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Exploring Halophilic Fungi in Indoor Air: Diversity, Salt Tolerance, and Ecological Implications

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ABSTRACT

Indoor environmental air containing diverse fungal communities. Inside environment is never been investigated for halophilic fungi. In this study, the existence, diversity, and salt tolerance of halophilic fungi were assessed from indoor air samples collected from different points inside the laboratory. A total of 87 isolates were isolated using culture-based methods. Halophilic fungi growth was screened on a range of salt concentrations (0.2 to 2.5 M NaCl). The fungal isolates grow on the elevated NaCl concentrations were classified as moderate halophiles. In the diversity analyses of indoor fungal species, it was revealed that the moderate fungal richness of a species with high evenness across sampling sites. However, statistical analysis showed that abundance vary regionally. On molecular level identification (ITS rDNA sequencing) the isolates of halophilic fungi were confirmed to the genus of Aspergillus. including A. versicolor, A. sydowii, and A. terreus.

Keywords: Indoor environments, halophiles, salt tolerance, built environment..

INTRODUCTION

Indoor environments in which the complex interactions occur between humans and diverse microorganisms. An individual typically spend approximately 90% of their time in enclosed spaces. As the situations, in indoor environments there is establishing an interaction among the microbes and human beings. These areas are home to complex microbial populations that include bacteria, fungus, viruses, and archaea. In indoor environments also present chemical pollutants and allergens. Fungus is a major and constant component of indoor bioaerosols and settled dust (1). Many of the indoor fungi appear to be harmless. But, some of the fungi are responsible for allergic responses, respiratory disorders, opportunistic infections, and the deterioration of building materials, highlighting the importance of understanding indoor fungal ecology (2,3).

Recent developments in molecular based and culture-independent sequencing techniques, it is possible to know that indoor fungal communities are extremely diverse. The indoor fungal communities have different composition than those found in outside fungal communities. Studies analyzing the structure of fungal communities have shown distinct and clear biogeographical patterns. One notable pattern was that the temperate regions of the world had a significantly greater diversity of indoor dust fungal communities than the tropical regions. Additionally, a notable finding was that phylogenetic similarity was largely impacted by the latitudinal location of the sampling site (1). Xerophilic fungal taxa are among the most commonly found components of indoor environments have developed a capability to grow at low water activity. These xerophilic fungi have a strong adaption mechanism in that they used their biochemical pathways to function in environments where water is available in less quantity. Aspergillus and Penicillium are the members, frequently found in indoor air and home dust,which are able to survive in dry environments (4-6). Prominent components of indoor dust mycobiomes having a presence of species like Aspergillus penicillioides and Aspergillus restrictus, which have a great ability to adapt to conditions with low water supply (7). Despite their prevalence, these fast-growing mesophilic taxa and xerophilic species are frequently underrepresented in surveys.

The overlap of xerophilic and halophilic fungi is an important yet understudied aspect of indoor fungal biology. These two physiological features of these fungi are not interchangeable, as certain fungi that thrive in dry settings also exhibit halophilic or halotolerant behavior. This dual adaptation is demonstrated in a number of indoor-associated organisms from the genera *Aspergillus* and *Penicillium* in different studies. The properties are frequently noted for their xerophilic activity and also exhibiting tolerance or dependence on high salinity (8, 9).

Halophilic fungi are the organisms that require, or exhibit optimal growth under, saline conditions due to specialized physiological adaptations that enable them to withstand osmotic and ionic stress (8,10). As halophiles are naturally found in hypersaline environments such as salterns, salt lakes, and marine systems and the halophilic and halotolerant fungi have been isolated from a wide range of habitats, including soils, plant surfaces, food substrates, sediments and built environments (11).

Halophilic fungi have developed survival mechanisms that allow them to withstand osmotic stress resulting from elevated levels of NaCl solution are cell wall modifications, extremely adaptable metabolisms, modifications to the structure of enzymes, the synthesis of natural products, some of its bio macromolecules have different structural characteristics, and they may have adopted some newer mechanisms in high salt concentrations or extreme conditions (10,11). In one of the studies, it has been demonstrated that the adaptability behaviour of *Aspergillus penicillioides* in which this obligatory halophile need salt for the growth. Similarly, *A. penicilloides* can grow in a variety of saline habitats, including marine, athalassohaline, and polyhaline systems. (12).

There is growing evidence reported that xerophilic and halophilic fungi live in transitional habitats that connect terrestrial, air, and marine systems. It has been demonstrated that a variety of xerophilic and halophilic fungal communities are supported by marine surface waters, which are constantly in contact with the atmosphere. In the study it has been also shown that several of these taxa match those that have been identified in indoor settings, indicating possible connections between indoor, airborne, and marine fungal reservoirs (7).

Halophiles can withstand in saline micro-niches created by built environments. The halophilic communities are supported by localized high-salinity conditions created by salt efflorescence on walls, masonry, and other building materials. Such niches are inhabited by halophilic bacteria, which may have the impacts on material discolouration, biodeterioration, and structural degradation, reported in the investigations of both historic and modern buildings (13,14). While the halophilic and halotolerant fungi have also been identified, their diversity and ecological roles are still largely unknown, despite the fact that bacterial halophiles are the most commonly described in these settings (13). Furthermore, data suggests that airborne fungus can persist and spread throughout indoor settings independently of localized sources, suggesting that dispersal dynamics alter indoor fungal communities (15).

The halophilic fungi are becoming more widely known in a variety of settings, and little information is known about their prevalence and diversity in indoor air. A physiologically separate element of the indoor mycobiome may be excluded in conventional indoor fungal investigations, because it is often employing standard culture media and incubation conditions that are not specifically designed for the investigations of halophilic species. Moreover, there are relatively few of the systematic studies that specifically target halophilic fungi in indoor air, especially across disparate geographic and climatic contexts. Closing this gap is essential to defining the ecological range of halophilic fungi in constructed habitats and to developing a more thorough understanding of indoor fungal diversity.

Materials and Methods

Sampling of indoor environments: In the present study the indoor samples were taken across the laboratory (each corner and working area) to isolate the fungal species under different physicochemical conditions. Settle plate method used to trap the airborne spores on plain Potato Dextrose Agar plate at standardized heights of 1–1.5 meters. The isolates were purified again by aseptic techniques. The isolated colonies were further inoculated on to the salt containing Sabouraud dextrose agar plates. The isolates were inoculated in triplicate for assessment of halophiles (16, 17, 18).

Screening of halophiles: The isolated halophilic fungi from indoor environments were inoculated on to the SD agar containing varying concentration of salt NaCl (5% - 25%). Plates were incubated at 28 ± 2 °C for 7–14 days, allowing both fast-growing and slow-growing halophiles to develop. Distinct colonies were sub-cultured onto fresh saline agar. Morphological characteristics such as pigmentation, colony texture, and growth rate was noted (17).

Morphological and Microscopic Characterization

All isolated halophiles were stained with lactophenol cotton blue. Hyphal structures, conidiophores and their arrangements, and spore morphology was observed under a compound microscope and same were recorded.

Salt Tolerance Assay

In salt tolerance assays pure cultures were inoculated onto Sabauraud's Dextrose Agar (PDA) supplemented with graded concentrations of salt NaCl (0.2 – 2.5 M NaCl) and incubated at 28 ± 2 °C for 7 days. Radial colony diameters were measured to quantify growth responses, and isolates were classified as slight halophiles (optimal growth at 0.2 – 0.5 M NaCl) or moderate halophiles (sustained growth at 0.5 – 2.5M NaCl).

Molecular Identification

The molecular identification of halophilic fungal isolates was used to achieve exact taxonomic resolution beyond physical features. A commercial fungal DNA isolation kit (QIAGEN QIA quick PCR Purification Kit) was used to extract genomic DNA, and universal primers ITS1 and ITS4 were used to amplify the ribosomal DNA's Internal Transcribed Spacer (ITS) region. After PCR products were purified and sequenced, BLAST was used to compare the obtained sequences to the NCBI GenBank database in order to identify species. Future Biotech Lab carried out molecular identification using Clustal W multiple alignment software to identify sequences.

Diversity Assessment of Halophilic Fungi

The variety of halophilic fungus isolated from indoor environments was studied using both culture-dependent and molecular methods. While diversity indices like the Shannon–Wiener Index (H') and Evenness (E) were computed to assess community complexity and distribution, species richness (S) was assessed by counting unique taxa recovered from each sampling location (16, 19).

Statistical Analysis

To ensure reproducibility and dependability, all tests were conducted in triplicate and findings given as mean ± standard deviation (SD). To assess the organization of fungal communities in various indoor conditions, diversity indices such as species richness (S), Shannon-Wiener Index (H'), and Evenness (E) were computed. One-way Analysis of Variance (ANOVA) was used to compare diversity indices between sampling sites, and Tukey's post hoc test was used to identify significant differences ($p<0.05$). SPSSv.25 was used for statistical calculations (16, 20).

Results

87 fungal isolates were isolated from five indoor sampling sites, comprising four laboratory sampling points and one central working area (Table 1). The most isolates were collected from Laboratory sample point 3 (n = 21), followed by Laboratory sample points 1 (n = 19) and 2 (n = 18). The lowest number of isolates were found at Laboratory sample point 4 (n = 12) (Figure 1).

The isolates were tested for salt tolerance; growth was seen across a wide salinity range; fungi capable of growing between 0.2 and 2.5 M NaCl were discovered at all sample sites. One isolate (HLSP 01) from Laboratory sample point 1 showed olive-green, velvety colonies and grew best at 2.5 M NaCl, indicating that it is a moderate halophile. Similarly, three screened halophilic isolates (HCWA 03) with olive-green, brown, and brown-green colony morphologies were found in the central working area. The optimal growth conditions for these isolates were 2.5 M NaCl.

The salt tolerance range of recovered isolates was constant across all test locations (0.2-2.5 M NaCl), demonstrating the existence of fungi that can tolerate moderate salinity in indoor air. Isolates from both laboratory sample points and the core working area were mostly classed as moderate halophiles based on their physical traits and growth behavior in saline environments.
The genus Aspergillus was found to be the leading fungal genus among all screened halophilic isolates from indoor sites (Table 1).

Sampling Site	Total Isolates(n)	Representative isolates	Number of Halophiles Screened	Colony Morphology	NaCl tolerance range (M)	Optimal NaCl (M)	Halophilic behavior	Dominant genus
Laboratory sample point 1	19	HLSP	01	Olive-green, velvety	0.2 – 2.5	2.5	Moderate halophile	*Aspergillus*
Laboratory sample point 2	18	--	--	--	0.2 – 2.5	--	--	--
Laboratory sample point 3	21	--	--	--	0.2 – 2.5	--	--	--
Laboratory sample point 4	12	--	--	--	0.2 – 2.5	--	--	--
Central Working area	17	HCWA	03	Olive-green, velvety, Brown, Brown-green	0.2 – 2.5	2.5	Moderate halophile	*Aspergillus*

Table 1. Distribution, salt tolerance characteristics, and dominant genera of halophilic fungi isolated from indoor air sampling sites.

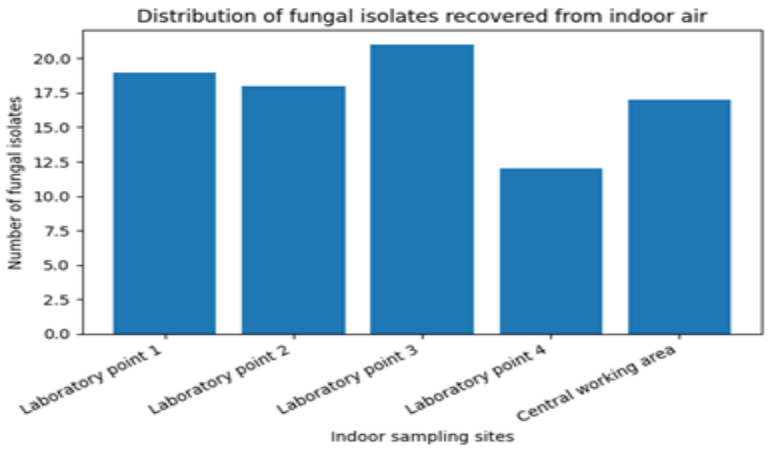

Figure 1. Distribution of fungal isolates recovered from indoor air sampling sites

Shannon-Wiener diversity index(H') and Evenness(E): The Shannon-Wiener diversity index (H') and Evenness (E) were used to evaluate the distribution of halophilic fungi across various indoor laboratory sites. The total fungal counts collected from Lab Spot 1, Lab Spot 2, Lab Spot 3, Lab Spot 4, and the Central Working Area were 19, 18, 21, 12, and 17 respectively. Based on these findings, the overall Shannon-Wiener Index was, H' = 1.593, indicating moderate diversity in the indoor environment. The Evenness score of E = 0.990 indicated that fungal species were distributed fairly evenly among the studied sites, with no particular area dominating the community structure.

When comparing sites separately, Lab Spot 3 contributed the most to diversity due to its higher count (21), whereas Lab Spot 4 Area contributed the least (12), indicating lower fungal richness. Despite the disparities in absolute counts, the strong Evenness value indicates that the fungal community was well distributed across sites. These findings show that halophilic fungi are not limited to a particular microhabitat, but rather spread throughout several indoor niches, adding to overall microbial diversity (Table 2).

Location	Count	Shannon–Wiener Index H'	Evenness E	Mean	SD	ANOVA
Lab Spot 1	19	1.593	0.990	17.4	3.36	F (4,10) = 7.76 P<0.004
Lab Spot 2	18					
Lab Spot 3	21					
Lab Spot 4	12					
Central Working Area	17					

Table 2. Diversity Indices Across Indoor Environments

Post Hoc Analysis: Tukey's HSD test was used to compare pairwise fungal abundance levels across the four laboratory sites and the central working area. The majority of comparisons were not statistically significant ($p > 0.05$), showing that fungal distributions were largely similar across Lab 1, Lab 2, Lab 3, and the Central Area. Lab 1 did not differ substantially from Lab spot 2, Lab spot 3, Lab spot 4, or the Central Area, although Lab 2 was statistically equivalent to Lab spot 3 and the Central Area. Similarly, Lab spot 3 demonstrated no substantial change from the Central Area.

Three pairwise comparisons showed significant results ($p < 0.05$). Lab spot 2 had a greater mean abundance than Lab spot 4 (mean difference = 5), whereas Lab spot 3 differed significantly from Lab spot 4 (mean difference = 7), and Lab spot 4 was significantly lower than the Central Area (mean difference = 7). These findings indicate that, whereas most sites had comparable fungal populations, Lab spot 4 consistently showed lower abundance than Lab spot 2, Lab spot 3, and the Central working area, demonstrating localized variation in fungal colonization within the indoor environment.

Comparison	Mean difference	Significance
Lab1 vs Lab2	2	Not Significant
Lab1 vs Lab3	4	Not Significant
Lab1 vs Lab4	3	Not Significant
Lab1 vs Central	4	Not Significant
Lab2 vs Lab3	2	Not Significant
Lab2 vs Lab4	5	Significant
Lab2 vs Central	2	Not Significant
Lab3 vs Lab4	7	Significant
Lab3 vs Central	0	Not Significant
Lab4 vs Central	7	Significant

Table 3: Tukey's HSD Pairwise Comparisons of Fungal Abundance

A one-way ANOVA was used to compare mean values across five groups (Lab spot 1, Lab spot 2, Lab spot 3, Lab spot 4, and Central working area). The analysis found a significant difference in group means ($F(4, 10) = 7.76$, $p < 0.004$), showing that one group differed considerably from the others.

Molecular Identification: The molecular characterization of halophilic fungal isolates recovered from the indoor environment was performed using ITS rDNA sequencing. BLAST analysis against GenBank revealed that all isolates shared high sequence similarity with members of the genus Aspergillus.

The halophilic fungus isolated from indoor samples belong to the genus Aspergillus, as evidenced by their consistently high similarity scores (≥99%). These data indicate that Aspergillus species are prominent colonizers in the most wet indoor microhabitats, demonstrating their ecological plasticity and tolerance to osmotic stress (Table 4).

Isolate Code	Closest Match (GenBank)	Accession No.	% Similarity
HCWA	*Aspergillus versicolor*	OR259030.1	99%
HLSP	*Aspergillus versicolor*	OR259030.1	99%
HCWA	*Aspergillus sydowii*	MN413179.1	100%
HCWA	*Aspergillus terreus*	MT558939.1	100%

Table 4: Molecular identification of halophilic fungal isolates based on ITS rDNA sequencing.

DISCUSSION:

This study provides clear evidence that halophilic and halotolerant fungi are present in indoor air across multiple laboratory and working environments. Fungal isolates from all sampling sites were capable of growth across a broad salinity range, and representative isolates showed optimal growth at elevated NaCl concentrations, confirming moderate halophilic behavior. These findings demonstrate that indoor air can support salt-adapted fungi and that halophilic traits are not restricted to classical hypersaline environments (1,9,10).

Indoor fungal communities are commonly described as being dominated by mesophilic and xerophilic taxa, reflecting the generally low water activity and intermittent humidity characteristic of built environments (1,5,21,22). The dominance of Aspergillus observed in the present study is consistent with numerous indoor air and dust surveys that report Aspergillus among the most frequently detected genera (1,5,15,22). However, most previous studies relied on conventional media and incubation conditions, which are not optimized for halophilic fungi. In some other studies, salt tolerance testing has been widely applied in extremophilic fungal studies, where genera such as Wallemia, Aspergillus, and Cladosporium have demonstrated remarkable growth under hypersaline conditions (16, 18). By employing saline growth conditions, the present study reveals that a subset of indoor Aspergillus populations exhibit true halophilic or pronounced halotolerant characteristics, supporting earlier suggestions that halophilic fungi are systematically underestimated in indoor surveys (8,9,13).

Halophilic fungi have traditionally been associated with hypersaline lakes, salterns, marine systems, and salt-affected soils (10,11,12). Nevertheless, accumulating evidence indicates that halophilic and halotolerant fungi occupy a much broader ecological range, including food products, dust, building materials, and indoor environments (9,7,13,23,24). Studies documenting halophilic fungi on salt-attacked masonry and historical buildings have demonstrated that built environments can generate saline micro-niches favorable for halophilic microorganisms (13,14,25). The recovery of halophilic fungi from indoor air in the present study extends these observations and suggests that indoor air may act both as a dispersal medium and as a reservoir for salt-adapted fungal propagules.

Indoor environments where fluctuating humidity, periodic desiccation, and low water activity, conditions that select for stress-tolerant fungi (5,15,21). In addition, salt accumulation on indoor surfaces, in settled dust, or through building materials can create localized osmotic stress conditions similar to those found in saline environments (14,25). likewise selective pressures have been projected to explain the presence of halophilic and xerophilic fungi in marine surface waters that are continuously exposed to atmospheric conditions (7). Accordingly, these findings support the view that halophily and xerophily represent overlapping adaptive strategies rather than strictly habitat-specific characters (9,11,23).

The diversity analysis in the present study revealed that the moderate fungal diversity with high evenness found in across indoor sampling sites. Which also indicating that halophilic fungi were distributed across multiple indoor microhabitats rather than being limited to a single location. Although the Laboratory sample point 4 consistently exhibited reduced fungal abundance and differed significantly from other sites, most sampling locations showed comparable fungal populations. The similar findings of relatively uniform fungal distribution across indoor spaces have been reported in large-scale indoor studies, where fungal community composition was influenced more by regional and environmental factors than by room function alone. These findings suggest that halophilic fungi, once introduced indoors, may persist broadly across indoor environments (1,15).

In the molecular identification study, all sequenced halophilic isolates confirmed that they are belonged to the genus *Aspergillus*, with high sequence similarity to *A. versicolor, A. sydowii, and A. terreus*. These species are recognized for their ecological adaptability, stress tolerance, and ability to colonize varied surfaces, including interior materials, marine settings, and saline habitats (12,23,24,26,27). In several studies particular for *Aspergillus sydowii* and *A. versicolor*, have been reported from both indoor environments and saline or marine-

associated systems, with supporting their classification as versatile and stress-adapted fungi (7,12,27). The dominance of Aspergillus among halophilic isolates suggests that this genus may play a key role in colonizing osmotically challenging indoor niches.

Halophilic and halotolerant fungal species may underestimate while using culture-dependent methods as a fungal diversity, non-culturable or slow-growing taxa (3,7). In addition, molecular identification and broader sequencing was performed on a fewer representative isolate. Seasonal variation and environmental parameters such as relative humidity, ventilation, and surface salinity were not assessed and may effect halophilic fungal distribution (5,21). The selective isolation strategy employed here for a significant methodological strength, enabling targeted detection of halophilic fungi that are often overlooked in conventional indoor air studies would minimizes the limitations (9,13).

Both culture-based and culture-independent approaches needs for more characterization of indoor halophilic fungal communities. Functional studies such as examining osmoadaptation mechanisms, stress-response pathways, and potential implications for indoor air quality. Material biodeterioration would further advance understanding in this area (11,14,25). Expanding such investigations across different climatic regions and building types will be essential for determining the broader ecological significance of halophilic fungi in indoor environments.

CONCLUSION

The present study confirms that halophilic and halotolerant fungi are present in indoor air adding to the fungal diversity across laboratory and working environments. Interestingly, we found that the moderately halophilic Aspergillus species are well adapt to indoor conditions. These physiological groups are not be studied in the traditional indoor fungal survey and need to be overlooked. Similarly in the future by incorporating halophilic growth conditions the more diversity of halophilic fungi may isolate and the mechanism of adaptation in built environments is getting in better way..

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