Research in Microbiology 174 (2023) 104080

Contents lists available at ScienceDirect

Research in Microbiology

journal homepage: www.elsevier.com/locate/resmic

Review

Archaea: current and potential biotechnological applications

David Aparici-Carratalá, Julia Esclapez, Vanesa Bautista, María-José Bonete, Mónica Camacho*

Department of Biochemistry and Molecular Biology and Soil Science and Agricultural Chemistry, Biochemistry and Molecular Biology Area, Faculty of Science, University of Alicante, Carretera de San Vicente del Raspeig s/n, San Vicente del Raspeig, Alicante, 03690, Spain

A R T I C L E I N F O

Article history:
Received 29 January 2023
Accepted 10 May 2023
Available online 15 May 2023

Keywords:
Biotechnology
Applications
Extremophiles
Archaea
Extremozymes

A B S T R A C T

Archaea are microorganisms with great ability to colonize some of the most inhospitable environments in nature, managing to survive in places with extreme characteristics for most microorganisms. Its proteins and enzymes are stable and can act under extreme conditions in which other proteins and enzymes would degrade. These attributes make them ideal candidates for use in a wide range of biotechnological applications. This review describes the most important applications, both current and potential, that archaea present in Biotechnology, classifying them according to the sector to which the application is directed. It also analyzes the advantages and disadvantages of its use.

1. Introduction

Throughout the history of living beings, there have been numerous classifications that have been made of them. Woese et al. [1] proposed a superior category to the "Kingdom" known as "Domain", classifying living beings into three Domains: *Eukarya*, *Bacteria*, and *Archaea*. They were established following molecular criteria, comparing 16S rRNA sequences. Archaea are single-celled prokaryotic microorganisms and resemble both bacteria and eukaryotes. However, many distinctive features characterize archaea. Four archaeal superphyla are known: *Euryarchaeota*, TACK, DPANN, and Asgard (Fig. 1) [2–5].

Nevertheless, the formal classification of archaea is continually under review due to the constant evolution of microbial identification techniques and the discovery of new members of this Domain. There is another way to classify archaea regardless of their taxonomy, based on their habitat and metabolism, according to their way of life. The main limitation of this physiological classification is that it only includes the extremophile archaea, which develop in places where the environmental conditions are "extreme", which differ from the "standard or normal ones" to which most known microorganisms are adapted. Despite this, this classification is in common use. However, there is no clear physiological classification, since it varies from one author to another. In this review, a general classification created by the authors at their own initiative, with all the information collected, will be used. In this way, the archaea would be divided as shown in Table 1.

Biotechnology is a multidisciplinary science whose primary purpose is to generate and/or improve processes of interest to living beings and the environment [7]. The biotechnological applications are so diverse that they have been classified according to different criteria. The most used classification is based on a color code, where each color represents the sector to which the application is directed. There is a consensus that up to eleven colors can be differentiated. Only eight will be considered (Table 2) because they are related to archaea.

- **Gray biotechnology:** Focused on ecosystems and environmental sciences.
- **Red Biotechnology:** Called Health Biotechnology. Related to biosanitary applications.
- **Golden Biotechnology:** Focused on bioinformatics/bioelectronics and nanotechnology.
- **Green Biotechnology:** Known as Plant Biotechnology. Dedicated to the agricultural sector.
- **Yellow Biotechnology:** Use of living organisms and/or biomolecules in the food industry.
- **Blue Biotechnology:** Applications in marine or aquatic environments: Called Marine Biotechnology.

* Corresponding author.
E-mail addresses: dac42@alu.ua.es (D. Aparici-Carratalá), julia.esclapez@ua.es (J. Esclapez), vanesa.bautista@ua.es (V. Bautista), mjbonete@ua.es (M.-J. Bonete), camacho@ua.es (M. Camacho).

https://doi.org/10.1016/j.resmic.2023.104080

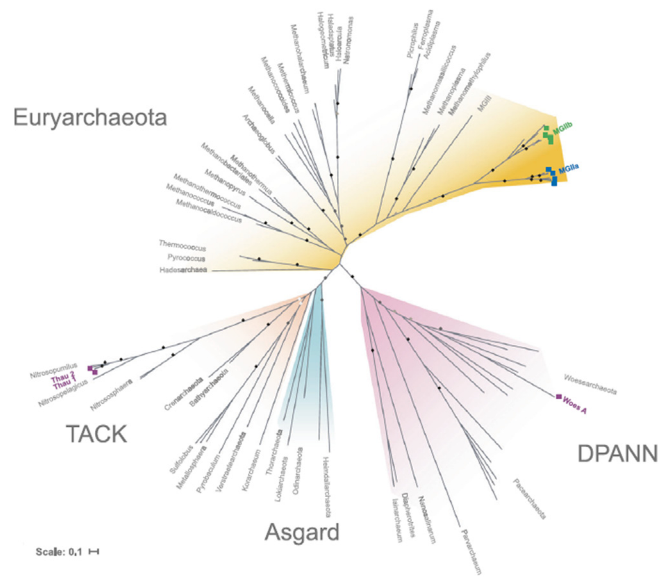

Fig. 1. Phylogenetic tree based on 15 conserved ribosomal archaeal genes. Superphyla present in the *Archaea* Domain. Modified from Orellana et al. [6].

- **White Biotechnology:** Called Industrial Biotechnology. Focused on industrial processes.
- **Brown biotechnology**: Focused on veterinary medicine.

Archaea did not initially have a prominent role in Biotechnology; however, they are currently garnering increasing attention. This review compiles the main applications, both current and potential, that archaea present in Biotechnology. It classifies them according to the sector to which the application is directed and analyzes the advantages and disadvantages of its use.

2. Gray biotechnology

Archaea have great potential to be used in the bioremediation of contaminated environments due to their high resistance and the ability of these organisms to grow in these places. Besides, since temperature, pH, and salinity play a fundamental role in microbial growth, in environments with extreme conditions, many microorganisms cannot grow, which significantly limits the bioremediation process. In this sense, the representatives of *Archaea* are ideal candidates for this function. They use pollutants as substrates to obtain carbon and energy.

One of these environments is the saline wastewater generated by industrial sectors, such as the oil or textile industry. They are often contaminated with toxic substances, which seriously threaten the health and the environment. Therefore, they must be degraded before these waters are reused in other processes or released into the environment [8]. Haloarchaea are potential candidates to bioremediate this type of water due to their ability to grow in high salinity. Numerous investigations show that organic contaminants, e.g., aromatic hydrocarbons, petroleum, or nitrates and nitrites, can be removed by this strategy [9–16]. Methanogenic archaea also participate in the anaerobic treatment of sewage sludge and agricultural and industrial waste, as well as in the treatment of oil spills or the removal of methanol and chlorinated compounds from wastewater [10,17,18]. Similarly, hyperthermophilic archaea can eliminate the phosphate and arsenate present in these waters, in addition to nitrogen [19], as occurs with ammonia-oxidizing archaea [20]. Acidophilic archaea remove sulfur compounds [21]. Metallophiles remove heavy metals, and radiophiles bioremediate nuclear waste [22,23]. Additionally, some archaea resist antibiotics and can degrade them [17]; other archaea also eliminate xenobiotics, such as pesticides [24].

In many cases, remediation is done by physical and electrochemical methods, having the disadvantage that they are expensive, energy-intensive, and inappropriate for large volumes. Biological systems could be a more profitable alternative for the decontamination of ecosystems. However, its application in bioremediation has only been tested on a small scale, mainly due to the need to develop bioreactors and a process monitoring and control strategy, although it is currently being investigated so that archaea can be used [8].

3. Red Biotechnology

3.1. Drug administration

Liposomes are vesicles composed of concentric lipid bilayers alternated with aqueous compartments that allow them to house compounds inside. When archaeal membrane lipids are used in the preparation of liposomes, one with different attributes is obtained, known as "archaeosome" [25]. The physicochemical properties of these lipids are transferred to the archaeosomes, which show remarkable stability in stressful situations (Fig. 2), given that the lipids of archaea are less vulnerable than those of microorganisms belonging to other Domains [15,26]. As a consequence of these characteristics, the use of archaeosomes for biotechnological applications has been proposed.

Archaeosomes have greater encapsulation efficiency than liposomes, which increases their hermeticity and useful life. Such is their stability that some can be stored in oxygen with hardly any degradation [25]. That, together with the fact that the cells of the mammalian immune system react against the lipids present in the archaeal membranes [28], makes archaeosomes ideal agents to be used in the administration of vaccines, acting as scaffolds for antigen presentation, or directly as adjuvants to stimulate the immune system, and are biocompatible [18]. In addition, the uptake of

Table 1
Physiological classification of archaea.

Condition (dependence)	Category	Characteristic
Temperature	Thermophiles	High optimum growth temperature, between 45 and 80 °C.
	Hyperthermophiles	Optimal growth at very high temperatures >80 °C.
	Psychrophiles	Low optimum growth temperature ≤15 °C.
pH	Acidophiles	Growth in places with low pH ≤ 5.
	Alkaliphiles	Very high optimum pH values ≥ 9.
Other features	Halophiles	Environments of high salinity (3–4 M NaCl).
	Piezophiles	Places of high pressure (>1 atm) for their development.
	Radiophiles	Survival to large amounts of radiation.
	Methanogens	Methane production as a byproduct of their energy metabolism.
	Metallophiles	Environments with high concentrations of heavy metals.

Table 2
Classification of Biotechnology by colors.

GRAY	RED	GOLDEN	GREEN
ENVIRONMENTAL 	MEDICINE HUMAN HEALTH 	BIOINFORMATICS NANOTECHNOLOGY 	AGRICULTURAL VEGETABLE 
YELLOW	**BLUE**	**WHITE**	**BROWN**
FOOD PROCESSES 	MARINE AQUACULTURE 	INDUSTRIAL PROCESSES 	VETERINARY 

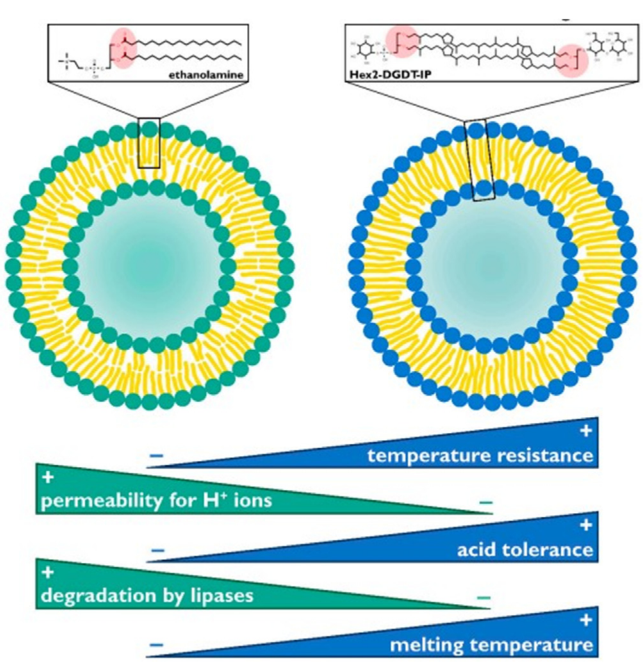

Fig. 2. Properties of archaeosomes. Compilation of some of the properties of archaeosomes compared to conventional liposomes. Modified from Rastädter et al. [27].

archaeosomes by phagocytic cells occurs more easily than that of conventional liposomes, and the application of immunostimulators is not required [25,28]. Therefore, its robustness is a crucial advantage over liposomes in processing and storage.

In the same way, their stability against low pHs and their ability to store drugs enables them to administer in the pharmaceutical industry, even for those that must be administered orally, since they protect against degradation by bile salts in the stomach [29]. Several studies with mice support such applications [30]. They have also been used as insulin transporters and for intravenous drug delivery in cancer therapy [27]. Moreover, archaeosomes have also been proposed in gene transfection in mammalian cells [7,27]. Furthermore, they can be formed at any temperature in the physiological range, facilitating the encapsulation of thermally labile compounds [25]. Nonetheless, the production of archaeal lipids is restricted by the small scale of archaeal fermentation bioreactors and the concentrations of biomass obtained [7], factors that limit its use on a large scale.

Archaeal gas vesicles (GVs) are also emerging as potential drug-delivery vehicles and in vaccine development. The vesicles are synthesized by four species of halophilic archaea (*Halobacterium salinarum*, *Haloferax mediterranei*, *Halorubrum vacuolatum*, and *Haloquadratum walsbyi*) to regulate their buoyancy in conditions of low levels of dissolved oxygen. GVs are essentially composed of two proteins located around additional ones. The main proteins are GvpA, with a structural role, and GvpC, located on the surface and with a stabilizing function [7,15]. GV are not toxic and are effective, stable, immunogenic, and permeable. This biological stability allows them to present the epitope of interest sustainably [31].

Unlike archaeosomes, recombinant GVs are scalable due to their easy production and processing since the cells are efficiently lysed in low salt concentrations. Also, they remain stable without refrigeration over long periods [7] and are resistant to chemical or enzymatic degradation [32], making them ideal for manufacturing vaccines aimed at developing countries that lack the systems required for their storage. Moreover, they can be administered to immunosuppressed people. Despite this, its production has only been recorded on a laboratory scale.

3.2. Blood tests

Glucose dehydrogenase (GlcDH) (EC 1.1.1.47) from *Hfx. mediterranei* is involved in the first step of the modified Entner-Doudoroff pathway, that catabolizes glucose to pyruvate [33]. That also occurs in other archaea, such as the thermoacidophile *Sulfolobus solfataricus*, whose GlcDH is used in commercial kits for analytical purposes, specifically in determining blood glucose [26,34].

3.3. Antimicrobials

"Archaeocins" are proteins secreted by certain archaea with potent antimicrobial activity and different structures from bacterial antibiotics [35]. They exert their effect against archaea related to the archaeocin-producing strain to compete against them, although it is thought that they are also active against the rest of the Domains [36]. To date, only two classes of archaeocins have been found: sulfolobicins, generated by thermoacidophiles of the order *Sulfolobales*, and halocins, produced by haloarchaea of the order *Halobacteriales*. For example, the KPS1 halocin is active against certain Gram-positive and Gram-negative bacteria [37].

A peptide similar to cationic antimicrobial peptides (CAMP), with activity against bacteria, fungi, and protozoa, was identified in *Sfb. islandicus*. This peptide was shown to have a broad-spectrum antibiotic character [38].

However, the use of all these compounds as drugs in biomedicine has been hampered by the lack of knowledge about them due to their recent discovery and not knowing positive results with their application. Besides, haloarchaeal cultivation is challenging because high concentrations of salt are required, leading to bioreactors' corrosion. So, corrosion-resistant materials must be used, thus increasing the cost of the process. Also, the technical difficulties presented by the subsequent purification of the compound of interest must be overcome since a high salt content often does not allow the use of chromatography. Instead, the lysis of the cells can be carried out simply by adding ultrapure water. Likewise, halocins are relatively stable at high temperatures and pH variations and can be stored for long periods [11].

3.4. Disease prevention and/or treatment

The great metabolic diversity of archaea and their low nutritional requirements make them helpful tools for discovering new drugs. For example, although the action spectrum of halocin H6 was initially thought to be restricted to archaea, Lequerica et al. [39] found that it can also inhibit the Na^+/H^+ exchanger (NHE) in mammalian cells. This exchanger is present in all Domains and is involved in the regulation of cytosolic pH. Instead, its dysfunction is associated with heart disease and high blood pressure [13,40]. If halocin H6 is used in these circumstances, cation overload in cardiac cells is avoided and damages the myocardium [39]. Therefore, halocin could be used as a cardioprotective and for the treatment of NHE hyperactivity, and to control blood pressure; since it has a higher specificity of action than other NHE inhibitors and produces fewer side effects [41], although further investigation is still required.

Many studies support the therapeutic properties of some substances from halophilic archaea. For example, Safarpour et al. [42] showed that some metabolites from a strain of *Hbt. salinarum* had a powerful cytotoxic effect against human prostate cancer cell lines, while it did not affect the control group of normal cells.

Secondly, haloarchaea represent a natural source of carotenoid pigments, known for their antioxidant and photoprotective properties [43]. Studies carried out *in vivo* and *in vitro* verify that carotenoids from haloarchaea have chemopreventive and anticancer effects. Indeed, *Hbt. halobium* carotenoids reduce the viability of the cancer cell line HepG2 (liver carcinoma) and the carotenoids from *Haloplanus vescus* and *Halogeometricum limi* [35]. Additionally, studies with extracts of the latter archaea suggest that they can act as erythroprotective agents [44]. The most abundant carotenoid in most species of haloarchaea analyzed is bacterioruberin (BRU), a C50 carotenoid-based on lycopene, although they also synthesize β-carotene, canthaxanthin, and many others in lower concentrations. BRU can prevent human skin cancer by repairing DNA strands damaged by ionizing UV radiation [7,23]. *Hfx. volcanii* BRU is also used in assisted reproduction to increase artificial insemination yields [45]. Therefore, it is necessary to study the potential of haloarchaea to produce pigments useful in biomedicine in the treatment and/or prevention of diseases. Being haloarchaea, The carotenoid extraction protocol from haloarchaea is simple and cheaper than other sources; its synthesis does not negatively impact the environment. However, to favor that these haloarchaea accumulate carotenoids massively, low concentrations of NaCl are required, leading to prolonged growth rates and, sometimes, cell lysis [14].

Just as certain archaeal compounds serve to prevent and/or treat specific diseases, the archaea are also capable of it. In this case, as they are used as probiotics, they are called "archaeobiotics". For example, the methanogenic archaea *Methanomassiliicoccus luminyensis* has been suggested as a biological therapy for trimethylaminuria disease (TMAU) or fish odor syndrome and the prevention of cardiovascular diseases and atherosclerosis. TMAU is a metabolic abnormality caused by the accumulation of trimethylamine (TMA) caused by the body's inability to break it down [46]. That increases TMA levels in the blood and is excreted through body fluids such as sweat, saliva, and urine (Fig. 3). Sometimes the individual has psychological and stress problems, leading to isolation. One possibility is the use of archaea to limit TMA accumulation. Mihajlovski et al. [47] demonstrated the existence of the archaeon *M. luminyensis* in the human intestine and, subsequently, Brugère et al. [48] showed that this methanogen used methylated compounds (including TMA) as acceptors final electrons to produce methane. Then, its use as a therapeutic agent has been raised, applying this methylotrophic archaeon intestinally in patients with TMAU, metabolizing and depleting this metabolite in coordination with its production by the microbiota [7] (Fig. 3).

Also, using these archaeobiotics would reduce the risk of cardiovascular disease and atherosclerosis. The explanation is that by reducing TMA levels in the blood, they contribute to a lower formation of trimethylamine oxide (TMAO), a metabolite with proatherogenic properties [7,46] (Fig. 3). The use of archaea to treat TMAU has several advantages, such as its identification occurring in the same place where it is to be applied because its sensitivity to certain antimicrobials would allow its eradication if it does not work. Furthermore, the methane generated by its metabolism is biologically inert in humans, in the amounts that would be derived from TMA. It is also possible to administer only the enzymes

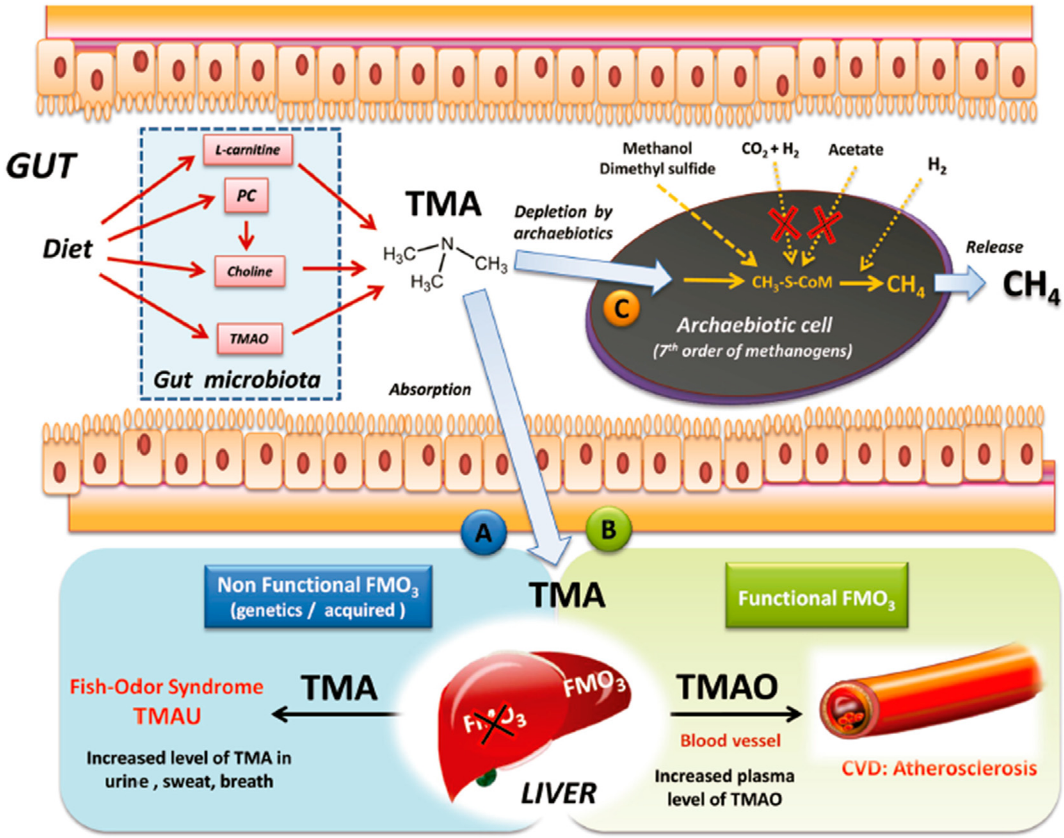

Fig. 3. Archaebiotics concept. Origin of TMA and its fate in the body [48].

involved in the metabolism of TMA instead of using living cells [49]. Despite these advantages, several obstacles must be overcome to use them in treating these diseases. They must be transported to the intestine in anoxic conditions since they are vulnerable to the presence of oxygen. Also, it is necessary to study whether its administration occurs at adequate levels to deplete TMA from a clinical point of view and the possible side effects [48]. Therefore, this archaeon has not been used as a human biotherapeutic product.

Many bacteria produce diketopiperazines, also called cyclic dipeptides, and about a decade ago, it was discovered that the haloarchaea *Haloterrigena hispanica* was also capable of producing them [50]. Diketopiperazines have many applications for medical purposes, but the most interesting function is their ability to activate and inhibit "quorum sensing" systems, through which bacteria control their phenotypic expression based on cell density. That makes these substances a potential therapy for those pathogens that use this genetic information modification system and that cause infections in humans, as is the case of *Pseudomonas aeruginosa* in patients with cystic fibrosis [43].

3.5. Disease diagnosis and drug efficacy testing

Archaea have also been indicated for disease diagnosis. Haloarchaeal GVs have been tested as contrast agents in medical diagnostics by ultrasound and magnetic resonance [32,51]. A protein from *Hbt. halobium* helps diagnose colon or rectal cancer patients due to its ability to induce an immune response and the production of antibodies that also recognize the product of an oncogene associated with this disease [26]. Moreover, haloarchaea are sensitive to cytostatic drugs whose target is DNA topoisomerase II and cytoskeleton components, which makes them suitable for efficacy tests and drug preselection [52].

4. White Biotechnology

The synthesis of proteins and enzymes, active in extreme conditions, by the extremophilic archaea, has revolutionized industrial Biotechnology, making them ideal candidates to perform biocatalytic functions; since they develop under extreme conditions. Some industries that have benefited are the textile, leather, paper, pharmaceutical, and food industries. However, most commercially available enzymes are bacterial or fungal, and very few are from *Archaea*. Nevertheless, they are currently in the spotlight for survival in conditions where fungal and bacterial enzymes are denatured. For this reason, archaeal enzymes such as proteases, lipases, amylases, cellulases, and more have already been tested [53]. Many of them are already being used in large-scale industrial processes.

4.1. Cosmetic industry

Archaeosomes, mentioned above, can also be used in the cosmetic industry due to their ability to store active agents and penetrate the skin, as occurs with conventional liposomes. That makes them good candidates for use as molecule vectorization systems in skin care creams [27]. Also, the exopolysaccharides (EPS) produced by the haloarchaea *Haloterrigena turmenica* have a potential application in cosmetics since they have a greater moisture retention capacity than hyaluronic acid [54], used in this type of industry. Moreover, the metabolites that protect radiophilic archaea from ultraviolet radiation could be used in sun creams [55], as occurs with carotenoids, due to their protective capacity against photooxidation [52].

4.2. Gas industry

One of the most successful applications of archaea is the production of biogas since they are the only microorganisms capable of generating it. That comes from the anaerobic degradation of organic waste carried out by methanogen archaea to obtain energy, and its main component is methane, although they also include carbon dioxide and other gases in smaller proportions [7,26]. This process occurs spontaneously in nature, and the gases generated are released into the atmosphere. This same situation can be reproduced in industrial plants. In this case, the archaea are introduced into fermenters or bioreactors, with the organic waste and water, obtaining biogas and a secondary product called digestate, which has applicability in Green Biotechnology. Biogas can be transformed into electrical and thermal energy [52] or, after reducing the percentage of carbon dioxide, it is incorporated into the natural gas network in the form of biomethane [19]. The latter is a gas considered a renewable energy source and has multiple uses [17].

Nowadays, it is possible to find urban plants that use this biogas as an energy source [21]. Therefore, biogas can substitute natural gas due to its higher calorific value and less polluting effect. However, from an economic point of view, the supply of biogas supposes a higher production cost than other energy production installations since its storage is quite expensive and complex. During production, a strong odor is generated in the surroundings, and eliminating them, would further increase its price. Also, its uncontrolled production could represent a serious threat, given that the main components of biogas, methane and carbon dioxide, are potent greenhouse effect gases. For this, biogas is still far from replacing other non-renewable energies, although numerous companies currently specialize in its production.

Methanogenic archaea also produce biohydrogen, rather than consuming it, when the amount of hydrogen available in the medium is limited. Its production depends fundamentally on fossil fuels, while only a tiny part originates from electricity and renewable resources. Given the demand for H_2 and the reduction of CO_2 emissions, biohydrogen becomes important [7,56]. Although it has these advantages, the biological production of H_2 presents drawbacks due to its storage and supply, although formate is already a solution [19]. However, this application is still restricted to the laboratory scale.

4.3. Textile industry

Archaea have many applications in the textile industry that secrete halocins. They are used during the leather tanning process, a method through which animal skins are transformed into leather. The first stage consists of preserving the skin by introducing it into baths with a high salt concentration, which develops harmful halophilic microorganisms that can damage the tissues, affecting the quality of the products obtained. Due to their antimicrobial activity, halocins can be used to prevent the unwanted growth of these microorganisms [11,17].

Archaeal enzymes are also important in cleaning fabrics made by the textile industry. For example, cellulases from *Hbt. salinarum* could be used to manufacture detergents for washing fabrics made from cotton, which generally deteriorate quickly after many washes. However, adding cellulases prevents this fabric alteration and allows it to maintain the same characteristics [21]. Also, serine proteases and peptidases obtained from hyperthermophilic archaea of genera such as *Desulfurococcus*, *Thermococcus*, and *Pyrococcus* are part of the detergents for washing fabrics at more than 80 °C [34], the temperature at which other enzymes are denatured. In contrast, psychrophile-derived proteases have been proposed for use in detergents designed for cold water washing [57].

Moreover, as previously pointed out, there are haloarchaea capable of removing azo dyes from wastewater. This same biodecoloration principle can be applied to fabrics dyed with this type of dye, commonly used in the textile industry [12].

4.4. Plastic industry

Until now, archaea with the ability to degrade plastic have not been found; however, they can produce compounds with similar characteristics, polyhydroxyalkanoates (PHA). These compounds, among which PHB is found, are produced by bacteria and some species of haloarchaea as an internal reserve of carbon and energy in the presence of an excess of them. They are polymers composed of hydroxy fatty acids and accumulate as cytoplasmic inclusions formed by a polyester core and a cover made of phospholipids and proteins (Fig. 4) [11].

Its application in Biotechnology lies in the fact that PHA exhibits thermoplastic characteristics and elastomeric properties similar to synthetic plastics. However, the latter's production is based on non-renewable resources made from petroleum, which are not biodegradable, causing environmental pollution problems. PHAs are biodegradable [17], so they are ideal for replacing conventional plastics in many applications. For example, they could be used in medicine to make artificial blood vessels or wound dressings and disposable for doctors [59]; also, they present applications in the packaging and food industry, in pharmacy, and agriculture [15,60]. Among the haloarchaea, *Hfx. mediterranei* is the most promising for the industrial production of bioplastics due to its accumulation of large amounts of PHA [59]. In some cultures of this archaea, up to 55% and 65% of the dry weight of cells has been obtained as PHA [43,61], and patents have been issued for its commercial production [13]. Its use for the production of biopolyesters has many positive aspects. It can form a wide variety of homo and heteropolymers with different physical and chemical properties by only modifying the carbon sources and substrates of the culture medium; this allows for obtaining thermoplastics with the desired properties. In addition, the extreme salinity conditions required for its growth allow sterility to be minimal, which offsets the high production cost due to the large amounts of salt required. Also, its lysis can be easily performed in hypotonic media, thus releasing the PHA granules for later recovery [52], which is also simple; the PHA pellet can be recovered by low-speed centrifugation [59].

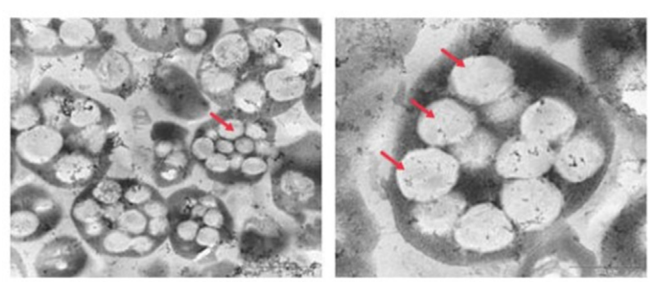

Fig. 4. PHB granules. Transmission electron microscopy image showing PHB granules included in the cytoplasm of the haloarchaea *Hfx. mediterranei* (marked with red arrows). The photograph was taken at the Technical Research Services of the University of Alicante (Spain). Modified from Bautista [58]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Additionally, they can be grown using cheap carbon sources such as sugars and starch [15]. However, the yield of PHA production using haloarchaea is still lower than that achieved using bacterial strains [7]; the production costs of plastics of petrochemical origin are still lower than those of archaeal origin, so they are not economically beneficial. Therefore, despite the efforts made to improve the efficiency of the process, the bioplastics production by haloarchaea has only been carried out on a pilot scale and has not yet been industrialized.

4.5. Mining industry (biomining)

The extraction of metals from minerals and waste materials is usually carried out through pyrometallurgy and hydrometallurgy, chemical processes that use high temperatures which generate environmental pollution. However, a new technique known as "biomining" is rising and represents an alternative method to overcome such problems [62]. Biomining uses microorganisms, which play a significant role in the mobilization and transformation of metals. The archaea can modify the oxidation state of metals and cause their solubilization or biomineralization, so they are studied as a possible option to use in biomining.

One option to extract metals is to use the same archaea used in the bioremediation of metal-contaminated environments since they can accumulate metals and release them with their lysis. However, the two main techniques used in biomining are bioleaching and bio-oxidation. The first consists of the recovery of metals through their solubilization, converting an insoluble form of the metal to a soluble form by biological catalysis; however, the second covers the pretreatment of minerals that occlude the target metals (usually gold and other precious metals) to eliminate them and facilitate their subsequent mobilization [62]. Initially, this process was carried out with mesophilic microorganisms. However, the archaea speed up the process and improve the dissolution of sulfurous minerals, increasing the obtainment of sulfate and metals. The most used in biomining are thermophiles and acidophiles, although not exclusively. Some of the most representative species belong to the genera *Acidianus*, *Ferroplasma*, *Sulfolobus*, or *Metallosphaera*. One of the species with the most significant potential for bioleaching is *Sfb. metallicus*, since it solubilizes the chalcopyrite ($CuFeS_2$) films that form in some minings operations, recovering the copper and iron of it and exposing the precious metals so that they can be exploited [34,52].

Furthermore, *Ferroplasma acidiphilum* oxidizes ferrous ions [62] and the hyperthermophilic archaeon *Pyr. furiosus* can bioleaching gold and other metals [22]. Studies show that many archaeal species could efficiently carry out the biomining process.

5. Yellow Biotechnology

In the food industry, extremozymes have been tested, for example, for meat tenderization, fruit juice clarification, etc. [9,13,19]. Specifically, barophilic proteins have been proposed to produce and sterilize foods requiring high-pressure conditions [23,63]. Other compounds of archaeal origin can also be used. For example, the archaeosomes, due to their stability, have even been proposed to encapsulate additives and ingredients [7]. Also, the carotenoids produced by haloarchaea can be used in feeding as additives and colorants [7,14].

Haloarchaea can be applied in food industry processes that require large amounts of salt. For example, they are ideal candidates for preparing fermented salty foods, such as "jeotgal" (fermented seafood), "kimchi" (fermented cabbage), or the Thai fermented fish sauce "nam-pla", which are widely used in East Asian countries. In the case of fermented fish sauces, it is an expensive and long-lasting process that requires large spaces. Many microorganisms isolated from these fermented products are from the haloarchaeal family *Haloferacaceae*, which participate through their proteases. However, some studies show that the exogenous addition of halophilic archaea speeds up the process and increases the quality of the final product, improving its flavor and aroma [9,15]. So, the use of haloarchaea could be potentially beneficial for the fermented salty food industries, mainly reducing their total cost. At the same time, halocin secretion by haloarchaea also has a potential application in the food industry as a preservative for salty foods, preventing spoilage by inhibiting the growth of other halophilic microorganisms [17].

Archaea are also an ideal candidate for the production of lactose-free milk, which is vital so that people who are lactose intolerant can obtain the calcium and vitamin D present in milk. This process requires the use of lactases from microbial sources and represents an additional step in milk processing, increasing the potential for contamination. However, using thermostable lactases from thermophilic and hyperthermophilic archaea allows solving this problem by acting under pasteurization conditions so that they can eliminate lactose while the temperature is high enough to avoid the growth of harmful microorganisms [64].

The EPS produced by archaea (predominantly thermophiles and halophiles) have numerous applications in the food industry as gelling or emulsifying agents due to their high viscosizing and thickening power [43,52,59], as occurs with biosurfactants produced by haloarchaea [13]. Also, various EPS can be applied as a coating on foods, especially fruits and vegetables, forming a film that can prevent surface darkening, dehydration, and loss of quality due to oxidation or oil diffusion, thus extending the shelf life of these foods [61]. They have the advantage of being stable at high salt concentrations, unlike other polysaccharides of high commercial value, such as xanthan, and some are resistant to extreme pH and temperatures [15,26]. Although their composition and quantity are genetically determined, they are highly influenced by the substrates and the growing conditions.

Furthermore, many industrially produced EPS are synthesized by pathogens [43]. In this sense, the archaea are a safer alternative source since archaeal pathogens have not been discovered to date. The problem is that most of the research on the archaeal EPS has been led at explaining its biological function and not so much at seeking its applicability. Besides, they have higher production costs and worse physicochemical properties than EPS of vegetable origin. Summarizing all this, the EPS produced by *Archaea* is not economically profitable for companies, and, despite its power, at present, no EPS produced by *Archaea* has a commercial application [59].

6. Blue Biotechnology

In the past, antibiotics were used to fight against outbreaks of diseases produced in aquaculture. During the last decades, the inclusion of probiotics in animal feed has been proposed to replace antibiotics due to their possible side effects and the appearance of resistant pathogens. Nonetheless, the biggest challenge is the incompatibility of probiotics with industrial processing since the high temperature and pressure at which foods are processed limits the viability of beneficial microorganisms and compromises their essential probiotic characteristics to improve the health of aquatic organisms. Other alternatives have been sought to face this situation, such as using archaeobiotics due to their compatibility with the conditions of industrial processing and their participation in many biological functions related to animal health, such as the growth of gut microbiota [65].

Patents have already been commercialized using archaea as a probiotic supplement for feed in aquaculture practices and livestock. In the case of farm animals, the feed supplementation with archaea has improved their growth rate and immunity and decreased susceptibility to parasite infections. In contrast, aquaculture has shown greater digestion and absorption of nutrients, an increase in its growth rate, the modification of the composition of the intestinal microbiota, and an improvement in the impact that has their fecal matter on the environment by reducing the contaminants present in it [65].

These results highlight their importance as food supplements in animal feed. That is why archaea are emerging as next-generation probiotics in animal feed and have already been used by some European aquaculture feed companies, such as TwentyGreen®, representing a promising alternative to replace conventional probiotics. However, archaea use in aquaculture has been hindered for various reasons. Some of them are its difficult cultivation compared to a bacterial culture, the lack of information on the species of archaea that are part of the intestinal microbiota of fish and its colonization rate in the intestinal mucosa, a higher price compared to conventional probiotics, and the lack of knowledge of the possible side effects that the increase in archaea could have on the body.

7. Green Biotechnology

7.1. Biofertilizers

The treatment of organic waste with methanogenic archaea is not only beneficial in terms of biogas production, but also the post-digestion matter obtained (digestate) can be reused as fertilizer [56]. These biofertilizers are very effective in helping return organic carbon to the soil while reducing the demand for mineral fertilizer production. The study carried out by Koszel and Lorencowicz [66] showed that alfalfa plants treated with digestate from a biogas plant showed a more significant increase in macroelements in the leaves than plants treated with mineral fertilizers, showing that they are an excellent natural alternative for use in agriculture.

Furthermore, although the participation of ammonia-oxidizing archaea (AOA) in the composting process has not been determined, adding AOA to a compost sample accelerates the process and decreases its duration. It also increases the nitrogen content of the final product obtained, which is usually low due to its conversion to ammonia by microorganisms and the volatilization of nitrate as a potent greenhouse gas, nitrous oxide. Therefore, it improves the composting process's performance and industrial value while increasing the number of nitrogen forms useable by plants [67]; they also have a favorable effect on the environment by limiting the release of this gas. Despite this, they have not yet been used for this purpose.

7.2. Drought-resistant crops

Due to the increase in temperatures due to global warming, research is being carried out on obtaining crops with more excellent resistance to drought and heat since heat stress leads to the formation of reactive oxygen species (ROS). The accumulation of ROS is toxic to cells and causes plant death. For this reason, many laboratories have proposed inserting hyperthermophilic archaeal genes in the genome of plants to deal with this situation [34]. Indeed, some studies show that introducing and overexpressing the gene that encodes the enzyme superoxide reductase in a plant cell culture improves the tolerance to heat, eliminates ROS, and decreases mortality [19]. Also, *Archaea* could be used as plant growth promoters due to their ability to solubilize different chemical compounds, such as phosphorus [22], fix nitrogen, and produce siderophores (iron chelators) [68]. Although these reports show that archaea are potentially useful in this field, more studies of their role in agriculture are needed before they can be put into practice.

8. Golden Biotechnology

8.1. Bioelectronics

The extremophilic protein with greater relevance in this field is the bacteriorhodopsin (BR) produced by haloarchaea, which has become very important at a commercial level, being produced by many companies for numerous applications related to bioelectronics. BR is linked to a chromophore (retinal), which allows it to capture photons [69]. It is resistant to thermal, chemical, and photochemical degradation due to its structure in the form of a hexagonal network, and it has excellent thermodynamic and photochemical stability [15]. Its catalytic cycle and photoelectric, photochromic, and protonkinetic molecular functions make this protein a key molecule with potential applications in Biotechnology. Also, it is possible to immobilize it in a simple way on solid supports. Such attributes have led to the use of this resource as a spatial light modulator, holographic medium, high-resolution monitor, optical biocomputation of neural networks, and optical switches [18,69], as well as computer memories with a greater storage and processing capacity than CD-ROMs.

It can be used in the construction of photovoltaic cells that convert solar into electrical or chemical energy [13] and as a non-toxic miniaturized computer element (biochip) to replace silicon chips [52]. Other applications of BR are the creation of artificial retinas and the development of biological camouflage for military clothing and concealment. The first is based on the fact that BR is an analog of visual rhodopsin in mammalian eyes and is used to obtain implants for people with retinal disorders or similar diseases [7], while the second uses the BR's ability to be undetectable by certain detectors as a result of diffraction and effective hiding of light [70]. A widely used and well-known application is as a biosensor since BR is highly susceptible to being stimulated by external agents [71].

Although several haloarchaea are capable of producing BR, *Hbt. salinarum* is the model organism for its large-scale production; under limited conditions of oxygen and the presence of light, 75% of the total dry mass of its membrane is made up of this protein [7]. However, many of these applications are no longer carried out due to the increasing technological innovation and the development of new materials in this science. Therefore, it is only available in small quantities and at very high prices.

8.2. Nanobiotechnology

Nanobiotechnology is a multidisciplinary science that manipulates matter from living organisms to create new structures, materials, and devices on an atomic scale. This aspect has found biotechnological applications in different fields due to its innovative functions and properties, such as increased solubility, high volume/surface ratio, and multifunctionality [15].

Many archaeal cellular components may provide a cost-effective way of producing nanoparticles. BR is a component with great potential to be integrated into nanostructures due to its small size. Given its ability to act as a light sensor, this protein can collect energy from sunlight, which could be consumed directly or stored in light batteries [70]. Also, there are reports demonstrating the potential of haloarchaea to intracellularly synthesize silver nanoparticles, which have been shown to have antibacterial activity against gram-positive and gram-negative bacteria [15,72]. This fact, together with their lower toxicity than other bactericides, shows that they are suitable candidates for applications related to Red

Biotechnology, although they also have applications as optical receptors and electric batteries [9].

The S-layer glycoproteins located in the membrane of *Archaea* are also attractive for their use in Nanobiotechnology due to their physicochemical properties, such as the ability to assemble into matrices and surfaces or their porous nature. All the pores present in the S-layer have the same size and morphology, and the functional groups are in defined positions, which have been used for the manufacture of isoporous ultrafiltration membranes [73], bioanalytical sensors, affinity membranes, and immobilization matrices for the union of functional molecules [11,52].

9. Brown biotechnology

Many technologies and drugs designed for human medicine subsequently cross barriers and gain importance in veterinary medicine. Halocins, named above, are not an exception. To date, there are no examples of their use in human or veterinary medicine, but some of their potential medical use is known. Such is the case of the halocin H6, which has therapeutic benefits after organ transplant surgeries in dogs, protecting the canine myocardium from experiencing ischemia after myocardial revascularization, and decreasing ventricular ectopic beats and infarct size [28,43].

10. Other biotechnological applications

10.1. Molecular Biology

Extremozymes from thermophilic and hyperthermophilic archaea have revolutionized Biotechnology, proving to be very useful in Molecular Biology laboratories, especially those that require high temperatures. Thermostable DNA polymerases, utilized in Polymerase Chain Reaction (PCR), are one of the best examples of applying thermostable enzymes. The first thermostable DNA polymerase used in PCR was the "Taq polymerase", obtained from the thermophilic bacterium *Thermus aquaticus*. Although its use was a significant advance in the development of PCR, it presents several problems; possibly the most notable is the absence of 3′-5′ exonuclease activity (error-correcting) [74]. For this reason, more reliable alternatives are used, that is, enzymes that present this polymerization activity and proofreading. Such is the case of the DNA polymerases "*Pfu*", "*Pwo*", "*Vent*" (or "*Tli*"), and "*Tkod*", extracted from the hyperthermophilic archaea *Pyrococcus furiosus*, *Pyrococcus woesei*, *Thermococcus litoralis* and *Thermococcus kodakarensis*, respectively.

These enzymes have a low error rate (up to ten times less than *Taq* polymerase) [69,75], a high processivity, and a high extension rate, which allows the obtaining longer and more specific amplification products and, therefore, an improvement in PCR [19,63]. Today, countless commercially available thermostable DNA polymerases from Archaea are being used for PCR [17]. Since the discovery of the first DNA polymerase more than 60 years ago, they have been grouped into seven families: A, B, C, D, X, Y, and RT, with different biochemical properties [76]. The four enzymes mentioned above are part of the B family of DNA polymerases, characterized by high yield and high fidelity properties that make it possible to use them in other applications, such as high-fidelity PCR, cloning, DNA sequencing, and site-directed mutagenesis. In contrast, the Y family of DNA polymerases, in which the Dpo4 enzyme from *Sfb. solfataricus* is found, play important roles in error-susceptible PCR and random mutagenesis due to their low fidelity [74].

However, DNA polymerases are not the only thermostable enzymes extracted from archaea with utility in Molecular Biology. DNA and RNA ligases are also of interest, especially useful in the Ligase Chain Reaction (LCR) due to their ability to form phosphodiester bonds in nucleic acids. These enzymes can be an alternative to *Taq* DNA ligase because they are more active at high temperatures [19]. Similarly, archaeal restriction enzymes with novel recognition sites have been isolated and are now produced commercially. For example, the enzymes MaeI, *Mae*II, and *Mae*III from the methanogenic archaeon *Methanococcus aeolicus*, or HcuI, *Hh*II, and HsaI from the haloarchaea *Hbt. cutirubrum*, *Hbt. halobium* and *Hbt. salinarum*, respectively, although there are many more examples [15,52].

10.2. Tire recycling

The production of enzymes that cause the desulfurization of rubber by *Pyr. furiosus* has led to the study of its possible application in tire recycling. In addition, the material obtained from this reaction has mechanical properties that make it ideal for coating highway guardrails and reducing injuries in motorcycle accidents [34,77]. However, they have not been used for any of these applications.

10.3. Antifouling

Due to their ability to block quorum sensing systems in bacteria, diketopiperazines can also be used to prevent the formation of biofilms and biofouling [43], especially in the shipping industry, where these types of events occur frequently. Similarly, haloarchaeal proteases, miscible in organic solvents, are potentially useful as an additive in antifouling coating paints to prevent biofouling in submarines, water intakes, and pipelines [15].

11. Conclusions

Based on all the information in this review, it can be concluded that *Archaea* are a resource that is just beginning to be exploited in Biotechnology, and there are still many applications that remain unknown. However, they have current applications in the different sectors to which this science is directed, thanks to their characteristics and adaptations to extreme conditions. However, improvements are still needed to replace the processes already established and used today. Probably, with the development of technologies based on "omics" and bioinformatics, the improvement of exploration techniques for inhospitable places, and the sequencing of a new generation of genomes, new archaea with surprising characteristics will be discovered that will enlarge their field of investigation.

Author contributions

Conception and design of the review, D.A.-C., M.C.; Data collection and evaluation, D.A.-C., J.E., V.B., M.-J.B., M.C.; Writing—original draft, preparation of tables, figures and validation, D.A.-C., V.B., M.-J.B.; Visualization, review, and editing: D.A.-C., J.E., M.C.; Structure, and supervision: D.A.-C., M.-J.B., M.C.

Funding

This research was funded by Universidad de Alicante, VIGROB-016.

Conflict of interest

The authors declare that they do not have any conflict of interest.

References

[1] Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 1990;87(12):4576–9. https://doi.org/10.1073/pnas.87.12.4576.

[2] Baker BJ, De Anda V, Seitz KW, Dombrowski N, Santoro AE, Lloyd KG. Diversity, ecology and evolution of Archaea [published correction appears in Nat Microbiol. 2020 May 19;:]. Nat Microbiol 2020;5(7):887–900. https://doi.org/10.1038/s41564-020-0715-z.

[3] Guy L, Ettema TJ. The archaeal 'TACK' superphylum and the origin of eukaryotes. Trends Microbiol 2011;19(12):580–7. https://doi.org/10.1016/j.tim.2011.09.002.

[4] Seitz KW, Dombrowski N, Eme L, Spang A, Lombard J, Sieber JR, et al. Asgard archaea capable of anaerobic hydrocarbon cycling. Nat Commun 2019;10(1):1822. https://doi.org/10.1038/s41467-019-09364-x. Published 2019 Apr 23.

[5] Whitman WB, Oren A, Chuvochina M, da Costa MS, Garrity GM, Rainey FA, et al. Proposal of the suffix -ota to denote phyla. Addendum to 'Proposal to include the rank of phylum in the International Code of Nomenclature of Prokaryotes'. Int J Syst Evol Microbiol 2018;68(3):967–9. https://doi.org/10.1099/ijsem.0.002593.

[6] Orellana LH, Ben Francis T, Krüger K, Teeling H, Müller MC, Fuchs BM, et al. Niche differentiation among annually recurrent coastal Marine Group II Euryarchaeota. ISME J 2019;13(12):3024–36. https://doi.org/10.1038/s41396-019-0491-z.

[7] Pfeifer K, Ergal İ, Koller M, Basen M, Schuster B, Rittmann SKR. Archaea biotechnology. Biotechnol Adv 2021;47:107668. https://doi.org/10.1016/j.biotechadv.2020.107668.

[8] Mainka T, Weirathmüller D, Herwig C, Pflügl S. Potential applications of halophilic microorganisms for biological treatment of industrial process brines contaminated with aromatics. J Ind Microbiol Biotechnol 2021;48(1–2). https://doi.org/10.1093/jimb/kuab015. kuab015.

[9] Amoozegar MA, Siroosi M, Atashgahi S, Smidt H, Ventosa A. Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes. Microbiology (Read) 2017;163(5):623–45. https://doi.org/10.1099/mic.0.000463.

[10] Ding JY, Lai MC. The biotechnological potential of the extreme halophilic archaea *Haloterrigena sp*. H13 in xenobiotic metabolism using a comparative genomics approach. Environ Technol 2010;31(8–9):905–14. https://doi.org/10.1080/09593331003734210.

[11] Haque RU, Paradisi F, Allers T. *Haloferax volcanii* for biotechnology applications: challenges, current state and perspectives. Appl Microbiol Biotechnol 2020;104(4):1371–82. https://doi.org/10.1007/s00253-019-10314-2.

[12] Kiadehi MSH, Amoozegar MA, Asad S, Siroosi M. Exploring the potential of halophilic archaea for the decolorization of azo dyes. Water Sci Technol 2018;77(5–6):1602–11. https://doi.org/10.2166/wst.2018.040.

[13] Litchfield CD. Potential for industrial products from the halophilic Archaea. J Ind Microbiol Biotechnol 2011;38(10):1635–47. https://doi.org/10.1007/s10295-011-1021-9.

[14] Rodrigo-Baños M, Garbayo I, Vílchez C, Bonete MJ, Martínez-Espinosa RM. Carotenoids from haloarchaea and their potential in biotechnology. Mar Drugs 2015;13(9):5508–32. https://doi.org/10.3390/md13095508. Published 2015 Aug 25.

[15] Singh A, Singh AK. Haloarchaea: worth exploring for their biotechnological potential. Biotechnol Lett 2017;39(12):1793–800. https://doi.org/10.1007/s10529-017-2434-y.

[16] Voica DM, Bartha L, Banciu HL, Oren A. Heavy metal resistance in halophilic Bacteria and Archaea. FEMS Microbiol Lett 2016;363(14):fnw146. https://doi.org/10.1093/femsle/fnw146.

[17] Gill R, Kaur R, Rani N, Kaur S. Recent biotechnological applications of archaeal domain. In: Pirzadah T, Malik B, Hakeem K, editors. Plant-microbe dynamics: recent advances for sustainable agriculture. New York: CRC Press; 2021. p. 135–50.

[18] Schiraldi C, Giuliano M, De Rosa M. Perspectives on biotechnological applications of archaea. Archaea 2002;1(2):75–86. https://doi.org/10.1155/2002/436561.

[19] Straub CT, Counts JA, Nguyen DMN, Wu CH, Zeldes BM, Crosby JR, et al. Biotechnology of extremely thermophilic archaea. FEMS Microbiol Rev 2018;42(5):543–78. https://doi.org/10.1093/femsre/fuy012.

[20] Yin Z, Bi X, Xu C. Ammonia-oxidizing archaea (AOA) play with ammonia-oxidizing bacteria (AOB) in nitrogen removal from wastewater. Archaea 2018;2018:8429145. https://doi.org/10.1155/2018/8429145. Published 2018 Sep. 13.

[21] Archaeas De la Cruz J. Utilización Tecnológica. [WWW Document]. 2018. URL, https://steemit.com/stem-espanol/@josedelacruz/archaeas-utilizacion-tecnologica.

[22] Naitam MG, Kaushik R. Archaea: an agro-ecological perspective. Curr Microbiol 2021;78(7):2510–21. https://doi.org/10.1007/s00284-021-02537-2.

[23] Raddadi N, Cherif A, Daffonchio D, Neifar M, Fava F. Biotechnological applications of extremophiles, extremozymes and extremolytes. Appl Microbiol Biotechnol 2015;99(19):7907–13. https://doi.org/10.1007/s00253-015-6874-9.

[24] Del Giudice I, Coppolecchia R, Merone L, Porzio E, Carusone TM, Mandrich L, et al. An efficient thermostable organophosphate hydrolase and its application in pesticide decontamination. Biotechnol Bioeng 2016;113(4):724–34. https://doi.org/10.1002/bit.25843.

[25] Jacquemet A, Barbeau J, Lemiègre L, Benvegnu T. Archaeal tetraether bipolar lipids: structures, functions and applications. Biochimie 2009;91(6):711–7. https://doi.org/10.1016/j.biochi.2009.01.006.

[26] Pérez MA. Microorganismos halófilos en ambientes salinos de Andalucía: estudio taxonómico numérico y molecular. Spain: University of Granada; 2014.

[27] Rastädter K, Wurm DJ, Spadiut O, Quehenberger J. The cell membrane of *Sulfolobus spp*.-homeoviscous adaption and biotechnological applications. Int J Mol Sci 2020;21(11):3935. https://doi.org/10.3390/ijms21113935. Published 2020 May 30.

[28] Irwin JA. Extremophiles and their application to veterinary medicine. Environ Technol 2010;31(8–9):857–69. https://doi.org/10.1080/09593330.2010.484073.

[29] Li Z, Chen J, Sun W, Xu Y. Investigation of archaeosomes as carriers for oral delivery of peptides. Biochem Biophys Res Commun 2010;394(2):412–7. https://doi.org/10.1016/j.bbrc.2010.03.041.

[30] Li Z, Zhang L, Sun W, Ding Q, Hou Y, Xu Y. Archaeosomes with encapsulated antigens for oral vaccine delivery. Vaccine 2011;29(32):5260–6. https://doi.org/10.1016/j.vaccine.2011.05.015.

[31] DasSarma S, Karan R, DasSarma P, Barnes S, Ekulona F, Smith B. An improved genetic system for bioengineering buoyant gas vesicle nanoparticles from Haloarchaea. BMC Biotechnol 2013;13:112. https://doi.org/10.1186/1472-6750-13-112. Published 2013 Dec 21.

[32] Hill AM, Salmond GPC. Microbial gas vesicles as nanotechnology tools: exploiting intracellular organelles for translational utility in biotechnology, medicine and the environment. Microbiology (Read) 2020;166(6):501–9. https://doi.org/10.1099/mic.0.000912.

[33] Bonete MJ, Pire C, Llorca FI, Camacho ML. Glucose dehydrogenase from the halophilic Archaeon *Haloferax mediterranei*: enzyme purification, characterisation and N-terminal sequence. FEBS Lett 1996;383(3):227–9. https://doi.org/10.1016/0014-5793(96)00235-9.

[34] Gómez P, Pérez M. Archaeabacterias hipertermófilas: vida en ebullición. Rev Complut Cienc Vet 2007;1(2):560–72.

[35] Corral P, Amoozegar MA, Ventosa A. Halophiles and their biomolecules: recent advances and future applications in biomedicine. Mar Drugs 2019;18(1):33. https://doi.org/10.3390/md18010033. Published 2019 Dec 30.

[36] Atanasova NS, Pietilä MK, Oksanen HM. Diverse antimicrobial interactions of halophilic archaea and bacteria extend over geographical distances and cross the domain barrier. Microbiologyopen 2013;2(5):811–25. https://doi.org/10.1002/mbo3.115.

[37] Kavitha P, Lipton AP, Sarika AR, Aishwarya MS. Growth characteristics and halocin production by a new isolate, *Haloferax volcanii* KPS1 from Kovalam solar saltern (India). Res J Biol Sci 2011;6:257–62. https://doi.org/10.3923/rjbsci.2011.257.262.

[38] Notomista E, Falanga A, Fusco S, Pirone L, Zanfardino A, Galdiero S, et al. The identification of a novel *Sulfolobus islandicus* CAMP-like peptide points to archaeal microorganisms as cell factories for the production of antimicrobial molecules. Microb Cell Fact 2015;14:126. https://doi.org/10.1186/s12934-015-0302-9. Published 2015 Sep 4.

[39] Lequerica JL, O'Connor JE, Such L, Alberola A, Meseguer I, Dolz M, et al. A halocin acting on Na+/H+ exchanger of haloarchaea as a new type of inhibitor in NHE of mammals. J Physiol Biochem 2006;62(4):253–62. https://doi.org/10.1007/BF03165754.

[40] Besse A, Peduzzi J, Rebuffat S, Carré-Mlouka A. Antimicrobial peptides and proteins in the face of extremes: lessons from archaeocins. Biochimie 2015;118:344–55. https://doi.org/10.1016/j.biochi.2015.06.004.

[41] Soda IM. Aplicaciones de las halocinas producidas por Arqueas halófilas. Ciencia e Invest 2005;8(2):101–6. https://doi.org/10.15381/ci.v8i2.6745.

[42] Safarpour A, Ebrahimi M, Shahzadeh Fazeli SA, Amoozegar MA. Supernatant metabolites from halophilic archaea to reduce tumorigenesis in prostate cancer *in-vitro* and *in-vivo*. Iran J Pharm Res 2019;18(1):241–53.

[43] Charlesworth JC, Burns BP. Untapped resources: biotechnological potential of peptides and secondary metabolites in archaea. Archaea 2015;2015:282035. https://doi.org/10.1155/2015/282035. Published 2015 Oct 4.

[44] Hou J, Cui HL. In vitro antioxidant, antihemolytic, and anticancer activity of the carotenoids from halophilic archaea. Curr Microbiol 2018;75(3):266–71. https://doi.org/10.1007/s00284-017-1374-z.

[45] Zalazar L, Pagola P, Miró MV, Churio MS, Cerletti M, Martínez C, et al. Bacterioruberin extracts from a genetically modified hyperpigmented *Haloferax volcanii* strain: antioxidant activity and bioactive properties on sperm cells. J Appl Microbiol 2019;126(3):796–810. https://doi.org/10.1111/jam.14160.

[46] Gaci N, Borrel G, Tottey W, O'Toole PW, Brugère JF. Archaea and the human gut: new beginning of an old story. World J Gastroenterol 2014;20(43):16062–78. https://doi.org/10.3748/wjg.v20.i43.16062.

[47] Mihajlovski A, Alric M, Brugère JF. A putative new order of methanogenic Archaea inhabiting the human gut, as revealed by molecular analyses of the *mcrA* gene. Res Microbiol 2008;159(7–8):516–21. https://doi.org/10.1016/j.resmic.2008.06.007.

[48] Brugère JF, Borrel G, Gaci N, Tottey W, O'Toole PW, Malpuech-Brugère C. Archaebiotics: proposed therapeutic use of archaea to prevent trimethylaminuria and cardiovascular disease. Gut Microb 2014;5(1):5–10. https://doi.org/10.4161/gmic.26749.

[49] Fadhlaoui K, Arnal ME, Martineau M, Camponova P, Ollivier B, O'Toole PW, et al. Archaea, specific genetic traits, and development of improved bacterial

live biotherapeutic products: another face of next-generation probiotics. Appl Microbiol Biotechnol 2020;104(11):4705–16. https://doi.org/10.1007/s00253-020-10599-8.
[50] Tommonaro G, Abbamondi GR, Iodice C, Tait K, De Rosa S. Diketopiperazines produced by the halophilic archaeon, *Haloterrigena hispanica*, activate AHL bioreporters. Microb Ecol 2012;63(3):490–5. https://doi.org/10.1007/s00248-011-9980-y.
[51] Bulte JWM. Gas vesicles as collapsible MRI contrast agents. Nat Mater 2018;17(5):386–7. https://doi.org/10.1038/s41563-018-0073-x.
[52] Oren A. Industrial and environmental applications of halophilic microorganisms. Environ Technol 2010;31(8–9):825–34. https://doi.org/10.1080/09593330903370026.
[53] Littlechild JA. Archaeal enzymes and applications in industrial biocatalysts, vol. 2015. Archaea; 2015. p. 147671. https://doi.org/10.1155/2015/147671. Published 2015 Sep. 30.
[54] Squillaci G, Parrella R, Carbone V, Minasi P, La Cara F, Morana A. Carotenoids from the extreme halophilic archaeon *Haloterrigena turkmenica*: identification and antioxidant activity. Extremophiles 2017;21(5):933–45. https://doi.org/10.1007/s00792-017-0954-y.
[55] Gabani P, Singh OV. Radiation-resistant extremophiles and their potential in biotechnology and therapeutics. Appl Microbiol Biotechnol 2013;97(3):993–1004. https://doi.org/10.1007/s00253-012-4642-7.
[56] Enzmann F, Mayer F, Rother M, Holtmann D. Methanogens: biochemical background and biotechnological applications. AMB Express 2018;8(1). https://doi.org/10.1186/s13568-017-0531-x. 1. Published 2018 Jan 4.
[57] Coker JA. Recent advances in understanding extremophiles. F1000Res, vol. 8. F1000 Faculty Rev-1917; 2019. https://doi.org/10.12688/f1000research.20765.1. Published 2019 Nov 13.
[58] Bautista V. Caracterización génica y bioquímica de una ciclodextrin glucanotransferasa, enzima implicada en el metabolismo del almidón en *Haloferax mediterranei*. Spain: University of Alicante; 2010.
[59] Poli A, Di Donato P, Abbamondi GR, Nicolaus B. Synthesis, production, and biotechnological applications of exopolysaccharides and polyhydroxyalkanoates by archaea. Archaea 2011;2011:693253. https://doi.org/10.1155/2011/693253.
[60] Albuquerque PBS, Malafaia CB. Perspectives on the production, structural characteristics and potential applications of bioplastics derived from polyhydroxyalkanoates. Int J Biol Macromol 2018;107(Pt A):615–25. https://doi.org/10.1016/j.ijbiomac.2017.09.026.
[61] Vijayendra SV, Shamala TR. Film forming microbial biopolymers for commercial applications–a review. Crit Rev Biotechnol 2014;34(4):338–57. https://doi.org/10.3109/07388551.2013.798254.
[62] Castro C. Interacción de una arquea termófila con la superficie mineral y su influencia en la biolixiviación de minerales. Argentina: National University of La Plata; 2016.
[63] Arora NK, Panosyan H. Extremophiles: applications and roles in environmental sustainability. Environ Sustainability 2019;2(3):217–8. https://doi.org/10.1007/s42398-019-00082-0.
[64] Li B, Wang Z, Li S, Donelan W, Wang X, Cui T, et al. Preparation of lactose-free pasteurized milk with a recombinant thermostable β-glucosidase from *Pyrococcus furiosus*. BMC Biotechnol 2013;13:73. https://doi.org/10.1186/1472-6750-13-73. Published 2013 Sep. 21.
[65] Chuphal N, Singha KP, Sardar P, Sahu NP, Shamna N, Kumar V. Scope of archaea in fish feed: a new chapter in aquafeed probiotics? Probiotics Antimicrob Proteins 2021;13(6):1668–95. https://doi.org/10.1007/s12602-021-09778-4.
[66] Koszel M, Lorencowicz E. Agricultural use of biogas digestate as a replacement fertilizers. Agric Agric Sci Procedia 2015;7:119–24. https://doi.org/10.1016/j.aaspro.2015.12.004.
[67] Xie K, Jia X, Xu P, Huang X, Gu W, Zhang F, et al. Improved composting of poultry feces via supplementation with ammonia oxidizing archaea. Bioresour Technol 2012;120:70–7. https://doi.org/10.1016/j.biortech.2012.06.029.
[68] Yadav AN, Verma P, Kaushik R, Dhaliwal HS, Saxena AK. Archaea endowed with plant growth promoting attributes. EC Microbiol 2017;8(6):294–8.
[69] Alquéres SMC, Almeida RV, Clementino MM, Vieira RP, Almeida WID, Cardoso AM, et al. Exploring the biotechnological applications in the archaeal domain. Braz J Microbiol 2007;38:398–405. https://doi.org/10.1590/S1517-83822007000300002.
[70] Saeedi P, Moosaabadi JM, Sebtahmadi SS, Mehrabadi JF, Behmanesh M, Mekhilef S. Potential applications of bacteriorhodopsin mutants. Bioengineered 2012;3(6):326–8. https://doi.org/10.4161/bioe.21445.
[71] Ashwini R, Vijayanand S, Hemapriya J. Photonic potential of haloarchaeal pigment bacteriorhodopsin for future electronics: a review. Curr Microbiol 2017;74(8):996–1002. https://doi.org/10.1007/s00284-017-1271-5.
[72] Srivastava P, Braganca J, Ramanan SR, Kowshik M. Green synthesis of silver nanoparticles by haloarchaeon *Halococcus salifodinae* BK6. Adv Mater Res 2014;938:236–41. https://doi.org/10.4028/www.scientific.net/amr.938.236.
[73] Schuster B, Sleytr UB. S-layer ultrafiltration membranes. Membranes 2021;11(4):275. https://doi.org/10.3390/membranes11040275. Published 2021 Apr 8.
[74] Zhang L, Kang M, Xu J, Huang Y. Archaeal DNA polymerases in biotechnology. Appl Microbiol Biotechnol 2015;99(16):6585–97. https://doi.org/10.1007/s00253-015-6781-0.
[75] Counts JA, Zeldes BM, Lee LL, Straub CT, Adams MWW, Kelly RM. Physiological, metabolic and biotechnological features of extremely thermophilic microorganisms. Wiley Interdiscip Rev Syst Biol Med 2017;9(3). https://doi.org/10.1002/wsbm.1377. 10.1002/wsbm.1377.
[76] Redrejo-Rodríguez M, Ordóñez CD, Berjón-Otero M, Moreno-González J, Aparicio-Maldonado C, Forterre P, et al. Primer-independent DNA synthesis by a family B DNA polymerase from self-replicating mobile genetic elements. Cell Rep 2017;21(6):1574–87. https://doi.org/10.1016/j.celrep.2017.10.039.
[77] Bredberg K, Persson J, Christiansson M, Stenberg B, Holst O. Anaerobic desulfurization of ground rubber with the thermophilic archaeon *Pyrococcus furiosus*-a new method for rubber recycling. Appl Microbiol Biotechnol 2001;55(1):43–8. https://doi.org/10.1007/s002530000499.