



RESEARCH ARTICLE

HALOPHILIC ASPERGILLUS SPECIES IN EXTREME ENVIRONMENTS: PHYSIOLOGY, ADAPTATIONS, AND APPLICATIONS

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Abstract

Salt is essential for growth at certain limits, but life dwelling in harsh, salty environments are the halophiles. Studies on the halophilic bacteria, archaea, and fungi are reported around different locations, but the fungus *Aspergillus* species is a predominant halophilic eukaryote reported in various research works. They have been reported from an environment with high salt concentrations. Halophilic *Aspergillus* species grow better in harsh conditions and have an exciting opportunity for research and application ahead of them. Their potential in biotechnology, together with their special capacity for adaptation to harsh settings, makes them precious in many sectors from environmental science, to biotechnology, and even in bioremediation settings. Therefore, this work review the physiology and unique adaptations of halophilic *Aspergillus* species in extreme environments, biomolecule produces and their applications in different areas and future prospects.

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Introduction:-

Ascomycetae is a group of mycobiota containing the *Aspergillus* genus. It dwells in hypersaline environments and is considered a halotolerant halophilic filamentous fungus.

Aspergillus species are discovered in hypersaline environments and are classified as moderate to extremely halotolerant and halophilic fungi. They are being identified as new and uncommon species, including those that were previously known. (Gunde-Cimerman & Zalar, 2014).

The halophilic *Aspergillus* species bearing conidiophores are propagated asexually. Halotolerant *Aspergillus* contains a well-developed cell wall, septate hyphae, nucleus, smaller vacuoles, thick granular cytoplasm, and large mitochondria of various shapes. The cytoplasmic membrane is undulated in tandem with the hyphal wall, and lipid globules and melanin granules develop at the front of the cell membrane. Many lysosomes emerged in the hyphal cytoplasm (EL-MeLEigy MA, n.d.). According to the study, the *Aspergillus* species has larger conidia, can tolerate dry circumstances, and can endure high salt. The thicker hyphae of halophilic *Aspergillus* maintain the cell's ionic balance also reported (Jiménez-Gómez et al., 2022).

The halophilic *Aspergillus* species is commonly found in soil, decaying organic matter, and indoor environments. These filamentous fungi are found in the different geographical regions of around world, for eg. fungi from the great Sebkhah of Oran, Algeria (Chamekh et al., 2019), Miani-Hor Mangrove Forest Soil from Pakistan (Khan et al., 2020),

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Gulf sediment located in North America (González-Martínez et al., 2017), Bonna sediment located in New England (Corral et al., 2018a), flora of Salt mines and salterns in Jilin, China (Qiu et al., 2020). The halophiles live in association with a plant growing around salt mines, the *Medicago sativa* L. Plant (González-Abad et al., 2019a) and fibrous waste material of (Liu et al., 2017).

This salt-loving species is divided into the ranges of salt concentrations found naturally and in artificial environments. Dead sea marine environments and salt lakes contain moderate to extreme salt concentrations (Chamekh et al., 2019; Lee, 2013). They can grow well in various substrates and to various environmental conditions, such as different temperatures, low water conditions, pH levels, and different salt concentrations (Pitocchi et al., 2020). The halophiles are categorized into moderate and extreme salt-loving species. The species sustain and adapt themselves to environments or niches that contain moderate to extreme salt extents.

The majority of halophilic fungi are included in Ascomycota, of which the most important species is *Aspergillus* species. The exact classification of the Halophilic *Aspergillus* genus may vary depending on the specific species and recent phylogenetic analyses. But, in many studies, different species of *Aspergillus* like *A. flavus*, *A. niger*, *A. terreus*, *A. versicolor*, *A. restrictus*, *A. glaucus*, *A. chevalieri*, *A. alternate*, *A. destruens*, *A. loretoensis*, *A. atacamensis*, *A. salisburgensis*, *A. sclerotialis*, *A. Montevidenis*, *A. cristatus*, *A. ruber* (previously *Eurotium rubrum*), *A. Subalbidus*, *A. candidus*, (Gunde-Cimerman & Zalar, 2014), *Aspergillus niger*, *Aspergillus nomius*, *Aspergillus* sp. SFB81 and many more species were reported as halophiles in different studies (Wingfield et al., 2023).

Molecular adaptations:

Extremolytes and extremozymes are produced by halophilic fungi in extreme environmental conditions to maintain the osmotic stress (Raddadi et al., 2015).

Exposure to high salinity causes fungi to experience osmotic stress and ionic stress. In high salinity, halophilic fungi adapt mechanisms like salt in, accumulation of organic osmolytes, low salt, salt out, and maintaining the proper membrane fluidity (Pérez-Llano et al., 2020a).

In high osmolarity, some fungi can prevent water loss by accumulating K^+ ions in their cells while excluding Na^+ ions (Plemenitač et al., 2014).

At molecular level mechanisms, the proteins in the plasma membrane contain acidic amino acids at the surface. Proteins make a protective barrier with the help of water, remain active in ionic stress, and prevent dehydration and precipitation of the molecules.

In one of the studies of halophiles *A. sydowii*, Yordanis Pérez-Llano et al. mentioned the balance of ions during osmotic stress is independent of osmotic conditions.

Organic osmolytes or compatible solutes are produced by the genus *Aspergillus* inside the cell but are released extracellularly, and they are extractable. The osmolytes that maintain the osmotic balance and do not alter the activity of enzymes are polyols, noncharged solutes (sucrose, trehalose), anionic solutes (β -glutamate, hydroxybutyrate), zwitterionic solutes (betaine, ectoine), and dimethylsulfoniopropionate. In *Aspergillus niger*, glycerol, erythritol, mannitol, and trehalose have been reported, and glycerol is the only osmolyte reported in most of the hypersalinity (Pérez-Llano et al., 2020b). Some fungi can accumulate osmolytes like polyols, sugars, and amino acids as compatible organic solutes (Roberts, 2005).

Halophiles can cause structural modification in cell envelope due to the presence of pigments and/or hydrophobins (Pérez-Llano Y, Gostinčar C).

Metabolic Adaptation:

The metabolic adaptation of *A. sydowii* is considered as a model for the study of adaptations of halophilic *Aspergillus* species, as reported by Jimenez Gomez et al. In the study, at saturated NaCl conditions, glutamate presence was reported and some amino acids like serine, glycine, alanine, etc levels couldn't change while sulphur containing amino acids like methionine, cysteine and also proline were not found. Tridecanoic acid and palmitic acid were reported in both conditions of *A. sydowii* found in mycelia. Unsaturated fatty acids with an 18-carbon chain

reported at the maximum level for supporting fungal growth in hypersaline growth. In the saturated NaCl concentration, glucanoyltransferase-domain-containing protein and β -1,3 glucan biosynthesis were found. In one of the study movement of glucose, amino acids, and Na^+ facilitate by the membrane protein. (Jiménez-Gómez et al., 2022).

Enzymes by Halophilic *Aspergillus* species: (Hydrolases, Oxidoreductase)

Halophilic *Aspergillus* species produce extracellular biocatalysts under osmotic and ionic stress.

Hydrolases involved in the metabolic pathway use water to break down the molecules, and oxidoreductases oxidize and reduce substrates by transferring electrons from one substrate to the other.

Hydrolases are water-soluble which have polythermophilicity, and can maintain high stability (Datta et al., 2010; Primožič et al., 2019; Ruginescu et al., 2020; Yin et al., 2015; Zain Ul Arifeen & Liu, 2018). This halophilic filamentous fungus produces different extracellular enzymes, has industrial uses, and can be easily extracted (Datta et al., 2010; Fuciños et al., 2012; Primožič et al., 2019).

An extracellular alginate lyase have a potential application such as in the biomedical sector generated by *Aspergillusoryzae* (Singh et al., 2011).

Halophilic fungi produces hydrolases like amylase, lipase, cellulase, protease, xylanase, pectinase, and others (Ruginescu et al., 2020; Zhang et al., 2018).

These enzymes are use in the industries for biofuel production, bioremediation, food, cosmetics, detergent, and pharmaceutical processes, and in various sectors (A. Ali Imran, 2014; Amoozegar et al., 2019; González-Abra delo et al., 2019b; Mudau & Setati, 2006).

Halophilic *Aspergillus* species also produces enzymes oxidoreductases (manganese peroxidases, lignin peroxidases, laccases) which can degrade lignin.

These lignolytic enzymes decolorize dyes, repair coloured effluents, decompose other organic contaminants, and help with bioremediation. (Bonugli-Santos et al., 2012).

Table 1:- Different enzymes from halophilic *Aspergillus* species and their applications.

Enzymes	<i>Aspergillus</i> species	Industrial applications	References
β-galactosidase	<i>Aspergillus tubingensis</i> GR1	Many Industrial and Medical Applications	(Raol et al., 2015)
Cellulase	<i>Aspergillus flavus</i> , <i>Aspergillus restrictus</i> , <i>A. subramanianii</i> A2, <i>A. terreus</i> S11, and <i>A. flavus</i> KUB2	Biofuel Production	(A. Ali Imran, 2014; Chamekh et al., 2019; Namnuch et al., 2021)
Amylase	<i>Aspergillus gracilis</i> , <i>Aspergillus penicillioides</i> , <i>Aspergillus penicillioides</i> TISTR3639, <i>Aspergillus gracilis</i> TISTR 3638, <i>A. subramanianii</i> A2	Saline Waste Water treatment	(A. Ali Imran, 2014; I. Ali et al., 2014a, 2015; Namnuch et al., 2021)
Lipase	<i>Aspergillus gracillus</i> , <i>Aspergillus restrictus</i> , <i>A. subramanianii</i> A2, <i>A. terreus</i> S11	bioremediation of saline oil spills	(A. Ali Imran, 2014; Namnuch et al., 2021) 39
Xylanase	<i>A. gracilis</i> <i>Aspergillus</i> sp. Av10, <i>Aspergillus</i> sp. Sh 86, <i>A. niger</i> G 2-11, <i>A. niger</i> S87, <i>A. niger</i> K6-11, and <i>A. flavus</i> KUB2	Food industry, bakery, paper and pulppharmaceutical, inbio-refinery, andtextile.	(A. Ali Imran, 2014; Kutateladze, 2009; Phuyal et al., 2023)
Protease	<i>Aspergillus restrictus</i> , <i>A. subramanianii</i> A2	fish sauce production	(A. Ali Imran, 2014; Namnuch et al., 2021)
β-glucosidase	<i>Aspergillus sydowii</i> BTMFS55	food and pharmaceutical	(Madhu et al., 2009)

		industry	
Pectinase	Aspergillus sp. Sh 86, A. niger G 2-11, Aspergillus niger S87, Aspergillus sp. Av 10, Aspergillus niger K6-11.	Fruit ripening	(Kutateladze, 2009)
Esterase	A. sydowii EXF-12860, A. destruens EXF-10411	Bioremediation	(González-Abradelo et al., 2019a)
Chitinase	A. flavus	Important food and pharmaceutical industries, antimicrobial agents, elicitors, lysozyme inducers, and immune enhancers	(Beltagy et al., 2018; Pradeep Kumar, 2021)
Alginate lyase	A. oryzae	Biomedical industry	(Singh et al., 2011)
Collagenase	Aspergillus oryzae	Healthcare	(Chamekh et al., 2019; Moubasher, 2018)
Other enzyme			
Lignin Peroxidase, Manganese peroxidase	Aspergillus sclerotiorum CBMAI 849	decolorization of dyes, remove colour from waste water, breakdown of other organic pollutants, and bioremediation	(Bonugli-Santos et al., 2010)
Peroxidase, laccase, Catalase, glutathione peroxidase and superoxide dismutase	Aspergillus sydowii EXF-12860,		(González-Abradelo et al., 2019a; Jiménez-Gómez et al., 2020)
Peroxidase, laccase	Aspergillus destruens EXF-10411		(González-Abradelo et al., 2019a)

Bioactive compounds by halophilic Aspergillus species: (Antimicrobials, anticancer and Antioxidants)

The halophilic Aspergillus, the dominant genus, is living in man-made and naturally found hypersalinity. They are extraordinary producers of diverse bioactive compounds called secondary metabolites or natural products (Corral et al., 2018b)(44)(Corral et al., 2018b). They produce bioactive compounds like antimicrobials, anticancer, and antioxidants used in the case of bacterial infections, cancer treatments, and food prevention.

Halophiles produce bioactive compounds like bisvertinolone, 2-hydroxycircumdatin C, ergosterol, etc. and some novel compounds (Corral et al., 2018b; Zheng et al., 2013) (Table 2).

Halophilic fungus produces secondary metabolites that can be used for survival, growth, and communication, and they have industrial and clinical (Cui et al., 2009; Xiao et al., 2013).

The halophilic Aspergillus species can also produce a natural product that is effective against human and plant pathogens such as bacterial and fungal infections (Table 2).

Some of the secondary metabolites form by halophilic Aspergillus species not yet been identified.

Bioremediation: recycling of environmental pollutants

Copper, silver, zinc, nickel, lead, mercury, arsenic, cadmium, chromium, and selenium are a few of the heavy metals that are harmful to life at very low concentrations (Han, 2002). Most industrial processes associated with metal fabrication, leather tanning, and other landfill hazardous waste sites also produce leachates. Leachates are the main sources of heavy metals, which accumulate in nature and are not converted into degradable form (Reed, 1994; Schalscha, 1998). Bioremediation is a cutting-edge, environmentally friendly, and rapidly expanding technology that makes use of the capacity of bacteria, fungi, algae, or plants to extract heavy metals from contaminated places and either reduce them into less dangerous forms or recover them (Fenner, 2013). Amongst all biological forms, the fungus is the most promising microlife and gives the best results in bioremediations of recalcitrant compounds (Vijayaraghavan, 2006a). Therefore, mycoremediation, or the use of fungus, is a dependable and

effective method, as they are natural decomposers and produce several extracellular oxidoreductase enzymes (Bonugli-Santos et al., 2010; González-Abradelo et al., 2019b).

Table 2:- List of Bioactive compounds produced by halophilic *Aspergillus* species.

Activity	Species	Compounds	References
Antimicrobial	<i>Aspergillus protuberus</i> MUT 3638	Bisvertinolone	(Corral et al., 2018b)
	<i>A. gracilis</i> , <i>A. penicillioideus</i> , and <i>A. flavus</i>	crude product	(A. Ali Imran, 2014)
	<i>Aspergillus flocculosus</i> PT05-1	Ergosteroids, Pyrrrole derivatives:	(Zheng et al., 2013)
	<i>Aspergillus terreus</i> PT06-2	Terremide A & B, Terrelactone A	(Briard et al., 2019; Wang et al., 2011)
	<i>Aspergillus terreus</i> Tsp22	Crude Product	(Lebogang, n.d.)
Antioxidant	<i>A. ochraceus</i> EN31	2-hydroxycircumdatin C	(Cui et al., 2009)
	<i>A. wentii</i> EN-48	Methyl 4-(3,4-dihydroxybenzamido) butanoate, 5-O-methylsulochine, 4-(3,4-dihydroxybenzamido) butanoic acid	(Li et al., 2014)
Anticancer	<i>Aspergillus</i> sp. Nov. F1	Ergosterol, Rosellichalasin, Cytochalasin E	(Xiao et al., 2013)
	<i>Aspergillus flocculosus</i> PT05-1	New ergosterol derivative, 7-noregosterolide, 3b-hydroxyergosta-8, 24(28)-dien-7-one.	(Zheng et al., 2013)

The polyextremophilic α -amylase obtained from halophilic *Aspergillus gracilis* TISTR 3638 can be effectively applied for saline wastewater remediation in mild acidic and temperature conditions and at lower water activity (I. Ali et al., 2014b). *Aspergillus* species like *A. tubingensis* GR1 isolate efficiently produce β -galactosidase by using agro-based waste, largely a part of unused crop residues. These halophiles can solve the reuse of agro-based waste for commercial purposes (Raol et al., 2015). Halophilic mycobiota like *A. sydowii* and *A. destruens*, due to mycotreatment, biodegrade and adsorb the xenobiotic compound polycyclic aromatic hydrocarbons (PAH) and pharmaceutical compounds (PhC) from saline biorefinery wastewater (González-Abradelo et al., 2019b). A marine fungus, *A. sclerotiorum* CBMAI849, reported that recalcitrant molecules like pyrene and benzopyrene were efficiently metabolized, and it shows the *Aspergillus* species bioremediate the recalcitrant compound in saline conditions (Passarini et al., 2011). In the study of biosorption by obligate halophilic fungi using *Aspergillus flavus*, *Aspergillus gracilis*, *Aspergillus penicillioideus* (sp. 1), *Aspergillus penicillioideus* (sp. 2), and *Aspergillus restrictus*, amongst them *A. flavus* showed 86% efficiency of adsorption (Bano et al., 2018).

Conclusion:-

Halophilic and halotolerant fungi are the mycobiota living in harsh salinity conditions where many species exist. The present study aims to explore the dominant halophilic *Aspergillus* species that inhabit artificial and natural salty environments. Halophilic *Aspergillus* species developed adaptive mechanisms at molecular and metabolic levels, and more studies on adaptation mechanisms species-wise are needed. Halophilic *Aspergillus* species produce different biomolecules which are unique and helpful in different biotechnology and healthcare areas. The halophilic *Aspergillus* species produce biocompounds that are helpful in the recycling of environmental pollutants. To the best of our knowledge study on bioremediation of arid and semiarid regions has not been done so far and there is need to remediate the soil of this regions using halophilic fungi or their biocompounds. Therefore, future research on the isolation of more halophilic *Aspergillus* species and searching for new industrial potential biomolecules have to be explored.

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