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HALOPHILIC FUNGI: VERSATILE MICROORGANISMS FOR BIOTECHNOLOGICAL ADVANCEMENTS

Shamsuddin Iqbal[1]*, Muhammad Sharif Hasni[1], Yasmeen Malik[1,2], Salman Baloch[1], Rida Aamir[1]

[1]**Institute of Biochemistry,** University of Balochistan, Quetta, 87300, Pakistan

[2]**Mines and Minerals Development Department,** Government of Balochistan, Pakistan

***Corresponding Author:** Shamsuddin Iqbal. Email: shms3853@gmail.com

Abstract

A genus of fungus known as halophilic fungi has evolved special adaptations to thrive in high-salt settings including salt flats, salty lakes, and marine ecosystems. Their potential uses in a variety of industries, including biotechnology, bioremediation, and medicines, have been shown by recent investigations. Halophilic fungi are employed in biotechnology to create enzymes that may be used in industrial processes, such as amylase, protease, and lipase. Halophilic extracellular proteases exhibit great potential in industries like detergents, leather, peptide synthesis, and biodegradation, amylases for biofuel production and beverage industries thanks to their robust properties and ability to withstand harsh industrial conditions. They can also support bioremediation by reducing pollutants like polycyclic aromatic hydrocarbons (PAHs) and oil spills with the help of halophilic lipases, offering a more eco-friendly option to traditional remediation techniques. Halophilic fungi generate secondary metabolites having potential medicinal uses, such as antibacterial, antifungal and anticancer drugs. More investigation into halophilic fungi is necessary given their potential to offer environmentally acceptable solutions to industrial and environmental problems.

Keywords: *Antibacterial, Bioremediation, Biotechnology, Halophilic, Polycyclic aromatic hydrocarbons (PAH)*

INTRODUCTION

There are a variety of hypersaline environments found around the world, including saline soil, salty water, and salted food. Nevertheless, hypersaline soils display a wide range of diversity and heterogeneity in terms of their composition and nature, which can consist of various minerals at different depths (1). Most of the microorganisms discovered in these environments are archaea and bacteria, with fungi being mostly unknown. Fungi are widely distributed and play a crucial part in splitting up complex substances in uncomplicated forms necessary for nutrient recycling on Earth. Although a few documented cases, fungi have been discovered in a variety of severe habitats, such as icebergs, dunes, acidic pit drainages, boiling springs, saline marshes, hydrothermal wells, and deep ocean sediments. These conditions are typically not favourable for most eukaryotic species (2). Surprisingly, a number of fungal species from harsh conditions have generated brand-new natural chemicals with a variety of unidentified chemical compositions. As a result, researching fungi in extreme environments presents a significant opportunity for identifying new bioactive compounds (3). Salterns are examples of such extreme hypersaline environments where salts are produced for human consumption by evaporating seawater. Salterns possess distinctive physicochemical characteristics, such as varying salinities, low oxygen levels, and intense ultraviolet radiation (4).

The microbial population that thrives in hypersaline habitats is a valuable resource due to its ability to adapt to very harsh environments and produce the utmost biometabolites that are currently required for biotechnological applications faced with challenges in changing environments (5). To understand their behaviour, scientists have developed a classification system that categorizes halophiles based on their salt tolerance levels. The system comprises three types: Mild (Moderate) halophiles that prefer salt concentrations ranging from 3-15% NaCl, Intense (Extreme) halophiles that prefer less than 25% NaCl, and halotolerant (Slight) organisms that can grow optimally at 2.5% NaCl (6). Due to its potential biotechnological uses, halophiles have drawn researchers' interest. These halophilic organisms have been found to produce a variety of useful substances, including surfactants, bioactive compounds, enzymes, and pigments. They have also been shown to be effective in degrading metals and removing pollutants from saline water. Recent studies have revealed that halophilic fungi are particularly diverse in hypersaline environments. In 2000, a study was conducted to investigate the morphological and molecular characteristics of halophilic fungi in a saline environment. The study shed light on the fascinating adaptations of these fungi to high salt concentrations and their potential for biotechnological applications. Further research on halophilic fungi could reveal new insights into their unique properties and open up new avenues for their practical use (7).

Current investigations on halophilic species have mainly employed PCR-based techniques to target 16S rRNA genes (8). However, these studies have only revealed limited information on the determination and proportional prevalence of different halo-biotic microorganisms from various origins. In situ studies proved that a number of the majority widespread segregated and researched salt-loving organisms, such as Haloarcula, perhaps not be significant in their natural environment, making up less than 0.1% of the community (9). In contrast, they often appear in isolation studies. Recent comparative genomic and proteomic analyses have revealed that halophiles possess unique molecular signatures that correspond to their adaptations to the environment. In contrast to non-halophiles, halophiles have more stable chromosomes and contain fewer transposable IS elements, as evidenced by studies (10). Profiling of halophilic proteomes revealed unique features such as minor hydrophobicity, high redundancy of acidic dregs, low cysteine presence, minute tendency to create spiral, plus maximum propensity to form curled shapes similarly, it has been noted that halophilic microorganisms exhibit distinct patterns of dinucleotide and codon usage (11).

Halophiles have adapted different techniques to survive in high salt environments, including the accumulation of compatible solutes (12). Some halophiles use the "salt in" strategy, which involves the accumulation of significant quantities of Na+, and K+ in cytoplasm using energy-dependent ionic pumps and protein transporters (13). In contrast, the "low salt in" strategy involves maintaining low salt concentrations in the cytoplasm and using compatible solutes to survive in hypersaline conditions (12). Acidic proteins produced by halophiles can increase solvation and prevent protein clapping, precipitance, and denaturation in high-salinity environments (14). While extremely halophilic archaea accumulate high levels of potassium, eukaryotes encompassing fungi among others are not able to bear such elevated intracytoplasmic electrolytic proportions, and instead produce and accumulate glycerol, trehalose, and other compatible solutes (12). Halophilic fungi are an important group of halophiles that have the potential for biotechnological applications, and their extracellular metabolites are easily extracted, thus demonstrating superior proficiency with respect to number and standard for application in biotechnology (15). Therefore, it is important to investigate the biotechnological potential of halophilic fungi to attract the industrial and academic world to enhance research on their potential for use in biotechnology.

DISCOVERY AND DISTRIBUTION OF HALOPHILIC FUNGI

The discovery of halophilic fungi as thriving occupants of hypersaline surroundings was made known. This revelation followed their detection in artificial solar salterns located in Slovenia, as reported by (16). Over time, there have been multiple accounts of halophilic fungi in different regions worldwide. Recently, a standard has been established for identifying these fungi. As per this criterion, fungi found in hypersaline environments with a salt concentration up to 1.69 molarity, which is capable to thrive in a culture dish on or more than 2.9 M salt concentration, ought to be classified as salt loving fungus, according to (17). Many important variables, including as the sample site and time, dissolved oxygen content, water holding capacity, and the accessibility of organic and inorganic resources, have a substantial impact on the distribution, development, and lifespan of these fungi have identified these as crucial elements for the geographical distribution of these fungi (18). Halophilic fungi exhibit adaptability to hypersaline environments regardless of the salt concentration range, as they can thrive in various saline habitats, ranging from freshwater to hypersaline waters, as well as in saturated natural or man-made salterns.

According to Oren et al., (2013) halophilic fungi's ability to inhabit such diverse environments is indicative of their resilience and adaptability (12). Phylogenetic Evaluation within this groups have revealed fascinating remote connections with other species or genera, according to (5). Prominent specimen , as identified, include the dark yeast like including similar melanosomes-present fungi of the Cladosporium genre, several microbes of the distorted A. flavus and P. notatum, the heterothalic aspergilus Emri-cella and aspergilus euro-tium, specific non-pigmented yeasts, and Wallemiaceae (18).

GENOMIC ANALYSIS OF HALOPHILIC FUNGI

Halophilic microorganisms are valuable resources for studying the basic principles of life and survival in harsh environment, as well as for understanding their role in major biogeochemical cycles (19). Their study can aid in the identification of genes that are best suited for white biotechnology, by offering insight into salt stress survival and the genetic mechanisms that enable organisms to thrive in high-salt environments (17). By studying halotolerant and obligate halophile fungi like Hortaea werneckii and Wallemia ichthyophaga, researchers have discovered novel molecular mechanisms that allow these organisms to adapt to and survive in high-salt environments (20). These fungi have developed distinct ways to combat high salt concentrations, and their comparative analysis has provided valuable insights into the genetic and biochemical mechanisms that enable organisms to thrive under extreme conditions. For example, the analysis of these fungi has revealed unique molecular mechanisms that are used to counteract the effects of high salt concentrations (20). Such findings have important implications for the development of biotechnological applications that can function in high-salt conditions.

BIOTECHNOLOGICAL RESEARCH ON HALOPHILIC FUNGI

Since the year 2000, a significant amount of research on halotolerant fungal species have concentrated on their molecular and structural resilience in hypersaline environments. These researches have provided insight into the genetic and biochemical mechanisms that allow these fungi to survive and thrive in high-salt conditions. Despite the significant research on the adaptations of halophilic fungi in hypersaline environments, there is comparatively less information available on their biotechnological applications in contrast to salt-loving bacteria. Studies by Oren-2010, Tiquia-2010, Mormile 2010, and Tiquia-Arashiro and Rodriges-2016a) (Bano' et al. 2018, kumar et al 2021) have highlighted the biotechnological potential of halophilic bacteria (21-24). However, this review aims to summarize majority of published applications of halophilic fungi.

BIOACTIVE COMPOUNDS SYNTHESIS BY HALOPHILIC AND HALOTOLERANT FUNGI

The researcher conducted a study to investigate the potential of 43 fungal species, obtained from different environments, to metabolize and synthesize substances that exhibit biological potentials like hemolysis, antibacterial, and acetyl-cholinesterase prohibition (25). The outcomes of the study revealed that halophilic fungi have the ability to synthesize particular biologically active products within stressful situations which are not suitable for nascent species. Interestingly, it has been analyzed that the high sodium chloride concentration has developed enhanced hemolytic performance, which is attributable to the generation of bioproduct that are only soluble in organic solutions. Halophilic fungi, unlike non-halophilic species, do not produce proteins that cause hemolysis of erythrocytes. However, it was found that most halophilic fungi exhibited higher hemolytic activity under low water activity and cold conditions, indicating an immune response to stress. The antibacterial potential of the fungal species showed a similar pattern to the hemolytic activity, and the bioactive extracts or metabolites clearly inhibited the maturation of B. subtilis which is a gram positive bacteria. Among all no extracts demonstrated acetylcholinesterase inhibition. Therefore, the authors suggested that halophilic fungi, such as A. pullans., Var-melanoginum, Hortaea. W, T. salenum, and W. sebi are the most promising species for their haemolytic and antibacterial character, and are potentially utilized for industrial practices.

MEDICINAL USE OF HALOPHILIC FUNGI

The halophilic fungus Aspergillus protuberus isolated from Arctic Ocean abyssal marine sediments, exhibits antibacterial activity against A. baumanii, B. metallica, S. aureus, and K. pneumoniae (26). Aspergillus sp. nov. F1 produces Ergosterol, Rosellichalasin and Cytochalasin E which have potent cytotoxic activity to human tumor cell lines A549, Hela, BEL-7402, and RKO (27). Alternaria sp. produces alterperylenol and stemphyperylenol which have antibacterial activity against Clavibacter michiganensis and antifungal activity against Alternaria brassicicola and Pestallozzia theae (28). The antibacterial activities of three halophilic fungal strains, namely A. gracilis, A. flavus and A. penicillioidis, were evaluated through spectrophotometry and screen plating method to evaluate the effects against two different types of bacteria, gram negative E. coli and gram positive *B. subtilis* (29). The findings demonstrated that these strains were dynamic in creating antibacterial metabolites against both bacterial species tested. In contrast to a previous study conducted by Sepcic et al. (2011), which found that, halo-philic fungi did not show effectiveness against *E. coli*. Additionally, we investigated the antioxidant properties of these halo-philic fungi using thin-layer chromatography and a total phenolic content assay. Interestingly, every obligate halo-philic fungi demonstrated positive antioxidant potential. Notably, A. penicillioidis (species 2) exhibited the highest antioxidant capacity among the tested fungi. Furthermore, we delved into the antioxidant potential of these halophilic fungi through the utilization of thin-layer chromatography and a total phenolic content assay. Surprisingly, all of the obligate halophilic fungi exhibited positive antioxidant potential, suggesting their ability to combat oxidative stress. Among the tested fungi, Aspergillus penicillioides (identified as species 2) demonstrated the most robust antioxidant capacity, highlighting its notable role in scavenging free radicals and protecting against oxidative damage. The screened hydro-lases, including amylases, xylanases, cellulases, proteases, lipases and amylases, were investigated through plate-screening and enzyme assays, and each primitive Fungus extracts were acquired under conditions of 10% sodium chloride concentration, except for A. penicillioidis (sp. 2), which showed positive results for all of the screened enzymes.

In their study, Xiao's et al., (2013) obtained a mildly halophilic fungal genotype, A. species nov. F1. from a saltern located at Weihai, China. Researchers studied the generation of secondary metabolites, specifically cytotoxic compounds, through fungus under different salt concentrations (30). The results showed that an increase in salt concentration led to an increase in the production of cytotoxic compounds. Three cytotoxic compounds, ergosterol, rosellichalasin, and cytochalasin E, had been obtained through isolation from the combined ethyl acetate filterate derived from the broth and mycelium of the A. species. nov. F1 organism. The identification of the segregated products were carried out though 1-H and 13-C NMR spectrum analysis. Cytochalasin-E is highly abundant compound obtained (985 mg) succeeded though rosellichalasin-712 milligram and ergosterol-346 milligram. The unrefined and sterilized cytotoxic metabolites demonstrated anticancerous activity against various abnormal growths. A549, Hela, BEL-7402, and RKO cell lines were all sensitive to the crude extract, albeit data for the latter were not given. High toxicity was demonstrated by the purified compounds toward the human tumor cells like RKO. Ergosterol has a strong inhibitory effect on the RKO human colon cancer cell line. Halophilic fungal biocompounds, such as hydrophobins, offer pharmaceutical applications by enabling hydrophobic drug formulation and delivery, potentially replacing immunogenic synthetic surfactants and enhancing drug stability (31). In the future, halogenated drugs may have increased action against drug-resistant bacteria and OMIC technologies present new potential for the discovery of exclusive properties and/or novel biomolecules derived from halophiles in the future (32, 33).

In a study conducted by Jančič et al. (2016), a total of thirty strains of W. species. were acquired from multiple culture meadia. These strains were obtained from diverse hyper-saline ecosystem (34). Under carefully monitored circumstances, the strains were cultivated and developed to create secondary compounds from the appropriate medium, salt conc: (NaCl and MgCl2), and sugar (glucose). HPLC-diode microarray screening was utilized to detect about hundred various metabolites selected from a total of 200 filtrate of Wallemia species. In this study, NaCl had the greatest impact of all the measured solutes, according to instrument learning analyses. The biological activity of several substances was dramatically impacted by NaCl, according to mass spectroscopy data. The strains produced more hazardous metabolites when sodium chloride content was raised from 5 percent to 15 percent. Rhinosinusitus, asthma, pneumonia, bronchi disorder, and respiratory infections are among the disorder of respiration which these hazardous substances are known to induce. Given that Wallemia species. is frequently encountered in domestic particulate matter and as a contaminant in food, further studies in this area would be interesting to expand our understanding.

APPLICATION IN BIOREMEDIATION

Extraction of metals from halophilic fungi

Bano and colleagues (2018) conducted an experiment in which they tested the metal removal abilities of several obligate halophilic fungi including A. flavus, A. gracilus, A. penicillioidis (two strains designated specie. 1 and specie. 2), A. restrictus, and Halophilic Sterigmato-myces (24). The fungi were grown in 50 mL PDB containing 10% table salt (NaCl) and metallic salts including CdCl2•H2O, CuCl2•2H2O, Fe(NH4)2SO4•6H2O, MnCl2, Pb(NO3) 2, and Zn(NO3) 2 at a concentration of 1000 ppm. After 14 days, the filtrate and biomass were analyzed using atomic absorption spectroscopy and acid digestion method. Reference and controlled samples were employed to ensure accuracy in the results. The findings revealed that each fungus removed at least 67% of the metals on average, with Aspergillus flavus and Sterigmatomyces halophilus showing the highest activity at 85.6% and 83.3% metal removal, respectively. Aspergillus penicillioides (sp. 1) exhibited the least activity with only 67% metal adsorption. Overall, each fungus absorbed at least 63% of each metal, with Fe and Zn showing the highest metal removal rates at approximately 84%. Copper was the least absorbed metal with a rate of 63.2%.

BIODEGRADATION OF POLYCYLIC AROMATIC HYDROCARBONS

Pyrene degradation

Pyrene, a model heavy Polycyclic Aromatic Hydrocarbon (PAH), was effectively removed by Embellisia sp. KJ59 and Alternaria sp. KJ66 at different NaCl concentrations (0%, 2.5%, and 5% w/v). Embellisia sp. KJ59 achieved pyrene biodegradation rates of 98.6%, 96.6%, and 94.8% in the respective salinity levels, while Alternaria sp. KJ66 achieved rates of 95.6%, 95.2%, and 88.3%. Chromatograms illustrating pyrene degradation can be found in the Supplementary material. Notably, the study revealed that salinity did not significantly affect the degradation rate of pyrene by these fungi. Romero et al examined pyrene degradation by Fusarium solani and Rhodotorula glutinis, finding them to degrade 32% and 37% of pyrene in 17 days, respectively. In contrast, Embellisia sp. KJ59 and Alternaria sp. KJ66 showed more efficient pyrene removal within 14 days in both saline and non-saline media. These strains are promising for pyrene biodegradation in various environments (35). In a hypersaline environment (1 M NaCl) using PAHs as the sole carbon source, A. sydowii removed Phe and BaP similarly. Initially, within the first day, the mycelium absorbed a significant portion (70% for BaP and 75% for Phe), followed by partial desorption. After two days, degradation began, resulting in nearly 90% removal of both PAHs by day 10. Dead mycelium had little impact on Phe and BaP elimination (36).

Phenol biodegradation

Jiang et al. (2016) isolated a fungus from triggered sludge in a pharmaceutical industry in Wuhan, China, based on its ability to tolerate phenol and anthracene. The fungus was identified as Debaryomyces sp., with the closest resemblance to Debaryomyces hansenii and Debaryomyces subglobosus. The researchers assessed the capability of fungi to degrade phenol by adding 100-1200 mg/Litre of carbolic acid to the cultured media, supplemented with 5% NaCl. The findings showed that lower preliminary levels of phenol (up-to 499 mg/L) were degraded more quickly than higher concentrations, which took four or more days to degrade completely. The existence of heavy metallic ions Co and Ni inhibited phenol degradation, while Mn and Zn had little effect (37). The researchers also tested the effects of PH, dissolved oxygen, and salinity on phenol degradation and found that a neutral PH of 6.0, high dissolved oxygen levels, and low salinity (1% NaCl) facilitated the growth of the fungus and the degradation of phenol.

SYNTHESIS OF ENZYME

Cellulases

In Gunny et al.'s (2014) study, two fungal strains, A. terreus and P. species were isolated and evaluated for halo-tolerance by using saline cultured media (38). Aspergillus terreus showed great hydrolysis capability at hypersaline level and was selected for additional investigation. The researchers characterized unrefined cellulases from A. terreus for their ionic stability, heat and salinity. To test halo-stability, the enzymes were incubated for either 1 hour or 24 hours in varying salt levels. Enzymes sustainability enhanced upto 15% saline concentration, and then felt down at higher concentrations at both time intervals. Sustainability in heat was analyzed at various ttemperatures (0-78 °C) and salt conc: (0-3 M NaCl), and enzyme stability was found to increase with increasing salt concentration until 40 °C, after which enzyme activity gradually decreased. These results suggest that higher salt concentration increases enzyme thermostability. Finally, the performance of cellulases were assessed in the existance of various ionic-liquids ([BMIM][Ac], [EMIM][Ac], and [BMIM][Cl]). The activity of enzymes were discovered to increase up to 10% ionic liquid concentration, beyond which it decreased gradually.

Amylases

Ali et al. (2016) investigated the characteristics of alpha amylase from the halophilic fungus A. penicillioides (39). The researchers purified the enzyme using column chromatography and found its molecular mass about 42 kilo-Dalton by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. The enzyme exhibited polyextremophilic properties and was found to be alkalophilic, with a maximum activity at PH-9. However, enzyme performance decreased readily beyond PH-9. The alpha amylase showed increased activity up-to 78 °C, which is the optimum temperature reported so far for α-amylase from halophilic fungi, and still retained activity over 80% at 90 °C. The enzyme's activity also increased with increased salinity and exhibited optimum halophilic character at 30%. The addition of CaCl2 activated the enzyme, indicating that it could be a metalloenzyme. However, ZnCl2 greatly inhibited the enzyme, followed by moderate inhibition from EDTA and FeCl2. The enzyme was compatible with various commercial detergents and retained more than 80% of its residual activity in any of the diagnostic surfactant. Furthermore, alpha amylase extracted from A. penicillioides showed better performance in increasing salt concentrations and was more effective than commercial amylase and detergents in such conditions.

ECOLOGICAL PERSPECTIVE

Halophiles and halotolerant fungi, well-suited to hypersaline environments, play significant roles across various fields. In agriculture, the widespread use of herbicides and pesticides to control weeds and pests has led to the accumulation of these compounds and their derivatives in soil and water. Diuron, a phenyl urea herbicide (N-(3,4-dichlorophenyl)-N,N-dimethylurea), is extensively used in sugarcane, citrus, and coffee crops but presents challenges due to its high soil mobility, resistance to natural degradation, and strong toxicity. Furthermore, it can undergo biotic and abiotic reactions to produce more toxic metabolites with genotoxic and teratogenic effects, including 3,4-dichloro anilines, N-3,4-dichlorophenylurea, and N-(3,4-dichlorophenyl)-N-methylurea (40,41) Phenyl urea herbicides' environmental impact stems largely from biological processes, making bioremediation a promising tool for site restoration (42). Bioremediation of toxic material such as herbicides, pesticides, or any other chemical by using microorganism is an ecofriendly approach.

CONCLUSION

Halophilic fungi due to their instinct nature to survive in extremely harsh environment can provide the information about their genotype and phenotype. The bioactive mass from these fungi can be used in medicines and due to global warming the fertile land is converting into saline land; this problem can also be solved by halophilic fungi. Halophilic fungi can also be used for bioremediation and nano technology. We have few data regarding halophilic fungi as compare to other halophilic microbes so a detail research on their genes, bioactive mass and characteristics need to be done in order to evaluate their immense importance in ecosystem.

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