Haloarchaea: A Promising Biosource for Carotenoid Production

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Abstract

Haloarchaea are halophilic microorganisms belonging to the *Archaea* domain that inhabit salty environments (mainly soils and water) all around the world. Most of the genera included in this group are able to produce carotenoids at significant concentrations (even wild-type strains). The major carotenoid produced by the cells is bacterioruberin (and its derivatives), which is only produced by this kind of microbes. Nevertheless, the understanding of carotenoid metabolism in haloarchaea, its regulation, and the roles of carotenoid derivatives in this group of extreme microorganisms remains mostly unrevealed. Besides, potential biotechnological uses of haloarchaeal pigments are poorly explored. This work summarizes what it has been described so far about carotenoid production by haloarchaea, haloarchaeal carotenoid production at large scale, as well as the potential uses of haloarchaeal pigments in biotechnology and biomedicine.

Keywords

Haloarchaea · Isoprenoid · Carotenoids · Bacterioruberin · Natural biosources · Microbial blooms

13.1 Haloarchaea

Hypersaline environments represented by hypersaline lakes, soils, springs, solar salterns, and rock salt deposits are widely distributed. Organisms characterized by their high salt tolerance/requirements inhabit these ecosystems (Oren 2015). The organisms living under these conditions are usually termed "halotolerants/halophiles."

Halophilic microorganisms can be found in *Bacteria* and *Archaea* domains. However, microorganisms requiring high salt concentrations for optimal growth are mainly archaea grouped into the families *Halobacteriaceae* and *Haloferacaceae,* phylum *Euryarchaeota*, and *Archaea* domain (Gupta et al. 2016). These halophilic archaea are widely distributed in salty environments such as marshes or salty ponds from where NaCl is obtained for human consumption constituting the main microbial populations in such kind of ecosystems

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(Gupta et al. 2015; Oren 2010, 2013, 2014) (Fig. 13.1).

Halophilic archaea are mostly aerobic, although some species are able to grow anaerobically using nitrate as final electron acceptor (denitrification) (Torregrosa-Crespo et al. 2016). Most of the species are generally red-pigmented. To be alive under these extreme conditions (low water availability and high ionic strength), halophilic microbes have adopted different metabolic adaptations (Imhoff 1986):

(i) Amino acidic residues predominate in halophilic protein surface.
(ii) Cells accumulate high KCl intracellular concentrations to deal with high ionic strength or some osmolytes such as 2-sulfotrehalose (Desmarais et al. 1997).
(iii) Cellular bilayers have different compositions and structures (Mesbah and Wiegel 2012).

Due to these adaptations, haloarchaea have become a good and innovative source of different molecules of high interest in biotechnology such as enzymes able to be active at high temperature and high ionic strength (Madern et al. 2004; Bonete and Martinez-Espinosa 2011), PHB and PHA (Fig. 13.2), and carotenoids (Rodrigo-Baños et al. 2015). Besides, new roles for haloarchaea in wastewater bioremediation processes have also been reported (Bonete et al. 2015; Nájera-Fernández et al. 2012).

13.2 Haloarchaea-Based Biotechnology

Currently, biotechnology has great significance in many aspects, both industrial and on daily life. The use of several biomolecules such as enzymes as biocatalysts, antibiotics, and bioplastics is well established, and it has been the subject of numerous texts and revisions (Margesin and Schinner 2001). All halophilic microbes, particularly haloarchaea, show their specific metabolic pathways adapted to extreme conditions. Because of that, they are considered as natural sources from which natural biocompounds can be isolated and even produced at large scale. Consequently, and more and more with increasing intensity, there are functions that apply or intend to archaea-derived materials.

Halophilic archaea offer a multitude of actual or potential biotechnological applications. For example, the extremely stable lipids of membranes of these organisms represent a novel drug delivery system (Oren 2010; Patel and Sprott 1999; Schiraldi et al. 2002; Zhao et al. 2015). Bipolar structure of archaeal lipids offers opportunities for protein-lipid interactions (De Rosa et al. 1994). Liposomes with thermostability can be obtained with archaeal lipids (Gambacorta et al. 1995).

Self-assembling components from *Archaea* such as the S-layer glycoprotein and bacterioopsin are of interest for their nanotechnological potential (Oesterhelt et al. 1991; Sleytr et al. 1997). Polysaccharides secreted from haloarchaea could find use in the oil industry (Rodriguez-Valera 1992), while polymers secreted also from haloarchaea have been tested as a raw material of biodegradable plastics (Fernández-Castillo et al. 1986) (Fig. 13.2).

However, several technical difficulties have avoided large-scale industrial applications from archaeal cultures, and fermenters have to be resistant to corrosion by the media required for growth of halophiles. Two extreme halophilic archaea that produce poly-γ-glutamic acid and poly-β-hydroxybutyric acid, respectively (Hezayen et al. 2000), have been cultivated in a bioreactor composed of anticorrosion materials obtaining and accumulating poly-β-hydroxybutyric acid comprising up to 53% of the dry biomass.

Halophilic archaea have also been evaluated for bioremediation, in the treatment of wastewaters of textile industry, for degradation of organic pollutants (Margesin and Schinner 2001), and to accelerate remediation of oil-polluted saline environments (Banat et al. 2000).

Finally, halophilic enzymes can catalyze their respective reactions in non-aqueous environments, in water/solvent mixtures, at

Fig. 13.1 Aerial overview of the Santa Pola saltern ponds. This is an example of natural saline environments from where several extremophiles (halophilic *Bacteria* and *Archaea*) have been isolated

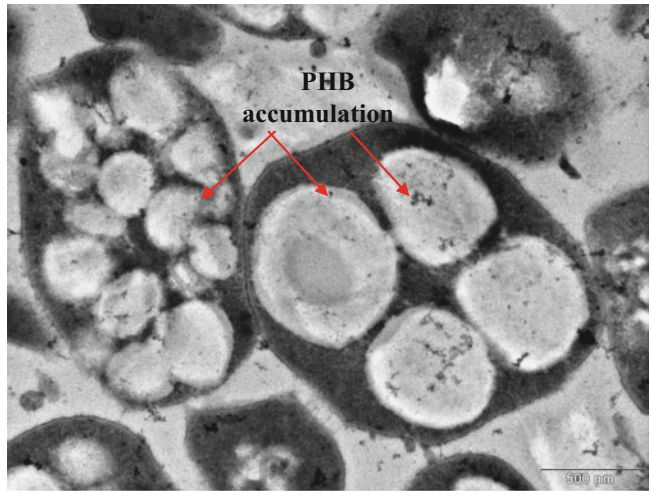

Fig. 13.2 *Hfx. mediterranei* cells. These cells can accumulate significant amounts of PHB when growing under specific conditions (courtesy: Vanesa Bautista)

extremely high pressures, at acid and alkali pH, at temperatures up to 140 °C, or near the freezing point of water (Adams et al. 1995).

13.3 Carotenoids from Haloarchaea

13.3.1 Biological Roles

Bibliography about carotenoids of extremophile microorganisms is scarce if we compare with all information available about carotenoid production from other organisms. Little has been written about carotenoid production by archaea and haloarchea (Naziri et al. 2014). At the end of the 1960s (Kelly and Jensen 1967; Schwieter et al. 1996), a study of carotenoid production from the *Haloferacaceae* family was described.

From that date up to now, it has been demonstrated that C_{50} carotenoids as bacterioruberin (which is usually the main carotenoid from halophilic archaea) and its precursors (2-isopentenyl-3,4-dehydrorhodopin (IDR), bis-anhydrobacterioruberin (BABR), and mono-anhydrobacterioruberin (MABR)) are synthetized by most members of the family *Haloferacaceae* (Kelly and Jensen 1967; Kushwaha et al. 1975).

Other carotenoids as β-carotene, lycopene, and phytoene are also produced by these species but at lower (Goodwin and Britton 1988) or very low concentrations as it happens with lycopersene, *cis*- and *trans*-phytoene, *cis*- and *trans*-phytofluene, neo-β-carotene, and neo–αcarotene. Probably they are used as precursors for the synthesis of other carotenoids including lycopene, retinal, and the members of the bacterioruberin group (Oren 2002).

The most widely analytical method used to identify and quantify carotenoids by halophilic archaea is spectrophotometry after separation or not by thin-layer chromatography or high-performance liquid chromatography. But there are some limitations that the coupling of HPLC with mass spectrometry can solve providing identification based on their molecular mass and their fragmentation with high sensitivity and selectivity (Rønnekliev et al. 1995; Van Bremen et al. 2012). Nuclear magnetic resonance combined to HPLC can help with isomer structure (Lorantfy et al. 2014). Besides, Raman spectroscopy has been used recently to identify common and less common carotenoids (α-bacterioruberin, salinixanthin, and spirilloxanthin derivatives) in model organisms belonging to the genera *Haloferax*, *Haloarcula*, and *Halobacterium* among others (Jehlička and Oren 2013b), and moreover it can be used to quantify carotenoids with a minimal volume of sample. Deeper research in techniques to identify carotenoids with high selectivity and sensitivity are required (Calegari-Santos et al. 2016).

Carotenoid regulation and metabolic pathways in haloarchea are still unknown (Tanaka et al. 2012), even if the first studies were described in the later 1970s, and at that time, synthesis of C_{40} carotenes in *Halobacterium* was described as follows: isopentenyl pyrophosphate leads to trans-phytoene, leads to trans-phytofluene, leads to ζ-carotene, leads to neurosporene, leads to lycopene, leads to gamma-carotene, and finally leads to β-carotene. Difference with pathway in higher plants is that the cis isomers of phytoene and phytofluene are not on the main pathway of carotene biosynthesis, as they are in plants (Kushwaha et al. 1976). Some research has shown that addition of C5 isoprene units to each end of the lycopene chain is the way in which bacterioruberin is synthesized (Kushwaha and Kates 1976; Kushwaha et al. 1975), but may be more than one biosynthetic pathway (Dassarma et al. 2001; Peck et al. 2001). Evidence support that lycopene cyclase (OE3983R) converts lycopene to β-carotene in *Halobacterium salinarum* str. NRC-1 (Peck et al. 2001), although the reactions ranging from lycopene to bacterioruberins are still not well known.

As it can be concluded from the previous section, bacterioruberin is the main carotenoid component responsible for the color of the red archaea of the families *Halobacteriaceae* and *Haloferacaceae*. This pigment is located in the cell membrane and has a rather different molecular structure. It has a primary conjugated isoprenoid chain length of 13 C=C units with no subsidiary conjugation arising from terminal groups, which contain four –OH group

functionalities only (Jehlička and Oren 2013a; Jehlička et al. 2013). Osmotic stress (D'Souza et al. 1997), compounds as aniline (Raghavan and Furtado 2005), low oxygen tension, and high light intensity (El-Sayed et al. 2002; Shand and Betlach 1991) are factors that induce its synthesis.

Bacterioruberin presents an important biological role as antioxidant and it protects cells against oxidative damage. This antioxidant activity is related to the number of pairs of conjugated double bonds, the length of the carbon chain, and the concentration (Albrecht et al. 2000; Miller et al. 1996; Tian et al. 2007). It contains 13 pairs of conjugated double bonds versus the nine pairs of conjugated double bonds of the β-carotene, which makes bacterioruberin a better radical scavenger than β-carotene (Saito et al. 1997; Yatsunami et al. 2014). Therefore, haloarchaea is resistant to strong light, to gamma irradiation, and to DNA damage resulting from radiography, UV irradiation, and H_2O_2 exposure (Kottemann et al. 2005; Shahmohammadi et al. 1998). What it is clearly stated up to now is that the carotenoids of halophilic microorganisms present higher antioxidant capacity than those produced by other microorganisms (extremophilic or not extremophilic).

Bacterioruberin increases membrane rigidity acting as a "rivet" in the membrane cells, a cause of its 4-hydroxyl substitutes in the structure, and also decreases water permeability acting as a barrier and allows permeability to oxygen and other molecules, which makes strains able to survive at low temperature or hypersaline conditions (Fang et al. 2010; Lazrk et al. 1988).

Other biological role of bacterioruberin is being part of rhodopsin complexes. Crystallographic studies have demonstrated that bacterioruberin sustains structural support related to archaerhodopsin that is a retinal protein-carotenoid complex found in the claret membrane of *Halorubrum* sp. as well as in other species (Cao et al. 2015; Feng et al. 2006; Li et al. 2000; Yoshimura and Kouyama 2008) and is used to obtain energy.

13.3.2 Production

Several microorganisms have been proposed as renewable, efficient factories for carotenoid production, microalgae being the most widely studied in that respect (Forján et al. 2015). However, little attention has been paid to the potential of haloarchaea as carotenoid producers in spite of their ability to synthesize and accumulate both C_{40} and C_{50} carotenoids (Rodrigo-Baños et al. 2015).

Several reasons probably explain the limited efforts paid in the use of haloarchaea for carotenoid production (Yatsunami et al. 2014): (a) C_{40} carotenoids have attracted most of the attention in research and development of carotenoid production technology due to their increasing commercial value and the increasing interest in the use of carotenoid producing microalgae to obtain them. However, C_{50} carotenoids which attain specific valuable chemical properties remain to be exploited. (b) No reports on scale-up of carotenoid production processes of haloarchaea have been published or are available. (c) Little information has been published regarding the biomass productivity of standard cultures of haloarchaea species; obtaining high biomass productivity values is a key issue to make a production process of a valuable compound feasible. (d) Though the biosynthetic pathway of bacterioruberin has been mostly described, deeper knowledge on the regulation of the key metabolic steps of the pathway should still be obtained. In addition, deeper knowledge on the influence of physical, chemical, and nutritional parameters on the haloarchaeal growth and on biosynthesis and accumulation of bacterioruberin should enable performing efficient processes of biomass production and pigment accumulation.

Consequently, the still scarce scientific information on biomass production and carotenoid accumulation by haloarchaeal species is an opportunity to study and determine metabolic, physiological, physical, and chemical conditions that might result in efficient production processes of carotenoid-enriched haloarchaeal biomass (Calegari-Santos et al. 2016).

In addition to it, if we have a look at the unique features occurring in the carotenoid producing haloarchaea species, the potentiality of these microorganisms emerges. For instance, haloarchaea species grow at high salt concentrations, and this becomes an advantage to avoid or limit bacterial growth other than the target archaeal species (De Lourdes Moreno et al. 2012). Furthermore, this is a competitive advantage for outdoor production if compared to production of non-halo-tolerant microalgae. The presence of salt is always problematic for many elements of the cultivation system, but a suitable salt concentration can be determined such that it enables growth and limits technical problems to the cultivation system derived from excess salt (Fig. 13.3).

One of the advantages of haloarchaea for production of C_{50} carotenoids is that their biosynthesis can be easily enhanced by transferring the cells from a culture medium of high salt concentration that favors growth (20–25% w/v) to a culture medium with a lower salt concentration (normally below 16% w/v) that favors rapid accumulation of bacterioruberin (D'Souza et al. 1997; Hamidi et al. 2014) (Fig. 13.4). That means that C_{50} carotenoid accumulation and fast cell growth are no compatible processes. Therefore, the feasible production of carotenoids from haloarchaea should be performed through a two-phase process consisting of biomass production under high salt concentration (first) and fast carotenoid biosynthesis and accumulation enhancement under low salt concentration (second).

Once pigments accumulate inside the haloarchaeal cells, the following step to complete the production process is extraction from the biomass. When carotenoid production is carried out from microalgal cells, extraction can become a key step in terms of process costs. Cells of many microalgal species are difficult to break due to a cell wall composition that is highly resistant to standard cell breaking tools, including the freezing-unfreezing of algal pellets in liquid nitrogen or the use of sonication, among others. One of the key advantages of haloarchaeal species for carotenoid extraction is that low salt concentrations induce cell lysis, which therefore avoids cost investments in terms of energy required to enable efficient cell breaking (Asker and Ohta 2002). This means that haloarchaeal cells might be suitable for maximizing pigment recovery eventually at lower costs compared to other microorganisms.

Among the factors that have been reported to influence the accumulation of carotenoids in halophilic archaea, pH, temperature, oxygen concentration, light irradiance, and salt concentration are included (Asker et al. 2002; Fang et al. 2010; Shand and Betlach 1991) (Fig. 13.4). But above the influence of the referred parameters on the accumulation rate of carotenoids, the first condition that it is required to make the process economically feasible is achieving high biomass productivities in the cultures of the haloarchaeal cultures. The few data available about biomass productivity of haloarchaeal cultures were obtained at laboratory scale and suggest biomass productivity values of about 0.08 g L d^{-1} (Rodrigo-Baños et al. 2015). These values are low if compared to those obtained in microalgal cultures. This in principle can be a disadvantage for large-scale production of carotenoids by haloarchaeal species. However, far from being taken as an unbeatable obstacle, the efficient massive production of haloarchaeal biomass must be taking as a challenge. In that respect, efforts might be paid to optimize the culture medium composition and reactor system that enable achieving higher biomass productivities at large scale.

Interestingly, the carotenoids of haloarchaeal species have been reported to accumulate intracellularly up to 20–25 mg g^{-1} (Hamidi et al. 2014). This compares well to the intracellular concentrations of carotenoids reported for several microalgal species. Moreover, such a level of intracellular accumulation of carotenoids, 2–2.5% on dry weight basis, is even higher than most of the data published for carotenoid accumulation of microalgae which are normally below 1% on dry weight basis, except for *Dunaliella salina* for β-carotene production.

As referred, the potential success of haloarchaeal species for carotenoid production lays in the biomass production improvement.

Fig. 13.3 Fermentador Biostat® B (B. Braun Biotech International) successfully used to grow haloarchaea under controlled conditions

There is still large room for improvement of the cultivation process at pre-industrial scale as the available production data in the literature come from laboratory experiences. The use of cheap, raw materials as source of nutrients; the optimization of the culture medium composition for large-scale production; the improvement of the cultivation systems; the development of production strategies at large scale based on two phases, biomass production (growth phase) and carotenoid accumulation (stress phase); and the development of extraction technology coupled to the cell lysis phase are all key factors to approach a feasible carotenoid production process by haloarchaeal species (Fang et al. 2010).

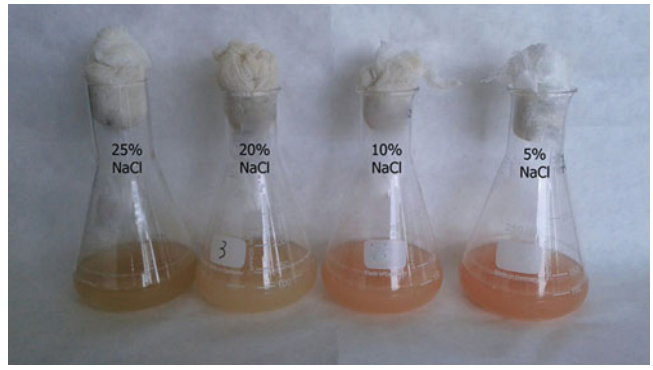

Fig. 13.4 Changes on the pigmentation of *Hfx. mediterranei* cells grown under different salt concentrations. Among the specific conditions that promote large accumulation of bacterioruberin-related pigments, salt concentration lower than 10% has been found to be highly efficient

13.4 Conclusions

Several studies demonstrated that some haloarchaeal species (wild-type strains) produce significant concentrations of carotenoids, which are highly marked demanding. Thus, haloarchaea constitute a promising biosource for carotenoid production at large scale by means of suitable bioprocess engineering tools, namely, specifically designed bioreactors.

The main reasons that make haloarchaea suitable for carotenoid production are as follows: (i) many haloarchaeal species possess high carotenoid production availability; (ii) haloarchaea can grow easily using suitable bioprocess engineering tools (bioreactor); (iii) downstream processes related to carotenoid isolation from haloarchaea are relatively quick, easy, and cheap; (iv) carotenoid production by haloarchaea can be improved by genetic modification or even by modifying several cultivation aspects such as nutrition, growth pH, or temperature; (v) carotenoids are needed to support plant and animal life and human well-being; and (vi) carotenoids are compounds highly demanded by pharmaceutical, cosmetic, and food markets.

There are not studies on the potential benefits of the carotenoids produced by haloarchaea on human health reported in the scientific literature up to now. Thus, more efforts should be made to address not only this question but also other open marks related to carotenoid synthesis and degradation in haloarchaea; such analysis would lead to a better understanding of the spatial distribution and function of different carotenoids and their derivatives in response to environmental and developmental signals. This knowledge may facilitate further progress in the field of carotenoid metabolic engineering in haloarchaea, and it would contribute to evaluate whether or not haloarchaea are good sources for carotenoid production at large scale.

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