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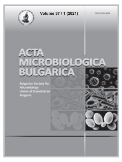

Review

Potential of Halotolerant and Halophilic Fungi as a Source of New Extracellular Enzymes and Antimicrobial Compounds

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Abstract

The scope of this review is the literature published over the last 10 years and presents information on studies conducted on microscopic fungi isolated from various natural saline ecosystems (aquatic and terrestrial). Another interesting point is that it presents a classification of halophilic fungi and the concepts of halotolerant and halophilic microorganisms. The main aim of this review is to show fungal diversity of different natural salinity ecosystems and the biotechnological potential of halophilic and halotolerant fungi as producers of biologically active substances (antibiotics, enzymes, polysaccharides). A careful study on halophilic and halotolerant fungi could offer new biologically active compounds that could be used in manyindustrial processes taking place in unfavorable conditions, such as high salt concentration, low water activity, high pressure or high temperature, etc.

Keywords: halophilic fungi, salt-adaptation, biodiversity, enzyme production, antimicrobial compounds

Резюме

Настоящият обзор представя преглед на литературата, публикувана през последните 10 години относно изследванията върху микроскопични гъби, изолирани от различни естествено солени екосистеми (водни и сухоземни). Едновременно с това, той запознава читателите с класификацията на тези гъби и концепцията за халотолерантни и халофилни микроорганизми. Проследено е разнообразието им в различни екосистеми с естествена соленост, както и техния биотехнологичен потенциал като продуценти на биологично активни вещества (антибиотици, ензими, полизахариди). Внимателното проучване на халофилните и халотолерантни гъби е предпоставка за получаването на нови биологично активни съединения с цел използването им в редица индустриални процеси, протичащи при неблагоприятни условия, като напр. висока концентрация на сол, ниска водна акти вност, високо налягане или висока температура и други.

Introduction

In recent years, there has been increasing interest in the study of extremophilic microorganisms, including halophiles. Intensified studies of halophilic eukaryotic microorganisms appear to have several advantages for science and society, as they: provide clues in our understanding of the targets, processesand networks involved in the complex field of salttolerance; increase our understanding of stress responses; identify genes that might enhance the properties of industrial microorganisms and of plants used in agriculture; contribute to a more complete understanding of 'simple' ecosystems that are characterized by the small diversity of their occupants and relatively few physical and chemical variables. As large areas throughout the world are exposed to salinisation, a better understanding of extremophilic fungi will help us to adapt to Earth's changing climate (Gunde-Cimerman *et al.,* 2009). The study of halophilic fungi contributes to the elucidation of the biodiversity of different ecosystems and clarifies the mechanisms of adaptation to adverse environmental conditions, such as increased salt concentration.

Halotolerant and halophilic microorganisms capable of living in a saline environment offer many actual or potential applications in various fields of biotechnology such as producers of new biologi-

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cally active substances (enzymes, antibiotics, polysaccharides, etc.). To date, the biotechnological applications of halophilic microorganisms have been associated mainly with the use of halophilic bacteria, while the broad potential of halophilic fungi is still poorly understood and therefore poorly used in practice (Ali *et al.,* 2014).

A large number of known microorganisms that grow and survive under normal conditions cannot give the desired results when applied in biotechnology due to the unfavourable conditions for their survival or for the activity required in this operation. Thus, understanding the hypersaline environment and the adaptation in their microbial inhabitants could potentially lead to new biotechnological applications or help to find resistance genes that can then be incorporated into food crops or other organisms to allow their optimal growth, or their products (such as enzymes) to be used in more extreme conditions (temperature, pressure, pH, and salinity) (Gunde-Cimerman *et al.,* 2009). Halotolerant microorganisms play an essential role in food biotechnology for the production of fermented foods and food additives. Other areas of application of these groups of extremophiles are the decomposition and/or transformation of a number of organic pollutants, as well as their participation in the production of alternative energy from bio-waste (Margesin and Schinner, 2001).

One of the main reasons for studying extremophiles, including halophilic microorganisms, is to understand their mechanisms of stress adaptation, as well as the possibilities for the biotechnological application of their metabolites capable of acting in extreme conditions. The low water activity and high concentration of salts in the hypersaline environment make these habitats an important source of halophilic microorganisms that can provide enzymes of industrial interest (Oren *et al.,* 2010).

There is a growing interest in the production of enzymes with extremophilic characteristics, as they can find application in industrial processes that take place under specific, adverse conditions, such as high temperature, acidic or alkaline pH, and high salt concentrations. Therefore, attempts are being made to discover or develop new extracellular enzymes, amylases, proteases, xylanases, cellulases and others with desired characteristics in order to meet the variety of applications (Ghadikolaei *et al.,* 2019). The ever-increasing antibiotic resistance determines the constant need for new antibiotics. Some of the halophilic fungi isolated so far show high antibacterial activity, which makes them attractive as potential producers of antibiotics. The existing information about halophilic fungi is still very fragmentary.

The aim of this review is to present the natural habitats and biodiversity of halophilic and halotolerant fungi, as well as their potential as sources of new biologically active substances with application in industrial processes taking place in unfavorable conditions such as increased salt concentration.

Halophilic and halotolerant fungi – natural habitats and biodiversity

For a long time, places with extreme living conditions were considered uninhabitable. Intensive research on these habitats has begun recently and a wide variety of extremophilic microorganisms have been found (Turk *et al*., 2011). Saline ecosystems (aquatic and terrestrial) are ch aracterized by the presence of specific flora, fauna, and microorganisms. There are two major types of biologically important environments in which the salt factor will interact with the microbial populations, soil and water. Soils containing >0.2% soluble salts are considered saline (Kaurichev, 1980). The range of salinities that can be found in soils is very wide and complex microflora develops within most of this range (Quesada *et al.,* 1982). Saline waters are much better studied and known than saline soils. Besides the ocean proper, there are many waters with salinity similar to that of seawater. Lakes are considered saline if they have >0.3% salinity (De Dekker, 1983).

Classification of halophilic fungi

Microbes that inhabit high-salt environments are adapted to high levels of ions, as well as to low water activity (w_a), and are described as halophilic or halotolerant, while the term xerophilic or xerotolerant is restricted to describing those organisms growing at w_a values imposed by ions other than inorganic ions (Grant, 2004). Halophiles are basically salt-loving organismsthat inhabit hypersaline environments. In a review article “Microbial life at high salt concentrations: phylogenetic and metabolic diversity”, Oren *et al.* (2008) wrote: “Salt dependence and salt tolerance are phenotypic characteristics generally included in the ‘polyphasic’ characterization of newly discovered microorganisms toward their description as new taxa with new names and the determination of their position within the microbial taxonomy”. According to the same publication, there is no precise definition of the term ‘halophilic’. Some authors use the term for all organisms that require some level of salt for growth, including concentrations around 35 g/L as found in seawater.

There are various definitions of halophilic fungi in the literature according to their relationship to salt, and there is still a debate as to which is most appropriate. The term halophilic for fungi was introduced as late as 1975 for a few foodborne species that showed superior growth on media with NaCl as thecontrolling solute (Pitt and Hocking, 1985). Some authors state that it is quite difficult to characterize the clear boundaries between the concepts: halotolerant and halophile fungi. Larsen (1986), and Kushner and Kamekura (1988) introduced three important definitions: moderate halophiles, extreme halophiles, and halotolerant microorganisms. Halophilic fungi can be classified according to their salt requirement and growth pattern. Gunde-Cimerman *et al.* (2005), proposed an operative definition of halophilic fungi according to their relationship to salt. In this study, halophilic fungi are defined as those fungi that can be cultured *in vitro* at a salt concentration of 3 M and can be isolated from natural habitats of salinity above 1.7 M.

Halophilic microorganisms, including microscopic fungi, have different mechanisms of adaptation and can multiply at high osmotic pressure and low level of w_a. They are able to maintain a balance between high osmotic pressure in the environment and low level of w_a outside the cell, compared to that within the intracellular (in the case of prokaryotes) or intercellular (in the case of eukaryotes) spaces (Le-Borgne *et al*., 2008). Fungi living in different saline environments are generally adapted to extreme conditions of low w_a, temperature, pH and salinity (Griffith, 1994).

Natural habitats and biodiversity of halophilic fungi

Fungal diversity of different saline environments has been studied by many researchers. Species diversity and potential application in the industry of halophilic and halotolerant fungi has arowsed increasing interest among scientists in the last decade. Halophilic fungi have been reported to exist in hypersaline and polyhaline marine environments, such as solar salterns, in different continents (Gunde-Cimerman *et al*., 2000; Nayak *et al.* 2012; Ali *et al*., 2018), Dead Sea (Buchalo *et al.,* 1998; Kis-Papo *et al*., 2003) desert soil (Gunde-Cimerman *et al*., 2005) and some sebkha (Jaouani *et al*., 2014), estuary and salinity lakes (Kopytina *et al.,* 2019), and mangroves (Nayak *et al*., 2012).

The Dead Sea is an extremely hypersaline environmenthostile to microorganisms. There is a high concentration of about 340‰ of total dissolved salts in its water, with levels of divalent ions such as magnesium and calcium being higher than those of monovalent ions. This specific ionic composition could be responsible for the low biodiversity of the Dead Sea biota (Buchalo *et al*., 1998; Kis-Papo *et al*., 2003). Buchalo *et al*. (1998) were the first to report *Gymnascella marismortui* species isolated from surface water samples from the Dead Sea as obligate halophilic fungi, and *Penicillium westlingii* and *Ulocladium chlamydosporum* isloated from deep waters at Ein Gedi as halotolerant (Buchalo *et al.,* 1998). Later, different autors published information for many more halotolerant and halophilic fungal species in Dead Sea samples, such as *Aspergillus* and *Cladosporium* spp., *Penicillium westlingii*, *G. marismortui* (Molitoris *et al*., 2000; Wasser *et al*., 2003; Nazareth *et al*., 2012; Nazareth*et al*., 2014). Fungal seasonal dynamics and interannual changes of the quantity and qualitative composition of filamentous fungi inhabiting the Dead Sea have been analyzed by Tova *et al.* (2017). Evidence that within 15 years (2000-2015) the turnover of fungal species diversity decreased from 34 species in winter 2000 to 2 species in summer 2015 was published in their article. Species diversity steadily decreased and highly and significantly correlated with declining water level as well as increasing density and salinity stress, which is currently 348 gL^{-1}. The two surviving species, *Aspergillus amstelodami* and *A. ruber,* increased in frequency across all sampling sites, down to the Dead Sea bottom (-291 m), due to their evolutionary adaptations to tolerate hypersalinity. However, even the species best adapted to highly increased salinity have decreased in frequency in the current extremely harsh conditions.

The Black Sea is an interesting saline ecosystem still poorly studied. In the literature there is scarce information about microbial and particular fungal diversity in some regions of the Russian, Georgian, Romanian, Trkey, Ukrainian and Bulgarian coastal waters (Kopytina, 2019). Micromycetes are represented by two ecological groups: 121 species of obligate water fungi (51 species of lower fungi, and 70 species of filamentous fungi (higher), 323 species of facultative water micromycetes (terrestrial fungi, which are able to function in marine water) (Dudka and Kopytina, 2011). The identification of isolated fungi from different geographical regions proved the existence of large amounts of fungi of the genera *Aspergillus, Penicillium, Alternaria* and *Cladosporium* (Kopytina, 2019).

Salt lakes are another natural habitat of halotolerant fungi. Episodic studies of the mycobiota inhabiting the salty estuaries (lakes) in the Black Sea basin have been carried out at different times, for example, on the Crimean Peninsula, Lake Pomorie in Bulgaria, Tekirgela estuary in Romania. Their salinity varies from 77.8 up to 340‰. In the Kuyalnitsky estuary, 44 species of fungi were found (in water - 5, in peloids - 42, on a tree blue - 14). In the bottom sediments of Lake Pomorie, 36 species have been identified (Smolyanyuk *et al*., 2011). In the peloids of the Tekirgela estuary (Romania), species of the families *Traustochytriaceae* and *Chytridiactae* have been identified (Kopytina, 2019). To our knowledge, there is no available information about the fungal biodiversity of Pomorie Lake water (Bulgaria). Mono Lake is a closed salt lake located in central California, particularly rich in sodium, potassium, phosphorus and boron. Soil and sediments contain very high levels of calcium and magnesium, but also barium, boron and strontium. In total, sixty-seven fungal species belonging to various taxonomic groups were isolated from sediment, soil and tufa samples, while fungi were not isolated from water samples. The highest number of species was found in the genera *Acremonium* (four species) and *Penecillium sodium*. Authors concluded that no very specific fungal flora was found in hypersaline environments (Steiman *et al*., 2004).

The structure and diversity of fungal communities was documented in young transient and mature hypersaline microbial mats from a tropical region (Puerto Rico). Traditional and molecular techniques revealed strong spatial and temporal heterogeneities in both microbial mats. As shown in the paper of Cantrell *et al*. (2013), some of the species isolated belong to the genera *Aspergillus, Cladosporium, Hortaea, Pichia* and *Wallemia*, which are often isolated from hypersaline environments. The most abundant clones belong to *Acremonium strictum* and *Cladosporium halotolerans*, which were not isolated in pure culture.

Another natural habitat in which halophilic fungi are found is solarsalterns - hypersaline extreme environments with unique physicochemical properties such as a salinity gradient. The hypersaline waters of solar salterns are referred to as thalassohaline, having salt concentrations greater than 3.5%, the concentration of salt in seawater (Gunde-Cimerman *et al*., 2000). Fungi isolated from salterns are represented by black yeasts (*Hortaea werneckii, Phaeotheca triangularis, Aureobasidium pullulans,* and *Trimmatostroma salinum*), *Cladosporium, Aspergillus*, and *Penicillium* species. Most studies on saltern-derived fungi focused on black yeasts and their physiological characteristics, including growth under various culture conditions (Chung*et al*., 2019).

A new halophilic fungal species, *P. imranianum*, isolated from a man-made solar saltern, located at Phetchaburi province, Thailand, has been recently reported. The authors claim that to the best of their knowledge, it is the most extremely halophilic *Penicillium* ever isolated (Ali *et al*., 2018). Studies by otherauthors have reported *G. marismortui* (Buchalo *et al*., 1998), *Wallemia ichthyophaga* (Zalar *et al*., 2005; Gunde-Cimerman *et al*., 2009), *Trichosporium* (El-Meleigy *et al*., 2010), *A. penicillioides* and *A. unguis* (Nazareth *et al*., 2012) as obligate halophiles.

Mangroves and salterns of Goa, India, are typical saline ecosystems where halophilic fungi have been found. According to authors, *Aspergillus and Penicillium species* predominate in the studied regions. The isolates are reported to have high levels of tolerance to sodium chloride and showed resistance to salts of heavy metals Pb^{2+}, Cu^{2+}, and Cd^{2+}. Species belonging to *Penicillium* showed the highest levels of halotolerance as well as resistance to heavy metals, the single isolate of a triverticillate morphotype of *Penicillium* obtained showing the highest resistance. Aspergilli also displayed fairly high halotolerance as well as metal resistance, with isolates of *A. niger* and *A. flavus* showing higher levels of resistance. Other isolates belong to the genera *Paecilomyces, Fusarium, Alternaria and Cladosporium*. Among these, *Paecilomyces* showed a fair amount of halotolerance; the others showed lower tolerance (Nazareth *et al*., 2012). During his study of mangroves and solar salterns of Goa (Nayak *et al.*, 2012), isolated and identified (characterized) high salt tolerant fungi belonging to the genus *Aspergillus*; some to *Penicillium,* and a few to *Eurotium* and *Hortaea*. Only one species, *A. penicillioides*, strongly required addition of salt to the growth medium and was termed as an obligate halophile. However, most isolates do not have an absolute requirement for added salt for growth. One hundred soil samples were collected from the Miani-Khor mangrove ecosystem of Balochistan, Pakistan, to study the microbial diversity of this salt area. Isolated halotolerant and halophilc fungi were identified by clasiccal and molecular methods. Different fungal species were found to belong to the genera *Aspergillus, Penicillium, Alternaria, Fusarium,* and *Pleosporaceae* (Khan *et al*., 2020).

Sebkhas are salt flat areas, coastal or interior, where, as the result of evaporation, salt and other evaporite minerals precipitate near or at the surface (Neuendorf *et al*., 2005). There are several publications about halotolerant and halophilic fungi isolated from The Great Sebkha of Oran (the largest sebkha in north-western Algeria), whose salt concentration is estimated at more than 100 g/ L^{-1} of dissolved salts (Benziane, 2013), Sebkha El Melah in Tunisia (Jaouani *et al*., 2014), and the sabkha in Kuwait (Al-Musallam *et al*., 2011). Fungal isolates were identified by morphological and microscopical observations and by molecular techniques. Halotolerant and halophilic fungi belonging to the genera *Penicillium*, *Trichoderma*, *Alternaria*, *Chaetomium* and *Wallemia* (showing high similarity (≥97%), *Chrysosporium*, *Tritirachium, Lecanicillium* and *Pleospora* showing a high similarity (100%) to unidentified species of these genera were described. Also, some fungi were found to belong to the genera *Aspergillus, Arachnomyces, Fusarium* and *Chaetomium.* Several species of the genera *Aspergillus* and *Penicillium* have already been reported as the most frequently isolated species in hypersaline environments. Surprisingly, the results show that only one strain of *Wallemia sp.* and the two strains of *G. halophilu*s are obligatorily halophilic. Most of the strains could grow at 12.5% NaCl and 5 strains (*A. subramanianii strain* A1, *Aspergillus* sp. strain A4, *P. vinaceum* and the two strains of *G. halophilus*) at 17.5%. The only strain that could grow at 20% was *Wallemia sp.* Taxonomic characterization of the isolates showed that the most dominant genera found in the sebkha were *Penicillium, Aspergillus*, their teleomorphic forms and *Fusarium* (Rajaa Chamekh*et al*., 2019). From Sebkha El Melah in Tunisia, Jaouani *et al.* (2014) isolated and characterized 21 fungi. They reported that this fungal community has similar composition to those reported in inhospitable habitats characterized by limitation of nutrients, moisture deficit, and exposure to high solar radiation. Alkali-halotolerant fungi have been isolated and assigned to 15 taxa belonging to 6 genera of Ascomycetes.

The Great Salt Plains (GSP) of Oklahoma was studied as a natural salinity habitat for halophilic and halotolerant fungi. This region is an inland terrestrial hypersaline environment where saturated brines leave evaporite crusts of NaCl (Evans *et al*., 2013). It was found that all of the isolates fall within the ascomycetes, with a predominance of *Trichocomaceae*, represented by *Aspergillus, Eurotium,* and *Penicillium* species. Representatives of *Anthrinium, Cladosporium, Debaryomyces, Fusarium*, and *Ulocladium* also were isolated. Overall, the isolates were widely halotolerant, with best growth observed at lower salinities and no halophilism. The fungal genera observed were all cosmopolitan, without strong specialization. The dominance of cosmopolitan fungal genera, such as *Aspergillus* and *Penicillium*, suggests that the hypersaline fungal community is a halotolerant subset of common soil fungi in these areas. The culturable fungal community at the GSP appears to follow the same trends, being composed mainly of *Aspergillus, Cladosporium, Fusarium,* and *Penicillium* species.

In the literature, there are several publications dedicated to ecology and fungal diversity in cold extreme habitats such as glaciers, the Arctic shores, and the Antarctic region (Abyzov 1993; Buchalo *et al*., 1998; Ma *et al*., 1999; Gunde-Cimerman *et al*., 2000; Ruisi *et al*., 2006). Gunde-Cimeran *et al*. (2005) surmise that fungal adaptations to low temperatures can be related to salt stress, suggesting a link between psychrophily and halophily. Resently published scientific papers indicate that fungal strains isolated from cold habitats such as glaciers have been observed to grow at diverse salt concentrations (NaCl 18-24%) (Hasan *et al*., 2018). For example, the fungal species most frequently isolated from ice, sediments and water samples collected from the Siachen glacier, Pakistan, were *Leotiomycetes* sp., followed by *Thelebolus, Penicillium, Cladosporium, Trichoderma, Periconia, Geomyces, Cryptococcus* and *Pueraria*. All isolates were found to be halophilic and able to tolerate NaCl concentration up to 10-20% (Hassan *et al*., 2017). From Tirich Mir glacier 46 fungal strains were isolated and characterized. Among them, one isolate of genus *Davidiella,* identified as *Davidiella tassiana* HTF9, showed growth in the presence of 18% NaCl (Rafiq *et al*., 2020). The investigation of the fungal biodiversity and halotolerant and halophilic fungal abundance of cold regions are only just beginning.

Extracellular enzymes and antimicrobial activity

The ability of many microorganisms to grow at different concentrations of NaCl and low a_w has been of great interest for microbiologists and biochemists in the last decade. Like each of their cellular components, their proteins are inherently more stable to salinity than those of conventional organisms. Microbiome of saline environments, especially halophilic fungi, has become one of the richest bioresources for industrially important molecules and enzymes. Halophilic microorganisms, including different groups of fungi, are recognized producers of secondary metabolites under harsh saline conditions, such as carotenoid pigments, retinal proteins, hydrolytic enzymes, and compatible solutes, such as macromolecules stabilizers, biopolymers, and biofertilizers (Amoozegar *et al.,* 2017). Table 1 presents some of possible areas of applications of halopfilic fungi.

Table 1. Potential biotechnological applications of halophilic fungi (Irman *et al*., 2016)

Application	Resources
Antibiotics produucers	Sepcic *et al*., 2010
Sorce of antioxidants	Ghosh *et al*., 2010; Ravindran, 2012
Environmental pollution indicators	Ali *et al*., 2014
Enzymes producers	Dalboge, 1997; Karbalaei-Heidari *et al*., 2011; Chakraborty *et al*., 2009; Vidyasagar *et al*., 2009

Extracellular enzymes produced by halophilic and halotolerant fungi

One of the important requirements for enzymes to find application in industry is that these bioactive catalysts maintain their stability and activity in an unfavorable environment, such as high temperature, presence of different salts, alkaline or acidic pH. Previous studies have shown that halophilic fungi are an important source of polyextremophilic metabolites. Their thermotolerant and halophilic properties allow them to be stable and applicable within wide ranges of pH and temperature in industrial processes (Dalboge, 1997; Setati, 2010).

As previously reported in the literature, extremozymes from halophilic fungi (such as *T. salinum, W. ichthyophaga, H. werneckii*, and *P. triangularis*) are interesting for industrial and biotechnological applications, since they can act at harsh conditions. Extremozymes from halophilic fungi, e.g. amylases, cellulases, lipases, and proteases, which possess the so-called polyextremophilicity (they are thermostable, tolerant to a wide range of pH, less susceptible to denaturation and tolerant to high salt concentrations), present a novel catalytic alternative for biotechnological applications (Delgado-García, 2012*)*. Physiological activities providing energy and metabolites for growth and other processes require the enzymes to be active under the prevailing conditions.

There are many scientific data in the literature on halophilic enzymes isolated from halophilic bacteria, while halophilic fungi are still poorly studied as sources of halophilic enzymes, but interest in them has grown in recent years (Mukhtar and Haq, 2012). Halophilic and halotolerant fungi isolated from different natural habitats can provide thermostable and salt-tolerant enzymes of industrial interest. Potential industrial applications of enzymes from halophilic fungi are in waste management (reduction of mixed waste or waste water remediation), textile, pharmaceutical, and food industries. For example, the demand for salt-tolerant lignocellulolytic enzymes for development ofvarious industrial processes such as production of biofuels is currently growing. Freshwater is a rare and valuable resource that could be replaced by sea water in certain biotechnological applications using salt tolerant enzymes (Arfi *et al*., 2013). Some enzymes of biotechnological interest isolated from halophilic fungi are presented in Table 2.

Laccases are phenoloxidases involved in the transformation of the recalcitrant fraction of organic matter in soil. These enzymes are also able to transform certain aromatic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and are known to be inhibited by chloride ions. *Chaetomium sp., Xylogone sphaerospora, and Coprinopsis sp.* fungi isolated from the Mediterranean coastal area have been found to produce halotolerant lacase (Qasemian *et al*., 2012).

Aspergillus caesiellus, a moderate halophilic thermotolerant fungal strain isolated from sugarcane fermentation in the presence of 2 M NaCl, has been reported as a producer of thermostable and salt-tolerant cellulases (Batista-García *et al*., 2014). Also, some unidentified halophilic fungal strains isolated from Great Sebkha of Oran from two species, *Aspergillus sp*. and *Chaetomium sp.*, were described as good producers of cellulose in the study of Chamekh *et al*. (2019). Some authors demonstrated that the marine white-rot fungus *Phlebia sp.,* when grown in the presence of 3% sea salt, secretes two different manganese peroxidases (MnP) distinct from the one produced in the absence of salt, suggesting that marine fungi may possess sets of alternative lignocellulolytic enzymes adapted to salt (Kamei *et al*., 2008).

To date, however, very little is known about the mechanisms employed by mangrove fungi to breakdown the available lignocellulosic biomass and how their enzymes has adapted to marine conditions, especially to the presence of high concentrations of salt. It has been shown that several marine ascomycetes encountered in mangrove are capable of wood decay and

Table 2. Examples of some valuable enzymes produced by halophilic fungi and their applicationin different industrial processes

Enzyme	Application	Halophilic fungal source	References
Proteases	degradation of plant and animal proteins; peptides synthesis	*P. chrysogenum*, *C. halotolerans* *C. sphaerospermum*, *C. cladosporioides*, *Engyodontium album*	Chamekh*et al*., 2019 Jaouani *et al*., 2014
Amylases	textile, laundry, pharmaceutical and food industries, wastewater remediation, degradation of starch	*A. gracilis*, *A. penicillioides*, *P. chrysogenum*, *C. cladosporioides*, *Engyodontium album*, *Alternaria alternata*	Chakraborty *et al*., 2009 Ali *et al*., 2014; Jaouani *et al*., 2014; Ali *et al*., 2015
Laccase	plant material delignification, lignin-degrading enzyme	*C. halotolerans*, *C. sphaerospermum*, *Penicillium sp.*	Jaouani *et al*.,2014
Lipases	Food and agro industrys, cleaning, biofuel, pharmaceuticals, textile cosmetic, perfumery, flavour industry, bioremediation	P. vinaceum, U. cynodontis Wallemia sp. *C. sphaerospermum* *E. album*	Jančič *et al*., 2016 Chamekh *et al*., 2019
Pectinases	Fruit juice industries, paper and pulp industries, food industries	Penicillium sp. K 3-17 (extreme halophile), *Penicillium* sp. K-5 A. terreus	Helan*et al*., 2013
Cellulases	Hydrolyses the cellulose, production of biofuel, waste management, paper industry	*A. calidoustus*, *G. halophilus*, *P. vinaceum*, *Aspergillus* sp., *Chaetomium* sp. *Ustilago cynodontis*, *Fusarium species*, *Chrysosporium* sp., *Arachnomyces* sp., *S. strictum*, *Aspergillus glaucus CCHA*, *Pestalotiopsis* sp. NCi6, *Aspergillus caesiellus*	Chamekh *et al*., 2019; Li *et al*., 2018; Arfi *et al*., 2013; Batista-García *et al*., 2014
Xylanases	Production of biofuel, waste management, paper industry, as a supplement in animal feed, for the manufacture of bread, food and drinks, textiles, bleaching of cellulose pulp, and xylitol production	*Pestalotiopsis* sp. NCi6	Wang *et al*., 2009; Arfi *et al*., 2013

show cellulose and xylan degradation activities (Bucher *et al*., 2004). Given the presence of sea water in these ecosystems, mangrove fungi are adapted to high salinity. *Pestalotiopsis sp.* NCi6, a halotolerant and lignocellulolytic mangrove fungus showed the ability to grow on a complex lignocellulosic substrate regardless of the presence of high concentrations of salt, but with variations in lignocellulolytic activities (Arfi *et al*., 2013). *A. caesiellus* H1 isolated from sugarcane bagasse fermentation in the presence of 2 M NaCl was characterized as a producer not only of thermostable cellulases but also of xylanases, MnP, and esterases. No laccase activity was detected in the tested conditions (Batista-García *et al*., 2014). The biotechnological potential and ability to produce extracellular enzymes (i.e., lipase, amylase, protease, and cellulase) of the 50 halophilic fungal strains isolated from Great Sebkha of Oran in north-western Algeria was tested by Chamekh *et al*. (2019). *Aspergillus sp.* strain A4, *Chaetomium sp.* strain H1*, P. vinaceum, G. halophilus,* and the two basidiomycetous *Wallemia sp.* and *U. cynodontis* have been reported as the most interesting species presenting the highest enzymatic index. *Wallemia sp*. has high lipase activity but no cellulolytic, amylolytic or proteolytic activity was detected. This result was also obtained by Jančič *et al*. (2016), who studied the enzymatic profile of the four species of *Wallemia (W. sebi, W. ichthyophaga, W. muriae* and *W. hederae)*. They found that *Wallemia spp*. secretes several enzymes including lipase and esterase, but no cellulolytic, amylolytic, and proteolytic activities were observed. Amylase, cellulase, invertase and xylanase were found to be secreted in significant amounts (creatingclear zones) by one straine of *P. imranianum* (extreme halophile) isolated by Ali *et al*. (2018), from a man-made solar saltern in Thailand. Polyextremophilic α-amylases obtained from halophilic *Engyodontium album,* obligate halophilic *A. gracialis* and from obligate halophilic *A. penicillioides* were purified and characterized (Ali *et al*., 2014; Lotrakul and Punnapayak *et al.*, 2014; Ali *et al*, 2015).

Fungi elaborate a wide variety of proteolytic enzymes than bacteria. Filamentous fungi have the potential to grow under varying environmental conditions such as time course, pH and temperature, utilizing a wide variety of substrates as nutrients (Haq *et al*., 2006).

Halophilic fungi showing antimicrobial activity

Antimicrobial resistance is a growing problem in the world today. Nearly three-quarters of all clinically relevant antibiotics are natural substances produced by bacteria or fungi. However, the antibiotics that are currently available are losing their effectiveness and increasing numbers of pathogens are becoming resistant. This means there is an urgent need for new antibiotics and new antimicrobial subsances (Katz and Baltz, 2016). Drug and antibiotic resistance is an issue that has developed over decades and determines the constant need for new antibiotics. Some of the halophilic fungi isolated so far show high antibacterial activity, which makes them attractive as potential producers of antibiotics. A series of recent studies has indicated that antibiotic activity from halophilic/halotolerant fungi is more effective at low water content or increased NaCl concentration (Sepcic *et al*., 2011). Quite recently, microbiologists have started to take a new and serious interest in the antimicrobial activity of fungi isolated from hypersaline ecosystems. Still there are very few reports related to antimicrobial agents produced by halotolerant and halophilic fungi. Table 3 presents information about the antimicrobial activity of some halophilic and halotolerant fungi.

Lebogang *et al*. (2009), carried out apreliminary study of fungi isolated from the soil of the saltpans in Botswana (saline and alkaline environment) and their anti-microbial properties. The most prevalent fungi were *Aspergillus, Fusarium, Dendryphiopsis* and *Phoma* species. Surprisingly, only one species of *Penicillium* was found. Bioactivity results indicate that all fungal isolates produce substances that inhibit the growth of at least one test microorganisms (*B. megaterium, S. aureus, E. coli, C. albicans, A. niger)*. Isolated *A. terreus* was found to exhibit the highest antimicrobial activity inhibiting the growth of all test microorganisms. The antimicrobial activity of *Phoma* species was observed to be the lowest. Other strains of the genus *Aspergillus* were also reported to have antimicrobial activity (Ali *et al*., 2014). It was demonstrated that the extremely halophilic fungal strain *P. imranianum* isolated from a man-made solar saltern in Phetchaburi province, Thailand, possesed antibacterial potential against gram-negative bacteria (*Acinetobacter* spp. and *E. coli*) compared to gram-positive ones (*S. aureus*). Its antibacterial activity was demonstrated by plate screening method (Ali *et al*., 2018).

A study of halophilic fungi from solar saltern (Helan Soundra Rani and Kalaiselvam, 2013) recorded *Cladosporium* sp.*, H. werneckii, A. alternata* isolates grown at 15% NaCl concentration. All isolates were screened for secondary metabolite production. Then, the potential of a secondary me-

Table 3. Antimicrobial activity ofsome halophilic and halotolerant fungi

Fungi	Antibacterial activity/test microorganisms	References
Cladosporium sp	*Salmonella typhi*, *Klebsiella pneumoniae*	Helan Soundra Rani and Kalaiselvam, 2013
H. werneckii	*Escherichia coli*, *Vibrio parahaemolyticus*	Helan Soundra Rani and Kalaiselvam, 2013
A. alternata	*E. coli*	Helan Soundra Rani and Kalaiselvam, 2013
A. protuberus	*Staphylococcus aureus*	Corral *et al*., 2018
A. flocculosus PT05-1	*Enterobacter aerogenes, Pseudomonas aeruginosa, Candida albicans*	Zheng *et al*., 2013 Wang *et al*., 2011
A. terreus PT06-2	*E. aerogenes*, *P. aeruginosa*, *C. albicans*	Zheng *et al*., 2013 Wang *et al*., 2011
P. imranianum	gram negative bacteria *(Acinetobacter* spp. and *E. coli)* gram positive one *(S. aureus).*	Ali *et al*., 2018

tabolite extract from *A. alternata* was observed by testing with human pathogens and it showed maximum inhibitory effect against *E. coli*. Extracts of *Alternaria alternata* were highly active against the pathogens *E. coli, S. typhi*, *V. parahaemolyticus*, *K. oxytoca*. Crude extracts from *Cladosporium sp* showed activity against *S. typhi, K. pneumoniae*, and *H. werneckii* fungus also showed inhibition against two pathogens, *E. coli* and *V. parahaemolyticus*. It was concluded that halophilic fungi from solar salterns are diverse, many produce compounds with antimicrobial activity, and could be suitable sources of new antimicrobial natural products (Helan Soundra Rani and Kalaiselvam, 2013).

The halophilic species of the genus *Aspergillus* are the most prolific and several strains of *Aspergillus* sp. have been isolated from Arctic sub-sea sediments from the Barents Sea. In particular, strain 8Na identified as *A. protuberus*, a polyextremophilic fungus able to grow in a wide range of pH, temperature and salinity (up to 25% (*w/v*)) showed antimicrobial efficacy against human pathogens. The strongest inhibitory action was observed against *S. aureus*. The molecule responsible for the activity was identified as *Bisvertinolone*, a compound member of the family Sorbicillinoid (Corral *et al*., 2018). *A. flocculosus* PT05-1 and *A. terreus* PT06-2, both isolated from sediment of Putian sea saltern of Fujian, China, showed antimicrobial activity against *E. aerogenes*, *P. aeruginosa*, and *C. albicans*. Strain PT05-1 produces 11 metabolites, among which two are new ergosteroids and pyrrole derivative compounds (Zheng *et al*., 2013), and strain PT06-2 produces the novel compounds Terrelactone A and Terremides A and B (Wang*et al*., 2011). All studiesof halophilic and halotolerant fungi until now indicate their great potential as producers of enzymes and antimicrobial metabolites.

Conclusions and perspectives

An attempt has been made to add new information on the biodiversity of salt-adapted fungi and their potential in biotechnology. Data persuasively prove the remarkable diversity of halophilic and halotolerant fungi. Published results demonstrate that species of a wide range of fungal genera can grow and form spores in saline environments. These extremophiles are ubiquitously present in natural ecological niches, such as salt lakes, inland seas, evaporation ponds of seawater, salterns, glaciers, the Arctic and Antarctic regions, etc. Taxonomic studies with culturable diversities of halophiles have revealed that the dominant genera are *Aspergillus, Cladosporium*, *Penicillium, Alternaria, Fusarium,* and *Chaetomium*. Many new species have been reported from various saline and hypersaline habitats located in different countries. The investigations of new natural sources of salt-adapted fungi as well as new information about their abundance will expand our knowledge of possible life performance under diverse and most extreme environmental parameters. In addition, understanding fungal adaptation to high salinity will provide new insight into the mechanisms that help organisms to survive under such extreme environmental conditions.

In this review, we have also focused on the capability of halophilic and halotolerant fungi to produce bioactive molecules. All data presented suggest that fungi isolated form different saline habitats are promising producers of extremozimes and new biologically active and antimicrobial substances for use in biotechnology and medicine. They produce unique biocatalysts that function under extreme conditions comparable to some industrial processes. Halophilic enzymes have a great economic potential in many industrial processes, including agricultural, chemical, and biotechnological applications. Haloenzymes, such as amylases, proteases, lipases, pullulanases, cellulases, chitinases, xylanases, pectinases, isomerases, esterases, dehydrogenases, etc. can be excellent candidates for use in the food, pharmaceutical, and detergent industries. They will be increasingly widely utilized in novel biocatalytic processes that are faster, more accurate, specific and environmentally friendly. Searching for novel biological active molecules of pharmacological importance from salt-adapted fungi would further provide new opportunities for discovery and identification of antimicrobial compounds with unique properties, and potential use in medicine.

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