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MINIREVIEW

Fungi in salterns

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Salterns are hypersaline extreme environments with unique physicochemical properties such as a salinity gradient. Although the investigation of microbiota in salterns has focused on archaea and bacteria, diverse fungi also thrive in the brine and soil of salterns. 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 gave attention to black yeasts and their physiological characteristics, including growth under various culture conditions. Since then, biochemical and molecular tools have been employed to explore adaptation of these fungi to salt stress. Genome databases of several fungi in salterns are now publicly available and being used to elucidate salt tolerance mechanisms and discover the target genes for agricultural and industrial applications. Notably, the number of enzymes and novel metabolites known to be produced by diverse saltern-derived fungi has increased significantly. Therefore, fungi in salterns are not only interesting and important subjects to study fungal biodiversity and adaptive mechanisms in extreme environments, but also valuable bioresources with potential for biotechnological applications.

***Keywords*:** salterns, halotolerant and halophilic fungi, black yeasts, biotechnological applications of fungi

Introduction

Microorganisms are found in extreme environments, including hot, cold, salty, dry, highly acidic, and alkaline habitats. Over the last few decades, microorganisms living in harsh conditions have gained increasing attention as promising resources for novel bioactive compounds and as research subjects to understand the evolution of life (Gostincar *et al.*, 2010; Rampelotto, 2013; Chavez *et al.*, 2015). The majority of known microorganisms in these environments are archaea and bacteria, whereas fungi remain largely undiscovered.

Fungi are ubiquitous and degrade complex substances into simpler forms critical for nutrient recycling on Earth. Although few cases have been reported, fungi have been discovered in glaciers, deserts, acidic mine drainages, hot springs, salt fields, hydrothermal vents, and deep sea sediments that are unfavorable to most eukaryotes (Ma *et al.*, 2000; He *et al.*, 2004; Wang *et al.*, 2007a, 2007b; Li *et al.*, 2009; Yang *et al.*, 2009; Stierle *et al.*, 2011; Jiang *et al.*, 2013). Remarkably, several fungal isolates from extreme environments have produced novel natural compounds with unknown chemical structures. For example, *Spiromastix* sp., isolated from deep sea sediment, and *Pseudogymnoascus* sp., obtained from an Antarctic marine sponge, produce depsidone acid and asterric acid derivatives, respectively, with previously undescribed structures (Niu *et al.*, 2014; Figueroa *et al.*, 2015). Therefore, the study of fungi in extreme environments offers an enormous opportunity to discover new bioactive compounds (Chavez *et al.*, 2015).

Salterns are hypersaline extreme environments that provide salts for human consumption after evaporation of seawater. They have unique physicochemical features, including a wide range of salinities, low oxygen, and intense ultraviolet radiation (Oren, 2009; Cantrell *et al.*, 2011). Fungi have long been considered inactive inhabitants of salterns. However, since initial reports of a fungal population in Slovenian salterns from the late 1990s to early 2000s (Tepsic *et al.*, 1997; Zalar *et al.*, 1999b; Gunde-Cimerman *et al.*, 2000), many saltern-derived fungi have been identified. Most of these fungal species have been investigated by Gunde-Cimerman and colleagues (Zalar *et al.*, 1999b; Gunde-Cimerman *et al.*, 2000; Turk *et al.*, 2004; Kogej *et al.*, 2005b, 2007; Butinar *et al.*, 2011; Lenassi *et al.*, 2013; Gunde-Cimerman and Zalar, 2014; Zajc *et al.*, 2014). Their extensive research has drastically extended our knowledge of halotolerant and halophilic fungi inhabiting salterns.

This review covers the physicochemical properties of salterns, diversity and community of saltern-derived fungi worldwide, and salt tolerance strategies and potential biotechnological applications of saltern-derived fungi.

Solar saltern systems

Structure and physicochemical properties of salterns

Solar salterns distribute globally along tropical and subtro-

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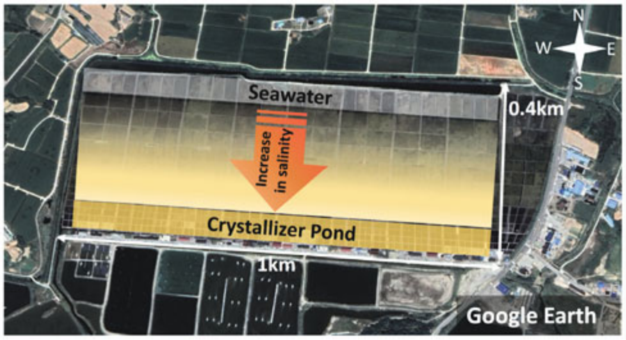

Fig. 1. An example of saltern structure (Gomso, Jeollabuk-do, Republic of Korea). Salterns are multi-pond systems, and salt is produced by seawater evaporating in stages. The salinity in individual ponds gradually increases and reaches its maximum in crystallizer ponds. This map was retrieved from https://www.google.co.kr/maps/@35.5955214,126.6152016,17z.

pical coasts and are composed of a series of separate shallow ponds that are typically less than 0.5 m in depth (Pedros-Alio, 2004). The first pond is filled with seawater by pumping or allowing flow, and then seawater evaporates in stages (Fig. 1). The physicochemical properties of seawater change due to evaporation in the flow-through multi-pond system consisting of evaporation and crystallizer ponds. In a few cases, individual ponds are not interconnected, and both evaporation and crystallization occurs in one pond (Maturrano *et al.*, 2006).

The brine in salterns originates from seawater and thus is dominated chemically by Cl^+ and Na^+ ions. In addition, Mg^{2+}, SO_4^{2-}, K^+, Ca^{2+}, Br^-, HCO_3^-, and F^- ions are present in saltern brines, and the chemical composition of the brine is altered by serial precipitation (Ventosa and Arahal, 2011). Precipitation of less soluble marine minerals such as $CaCO_3$ (aragonite and/or calcite), followed by $CaSO_4$ (gypsum), occurs in the early evaporation stage when the salinity of the brine increases to 2–4 fold that of seawater (Javor, 2002). In the last stage of salt crystal (halite) production in crystallizer ponds, NaCl concentrations surpass 30% (w/v) (Oren, 2009). After this stage, the brine depleted in NaCl, called bitterns, is usually treated as waste. However, if continuous evaporation of the brine is allowed to proceed (to approximately double concentration), additional soluble minerals, including potassium, magnesium, and lithium ions, precipitate (Javor, 2002; Ventosa and Arahal, 2011).

Although the pH of seawater is slightly alkaline owing to carbonate buffering systems, the pH of saline water in salterns is generally neutral (Gunde-Cimerman *et al.*, 2000; Zafrilla *et al.*, 2010; De Leo *et al.*, 2019). The pH of individual ponds in salterns can be regulated by salinity, temperature, and amount of carbonate ions. For example, pH was shown to gradually decrease from a less saline evaporation pond (pH 7.7) to a more saline crystallizer pond (pH 6.4) in a saltern in Egypt (Madkour and Gaballah, 2012).

Factors affecting properties of salterns

Microbial communities in individual ponds affect the physicochemical properties of salterns. For example, saltern crystallizer ponds tend to appear red when brine salinity reaches saturation. This red pigmentation that is caused by halobacteria and β-carotene-rich unicellular alga *Dunaliella salina* facilitates light absorption, increases temperature, and consequently enhances evaporation of brines (Oren *et al.*, 1992). Moreover, microbial mats formed in salterns normally seal the ponds, preventing brine leakage while also leading to anaerobic environments and reduction of evaporation (Javor, 2002). However, the linkages between fungal populations and saltern properties are largely unknown.

Nutrient availability and sediment composition are also associated with saltern attributes via microbial communities. Generally, nutrient availability increases as salinity increases. Comparative data of salinity, nutrient availability, and sediment composition in oligotrophic and eutrophic salterns were summarized in a previous report (Javor, 1983, 2002). Among the available nutrients, phosphate was reported to be a critical factor for growth of *D. salina*, a finding that was not evident in extremely oligotrophic salterns (Dolapsakis *et al.*, 2005). Additionally, the microbial mats do not develop as actively in calcium carbonate sediments as in siliciclastic or volcanic sediments because the large amount of $CaCO_3$ in salterns inhibits the uptake of minerals by microbes (Javor, 2002).

Climatic factors, including temperature, humidity, rainfall, wind, and duration of sunshine, are closely related to chemical parameters of salterns because these factors can determine evaporation rates. For example, high temperature promotes evaporation of brines, whereas low temperatures reduce not only evaporation but also microbial growth and metabolism. Temperature and irradiance are critical factors that control oxygen concentrations by regulating photosynthesis of microbes or brine density in salterns (Wieland and Kuhl, 2006).

Fungal species isolated from salterns

Most saltern-derived fungi studied by Gunde-Cimerman and colleagues were isolated from brine in active solar salterns in Sečovlje, Slovenia (Zalar *et al.*, 1999a; Gunde-Cimerman *et al.*, 2000; Turk *et al.*, 2004, 2007; Butinar *et al.*, 2005a, 2005b; Kogej *et al.*, 2005b, 2006; Vaupotic *et al.*, 2007; Lenassi *et al.*, 2013; Gunde-Cimerman and Zalar, 2014). Several additional fungal species from other salterns around the world, including Botswana, China, India, Israel, Puerto Rico, Spain, South Africa, and Thailand, have been reported (Table 1).

Isolation and identification

The isolation method used is critical for maximization of the number of fungal isolates obtained and an unbiased estimate of fungal population dynamics. Although it would be ideal to have a standardized protocol to isolate fungi in salterns, isolation methods widely vary depending on the research group. Four approaches have generally been used to isolate fungi from saline water in salterns: filtration, agar baiting, enrichment, and biofilm collection (Gunde-Cimerman *et al.*, 2000). Using membrane filters (0.45 μm) to filter water, fungal isolates can be collected and incubated on selective agar media at 20–30°C for 1 to 10 weeks (Nayak *et al.*, 2012). Selective agars include malt extract agar (MEA),

Table 1. Previously reported fungal isolates from salterns worldwide

Fungal isolates	Source	Location of salterns	Reference
Aspergillus fumigatus	Soil	Sečovlje, Slovenia	Tepsic *et al.* (1997)
Hortaea werneckii, *Phaeotheca triangularis*	Water	Sečovlje, Slovenia	Zalar *et al.* (1999a)
Trimmatostroma salinum	Water	Sečovlje, Slovenia / Spain	Zalar *et al.* (1999b)
Aureobasidium pullulans, *Cladosporium* sp., *Hortaea werneckii*, *Phaeotheca triangularis*, *Trimmatostroma salinum*	Water	Sečovlje, Slovenia	Gunde-Cimerman *et al.* (2000)
Eurotium sp. (*E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. repens*)	Water	Sečovlje, Slovenia / Eilat, Israel	Butinar *et al.* (2005b)
Scopulariopsis brevicaulis LMK002, *Scopulariopsis candida* LMK004/ 008, *Verticillium dahlia* LMK006	Soil	Florisbad, South Africa	Mudau and Setati (2006)
Aspergillus sp. (*A. candidus*, *A. caespitosus*, *A. flavus*, *A. flavipes*, *A. melleus*, *A. nidulans*, *A. ochraceus*, *A. penicillioides*, *A. unguis*), *Penicillium* sp. (*P. citrinum*, *P. chrysogenum*, *P. oxalicum*, *P. variabile*), *H. werneckii*	Water	Cabo Rojo, Puerto Rico	Cantrell *et al.* (2006)
Aspergillus japonicus, *Chaetomium globosum*, *Cladosporium cladosporioides*, *P. variabile*	Soil	Cabo Rojo, Puerto Rico	
Aspergillus niger, *C. globosum*, *C. cladosporioides*, *Nigrospora sphaerica*	Microbial mat	Cabo Rojo, Puerto Rico	
Candida quercitrusa JHSb	Soil	Qingdao, China	Wang *et al.* (2007a)
Aspergillus variecolor B-17	Soil	Jilantai, China	Wang *et al.* (2007b)
A. pullulans HN2-3	Soil	Qingdao, China	Chi *et al.* (2007)
P. citrinum B-57	Soil	Jilantai, China	Lu *et al.* (2008)
Emericella sp. (*E. stella-maris*, *E. filifera*)	Water	Sečovlje, Slovenia	Zalar *et al.* (2008)
Alternaria raphanin	Soil	Hongdao (Qingdao), China	Wang *et al.* (2009)
Alternaria alternata, *Aspergillus* sp., *Dendryphiopsis* sp., *Fusarium* sp., *Phoma* sp.	Soil	Botswana	Lebogang *et al.* (2009)
Aspergillus sclerotiorum PT06-1	Soil	Putian (Fujian), China	Wang *et al.* (2011a)
Aspergillus terreus PT06-2	Soil	Putian (Fujian), China	Wang *et al.* (2011b)
Aspergillus versicolor, *Aspergillus wentii*, *P. chrysogenum*, *Penicillium corylophilum*, *Penicillium griseofulvum*	Water	Ribander (Goa), India	Nayak *et al.* (2012)
A. candidus, *A. flavus*, *Aspergillus sydowii*, *A. versicolor*, *A. wentii*, *Eurotium amstelodami*, *H. werneckii*	Soil	Ribander (Goa), India	
A. penicillioides, *A. versicolor*, *H. werneckii*	Water	Santa Cruz (Goa), India	
A. flavus, *Aspergillus gracilis*, *A. penicillioides*, *Aspergillus restrictus* *Sterigmatomyces halophilus*	Soil	Ban Laem (Phetchaburi), Thailand	Ali *et al.* (2013)
Aspergillus sp. (*A. carneus*, *A. flavus*, *A. heteromorphus*, *A. nidulans*, *A. niger*, *A. tamari*, *A. terreus*, *A. tubingensis*), *A. pullulans*, *Cladosporium* sp. (*C. cladosporioides*, *C. dominicanum*, *C. sphaerospermum*), *Diatrypella pulvinata*, *Emericellopsis pallida*, *Eutypella scoparia*, H. werneckii, *Mycosphaerella* sp., *Nectria* sp., *Nigrospora oryzae*, *Penicillium simplicissimum*, *Periconia macrospinosa*, *Phellinum gilvus*, *Phialophora* sp., *Pichia guilliermondii*, *Preussia minima*, *Preussia pseudominima*, *Rhodosporidium* sp., *Scopulariopsis* sp., *Sporothrix* sp., *Trilirachium* sp., *Xylaria hypoxylon*	Microbial mat	Cabo Rojo, Puerto Rico	Cantrell *et al.* (2013)
Aspergillus flocculosus PT05-1	Soil	Putian (Fujian), China	Zheng *et al.* (2013)
Aspergillus sp. nov. F1	-	Weihai, China	Xiao *et al.* (2013)
Myrothecium sp. GS-17	Soil	Gansu, China	Liu *et al.* (2015)
A. tubingensis	Soil	Khambhat, India	Raol *et al.* (2015)
Yarrowia lipolytica N-6	-	Exportadora de Sal, Mexico	Alamillo *et al.* (2017)

marine agar (MA), yeast extract-peptone-dextrose (YPD) agar, potato dextrose agar (PDA), or Czapek dox agar (CDA), with various concentrations of NaCl and antibiotics such as chloramphenicol (50–100 mg/L) or streptomycin sulfate (0.3–0.5 g/L) added (Ali *et al.*, 2013). For agar baiting, agar blocks are placed in tubes and left in saline ponds. After several months, the blocks are collected and incubated on media as above. For the enrichment technique, nutrients (e.g., 1% glucose and 0.5–1.0% yeast extract) are added to water samples, which are then incubated at 25°C and 50 rpm for 1 week. Aliquots of the broth are then plated out on selective media (Mudau and Setati, 2006). Biofilms are collected from the surface of saline water and spread on selective media.

Agar baits and the dilution plate method can be used to collect fungi from soil samples (Tepsic *et al.*, 1997; Lebogang *et al.*, 2009). Agar blocks are incubated in soil for 30 days, and the soil is then diluted with sterile water or seawater. Both baits and diluted soil suspensions are incubated on selective media as above at 30–50°C up to 40 days.

Fungi in salterns have been identified using routine fungal identification techniques: cultural characteristics, microscopy, and sequencing of a genetic marker such as the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Gunde-Cimerman *et al.*, 2000; Butinar *et al.*, 2005b; Cantrell *et al.*, 2006; Ali *et al.*, 2013; Xiao *et al.*, 2013). It is often challenging to identify black yeasts, the dominant fungal species in salterns, by morphological characteristics because of their polymorphisms (de Hoog, 1999). Therefore, molecular tools are required for accurate identification of black yeasts.

Definition of halophilic and halotolerant fungi

In general, microorganisms growing only in the presence of salt are considered halophiles (Larsen, 1986). Halophilic microorganisms are classified into slight (optimum growth at 3% [w/v] NaCl), moderate (3–15% [w/v] NaCl), and extreme (25% [w/v] NaCl, but failing to grow below 12% NaCl) halophiles. In contrast, halotolerant microorganisms inhabit saline environments but survive without NaCl. Extremely halotolerant microorganisms refer to those that can grow in saline conditions above 15% (w/v) NaCl (Kushiner, 1978).

Occasionally, the term halophiles has been used for fungi differently from most prokaryotes: fungi thriving in hypersaline environments but viable without salt are designated halophilic fungi (Gunde-Cimerman *et al.*, 2009). For example, the black yeast *H. werneckii* grows at NaCl concentrations ranging from 0% (w/v) to saturation (32%), with optimum growth at 6–10% (w/v). In previous reports, this species was classified as either a halophilic or extremely halotolerant fungus (Gunde-Cimerman *et al.*, 2000; Kogej *et al.*, 2005b; Vaupotic *et al.*, 2007; Gunde-Cimerman and Zalar, 2014). In this review, we describe *H. werneckii* as an extremely halotolerant species and use the term 'obligate halophiles' for fungi requiring salt to grow.

Black yeasts in salterns

Black yeasts, including *H. werneckii*, *P. triangularis*, *A. pullulans*, and *T. salinum*, are melanized ascomycetous fungi belonging to the order Dothideales or Capnodiales and that are closely related phylogenetically to *Cladosporium* species. They are polymorphic fungi that undergo yeast-like, filamentous, and meristematic development, and hyphal forms are predominantly observed on solid agar media (de Hoog, 1999; Gunde-Cimerman and Zalar, 2014).

H. werneckii is the most extensively studied species among the four representative black yeasts in salterns. It is found in various saline environments, including seawater, sea sponges, mangrove plants, and salted foods (Mok *et al.*, 1981; Brauers *et al.*, 2001; Chen *et al.*, 2012; De Leo *et al.*, 2019). *P. triangularis* was first isolated from a humidifier (de Hoog *et al.*, 1997). *A. pullans* is a ubiquitous species that is frequently found in saline water but also flowers and fruits of various plants (Pinto *et al.*, 2018; Prasongsuk *et al.*, 2018). *T. salinum* was first described as a new species in the brine of Slovenian salterns (Zalar *et al.*, 1999b).

In synthetic medium, *H. werneckii* and *P. triangularis* grow at up to 25% (w/v) NaCl, whereas *A. pullulans* grows at concentrations only up to 10% (Turk *et al.*, 2004). *A. pullulans* displays optimum growth in medium without NaCl. *P. triangularis* cannot tolerate 30% (w/v) NaCl, whereas *H. werneckii* is able to grow at this NaCl level (Zalar *et al.*, 1999a). *T. salinum* grows optimally at salinities from 2–6% (w/v) but tolerates NaCl concentrations from 0% to 26% (w/v) (Kogej *et al.*, 2006).

Obligate halophilic fungi in salterns

The requirement for salt to grow is an unusual characteristic for fungi. Two fungal species, *Wallemia ichthyophaga* and *Aspergillus penicillioides*, have been reported as obligate halophiles isolated from salterns (Zalar *et al.*, 2005; Nazareth and Gonsalves, 2013). *W. ichthyophaga* is a non-melanized basidiomycetous fungus belonging to the order Wallemiales and the class Wallemiomycetes, and it has rarely been isolated from the environment, with only 24 strains isolated to date (Gostincar *et al.*, 2010; Gunde-Cimerman *et al.*, 2018). It is the most halophilic fungus ever described, requiring at least 10% (w/v) NaCl for growth. *W. ichthyophaga* shows optimum growth at 15–20% (w/v) NaCl, and can grow at NaCl saturation (30% [w/v]). It is also one of the most xerophilic (thriving at low water activity) fungal species, which is very rare among the Basidiomycota (Zajc *et al.*, 2014).

A. penicillioides is an ascomyceteous fungus present in diverse saline habitats, including the Dead Sea, salterns, estuaries, and mangroves (Nazareth and Gonsalves, 2013, 2014). When plated on regular fungal culture media such as CDA, PDA, Sabouraud agar, and MEA, *A. penicillioides* isolates from salterns in Goa, India, were unable to grow without the addition of 10% solar salt, and grew in up to 20% solar salt on CDA (Nayak *et al.*, 2012). This species has also been found in foods, optical instruments, and human skin (Ohtsuki, 1962; Castellani, 1964; Kozakiewicz, 1989).

Other fungi prevalent in salterns

In addition to black yeasts, species in the genera *Cladosporium*, *Aspergillus*, and *Penicillium* are commonly found in salterns. *Cladosporium* species are saprophytic and ubiquitous both outdoors and indoors. In a Slovenian saltern, *Cladosporium* sp. was predominantly isolated in media containing 50% sugar or combined sugar and salt, representing conditions with low water activity (Gunde-Cimerman *et al.*, 2000). *Cladosporium* species most frequently isolated from hypersaline water are *C. cladosporioides*, *C. herbarum*, and *C. sphaerospermum* (Gunde-Cimerman *et al.*, 2004). In microbial mats from the Cabo Rojo saltern in Puerto Rico, one of the most abundant clones is *C. halotolerans* based on culture-independent studies (Cantrell *et al.*, 2013).

Aspergillus and *Penicillium* species are also frequently detected in cultured isolates from saltern brines. For example, nine *Aspergillus* and five *Penicillium* halotolerant species were isolated from salt pond water in the Cabo Rojo saltern. *Aspergillus niger* was a dominant fungus in microbial mats at this site (Cantrell *et al.*, 2006). Moreover, *A. niger* and *Penicillium chrysogenum* were dominant among the 60 different species isolated from water samples collected in salterns of Slovenia, Israel, Spain, France, Namibia, Dominican Republic, and Portugal (Butinar *et al.*, 2011). Other *Aspergillus* species, including *A. sydowii* and *A. candidas*, have been isolated from salterns.

Eurotium species, teleomorphic fungi related to *Aspergillus*, are frequently isolated from hypersaline waters of salterns worldwide. The isolation frequency of individual *Eurotium* species differs based on salinity. *E. amstelodami*, *E. repens*, and *E. herbariorum* are dominant in brines with salinities above 17% (w/v), whereas *E. rubrum* and *E. chevalieri* are isolated at lower salinities (Butinar *et al*., 2005b). *E. amstelodami* and *E. herbariorum* are most widely distributed in salterns around the world (Butinar *et al*., 2011).

Adaptation of saltern-derived fungi to salt stress

To inhabit salterns, fungi must overcome osmotic (loss of water from cells and accumulation of solutes in the cytosol) and ionic (increasing levels of Na$^+$) stress in response to high salinity. Importantly, fungal adaption requires coping with fluctuating salinities as well as high salt concentrations (Gunde-Cimerman *et al.*, 2018). Adaptation of fungi to salterns under these conditions have been investigated mainly in black yeasts and the obligate halophile *W. ichthyophaga*.

The most representative strategy for salt adaptation, exhibited by *H. werneckii* and *W. ichthyophaga*, is utilization of compatible solutes. When grown in saline media, fungi accumulate compatible solutes in the cytosol to maintain intracellular Na$^+$ concentrations below toxic levels (Hohmann, 2002). The primary compatible solute is glycerol for both species (Petrovic *et al.*, 2002; Zajc *et al.*, 2014), and glycerol production is regulated by glycerol-3-phosphate dehydrogenase (Lenassi *et al.*, 2011). In addition to glycerol, these two species utilize distinct secondary compatible solutes: erythritol, arabitol, mannitol, and mycosporine-glutaminol-glucoside by *H. werneckii* (Kogej *et al.*, 2005a, 2007) and arabitol and mannitol by *W. ichthyophaga* (Zajc *et al.*, 2014).

Secondly, cell wall structure and melanization are associated with adaptation of saltern fungi to salinity stress. The melanization of cell walls play a role in effective intracellular retention of glycerol, attenuating their glycerol permeability in hypersaline environments (Kogej *et al.*, 2007). The cell wall of *H. werneckii* is melanized, and the degree of melanization is altered in response to various salinities. In transmission electron microscopy images, melanin can be detected as electron-dense granules. On media without salt, a thin layer of melanin granules forms in the outer cell wall of *H. werneckii*, whereas a dense layer of melanin granules appears at its optimum salt concentration (5% NaCl, w/v). When another black yeast, *T. salinum*, is grown without salt, large melanin granules are observed in its outer cell wall. This melanin layer becomes thicker and more compact with increasing NaCl concentrations (Kogej *et al.*, 2006). In a non-melanized fungus *W. ichthyophaga*, cell wall thickness increases in response to elevated salt concentrations (Kralj Kuncic *et al.*, 2010).

Thirdly, maintenance of ion homeostasis using numerous metal cation transporters is a salt adaptation strategy. Genome analysis of *H. werneckii* revealed enrichment of metal cation transporter homologues encoding a K$^+$ channel, Na$^+$/K$^+$ antiporter, Na$^+$/K$^+$-ATPase, and Na$^+$ simporter (Lenassi *et al.*, 2013). Indeed, *H. werneckii* maintains very low amounts of internal Na$^+$ and K$^+$ when exposed to high salt levels (Kogej *et al.*, 2005b). Similar to *H. werneckii*, another black yeast, *A. pullulans*, maintains low levels of cation concentrations in saline environments, indicating that it is a Na$^+$ excluder. In contrast, the *W. ichthyophaga* genome contains few metal cation transporter genes, and transcription of most transporter genes is salinity independent (Zajc *et al.*, 2013). Instead, genes encoding cell wall proteins called hydrophobins are significantly enriched in *W. ichthyophaga*, suggesting that preventing ion entry into cells by modulating its cell wall is an approach to salt adaptation used by this species.

Lastly, plasma membrane fluidity is regulated in response

Table 2. **Natural products from saltern-derived fungi**

Fungal isolates	Natural compounds	Description	Reference
S. brevicaulis LMK002, *S. candida* LMK004, and LMK008	Endo-1,4-β-mannanase	-	Mudau and Setati (2006)
V. dahlia LMK006	Endoxylanase, cellulase, and endomannanase	-	
A. pullulans	Alkaline protease	optimum activity at pH 9 and 45°C	Chi *et al.* (2007)
C. quercitrusa JHSb and *A. pullulans* HN2-3	Lipase	-	Wang *et al.* (2007a)
A. variecolor B-17	Variecolorquinones A and B	Novel quinone, cytotoxic	Wang *et al.* (2007b)
P. citrinum B-57	Pennicitrinone C and penicitrinol B	Novel citrinin dimers	Lu *et al.* (2008)
A. terreus Tsp22	Crude extracellular compounds	Antimicrobial	Lebogang *et al.* (2009)
A. raphani	Alternarosides A-C	Novel cerebrosides	Wang *et al.* (2009)
A. sclerotiorum PT06-1	Indole-3-ethenamide	Novel, cytotoxic	Wang *et al.* (2011a)
A. terreus PT06-2	Terremides A and B	Novel, antibacterial	Wang *et al.* (2011b)
	Terrelactone A	Novel compound	
Aspergillus sp. nov. F1	Cytochalasin E and rosellichalasin	Cytotoxic	Xiao *et al.* (2013)
A. flocculosus PT05-1	Ergosteroids and pyrrole derivatives	Antimicrobial	Zheng *et al.* (2013)
A. flavus, *A. gracilis*, and *A. penicillioids*	Crude extracellular compounds	Antibacterial	Ali *et al.* (2014)
A. flavus and *A. penicillioides*	Crude extracellular compounds	Antioxidant	
A. gracillis	Amylase and xylanase	-	
A. restrictus	Cellulase, lipase, and protease	-	
Myrothecium sp. GS-17	N-acetyl-2,4,10,17-tetrahydroxyheptadecylamine and -acetyl-3,5,11,18-tetrahydroxyoctadecyl-2-amine	Novel amides, cytotoxicity	Liu *et al.* (2015)
A. tubingensis GR1	β-galatosidase	-	Raol *et al.* (2015)
Y. lipolytica N-6	Crude extracellular compounds	Immunostimulatory and antioxidant	Alamillo *et al.* (2017)

to raised salt concentrations by *H. werneckii*. As mentioned above, glycerol is an important compatible solute, but membranes are highly permeable to this compound. Thus, glycerol retention mechanisms are critical for salt adaptation by *H. werneckii*. Compared to salt-sensitive *Saccharomyces cerevisiae* and the less halotolerant *A. pullulans*, the *H. werneckii* plasma membrane is more fluid (Turk *et al.*, 2004). However, fluidity of their plasma membranes are adjusted differentially in response to salt stress in these species. Above their optimum salinities, plasma membrane fluidity increases in *A. pullulans*, whereas it decreases in *H. werneckii* (Turk *et al.*, 2007).

Applications of saltern-derived fungi

Owing to their halophilic or halotolerant properties, saltern-derived fungi are considered to exhibit significant biotechnological potential. For example, halotolerance-linked genes in saltern fungi have been identified and heterologously expressed in salt-sensitive organisms to develop or improve their salt tolerance. Expression of an *A. pullulans* gene encoding 3′-phosphoadenosine-5′-phosphatase improved tolerance of *Arabidopsis thaliana* plants to salt and drought (Gasparic *et al.*, 2013). Because genomes of the extremely halotolerant *H. werneckii* and obligate halophile *W. ichthyophaga* are available (Lenassi *et al.*, 2013; Zajc *et al.*, 2013), additional genes from saltern-derived fungi will be identified with biotechnological applications.

Discovery of useful biosubstances from microorganisms is an important field in biotechnology. Although actual applications of saltern-derived fungi in biotechnology are currently very limited, the number of natural products originated from these fungi is gradually increasing (Table 2). Enzymes, including mannanases, amylases, xylanases, galactosidases, lipases, and proteases (Mudau and Setati, 2006; Chi *et al.*, 2007; Wang *et al.*, 2007a; Ali *et al.*, 2014; Raol *et al.*, 2015), are major compounds found in these fungi. Other bioactive compounds in these fungi include those that exhibit antimicrobial, cytotoxic, immunostimulatory, or antioxidant activities (Wang *et al.*, 2007b, 2009, 2011a, 2011b; Xiao *et al.*, 2013; Zheng *et al.*, 2013; Alamillo *et al.*, 2017). Importantly, several novel compounds have been identified in saltern-derived fungi, including *Alternaria raphanin*, *Aspergillus flocculosus*, *Aspergillus sclerotiorum*, *Aspergillus terreus*, *Aspergillus variecolor*, and *Penicillium citrinum* (Wang *et al.*, 2007b, 2011a, 2011b; Lu *et al.*, 2008; Zheng *et al.*, 2013; Liu *et al.*, 2015).

Conclusion

Owing to their unique physicochemical properties and particularly high levels and gradients of salinities, salterns can serve an ecological model to study halotolerant and halophilic fungi and their adaptation to salt stress. Although fungal communities in salterns remain relatively poorly understood compared to communities of archaea and bacteria, numerous studies support the idea that diverse fungi inhabit these extreme environments.

In addition to taxonomic research and physiological characterization, genomic and molecular studies of saltern-derived fungi have been conducted. Moreover, an increasing number of enzymes and novel metabolites have been identified in saltern fungi, and in *Aspergillus* species, particularly. Several *Aspergillus* sp. have been extensively used in industrial applications, and molecular techniques for *Aspergilli* are also well established. Therefore, the number of studies to discover natural compounds from saltern-derived *Aspergillus* sp. should continue to increase. Further investigation of differentiated properties of cosmopolitan species isolated from distinct ecological niches will be required to maximize utilization of saltern fungi. Overall, this integrated information will facilitate biotechnological applications of fungi from salterns.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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