



# Hypersaline environments as natural sources of microbes with potential applications in biotechnology: The case of solar evaporation systems to produce salt in Alicante County (Spain).

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## ABSTRACT

Extremophilic microbes show a unique metabolism due to the adaptations they display to deal with extreme environmental parameters characterizing the extreme ecosystems that they inhabit (high salt concentration, high temperatures, and extreme pH values, high exposure to solar radiation etc.). Halophilic microorganisms characterised and isolated from saltmarshes, brines, salted ponds, salty lagoons etc. have recently attracted attention due to their potential biotechnological applications (as whole cells used for different purposes like wastewater treatments, or their biomolecules: enzymes, antibiotics, carotenoids, bioplastics). Alicante county (southeast of Spain) accounts for a significant number of salty environments like coastal or inland salty ponds from where sodium chloride (NaCl) is obtained, marshes, salty lagoons, etc. The best system characterised so far from a microbiological point of view is "Salinas de Santa Pola", also termed "Salinas Bras del Port". However, there are many other salty environments to be explored, like the natural park of Torrevieja and la Mata lagoons, salty lagoon located in Calpe city or inland salted ponds like those located in the northwest of the county. This review summarises the most relevant biotechnological applications of halophilic microbes described up to now. In addition, special attention is focused on ecosystems such as the lagoons of Torrevieja or inland salt marshes as natural environments whose microbial biodiversity is worthy of being studied in search of new strains and species with the aim to analyze their potential biotechnological applications (pharmaceutical, food industry, biomedicine, etc.).

## 1. Introduction

"Hypersaline environments" are those with higher salt concentrations than seawater (around 3.5% w/v in seawater vs. up to 35% w/v in brines). This type of environment includes ecosystems like inland lakes, lagoons, salterns, marshes, or coastal/inland salty ponds and are relatively abundant and widespread in countries such as the United States of America, Mozambique, Argelia, Spain or Canada (Mcgenity and Oren, 2012).

Most of the knowledge about hypersaline ecosystems has been reported from Mediterranean salterns or inland lakes in countries like Israel or Spain. Thus, salterns such as those located in the south and southeast of Spain (Alicante, Murcia, or Huelva counties) have favourable features for their extensive study: easy accessibility to these environments, a clear gradient from seawater to salt saturation that allows

studies of the microorganisms at each location, constant salinity of each pond, etc. (Casamayor et al., 2002).

Some natural environments characterized by high salinity have been used for centuries to obtain NaCl and other salts, which are later used for different purposes. Over the centuries, humans have optimized the mining of salt, not only at the coast, but also inland, with the aim of extracting NaCl for cooking, food storage, road maintenance in winter, or to be used in chemical formulations. Saline production platforms are usually organized into multiple production ponds ranging from seawater to saturated salt; in these shallow ponds, water is periodically transferred from lower salinity ponds, called concentrators or heaters, to ponds called "crystallizers" in which salts precipitate (Ventosa et al., 2014).

Considering salt gradients across shallow ponds for NaCl production in solar evaporation systems, this review summarizes the current

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knowledge about microbial diversity in brines, their molecular adaptations to high salt concentration, and potential applications in biotechnology of halophilic microbes as well as of their biomolecules. The relevance of salty ecosystems located in the southeast of Spain as natural sources from which new microbial species and strains could be isolated is also discussed. Special attention is paid to those halophilic ecosystems located in Alicante County (Spain).

## 2. Salty ecosystems as natural sources of microbes with potential applications in biotechnology: the case of Alicante County (Spain)

The coastal and inland salt flats are hypersaline natural ecosystems that cover large expanses in the southeast of Spain in general and in Alicante County in particular. These ecosystems could be formed by i) natural salted lagoons, ii) by a mixture of natural lagoons and artificial ponds, or iii) by artificial ponds that are fed with sea water or brine (water with a high concentration of salt). The brine is usually extracted by pumping from saline aquifers (as is the case of the inland salt pans in the region of Alto Vinalopó, in the municipality of Villena, province of Alicante: N 38°38'11"/W 0°51'58") or from singular geological elements such as Cabezo de la Sal de Pinoso (N 38°24'14.5"/W 1°02'11.4"; declared in October 2021 by the Council as a natural monument).

The solar saltern "La Mata-Torre vieja" is a system formed by two large lagoons (N 38°01'00"/W 0°39'00") (Fig. 1). La Mata lagoon works like a large heater, and the Torre vieja lagoon behaves like a great crystallizer. La Mata lagoon is connected to the sea by a channel that allows the entry of seawater for the preconcentration of salt. Both lagoons are connected to each other by the Canal de Las Salinas. Torre vieja lagoon receives saline water from La Mata lagoon and brine waters from the washing of salt from the Cabezo de la Sal brine through a "brine pipeline". In this way, the production of salt in the lagoon of Torre vieja is possible thanks to both the brine from the Cabezo de la Sal (located in Pinoso municipality) and to the sea water (Fig. 1).

Another relevant example of a hypersaline environment located in the southeast of Spain is the "Santa Pola solar salterns" (N38°11'05"/W0°37'46"). This is a typical multi-pond solar saltern system with a discontinuous salinity gradient. The salt concentration is kept constant in each individual pond by a regulated artificial flow. These environments have focused the attention of three groups, mainly for decades: professionals of local management at the level of municipalities and

regional governments; professionals of environmental and tourist management; and agents related to mining.

Finally, another important saline ecosystem close to Santa Pola is "El Hondo Natural Park" (N38°10'55"/N/W0°45'09"), a Spanish protected natural area located between the municipalities of Crevillente and Elche. The natural park of El Hondo, together with that of the Salinas de Santa Pola, is part of the hydrographic basin of the Bajo Vinalopó river.

From the point of view of the local administration, these ecosystems are part of territories that usually have special protection due to their unique features and their microbial diversity, which is highly adapted to stressful conditions (high salt concentrations, low water, and oxygen availability, high sun radiation, alkaline pH in most cases). This is the case of ecosystems located in Torre vieja and Santa Pola, both protected as natural parks: Natural Park of the Lagoons of La Mata and Torre vieja and the Natural Park of the Salinas de Santa Pola (also termed "Salinas Bras del Port"). Santa Pola ponds constitute one of the best-studied saline systems in the world, both from the point of view of mining and tourism and from a microbiological and ecological point of view (Ventosa et al., 2014; Santos et al., 2010; Mojica et al., 2000). In relation to mining, these ecosystems, particularly salt precipitation ponds, are critical tools for obtaining salt, which is then used for human consumption, food conservation, cosmetics compound formulation, or winter road maintenance. It should be noted that the Valencian Community occupies one of the first places in salt production in Spain and the first in sea salt, being the salt pans of Bras del Port and those of Torre vieja (both in the province of Alicante), which contribute most significantly to this production in Europe. Regarding to environmental and tourism management, activities such as the sighting of migratory birds, or having baths in salt marshes and salt lagoons, have attracted a significant number of tourists, ecotourists, ecologists, and naturists for years (Mateo et al., 1997).

The 1970s and 1980s were a turning point in the way these brines were viewed and, by extension, the extremophilic ecosystems represented by salt marshes and lagoons, both coastal and inland. It was a time when biologists of various specialties intuited that the abundance and microbial diversity of the brines could be much richer than initially expected. Thus, a new era in the study of the microbiology of hypersaline environments has begun. In the specific case of the province of Alicante, the first studies on the microbiology of the salt pans of Bras del Port (Santa Pola) date back to the 1980s, studies led by researchers attached at the time to the University of Alicante.

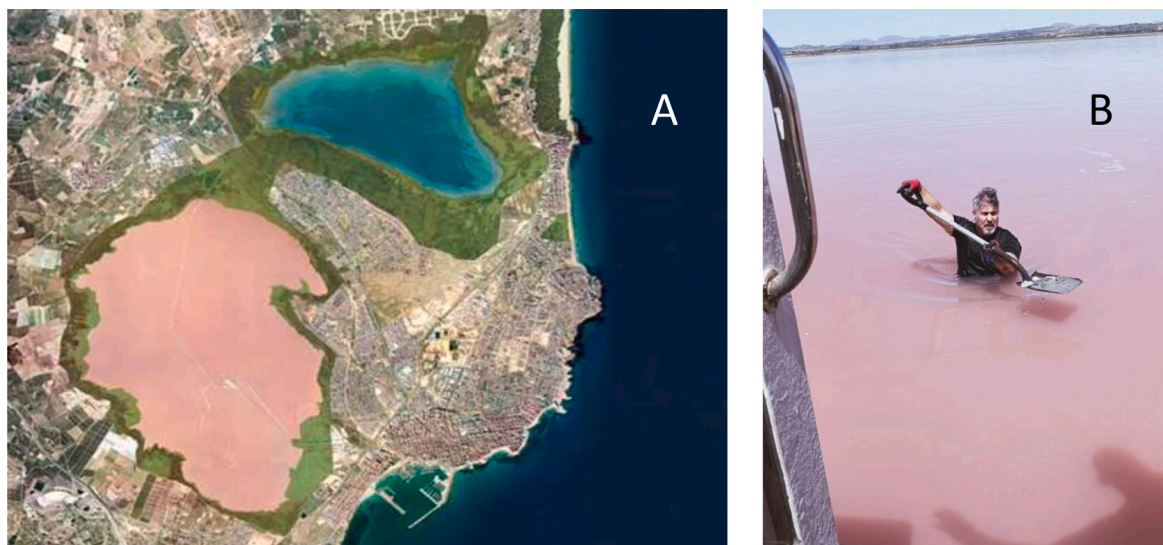


Fig. 1. A. Satellite image showing the salt lagoons of Torre vieja and La Mata (<https://www.google.es/maps/search/parque±laguna±torrevieja/@38.0057332,-0.7386368,10,859m/data=!3m2!1e3!4b1>). The lagoon on the right side corresponds to the heater, which is directly connected to the Mediterranean Sea; while the lagoon on the left corresponds to the precipitation ponds. B. Worker tacking samples from the center of the lagoon for quality monitoring and research.

Years later, in parallel to other studies carried out all over the world, other salt pans in the interior of the province of Alicante were also analysed from a microbiological point of view (Saleros de Penalva de Villena, north of the province of Alicante), finding that the brines of this area (60 km from the coast) are of marine origin and the microbial biodiversity is similar to that identified in the salt pans of Santa Pola (Zafrilla et al., 2010). Microbiological, biochemical, and biotechnological studies carried out to date on samples from the Santa Pola and Villena salt flats show the biotechnological potential of certain microbial species (bacteria, archaea, and microalgae) isolated from these ecosystems. Those applications are summarised in Section 5.

Despite new discoveries about the microbial ecology of these ecosystems, as well as the potential applications of some microbes and/or their molecules in biotechnology, there are still many hypersaline ecosystems in Alicante County that have not been studied at the microbiological or molecular level. Thus, the coastal salt flats of Calpe or the lagoons of La Mata and Torreveja (which stand out for their size and yield in salt production) have not been integrally characterized (sludge, water column, sediments, brines) neither at the level of physicochemistry nor at the levels of microbiology and biochemistry. Likewise, the natural park "El Hondo" or inland salt pans such as the Old Salt Pond in Villena or the salted baths termed "Salinetes" (located between the municipalities of Novelda and Petrer) have not been characterized to date.

### 3. Microorganism inhabiting hypersaline ecosystems

Microorganisms belonging to the three domains of life (*Archaea*, *Bacteria* and *Eukarya*) can be found in saline environments (Oren, 2002). The main feature characterizing them is their high salt requirement to be alive. According to it, they are classified into three different categories: weak halophiles (1–3% NaCl); moderate halophiles (3–15% NaCl); and extreme halophiles (15–30% NaCl). Microbial populations in these environments are dominated by halophilic microorganisms, also considered polyextremophiles, because they are well adapted not only to these high salt concentrations, but also to other extreme environmental parameters like extremely high and low pH or temperature values (Bowers et al., 2009).

The average salt concentration in seawater is 3.5% (w/v), whereas it can reach 35–40% (w/v) in salted ponds (concentrators and crystallizers). Along the salt gradient shown by coastal hypersaline environments, the higher the salinity, the lower the microbial diversity, but the higher the number of prokaryotic microbes showing an extreme halophilic profile. Thus, the abundance of halophilic archaea increases as the system is directed towards the crystallizers, due to the increase in their salinity, among other factors like solar radiation (Andrei et al., 2012). In contrast, the abundance of *Bacteria* domain members decreases along this salt gradient, being higher in the heaters. The number of viruses increases in the crystallizers, thus being a group that acts as a population controller of the *Archaea* domain (Rodríguez-Varela et al., 2009). The following subsections display relevant information about the most abundant halophilic microbial species in coastal and inland solar evaporation systems that produce salt.

#### 3.1. Archaea domain

Members of *Archaea* domain represent the main microbial populations in extreme halophilic environments, apart from abundant bacteria such as *Salinibacter ruber* (Woese et al., 1990). Extreme halophilic archaea are mainly classified within the phylum *Euryarchaeota*, class *Halobacteria* (Grant et al., 2001; Cavicchioli, 2011). Currently, up to 260 species of halophilic archaea have been characterised and grouped into 70 genera (Oren, 2020), three orders and six families: order *Halobacteriales* (families *Halobacteriaceae*, *Haloarculaceae*, and *Halococcaceae*), order *Haloferacales* (families *Haloferacaceae* and *Halorubraceae*) and order *Natrialbales* (family *Natrialbaceae*) (Fig. 2) (Gupta et al., 2016).

These species are, in general, aerobic chemoorganoheterotrophs, although some species can use  $\text{NO}_3^-$  or other compounds like  $\text{NO}_2^-$ ,  $\text{ClO}_3^-$  or  $\text{ClO}_4^-$  as final electron acceptors in an anoxic respiratory process, thus showing great metabolic diversity. Besides, most halophilic archaea species are characterized by their orange and red pigmentation due to the presence of carotenoids, as well as coloured membrane proteins like bacteriorhodopsin, halorhodopsin and other retinal proteins (Andrei et al., 2012).

The most abundant haloarchaea is *Haloquadratum walsbyi*, since it

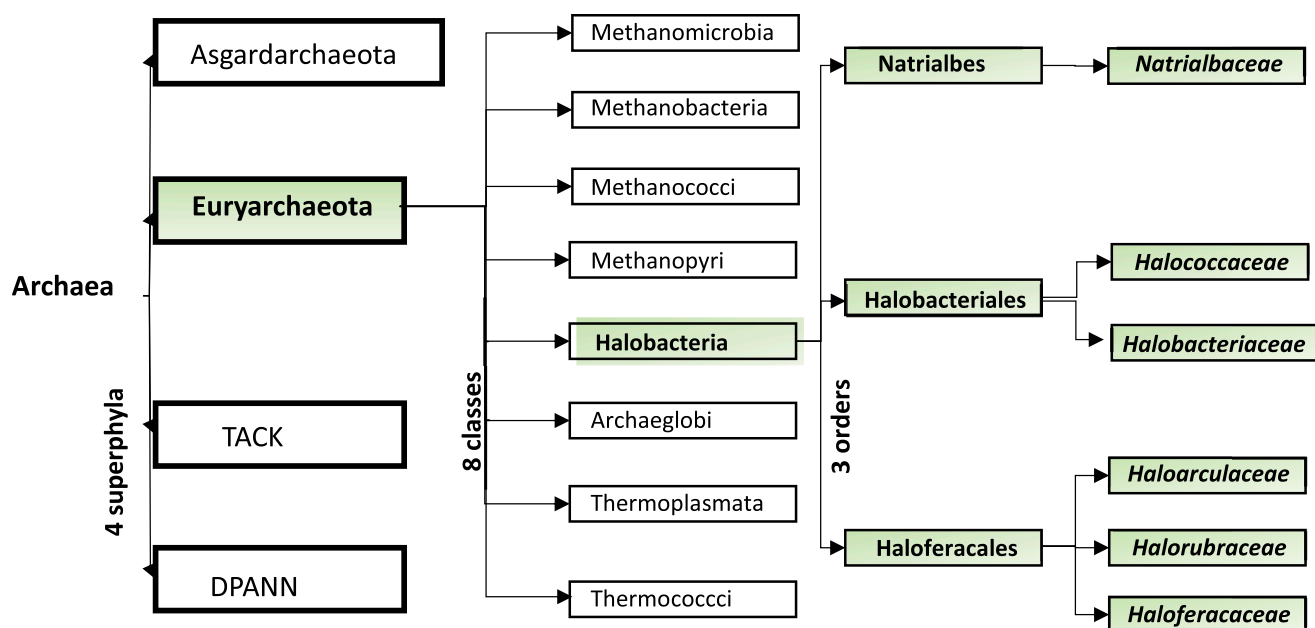


Fig. 2. Summary of the current classification of Archaea domain. Green color highlights the groups that constitute the main microbial populations in extrema halophilic environments like salted ponds, marshes, and coastal lagoons (Adapted from Gupta et al., 2016). TACK is a group of archaea acronym or Thaumarchaeota, Aigarchaeota, Thermoproteota and Korarchaeota, the first groups discovered. DPANN is a superphylum of Archaea first proposed in 2013 (They are known as nanoarchaea or ultra-small archaea due to their smaller size (nanometric) compared to other archaea).

can constitute up to 80% of the prokaryotic population in water samples close to saturation (Dyall-Smith et al., 2011). This microorganism shows square or rectangular shape and synthesises gas vesicles and polyhydroxybutyrate (PHA) granules (Stoeckenius et al., 1981). Its cytoplasm is well adapted to be functional in the presence of high levels of potassium chloride and requires a minimum salt concentration of 14% (w/v), being able to tolerate high molar concentrations of  $Mg^{2+}$ . Consequently, this microbe, as well as many other haloarchaea can live in extremely low situations of water activity (Bolhuis et al., 2006). The genome of this microorganism has a G + C content close to 48%, which is a lower percentage compared to the rest of the species of the *Halobacteriaceae* family (Oren, 2007). This microorganism was isolated simultaneously in 2007 in Australia (C23T strain) and Spain (HBSQ001 strain) (Burns et al., 2007). The sequence of the rRNA 16S gene presented a great similarity, with hybridization of 80%. The main difference between the two strains lies in the structure of their cell walls (Burns et al., 2007; Falb et al., 2008).

Along with the genus *Haloquadratum*, *Halorubrum* represents the second most abundant genus belonging to the class *Halobacteria* (Oren, 2020). Some species of this genus synthesise archaeorhodopsin-3, which is a photoreceptor that also acts through solar energy to create a proton motive force required for ATP synthesis (Bada et al., 2021). Species of this genus have been found in salted environments on different continents, such as Africa, Antarctica, Asia, and North America. The analysis of crystallizers belonging to the salt flats of Santa Pola (Spain) showed that 16 of the 17 colonies analyzed belonged to the genus *Halorubrum* (Benlloch et al., 2001). In Isla Cristina, Huelva (Spain), most of the sequences obtained by metagenomics were related to this genus, and the abundance of bacteriorhodopsin and halorhodopsin was related to the abundance of the sequences of the rRNA 16S gene (Fernández et al., 2014). Phylogenetic analysis of samples from different Spanish ponds showed that *Halorubrum* populations undergo rapid genomic variations (Mohan et al., 2014).

Species such as *Halorubrum lacusprofundi* are not only adapted to hypersaline environments but can also survive to low temperatures, such as those found in Antarctica. This species shows typical characteristics of halophilic microorganisms, with a high G + C content, an acidic proteome, and large extrachromosomal replicons. This acidic proteome allows more flexible protein formation, which is a key requirement at low temperature environments (Anderson et al., 2016).

Finally, the genus *Haloferax* becomes relevant in this type of environment due to its wide distribution and versatility in terms of molecular adaptations. Members of this genus are characterized by extreme pleomorphism, and a wide salt requirement compared to the rest of haloarchaea, since they can grow in the presence of 10–35% salt (w/v) (D'Souza et al., 1997). These species are Gram negative and show irregular morphology (rods, cups, or discs). Cytoplasmic membrane is covered by a hexagonal layer called the S layer, which is mainly formed by glycoproteins (Mengele and Samper, 1992). Most of the *Haloferax* species display aerobic metabolism, although there are certain species that behave as denitrifiers, using nitrate as an electron acceptor under aerobic conditions (Torregrosa-Crespo et al., 2020). Many of the species present a very special biotechnological interest since they can produce polymeric compounds as reserve materials (Lillo et al., 1990). Under certain growing conditions, some species are capable of accumulating polyhydroxyalkanoates (PHAs) as poly(3-hydroxy)butyrate (PHB) and poly(3-hydroxybutyrate-hydroxyvalerate) (PHBV) copolymer (Simó-Cabrera et al., 2021). As discussed in Section 4, *Haloarcula*, *Halorubrum*, and *Haloferax* members have focused the attention of researchers during the last two decades due to their potential biotechnological applications as PHAs or carotenoids producers under certain growth conditions (Asker and Ohta, 2002). In addition, some haloarchaea are more competitive in the environment thanks to the production of halocins, small molecules that inhibit the growth of other haloarchaeal species (Naor et al., 2013).

The haloarchaea previously mentioned were thought to be the most

abundant in extreme halophilic environments, but thanks to massive sequencing-based research, a group of small extremophile archaea (0.6) has recently been discovered. They represent a large percentage of the community ( $>10^6$  cells/mL representing up to 14%) and constitute a new phylum called Nanohaloarchaeota (Oren, 2020). Through metagenomic analysis, 3 species of the genus *Candidatus* have been identified from salt lakes and salt brines: *Candidatus nanosalina* and *Candidatus nanosalinicola* from Lake Tyrrel (Australia) and *Candidatus haloredivivus* from salt flats in Alicante (Spain) (Podell et al., 2013; Gomariz et al., 2015). Rhodopsin genes have been identified in all genomes of Nanohaloarchaeota members, assuming that these genes may be related to photoheterotrophic metabolism (Oren, 2020). Initially, it was thought that these microorganisms could live freely in this type of environment. However, recent studies have shown that they need to coexist with other types of microorganisms. For instance, *Candidatus antarcticus* requires the presence of *Halorubrum lacusprofundi* as a host for growth (Hamm et al., 2019).

### 3.2. Bacteria domain

The appearance of molecular techniques to study 16S rRNA genes as well as in situ fluorescence hybridization (FISH) approaches, allowed the detection of microorganisms of the *Bacteria* domain in hypersaline environments, dismantling the belief that these environments were only inhabited by the *Archaea* domain members (Antón et al., 2000). Thus, it has been reported that in hypersaline environments, the abundance of *Archaea* domain members is around 70–95%, while bacterial populations represent around 5–30% of the overall microbial community (Anton et al., 2008). The most abundant representative halophilic bacteria in this type of environment corresponds to the species called *Salinibacter ruber*, although there are other genera less represented, such as *Salisaeta*, *Salicola*, *Halomonas*, or *Chromohalobacter* (Antón et al., 2002).

The specie *Salinibacter ruber* belongs to the phylum *Bacteroidetes* and is generally found in extremely salted environments like crystallizers of saline environments. This bacterium shares many phenotypic traits with haloarchaea, as they are extreme halophiles, aerobic, and chemorganotrophs, in addition to accumulating intracellular potassium as a compatible solute (salt-in strategy). The strong resemblance of this type of adaptive mechanism between *S. ruber* and haloarchaea may be due primarily to horizontal gene transfer events, because of close evolutionary processes in the same type of extreme environments (Anton et al., 2002). This bacterium also shows reddish coloration due to the presence of carotenoid pigments such as salinixanthin and retinal pigments like xanthorhodopsin. Salinixanthin acts as a light antenna that transfers energy to the retinol group of xanthorhodopsin, which is a proton pump like bacteriorhodopsin (Gunde-Cimerman et al., 2018). This pigment can also be used as a biomarker and several studies have shown that the presence of salinixanthin can represent 5–7.5% of the total pigment content of natural brines (Oren and Rodríguez-Varela, 2001). The proteome of this *S. ruber* is also strongly acidic (average pI close to 5.92) due to the presence of high acidic amino acids content.

The membrane lipid composition of halophilic bacterial species is similar to that of the *Archaea* domain, showing haloarchaeal type-lipids such as phosphatidylserine, N,N-dimethylphatidylethanolamine or cardiolipin. The last lipid may represent up to 20% of the total lipid content, which may be an adaptation to the high salt concentration to optimize the functioning of the bioenergetic systems. In addition, a less common lipid called acylhalocapnine is also present in halophilic bacteria at concentrations that are generally modulated by the sodium and magnesium concentrations of the environment (Oren, 2013).

Most enzymes, the main example of which is NAD-dependent isocitrate dehydrogenase, require salt concentrations between 0.5–2 M for optimal activity, although they also work well at higher concentrations (Oren and Mana, 2002). In addition, changes in the salinity of the medium may have slight effects on membrane lipid composition (Lattanzio et al., 2009). In this discovery, using DGGE techniques (denaturing

gradient electrophoresis) the presence of two different phylotypes, *EBH-1* and *EBH-2*, was revealed, the first being the most abundant. Subsequently, 5 different strains of this first phylotype have been isolated: M1, M8 and M31 (from the salt flats of Campos de Mallorca), as well as P13 and P18 (from the salt flats of Bras del Port in Santa Pola) (Antón et al., 2002).

The great abundance of *S. ruber* has not only been determined in Spain, but it is also an important part of different environments in Turkey, Argentina, Mexico, Tunisia, Italy, and Israel. Despite this great abundance of *S. ruber* in almost all saline environments, there are areas where this domain does not follow the same pattern. In environments such as the Maras salt flats, studies have determined that the dominant bacterium is *Salicola maranensis* (Maturrano et al., 2006).

The family *Halomonadaceae* (belonging to the phylum *Proteobacteria*) also has a special interest in this type of environments due to its osmotic adaptation based on the accumulation of compatible solutes like ectoin. Species like *Halomonas elongata* can grow in a wide range of salt concentrations thanks to the production of compatible solutes that act as enzyme stabilizers and cell protectors (Schwibbert et al., 2011). Those molecules have focused attention worldwide thanks to their potential industrial uses (Ventosa et al., 1998).

### 3.3. Eukarya domain

Although the diversity and abundance of organisms in the Eukarya domain decline as salt concentration rises, some organisms can live in and adapt to salted environments. Species belonging to the genus *Dunaliella* are the most abundant eukaryotic organisms (algae belonging to the class *Chlorophyceae* and order *Volvocales*). These algae are characterized by lacking the rigid cell wall and by the presence of an outer cell membrane constitute by glycoproteins (Polle et al., 2009). Species of *Dunaliella* genus like *Dunaliella salina*, *D. parva*, *D. bardawil*, *D. pseudosalina* and *D. viridis* play a relevant role as primary producers of photosynthetic biomass in marshes and salted ponds (Polle et al., 2009). The two species that stand out the most in this type of environment are *D. salina* and *D. viridis*, which can withstand salt concentrations of 9–200 g/l to full saturation (Borovkov et al., 2020). The efficient adaptation of these species to high salt levels (even at complete saturation) allows them to dominate the ecosystem by lacking competitors (Buchheim et al., 2010). *Dunaliella* species have become sound at industrial and biotechnological levels due to their low production cost, high growth rate, and ability to grow under different sources of stress (high salinity, high solar radiation, and nutrient limitation) (Minhas et al., 2016). Within the potential biotechnological applications of *Dunaliella* spp., it is worth mentioning the production of carotenes ( $\alpha$ -carotene, all-trans  $\beta$ -carotene, 9-cis  $\beta$ -carotene, 15-cis  $\beta$ -carotene, and lycopene), proteins, fatty acids, and xanthophylls (zeaxanthin, lutein, a- and  $\beta$ -cryptoxanthin, violaxanthin and echinenone) (Ben-Amotz, 2002).

*Artemia salina* is also one of the most abundant eukaryotes in hypersaline environments. This is a micro branchiopod crustacean that usually inhabits lakes and brackish lagoons showing low microbial biodiversity and a simple trophic chain (Dhont et al., 2002). *A. salina* displays several adaptations to high salt concentrations. The main adaptation at the molecular level is a high efficiency in osmoregulation (which allows it to withstand concentrations ten times higher than seawater) (Clegg et al., 2002) (Clegg et al., 2002) and the capability of developing genetic diversity based on the region in which the species is located (regional endemism) (Gajardo and Beardmore, 2012). In addition, in environments where the lagoons dry out at certain stages, it is protected from extinction by forming embryos of encysted gastrula (cysts) that are highly resistant to severe dehydration (Muñoz et al., 2010). Genetic information relating to the environmental conditions that the population has previously faced is also stored in cysts (Gajardo and Beardmore, 2012).

Other components of the Eukarya domain, such as fungi, have not been identified as relevant components of hypersaline environments.

The most abundant fungal species described in hypersaline ecosystems are black and melanised fungi like *Hortaea werneckii*, *Phaeothea triangularis* and *Trimmatostroma salinum*. All these species show similar characteristics such as the presence of thick and melanised cell walls, slow and meristematic growth, and proliferation with endoconidiation, all these features being adaptations to these stressful environments (Gunde-Cimerman et al., 2000).

Finally, heterotrophic protists are a group that plays a significant role in population control in hypersaline environments. The presence of this type of species is mainly observed in ponds with intermediate concentrations of salt (between 10 and 15% salt w/v). Generally, these species are ciliates or flagellates. One of the species of this group identified in salted ponds is *Halocafeteria seosinensis*, which can grow with concentrations of 15% salt, although it tolerates up to 35%, and acts as a population controller (Park et al., 2006). On the other hand, several studies have tried to relate the presence of the heterotrophic protist *Fabrea salina* with the control of the *D. salina* population in areas with salt concentrations of around 9%, although there has not yet been enough evidence to demonstrate this control (Hong and Choi, 2015).

### 3.4. Halovirus

Hypersaline environments close to saturation are teeming with so-called extremophile viruses or haloviruses. Up to  $10^9$  viruses have been counted in a milliliter of saltwater sample (Atanasova et al., 2015) and several studies reported that microbial populations in extreme halophilic ecosystems are under control carried out by these haloviruses (Rodríguez-Varela et al., 2009).

Nearly 100 viruses have been identified as predators of halophilic microorganisms, of which 90 viruses infect haloarchaea, while the remaining 10 are capable of infecting bacteria or eukaryotes (Luk et al., 2014). The classification of these viruses is made according to their morphology, and belongs to different families myoviruses (52), siphoviruses (20), pleomorphic viruses (11), podoviruses (5), and lemon-shaped viruses (1) (Atanasova et al., 2015). These viruses are characterized by contractile (myoviruses) or non-contractile (siphoviruses and podoviruses) tails and linear double-stranded DNA (Sencilo and Roine, 2014).

Regarding bacteria, fewer than ten viruses have been identified that are capable of infecting bacteria of the genera *Pseudomonas*, *Halomonas*, *Salinivibrio*, *Salisaeta*, and *Salicola*. These viruses generally belong to the myovirus family, although viruses belonging to the podovirus family have also been identified (Atanasova et al., 2012).

## 4. Molecular adaptations of microorganisms to saline environments

Microorganisms that are adapted to life in extreme environments, such as extreme saline ecosystems, are not only adapted to high salt concentrations but are also usually adapted to a wide variety of factors, such as high irradiation and temperature, as well as low oxygen diffusion. Survival in those environments requires specialized adaptations at the cellular and enzymatical levels, to preserve the osmotic balance. This balance is very important since microorganisms can lose water, causing shriveling and fatal loss of cellular structure and function (Mongodin et al., 2005). Thus, proteins could suffer dehydration due to decreased water content within cells, promoting unfavorable interactions that can be averted by the modulation of their net charge. Consequently, the main difference between halophilic and non-halophilic proteins is the large proportion of glutamate and aspartate on the surface of halophilic proteins. Other features defining halophilic microbes and their proteins are a substantial number of protein charges and protein hydrophobicity compared to bacterial counter partners, as well as acidic proteomes (pI  $\approx$ 4,5) or high G + C content (Edbeib et al., 2016). The main molecular adaptations are summarised in table 1 and explained in detail in the following sections.

**Table 1**

summary of the molecular adaptations showed by the microorganisms to be alive in extreme saline environments.

Environmental parameter	Adaptation	Reference
High salt concentration	Salt-in strategy	Oren, 2000
	Synthesis of compatible solutes	Oren, 2002
Low oxygen availability	Synthesis of bacteriorhodopsin, gas vesicles or denitrification as respiratory pathway.	Hellingwerf, 2002 Oren 2012 Torregrosa-Crespo et al., 2018
	High solar radiation	Capability to regenerate intact chromosomes from dispersed fragments, synthesis of antioxidant enzymes, increase of the [Mn]/[Fe] ratio in the cell, or the production of carotenoids.
Competition for lack of resources	Indirect approach: microbes compete by nutrients, limiting the availability of nutrients for the rest of the microorganisms.	Ghoul and Mitri, 2016 Mazguene et al., 2018 Charlestown and Burns, 2015
	Direct approach: production of toxins and chemicals that inhibit the growth of other microorganisms (bacteriocins, archaeocins (halocins).	

#### 4.1. Adaptation to high salt concentration

For cell survival, the osmotic pressure of the cytoplasm must exceed that of the extracellular medium, since this pressure is essential as a driving force of the cell to maintain the cytoplasm at least isosmotic with respect to the extracellular medium (Kempf and Bremer, 1998).

The great diversity of microorganisms described from halophilic environments brings about a considerable diversity of mechanisms by which halophilic and halotolerant microorganisms can tolerate the great osmotic pressure generated by the saline environment (Oren, 1999a; Siglioccolo et al., 2011).

The first strategy, called "salt-in", is based on the intracellular accumulation of molar concentrations of potassium and chloride, which requires an efficient adaptation of the intracellular enzymatic machinery to high salt concentrations. The cytoplasmic proteins require high salt concentrations to be stable and active. To achieve this aim, these microorganisms have an acid proteome, which is a system that requires relatively little energy (Roberts, 2005). This strategy is used by a limited number of halophiles, with archaea of the order *Halobacteriales* being the main representative, which accumulate KCl in concentrations equal to or greater than the concentrations of NaCl from the extracellular medium. In *Bacteria* domain, the only order in which this strategy has been characterized is *Halanaerobial*, in which fermentative or homoacetogenic anaerobic bacteria are found (Oren, 2000). Protein composition and structure are also adapted to the high salt concentration. The proteome of halophilic archaea is highly acidic. Proteins have a low content of hydrophobic residues in surface such as lysine, while they have a high content of negatively charged residues such as aspartate. The interior of the proteins is rich in valine and low in isoleucine. The use of negative residues on the surface allows for a better organization of the water network on the protein surface and may be important in preventing aggregation (Gundee-Cimmerman et al., 2018).

The second strategy consists of the ejection of salts from the cytoplasm with the greatest efficiency possible, accompanied by the accumulation of the so-called compatible solutes to achieve an adequate osmotic balance (Oren, 2002). These solutes form structures that are excluded from the hydration layer of proteins, so they contribute to the stabilization of this layer by decreasing the coefficient of water activity (Held et al., 2010). In most extremophiles, these compounds are accumulated not only in response to high salt concentrations but also in response to temperature stress. The use of organic solutes requires a

lower adaptive response of the cellular enzymatic machinery than the "salt-in" strategy, although it requires a higher energy contribution (Oren, 1999b). This strategy is widely distributed in all three domains, although there are chemical differences in the solutes accumulated by each type of microorganism. The most abundant solutes in bacteria are ectoine and glycine betaine (Galinski, 1995). In the case of the algae *Dunaliella*, intracellular concentrations of glycerol have been found (Oren, 2002). Organic solutes have also been reported in the *Archaea* domain, in which most of the characterized members of the order *Halobacteriales* synthesize this type of solute even in the presence of high intracellular concentrations of KCl (Oren, 2000).

#### 4.2. Adaptation to low oxygen availability

These environments are also characterized by low oxygen diffusion, becoming a limiting factor for life in environments with an extreme high concentration of salts (up to saturation). To cope with this decrease in oxygen, a large part of polyextremophilic organisms use various strategies, such as the mechanisms based on bacteriorhodopsin. This molecule is an integral membrane protein located in the cell membrane of the *Archaea* domain, mainly in the order *Halobacteriales* (Haupts et al., 1999). This protein can absorb green light at an absorbance of 500–650 nm, with a maximum of 568 nm, and is able to convert light into an electrochemical gradient that is used to produce ATP (Hellingwerf, 2002). This machinery makes possible the production of ATP in a similar way to the electron transport chain, unlike the protons ejected in this case come from a photo excitable proton pump. In situations of low oxygen availability, bacteriorhodopsin is around 50% (v/v) of the overall cell membrane mass, thus demonstrating the great capacity of adaptation of these microorganisms to these situations and the relevance of this protein. (Haupts et al., 1999).

Another strategy to overcome oxygen depletion is the production of gas vesicles promoting cellular floating to make oxygen more accessible to cells. In addition to this general function, different specific functions of these structures have been identified: reaching higher light intensities for the proton pump, reducing the cytoplasmic volume to achieve a higher surface-to-volume ratio in archaea, and facilitating the dispersion of endospores in the case of anaerobic bacteria. Gas vesicles have been found in different species of the genera *Halobacterium*, *Haloferax*, *Haloplamus*, *Haloquadratum* and *Halorubrum* (Oren, 2012).

Because of the lack of oxygen in such environments, respiration is rarely 100% aerobic, which is the most cost-effective process for producing energy. Therefore, there are species that in the absence of oxygen can activate other metabolic pathways that allow them to use other electron acceptors apart from oxygen to respire and produce ATP. Within this context, denitrification reveals as the most important anoxic respiratory pathway carried out by microorganisms in which nitrate or nitrite are used as final electron acceptors instead of oxygen (Torregrosa-Crespo et al., 2018). Denitrification has been well studied so far in soils, however, the first studies on the nitrogen cycle in general and particularly on denitrification in saline environments have not been reported until the beginning of this century (Martínez-Espinosa et al., 2006; Martínez-Espinosa et al., 2011). Denitrifying halophilic microorganisms could be classified into two main groups according to the molecular machinery supporting denitrification: complete and partial denitrifiers. Complete denitrifiers are those microorganisms able to fully reduce nitrate to dinitrogen thanks to four consecutive enzymatic reactions. Partial denitrifiers only can drive a few of the four reactions thus contributing to the emission of intermediate products like N<sub>2</sub>O and NO, which are greenhouse gasses (Torregrosa-Crespo et al., 2016). Considering that haloarchaea constitute the major microbial populations in extreme salted ecosystems, several investigations aimed to evaluate which is the most abundant denitrifying phenotype (partial vs. complete). Recent results have revealed that denitrification carried out by haloarchaea is more widespread than initially thought, being partial denitrifiers important sources of nitrogenous gasses emission in arid and

semiarid salted ecosystems (Torregrosa-Crespo et al., 2018, 2019). On the contrary, complete halophilic denitrifiers have been considered as good model organisms for the design of bioremediation strategies to treat brines and salted wastewaters showing high content of nitrogenous compounds, heavy metals, and oxychlorides (Nájera-Fernández et al., 2012; Martínez-Espinosa et al., 2015; Miralles-Robledillo et al., 2019).

#### 4.3. Adaptation to high solar radiation

Radiation leads to the formation of multiple ions and electrons that modify biomolecules mainly through reactions involving reactive oxygen species (ROS). As an example, ROSs cause damage to cells due to nitrogenous base modifications or breaks in DNA strands (Halliwell and Gutteridge, 1999). Because of the adaptation to these DNA modifications, many haloarchaeal species show a genome constituted by several chromosomes and the cells show the potential ability to regenerate intact chromosomes from dispersed fragments, so there could be an overlap of genomic fragments (Soppa, 2014).

The main protection mechanism against this oxidative stress is based on antioxidant enzymes such as superoxide dismutase and catalase. Superoxide dismutase catalyses the conversion of oxygen into hydrogen peroxide and genes coding for this enzyme have been found in genomes of the genus *Halobacterium* (most of the species are strictly aerobic). In other anaerobic species, this enzyme is replaced by superoxide reductase (Confalonieri and Sommer, 2011).

Another mechanism of defense is the increase of the [Mn]/[Fe] ratio in the cell, which prevents the formation of iron-dependent ROS. In turn, the presence of Mn can also scavenge ROS (Daly et al., 2007).

Finally, most halophilic microbes inhabiting extreme salted environments highly exposed to sun radiation are characterized by pigmentation, mainly due to the production of carotenoids. Those pigments protect against solar radiation. Carotenoids are hydrophobic compounds containing at least 40 hydrocarbon groups that show antioxidant activity thus acting as scavengers of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Rodríguez-Baños et al., 2015). Some of the most abundant carotenoids produced by halophilic microorganisms are beta-carotene, lycopene, astaxanthin, and bacterioruberin, the last mainly produced by haloarchaea (Vilchez et al., 2011; Giani et al., 2020).

#### 4.4. Competition for lack of resources

Microbial populations constantly compete for nutrients and niches, and this competition is significant in extreme environments characterised by the low availability of nutrients. There are two different types of competition, indirect and direct. In the indirect approach, microbes compete by nutrients, limiting the availability of nutrients for the rest of the microorganisms. The second approach involves the production of biomolecules such as toxins and chemicals that inhibit the growth of other microorganisms (Ghoul and Mitri, 2016). Among the toxins, bacteriocins can be highlighted (Jack et al., 1995). These are proteinaceous or peptide toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains. Their homologs synthesised by archaea are called archaeocins, which are divided into two large groups: sulfolobocins (associated to the membrane and synthesised by members of the order *Sulfolobales*) and halocins (secreted to the outside of the cells and synthesised by members of the order *Halobacteriales*). In the case of hypersaline environments, halocins are of relevance. These proteins can also be divided into two large groups: halocin peptides also termed microhalocins, which are smaller than 10 kDa (Halocin S8, C8, R1, and A4), and halocin proteins, which are larger than 10 kDa (Halocins H1, H4, H6 and H7) (Mazguene et al., 2018).

The production of halocins is determined by a series of factors such as the composition of the medium, temperature, aeration, or pH, occurring at the beginning of the exponential phase and reaching optimal levels in the transition between this phase and the stationary

phase. Moreover, the activity of these proteins varies according to the species and can remain constant (Halocins H1, 28 and C8) or decline (Halocins H4, H6, KPS1, HA1, HA3 and H17) during microbial population growth (Mazguene et al., 2018). Bacteriocins are denaturated and inactivated in salted ecosystems whilst halocins require salt to be active. This pattern confers advantages to haloarchaea versus non-halophilic bacteria in halophilic ecosystems (Tadeo et al., 2009).

Based on the type of halocins, they could promote cellular growth inhibition or cellular death. Thus, the molecular mechanisms of action are varied: promoting changes in internal pH, or membrane potential, modulating proton motive force, modifying Na<sup>+</sup>/H<sup>+</sup> flux, or causing inhibition of the Na<sup>+</sup>/H<sup>+</sup> antiporter, etc. Halocins usually act against haloarchaea species that are also halocin producers, and very few have been shown to have adverse effects against bacterial species. These proteins are generally neutral or anionic, so they cannot interact with negatively charged bacterial membranes (Charlestoun and Burns, 2015).

### 5. Biotechnological applications of biomolecules from halophilic microbes

The use of extremophile microorganisms as cellular factories to produce biomolecules is gaining special interest in the discovery of new biomolecules with a wide variety of applications in many fields, from biomedicine to biotechnology. The continuous development of all "omics" and bioinformatics techniques has made it possible to study a multitude of genes involved in the synthesis of compounds for adaptation in response to extreme conditions. Extremophile microorganisms have been described as producing a wide variety of interesting compounds such as carotenoid pigments, biopolymers, bioplastics, hydrolytic enzymes, retinal proteins, and biofertilizers (Yin et al., 2015).

#### 5.1. Antioxidant pigments

Carotenoids are naturally occurring terpenoid pigments made of isoprene residues displaying a conjugated double polyene chain. They are hydrophobic compounds and are usually formed by a skeleton of 40 hydrocarbons, although most of the derivatives present in the *Archaea* domain have 50 carbon atoms. This chain can be terminated by rings or functional groups with oxygen (Jehlička et al., 2013). They can absorb light in the range of 300 to 600 nm and are responsible for the characteristic pigmentation shown by these microorganisms. The ability to absorb light at a certain wavelength is related to the number of functional groups and double bonds they present in their structure (Johnson et al., 1996).

Carotenoids can be classified according to two criteria: i) structure and presence or absence of oxygen atoms and ii) relation with vitamin A. Regarding its structure, two large groups can be distinguished: carotenes, which are composed exclusively of carbon and hydrogen (e.g. lycopene or β-carotene); and xanthophylls, which have oxygen atoms in their structure and may contain various carbonyl, epoxy, or hydroxyl groups, among others (e.g. xanthophyll, zeaxanthin, lutein) (Walter and Strack, 2011). The classification based on the relation with vitamin A makes it possible to identify several groups of pigments: non-pigmenting vitamin A precursors (β-carotene); pigments with partial vitamin A activity (cryptoxanthin); non-pigmenting vitamin A precursors (violaxanthin) and non-pigmenting vitamin A precursors (lutein or zeaxanthin) (Tanaka et al., 2012). The main biological function associated with these compounds is related to their antioxidant properties. Pigments such as xanthophylls are free radical scavengers of oxygen (ROS) and nitrogen (NOS) reactive species (Vilchez et al., 2011).

Most members of the *Haloferracaceae* family can synthesize C<sub>50</sub> carotenoids, among which bacterioruberin and its precursors (2-isopentenyl-3,4-dehydrorhodopin (IDR), bisanhydrobacterioruberin (BABR), and monoanhydrobacterioruberin (MABR) stand out. Other carotenoids also produced at lower concentrations are phytoene,

lycopene, and  $\beta$ -carotene (Goodwin and Britton, 1980). In several species of the genus *Halorubrum*, the bacterioruberin content has been quantified in more than 65% of the total carotenoids produced (Yatsunami et al., 2014). The synthesis of bacterioruberin is mainly induced by factors like low oxygen tension, high solar radiation, osmotic stress; and the presence of different compounds such as aniline (El-Sayed et al., 2002; D'Souza et al., 1997; Raghavan et al., 2005).

The production of carotenoids from haloarchaea has many applications in the field of biotechnology, especially in the food industry, as dyes for poultry, livestock, and fish feed. Other very promising applications are related to biomedicine (as antioxidants, antitumor, heart disease prevention agents, vitamin A precursors, and antibody production enhancers) (Vilchez et al., 2011; Naziri et al., 2014). Thus, a study using the tumoral HepG2 cell line of humans showed that the increase in the concentration of carotenoids extracted from the species *Halobacterium halobium* managed to decrease the viability of this cell line (Abbes et al., 2013).

More than 600 carotenoids are occurring naturally.  $\beta$ -carotenes, astaxanthin, canthaxanthin and lutein are highly marketed. So far, the commercialization of these compounds is carried out through chemical production, which has advantages and disadvantages compared to natural production. Thus, carotenoids chemically produced are characterised by excellent purity and consistency, with a relatively low production cost. However, the chemical synthesis of carotenoids involves the use of reagents, which are not environmentally friendly. Besides, the synthesis of pigments of greater structural complexity is expensive and high time-consuming. Considering all these disadvantages, the production of natural pigments using cells factories reveals as a promising approach to obtain carotenoids at a large scale (Naziri et al., 2014).

Although some haloarchaea species are good natural sources to obtain carotenoids, none of them has been used at large scale to obtain carotenoids. So far, the only halophilic organism successfully used for industrial production of carotenoids is *D. salina*. This species can produce a high percentage of the 9-cis derivative  $\beta$ -carotene. As example, Borovkov and co-workers studied the potential upscale production of carotenoids from controlled laboratory conditions to outdoor rafts using *D. salina*. The results obtained revealed a production  $600 \text{ mg m}^{-2}$  of carotenoids in autumn against  $200 \text{ mg m}^{-2}$  in summer, being 9-cis  $\beta$ -carotene the predominant carotenoid. This derivative was tested as part of a treatment for various diseases such as psoriasis, atherosclerosis, or retinitis pigmentosa (Borovkov et al., 2020).

## 5.2. Compatible solutes

Compatible solutes are organic compounds of low molecular weight acting as osmoregulatory molecules inside the cells. Their intracellular accumulation does not impair normal cellular processes and is promoted by transport from the cellular outside or by *de novo* synthesis (Liu et al., 2021).

The compounds that each microorganism accumulates depend mainly on the type of microorganism and its level of adaptation to osmotic stress. Weak tolerant species generally accumulate simple sugars such as sucrose and trehalose (Klahn et al., 2011). Moderate tolerant organisms accumulate glucosyl glycerol, which plays a very important role in the sustainability of cell division (Ferjani et al., 2003). Finally, the microorganisms that best adapt to this osmotic stress do so by accumulating ectoine, glycine and betaine (Klahn et al., 2011).

Among all the solutes that are produced in this type of environment to cope with osmotic stress, both ectoine and betaine stand out, with their multiple biotechnological applications. Regarding ectoine, its benefits are related to the stabilization of proteins and nucleic acids, the prevention of damage caused by high salt concentrations, temperature, desiccation and freezing. In addition, it allows to counteract the effects of UV-A radiation on the skin, so its main application is focused on the field of cosmetics as a dermatological additive in moisturizers. In the

field of biomedicine, this compound has been shown to inhibit the aggregation of  $\beta$ -amyloid in Alzheimer's, as well as its potential benefits in asthma disease (Oren, 2010). Other applications are related to the food industry, since it is used as a stabilizer in various foods (Detkova et al., 2007). The main microorganisms capable of producing this compound belong to the genus *Halomonas* (*H. elongata*, *H. boliviensis*, *H. salina*), in addition to other species such as *Chromohalobacter israelensis* and *C. salexigens* (Liu et al., 2021).

Betaine is of particular interest in the field of biomedicine, especially as a potential treatment for adipose infiltration of the liver at the onset of cirrhosis (Detkova et al., 2007). Its anticoagulant properties can be used to prevent the occurrence of thrombi and heart attacks (Messadek, 2005). Some of the microorganisms that can accumulate these compounds are *Halorhodopira halochloris* or *Thioalkalovibrio versutus* (Shivanand and Mugeraya, 2011).

## 5.3. Bacteriorhodopsin

Bacteriorhodopsin is an integral membrane protein found in species of the *Archaea* domain, within the so-called "purple membrane", mainly in the class *Halobacteria*. This protein can absorb green light at an absorbance between 500 and 650 nm, with a maximum at 568 nm (Trivedi et al., 2011). This light is used to generate an electrochemical gradient to produce ATP, so that protons are pumped outward using this solar energy (Li et al., 2018).

This protein has characteristics that make it a very interesting product for large-scale use. Despite being a protein present in microorganisms of hypersaline environments, it is stable in the absence of salt, maintaining its physico-chemical properties for long periods of time. It has a wide operating range in temperature (0–45 °C) and pH (1–11) (Li et al., 2018).

It was first discovered in the early 70 s in the archaea *Halobacterium salinarum*, developing its potential applications in the world of bioelectric from the year 2000, with its use as an artificial retinal prosthesis (Frydrych et al., 2000). The potential use of this protein in this field is determined by its photocycle, in which there is interconversion between the different forms of the protein (B state  $\leftrightarrow$  M state) (Li et al., 2018).

The different applications of bacteriorhodopsin in this field can be divided into two differentiated parts: photochemical and photoelectric applications. Regarding its photochemical applications, its use focuses on the production of optical memories. When the bacteriorhodopsin is excited by green light, the photocycle begins with the K state and ends with the O state. After the second excitation with red light, the cycle branches off into the P and Q states, in which these two states are considered as 1 in the binary code and the O state as 0. It is in this transition when data can be written and stored in these optical memories. In addition, the P and Q states are excitable with blue light, so the use of this light allows to erase the stored data (Li et al., 2018).

In the photoelectric section, a greater variety of applications can be found, among which motion biosensors, X-ray sensors and photovoltaic cells stand out. Motion biosensors are visual information systems that are based on biomaterials that are sensitive to light, and that detect optical flow to detect movement of objects. bacteriorhodopsin is used as a photodetector in these systems since its photoelectric response shows a wide dynamic range, as well as high resolution due to the high degree of photosensitivity that allows real-time processing to be achieved (Wang et al., 2005). In this same sense, new materials have been developed to improve the sensitivity of the X-ray detection technique. Bacteriorhodopsin is used as photodetectors for the detection of rays from ultraviolet to infrared, achieving real-time and usable detection (Pei et al., 2001).

## 5.4. Hydrolytic enzymes

Some of the enzymes used by halophilic organisms can function



under extreme physicochemical conditions, such as high salt concentrations or temperature. This efficient adaptation gives great interest to these proteins in the biotechnology industry, in processes with high levels of salinity where most catalysts are inhibited (van den Burg, 2003). The advantage of these enzymes lies in their structure, with many highly acidic amino acids on the surface, as well as a minor component of hydrophobic side chains. Both characteristics allow them to present greater flexibility and hydration (DasSarma et al., 2013).

The use of different enzymes from halophilic organisms has been enhanced, among which hydrolases, lipases, esterases, proteases and nucleases stand out. Hydrolases concentrate a group of enzymes that are widely distributed, among which are amylases, lipases, and proteases. Amylases catalyze the internal hydrolysis of  $\alpha$ -1,4-glucosidic bonds of starch in lower molecular weight products such as maltose or glucose (Souza, 2010). They have been able to obtain amylases with a high degree of purification of different species: *Haloferax mediterranei*, whose amylase requires a high concentration of salt (2–4 M) and a pH between 7 and 8; or *Hfx. sp. HA10*, which requires salt concentrations between 1 and 3 M, as well as a temperature close to 55 °C (Pérez-Pomares et al., 2018; Bajpai et al., 2015). The main applications that can be given to these proteins are related to the food industry, in baking processes, brewing and fruit juices, where they are added to degrade this starch (Dekhordi and Javan, 2012). The biofuel production industry also uses a significant number of amylases, to produce ethanol, in which starch must be processed to obtain sugars (Sánchez and Cardona, 2008). In the pharmaceutical industry, cyclodextrin glucosyltransferases are used to improve the lifetime of different drugs in an attempt to decrease the effective dose. These proteins generate cyclodextrins, which can form inclusion bodies to eliminate toxic compounds (Park et al., 2000).

Cellulases are coenzymes capable of hydrolysing 1,4- $\beta$ -D-glucoside bonds in celluloses and hemicelluloses. These enzymes can play a critical role in the discovery of new renewable energy sources. The use of lignin and cellulose residues presents certain economic limitations, especially in depolymerization processes, due to extreme pH and temperature conditions (Kasirajan and Maupin-Furlow, 2020). Therefore, enzymes obtained from haloarchaea such as *Hfx. sulfurifontis* can facilitate this process since they can work with a pH range of between 3 and 9, salt concentrations between 0 and 5 M, as well as temperatures between 20 and 80 °C (Malik and Furtado, 2018). In addition, these proteins have been used in other industries such as agriculture, for the control of plant growth; the extraction of carotenoids, accelerating the process of hydrolysis in plants; the food industry, for the extraction of fruit juices; preparation of detergents, reducing tissue damage (Bhat, 2000; Cinar, 2005; Karmakar and Ray, 2011).

Dehydrogenases represent another large group of enzymes widely used in different industries and that have managed to be isolated and produced with great efficiency from halophilic microorganisms. One group of them, alcohol dehydrogenases, have been potentiated in the production of biosensors or various compounds such as flavours or drugs (Goldberg et al., 2007). These are enzymes that work under extreme conditions, such as the isolated species *Haloarcula marismortui* (2 M KCl and temperatures up to 60 °C) (Timpson et al., 2012). Other examples are glucose dehydrogenases, which are widely used in the pharmaceutical industry to produce medicines or act as biocatalysts in processes to produce biofuel (Chansaenpak et al., 2021).

In the field of bioremediation and the treatment of contaminated water, an important group of enzymes from haloarchaea called nitrite and nitrate reductases come into play. These enzymes can be found widely distributed in haloarchaea, especially highlighting species such as *Hfx. mediterranei* or *H. marismortui* (Martínez-Espinosa et al., 2007). Therefore, contaminated water residues containing nitrites or nitrates are good sources for denitrification, a process that would allow their disposal. In addition, these enzymes can also be used to build biosensors to detect the presence of these polluting compounds (Martínez-Espinosa et al., 2006).

## 5.5. Bioplastics

The so-called polyhydroxyalkanoates (PHAs) represent a family of biopolymers that can be used as degradable bioplastics. They are polyesters that are stored by microorganisms, usually as a carbon source. Within this family, there are two compounds that stand out especially for their properties and uses in the industry: poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Chen, 2009).

These compounds are characterized by their complete biodegradability, biocompatibility, and sustainability, making them an ecological alternative to petrochemical-based plastics. In addition, the production of these compounds by halophilic microorganisms has several advantages that reduce production costs of fermentation and recovery: the high salt concentration at which halophilic microorganisms can grow prevents contamination by other microorganisms; saltwater sources can be used reducing the use of fresh water in production; their production can be carried out through a continuous process that increases efficiency; low-cost substrates, as waste from different industries (cellulose, kitchen waste, etc.) can be used; the recovery of PHAs can be performed by simple methods such as cell lysis by hypoosmotic shock (Quillaguamán et al., 2010).

The accumulation of PHAs has been described in various species of the bacterial domain as nitrogen fixers (*Rhizobium*). However, for all the properties discussed above, the halophilic species of the *Archaea* domain represent the most interesting group at an industrial level. *Hfx. mediterranei* is one of the species that have been most used to produce bioplastics, especially due to its high growth rate, metabolic versatility, its genetic stability, and its efficient transformation systems (Zuo et al., 2018). One of the advantages of this microorganism lies in the fact that it can synthesize PHBV without the need for a precursor in the medium. PHBV bioplastic is of greater interest than PHB for its versatility and flexibility, which allows it to be used in the manufacture of sanitary or packaging materials (Koller et al., 2007).

Moreover, halophilic bacteria are good PHAs producers. The species *Halomonas boliviensis* synthesizes PHB of high molecular weight under a wide range of culture conditions, salt concentrations from 0 to 25%, temperatures from 0 to 45 °C, as well as pH in the range from 6 to 11 (Quillaguamán et al., 2007).

## 5.6. Antimicrobial compounds

The above compounds represent the wide variety of compounds with the greatest interest that can be obtained from natural sources such as halophilic microorganisms from hypersaline environments. However, more compounds are becoming more important over time, including halocins and antimicrobial compounds.

One of the most serious health problems today is related to the continuous increase in antibiotic resistance of numerous bacterial species. For this reason, the search for new antimicrobial therapies becomes one of the fundamental objectives of science in the short term. Extreme environments, such as hypersaline, as well as the microorganisms that inhabit them, have become a promising new source for obtaining this type of compounds.

In saline soils from different parts of the world, bacterial species that produce molecules that have an action against other bacteria have been isolated. This is the case of the haloalkalophilic species *Nocardioopsis* sp. *AJ1*, in which it has been possible to study the production of two molecules, pyrrole (1,2-A (pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-) and actinomycin C2, which have antimicrobial action before various species such as *E. coli*, *S. aureus* or *P. aeruginosa* (Adlin-Jenifer et al., 2019).

In the *Archaea* domain, in the early 80 s a group of substances was discovered that were secreted by species of the genus *Halobacterium*. These substances, called halocins, are antimicrobial proteins and peptides that could cause the death of the microbiota found around these

species (Rodríguez-Valera et al., 1982). This antimicrobial activity is related to the domain of the ecological niche, in which it must compete with other species with similar adaptations and nutritional requirements (Shand and Leyva, 2007).

In this field, not only compounds with antimicrobial activity have been discovered, but also compounds that can be used as anticancer therapies. This is the case of carotenoids in the *Archaea* domain, discussed in previous sections. Halophilic and halotolerant species have also been put in the spotlight for their production of anti-cancer compounds. Different species of the genus *Bacillus*, *Halomonas*, *Vibrio* or *Marinobacter* have been shown to possess L-asparaginase and L-glutaminase activity. In a trial with human lymph blastocytes and myeloid leukaemia cell lines, it was possible to detect an improvement in the cytotoxicity of this compound against these cell lines (Ghasemi et al., 2017).

## 6. Conclusion

This work highlights the relevance of hyper/saline ecosystems. They host a great microbiological diversity that is still far from being fully described and exploited. In the southeast of Spain, these ecosystems represent an important area and have traditionally been used for the extraction of salt.

During the last three decades, the molecular adaptation and metabolism of the microorganisms that inhabit this extreme environment have been studied and promising biotechnological applications have been proposed. As examples of biotechnological applications, several molecules and processes have been here summarised apart from the CRISPR-Cas technology whose bases were firstly described from an haloarchaea isolated from Santa Pola brines.

Even though, more genomic, and proteomic studies are still necessary to discover the importance that these microorganisms can have in the balance of the ecosystem, the interactions among them, and in the discovery of new applications.

## Author contributions

Rosa María Martínez-Espinosa (RMME) conceived the global project and managed the funding. Guillermo Martínez (GMM) and Rosa María Martínez-Espinosa conducted the bibliometric and bibliographic analysis and integrated all the data into the local database. Carmen Pire (CP), GMM and RMME analysed the results and contributed equally to the writing of the original draft, review of the results and final editing. All authors have read and agreed to the published version of the manuscript.

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## Declaration of Competing Interest

The authors declare no conflict of interest.

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