also involved in the reinforcement of the cell membrane, and they can be part of the rhodopsin complexes (light-driven proton pump highly important for haloarchaea cells to obtain energy) [13].

The effect of several chemical compounds on the C_{50} carotenoids biosynthesis was first described from *Halobacterium cutirubrum* (*Halobacteriaceae* family) [31]. Few years later, it was described that bacterioruberin is in general synthesized from other C_{50} carotenoids, such as isopentenyldehydrorhodopin, bisanhydrobacterioruberin, and monoanhydrobacterioruberin [21]. The synthesis is induced by (i) low oxygen tension and high light intensity [34, 35]; (ii) osmotic stress [36]; and (iii) the presence of different compounds such as aniline (**Figure 1**) [37]. However, this general pattern has some exceptions, for example *Haloquadratum walsbyi*:

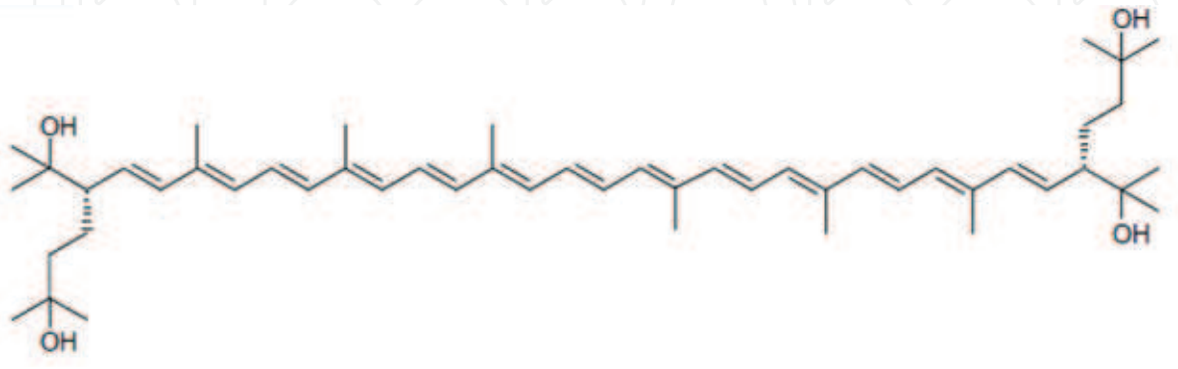

Figure 1. Chemical structure of bacterioruberin.

cells grown under osmotic stress did not experience changes in terms of either membrane lipid content or carotenoid production [38]. Composition of the total carotenoid fraction in haloarchaeal cells can also vary based on the nutritive factors within the culture media [39], the light intensity, oxygen tension, NaCl concentration [39, 40], and other physical-chemical parameters such as the pH value of the culture media.

2.3. Applications and prospects

Few studies stated that some haloarchaeal species (wild-type strains) produce significant concentrations of carotenoids, which are marked highly demanding [13]. Besides, carotenoid production by haloarchaea can be improved by genetic modification or even by modifying several cultivation aspects such as nutrition, growth pH, or temperature.

There are no studies on the potential benefits of the carotenoids produced by haloarchaea on human health reported in the scientific literature up to now. However, there are some patents in which the potential use of haloarchaeal carotenoids in biomedicine and biotechnology has been tested [13]. More efforts should be made not only to explore biotechnological uses of haloarchaeal carotenoids at large scale, but also to open marks related to carotenoid synthesis and degradation in haloarchaea. This knowledge will promote progress in the field of carotenoid metabolic engineering in these microbes, and it will contribute to evaluating the potential use of haloarchaea as sources for carotenoids production at large scale.

3. Enzymes

Enzymes from halophilic archaea are active and stable at high salinity conditions which are environments generally adverse to other enzymes. Compared to non-halophilic enzymes, they are characterized by a relatively higher usage of acidic residues, a low frequency of lysine, and a high occurrence of amino acids with a low hydrophobic character. This composition makes the proteins' surface acidic with a decrease in hydrophobic patches [41, 42]. Analysis of *Haloferax mediterranei* glucose dehydrogenase structure also reveals an absence of very mobile side chains on the surface that allow the formation of a highly ordered multilayered solvation shell. This feature is necessary under the water-limited conditions characterizing salty environments [42]. Halophilic enzymes present thermophilic character too; consequently, they are stable in a broad range of temperatures. Haloarchaea may endure high temperatures in their natural environment, and halophilic protein need to be not only soluble at high salt concentrations but thermostable as well [43, 44].

These unique characteristics make halophilic enzymes very attractive for biotechnological applications. They are also active and stable in media with low water activity as in the presence of organic solvents [45, 46], even at low salt concentrations if they are encapsulated in reverse micelles. Under these conditions, halophilic enzymes could be used in biotechnological applications in non-aqueous media [47, 48].

Many enzymes from haloarchaea with potential interest, such as glycosyl hydrolases, proteases, lipases, and esterases, have been characterized, but no large-scale applications have been reported yet. In this section, the main features characterizing haloarchaeal enzymes suitable to be used for biotechnological applications are described:

3.1. Glycosyl hydrolases

Glycosyl hydrolases are enzymes capable of hydrolyzing glucosidic bonds between carbohydrates. They are classified into 108 families based on amino acid similarities [49]. Among them, starch-hydrolyzing enzymes are of special interest since their substrate has attracted industrial attention in versatile processes, essentially in the food and detergent industries [50]. Hydrolysis of starch demands the coordinated activity of several enzymes. Most of the known starch-modifying enzymes can be found in the glycosyl hydrolase family 13 which includes α-amylases, pullulanases, α-1,6-glucosidases, branching enzymes, maltogenic amylases, neopullulanases, and cyclodextrinases [51].

Some halophilic amylases from Archaea have been characterized [51–55]. Most of them retain their activity at high temperatures. For example, the haloarchaeon *H. mediterranei* secretes an α-amylase showing optimum temperature between 50 and 60°C, but it retains 65% of the maximum activity at 80°C [55]. *H. mediterranei* also has a monomeric extracellular cyclodextrin glycosyltransferase working optimally at 55°C and 1.5 M NaCl, but it is active even at low salt concentrations as 0.5 M NaCl (retaining 65% of its activity) [56]. Cyclodextrins are interesting molecules because of their ability to form inclusion complexes with organic molecules, increasing their solubility in aqueous solutions.

As mentioned earlier, due to the low water activity in the environments inhabited by haloarchaea, many of their enzymes are functional in organic and hydrophobic solvents. For example, the amylase of *Haloarcula* sp. works optimally at 4.3 M salt and 50°C, but the enzyme does not lose its activity at low salt concentrations. Even in the absence of NaCl, it maintains more than 30% activity. The enzyme is also stable in benzene, toluene, and chloroform, showing its potential as a good candidate for industrial applications [45].

3.2. Cellulases and chitinases

Halophilic cellulases have recently generated interest by their application in biofuel production. Plant biomass, which is the starting material, consists mainly of cellulose, hemicellulose, and lignin. Especially the latter one is highly resistant to biodegradation processes, which involves the use of harsh pre-treatments (high temperatures and extreme pH conditions). Alkali pre-treatments can be done using alkaline salts, resulting in pH and salt concentrations like those found in alkaline saline lakes. Besides, ionic liquids (ILs) can efficiently solubilize cellulose, hemicellulose, and lignin under moderate temperatures. Thus, enzymes from halophilic archaea are good candidates to resist the extreme conditions of these processes [57].

Zhang et al. have identified and characterized a halophilic cellulase (Hu-CBH1) from the halophilic archaeon *Halorhabdus utahensis* [57], which is a heat-tolerant haloalkaliphilic enzyme. It

is active in salt concentrations up to 5 M NaCl, pH 11.5, and high levels of ILs. These results indicate that enzymes isolated from hypersaline environments are strong candidates for the development of IL-tolerant enzymes and cocktails capable of releasing monomeric sugars from IL-pre-treated biomass efficiently [57].

Two *Haloarcula* strains with cellulolytic activity were isolated from the saline soil of Yuncheng Salt Lake, China [58, 59]. Crude cellulase of strain LLSG7 was a multicomponent enzyme system, which showed endoglucanase, cellobiohydrolase, and β-glucosidase activities [59]. The cellulase secreted by strain G10 was an endoglucanase suitable for soluble cellulose degradation [58]. Both were highly active and stable over broad ranges of temperature, pH, and NaCl concentrations, and they displayed remarkable stability in the presence of non-polar organic solvents. The crude cellulase of strain LLSG7 was applied to hydrolyze alkali-pre-treated rice straw, and the enzymatic hydrolysate was used as the substrate for bioethanol fermentation by *Saccharomyces cerevisiae*. The yield of bioethanol obtained suggested it might potentially be used for its production [59].

Although several cellulases had been isolated previously, Sorokin and co-workers demonstrated for the first time that extremely halophilic archaea can grow in insoluble chitin and cellulose as a sole growth substrate in salt-saturated mineral media, indicating that euryarchaea participate in aerobic mineralization of recalcitrant organic polymers in environments saturated with salts [60].

As cellulases, chitinolytic enzymes have wide-ranging applications, such as the preparation of chitooligosaccharides and N-acetylglucosamines used in the pharmaceutical industry [61]. Chitinases are glycosyl hydrolases that catalyze the hydrolytic degradation of the β-1,4-glycosidic bonds present in chitin. It is one of the most abundant polysaccharides in nature besides cellulose and starch. The main natural chitin sources are the shells of crustaceans, insect exoskeletons, and fungal cell walls. A chitinolytic extremozyme from the halophilic archaeon *Halobacterium salinarum* showed the highest activity in the presence of 1.5 M NaCl, even retaining 20% of its activity in the absence of salt. It is an exo-acting enzyme with potential interest regarding the biodegradation of chitin waste or its bioconversion into biologically active products.

Hou et al. have identified the genes and enzymes involved in chitin catabolism in *H. mediterranei*, being the first time that this process has been described in haloarchaea. The study demonstrates that *H. mediterranei* can use colloidal or powdered chitin for both cell growth and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) biosynthesis (see Section 4), suggesting the potential of this strain for conversion of chitin into valuable bioplastics [62].

3.3. Proteases

Proteolytic enzymes are used to produce pharmaceuticals, foods, detergents, leather, silk, and agrochemical products. In terms of production, proteases represent the heart of the global market for enzymes [63].

Proteases from halophilic microorganisms have been reviewed recently [64]. In general terms, haloarchaeal proteases show optimum activity at high salt concentration, although some of

them can be stable and active at lower concentrations. The extracellular endopeptidase from *Halobacterium halobium* hydrolyzes polypeptides and oligopeptides with specificity for hydrophobic amino acids, especially proline [65]. The enzyme exhibits azocasein activity at low salt concentrations. This endopeptidase could be an important tool to be used in the food processing industry as well as in biomedical applications to produce peptides [65, 66].

Kim and Dordick studied the stability of a protease from *H. halobium* in different aqueous/organic solvent mixtures, and they observed that it correlates strongly with the salting-out ability of the solvent [46]. They concluded that solvents which act to increase the apparent hydrophobicity of the enzyme's core stabilize it in the same way as salting-out salts [46]. Finally, the p-nitrophenylphosphate phosphatase from *H. salinarum* was dissolved in an organic medium by creating a reverse micellar system at very low salt concentration and, under these conditions, the enzyme was active and stable [67]. The possibility of using these enzymes in hydrophobic medium increases the potential biotechnological applications.

3.4. Esterases and lipases

Esterases and lipases hydrolyze ester bonds between a fatty acid moiety and an esterified conjugate, such as a glycerol or phosphate. Lipases preferentially hydrolyze triglycerides composed of long-chain fatty acids while esterases usually hydrolyze water-soluble esters, including short-chain fatty acid triglycerides. Both enzymes have applications in food modification, detergent formulation, cosmetic, pharmaceutical, leather, textile, paper industries, biodiesel and biopolymer production, or pre-treatment of lipid-rich wastewaters [68]. These applications often require aggressive reaction conditions: high temperatures to sustain biocatalysis, organic solvents as part of the reaction mixtures, or presence of high salt concentration. Thus, extremophilic enzymes (especially halophilic enzymes) can be a very interesting option. The biotechnological applications of halophilic lipases and thioesterases have been reviewed recently [69]. Bhatnagar et al. isolated 54 Halobacteria from a salt lake in the Algerian Sahara and screened 35 of these strains [70]. Among them, strain TC6 (belonging to the *Natronococcus* genus) was selected for further study. It contains an extracellular lipase that was optimally active at 4 M NaCl, pH 7, and 50°C, and it was more active against the substrate p-nitrophenyl palmitate (C16). The enzyme hydrolyzed olive oil, indicating the presence of a true lipase, being the first one reported in archaea [71]. After that, a total of 118 halophilic archaeal strains were screened for lipolytic activity. Five isolates were selected and further characterized, indicating the presence of salt-dependent and temperature-tolerant lipolytic enzymes [72]. Camacho et al. studied the production of esterase and lipase in *Haloarcula marismortui* [73]: they observed high production rates of intracellular esterase and lipase using p-nitrophenyl valerate and p-nitrophenyl laurate, respectively. Two different esterases were detected as active enzymes at 0.5 and 5 M NaCl. Interestingly, in the absence of salt, esterase retained 50% of residual activity [73].

Moreover, Müller-Santos et al. cloned and overexpressed the *lipC* gene from *H. marismortui* [74]. The recombinant protein was purified and biochemically characterized. The enzyme exhibited preference for short-chain fatty acids and monoesters, and its optimum activity was observed in the presence of 3 M KCl while no activity was detected in the absence of salts.

An area of interest for the application of halophilic lipases is biofuel production. With this aim, a lipase from a haloarchaeal strain G41 was purified to homogeneity and characterized [75]. The enzyme displayed high stability and activity in the presence of hydrophobic organic solvents and showed preference toward long-chain substrates, which makes the enzyme suitable for biofuel production. The free and immobilized lipase from strain G41 was applied for biodiesel production, and 80.5 and 89.2% of yields were achieved, respectively [75]. This study demonstrated the feasibility of using lipases from halophilic archaea for biodiesel production.

4. Polyhydroxyalkanoates (PHAs)

4.1. Polyhydroxyalkanoates: definition, classification, and biosynthesis

PHAs are polyesters composed of hydroxy fatty acids, synthesized and stored as insoluble inclusions in the cytoplasm [76]. They serve as intracellular storage material of carbon source and energy. PHAs are produced in the stationary phase of growth, when the medium is deficient in some essential nutrients but a carbon source is available in excess. When it is running out, PHAs are depolymerized, and their degradation products are used for growth [77].

There is a wide variety of types of PHAs, depending on different aspects such as the microorganism strain, the growth conditions, or the carbon source used. To date, over 150 structural variations have been reported [78, 79]. There are different ways to classify these biopolymers: on the one hand, depending upon the number of carbon atoms in the monomers, PHAs are classified into two distinct groups [77, 79]: scl-PHAs (short-chain length PHAs), whose monomers consist of 3–5 carbon atoms, and mcl-PHAs (medium chain length PHAs), composed of monomers having 6–14 carbon atoms; on the other hand, PHAs can be classified depending on the type of monomers that form them [78, 79]: the homopolymers, made of identical monomers, include PHB (poly-3-hydroxybutyrate), P3HP (poly-3-hydroxypropionate), P4HB (poly-4-hydroxybutyrate), PTE (polythioester), PLA (polylactic acid), and PHV(polyhydroxyvalerate), among others; copolymers (also called heteropolymers), derived from more than one species of monomer like PHBV (polyhydroxybutyrate-valerate). In addition, the variety of PHAs is higher considering more aspects, such as the structure of their side chains (saturated or not) and the presence of aromatic or halogenated groups in their monomers [77].

PHAs are synthesized by four natural pathways [78]: pathway I, that converts sugar to acetyl-CoA, acetoacetyl-CoA to 3-hydroxybutyryl-CoA which is polymerized to PHB; pathway II, that begins from fatty acids to produce R-3-hydroxyacyl-CoA monomers for PHA synthesis via β-oxidation cycle; pathway III, that converts acetyl-CoA, malonyl-CoA to 3-ketoacyl-ACP into R-3-hydroxyacyl-CoA monomers; pathway IV converts butyric acid to S-3-hydroxybutyryl-CoA, then to acetyl-CoA to form PHA monomers.

All these metabolic pathways end with monomer polymerization to produce PHAs. The enzymes responsible for this reaction are PHA synthases. Therefore, it is considered that the key

enzymes for PHA production are the polyester synthases [76, 78, 80]. In the Bacteria domain, where these kind of proteins have been studied extensively, they are divided into four classes depending on the subunit composition and their substrate [76, 77, 80].

Class I and II are PHA synthases that consist of only one type of subunit (PhaC) with molecular masses between 61 and 73 kDa [80]. The difference lies in the substrate specificity: while class I PHA synthases utilize CoA thioesters of various 3-hydroxy fatty acids comprising 3–5 carbon atoms, class II PHA synthases use CoA thioesters of 3-hydroxy fatty acids with 6–14 carbon atoms [76, 80].

The PHA synthases of class III consist of two subunits, PhaC and PhaE, both with similar molecular weight (around 40 kDa). These kinds of enzymes prefer as substrate CoA thioesters of 3-hydroxy fatty acids with three to five carbon atoms [80].

Class IV PHA synthases are like class III but the PhaE subunit is replaced by PhaR (with molecular mass around 20 kDa). They use 3-hydroxy fatty acids with three to five carbon atoms as substrate [80, 81].

4.2. Haloarchaea as polyhydroxyalkanoate producers

Polyhydroxyalkanoates have been extensively studied in the Bacteria domain, from biochemical and molecular biology points of view. However, in the Archaea domain, knowledge is more limited, although haloarchaea seem to be good models to produce these biopolymers. PHAs have been found in strains belonging to the genera *Haloferax, Haloarcula, Natrialba, Haloterrigena, Halococcus, Haloquadratum, Halorubrum, Natronobacterium, Natronococcus*, and *Halobacterium* [76].

The archaeal PHA synthases are composed of two subunits, PhaE and PhaC, that are homologous to the class III PHA synthases from bacteria with only two differences: first, they present a longer C-terminal extension in the PhaC subunit; second, the PhaE subunit lacks the hydrophobic and amphiphilic amino acids for granule association and it is much smaller than its bacterial counterpart [82, 83]. All these evidences make that haloarchaea PHA synthases are classified as PHA synthases of class III but in a differentiated subgroup [83].

The advantages of using haloarchaea to produce PHAs are numerous: first, these microorganisms have simple growth requirements; second, the presence of high salt concentrations in their growth media prevents any kind of contamination from other organisms, so the requirements for sterile conditions can be reduced [14, 84]; moreover, the biopolymers obtained can be easily recovered by osmotic shock of cells using media with low salinity or even distilled water [14], so it is not necessary to use any solvents to extract them.

Different haloarchaea strains have been tested as potential PHA producers using numerous carbon sources, fermentation techniques, and downstream steps. The two most studied genera are probably *Haloarcula* and *Haloferax*. Within the genus *Haloarcula, Haloarcula* sp. IRU1 has been the most productive strain, obtaining PHB as biopolymer with a yield of 63% (w/w) of cell dry weight (CDW). Glucose was used as carbon source [85]. Other *Haloarcula* strains tested were *H. marismortui* and *Haloarcula hispanica*, but the yields obtained were 21 and 2.4% (w/w) CDW, respectively [82, 86].

With respect to the *Haloferax* genus, *H. volcanii* and *Haloferax gibbonsii* showed growth in the presence of glucose and yeast extract as carbon and nitrogen sources, producing PHB as biopolymer with yields of 7 and 1.2% (w/w) CDW [86]. However, *H. mediterranei* is probably the best-studied strain in terms of producing PHAs [84, 87, 88]. Besides the advantages that the haloarchaea present to produce PHAs, *H. mediterranei* has a relatively high growth rate and exhibits high production of the copolymer PHBHV instead of PHB [14].

PHBHV is a copolymer form of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). It presents improved properties compared to the homopolymer PHB, which is a brittle plastic that considerably limits its application [84]. Instead, PHBHV has similar properties to polypropylene: high impact resistance, toughness, and flexibility [14].

The main studies using *H. mediterranei* as model organism have been focused on the improvement of the accumulation of PHBHV and the reduction of the costs of production. Cheap carbon sources such as glycerol [89, 90], rice bran [91], rice-based ethanol stillage [92], cheese whey [93], and olive mill wastewater (the effluent of the olive oil industry) have been tested using multistage processes [94, 95] or only one-stage process (**Table 1**) [14].

	Olive mill wastewater [14]	**Hydrolyzed cheese whey [93]**	**Rice-based ethanol stillage [92]**	**Crude glycerol phase from biodiesel production [90]**	**Crude glycerol phase from biodiesel production and γ-butyrolactone [90]**	**Extruded rice bran and starch [91]**
Cultivation mode	Batch shake flasks	Batch reactor	Batch shake flasks	Fed-batch reactor	Fed-batch reactor	Fed-batch reactor
Biopolymer						
Type of biopolymer	PHBHV	PHBHV	PHBHV	PHBHV	PHBHV	PHBHV
3-HV content/ PHBHV (mol %)	6.5	1.5	15.4	10	11–12	–
Yield						
Protein (g L^{-1})	10	7.54	–	5.5	5.6	65.1
PHA concentration (g L^{-1})	0.2	7.92	16.42	16.2	11.11	77.8
PHA/CDM (%)	43	53	71	–	–	55.6

Table 1. Cultivation mode, type of biopolymer produced, and maximum polymer yields for cultivations of *H. mediterranei* in different waste sources.

4.3. Applications and prospects

PHAs have received considerable attention because of their industrial applications as biodegradable and biocompatible polymers [84]. These exhibit similarities with the conventional petrochemical-derived plastics, but they can be biodegradable in different environments [76]. The fields in which PHAs can be applied are varied: in the packaging industry, where they can compete with nondegradable polymers in the production of bottles and containers [96–98]; in biomedicine, as osteosynthetic materials, sutures, and wound dressing due to PHB's compatibility with the blood and tissues of mammals [98]; and in pharmaceutical applications for the controlled release of medicines [84].

Nowadays, there are numerous important companies that develop and commercialize different types of PHAs such as Biomer (Germany), PHB Industrial (Brazil), Bio-on (Italy) or Telles LLC (USA), which sell these biopolymers under the commercial name Metabolix.

In spite of all the advantages that these biopolymers present, their production on a large scale is still complicated: first, the PHAs production cost is still high (7–10 Euros/kilogram) [98]; second, petroleum, as a raw material for conventional plastics, has not increased its price dramatically in the last few years [99]; third, PHA processing is more difficult than petrochemical plastics due to their slow crystallization processes [100]; fourth, PHAs do not have consistent structures and properties compared to conventional plastics [99]. Therefore, nowadays PHAs are not products that can compete with plastics derived from the petrochemical industry yet.

The future objectives for PHA production remain the same as when these biopolymers were discovered: improving the productivity and reducing their costs, innovating in the use of waste carbon sources, improving genetically the microbial strains used, and using shorter downstream steps. In this sense, haloarchaea can be good models for the achievement of these objectives since their nutritional requirements are low and the processes to obtain these biopolymers are easier than in many bacterial species.

5. Conclusions

Haloarchaea (wild-type strains) can produce high concentrations of biocompounds that are of high interest for biotechnological purposes. Consequently, these microorganisms reveal new natural sources from which enzymes, pigments, and other secondary metabolites can be produced. Among other biocompounds of interest, halocins are of special relevance. These are bacteriocin-like substances (antibiotics) capable of killing sensitive halobacterial cells by affecting the bioenergetic steady state across the membrane [101, 102]. Potential uses of these specific antibiotics in biotechnology, pharmacy, and biomedicine produced by haloarchaea remain unexplored. The cells themselves are also promising systems to explore other uses such as biosensors or soil/water bioremediation strategies [8, 103]. Nevertheless, great effort must be made in the near future to scale-up the engineering tools required to produce biocompounds from haloarchaea at high concentrations or to use haloarchaeal whole cells for biotechnological purposes at large scale.

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