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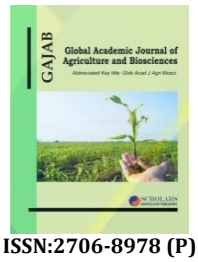

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Original Research Article

Isolation, Identification and Characterization of Fungi from Bathroom Sludge Effluents

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Abstract: Bathroom effluents which not channeled to a soak away pit comes out as bathroom sludge. These effluents when inhaled or in contact with humans can cause infection and disease. Fungi are one of the most ubiquitous organisms found in bathroom, bathroom floor and bathroom sludge effluents. Effluents on bathroom floor are washed off from condict pipes and come out as bathroom sludge. The identification and isolation of fungi isolated from bathrooms sludge from Enugu State University of Science and Technology female hostels was studied using standard methods. The organism was cultured using potato dextrose agar and the antibiotics chrolifinical was added to the agar medium to inhibit the growth of bacteria and support the growth of fungi. The Pd agar was used to isolate a pure culture of fungi. The fungi were further identified morphologically and microscopically using a fungi atlas as a guide. The identified organisms were *Candida albicans, Aspergillus nidulans Aspergillus niger, Aspergillus flavus* and *Mucor mucedo*. The germ test tube was further conducted on *C.albicans* to differentiate it from other species of Candidiasis and a positive *C.albicans* was confirmed. The proliferation rate of these organisms was observed and recorded in day 4 and day 7 respectively. *M.mucedo* has growth rate of + in 4 days and still maintained + in 7 days. All other identified fungi had ++ in day four and ++++ growth rate in day seven, suggesting that the more these organisms are exposed to the environment, they proliferates quickly and cause diseases. These research shows that bathroom sludge effluents are inhibitors of fungi and these effluents when inhaled or exposed to the environment in contact with humans can cause disease and infection and should be channeled to a waste water pit rather than exposed to the environment. Modern building structure should channel this bathroom sludge to a soak away pit rather than channel it to the environment. Cleaning of bathroom with detergent and disinfectant should be encouraged, although it only reduces the microbial load of the fungi but does not eliminate totally.

Keywords: Isolation, Characterization, Fungi, Bathroom, Sludge, Effluents.

INTRODUCTION

Fungi are capable of obtaining nutrients from their substrates and reproducing by producing minute spores, which enables them to disperse and colonize a wide range of environments [1]. Buildings in poor areas of developing countries are more likely to have fungal growth because they're often built without considering the environment, planning, or safety regulations [2].

Mucor species are known to contribute to food spoilage, and certain strains within this genus have been identified as causative agents of mucormycosis, particularly in individuals with underlying conditions like diabetes, leukemia, or immunodeficiency, who are predisposed to infection [3].

Exposure to fungal mycotoxins can occur through multiple routes, including inhalation of airborne contaminants, ingestion of contaminated food products, and dermal contact, leading to a diverse range of adverse health outcomes, deleterious respiratory disorders and gastrointestinal disturbances [4].

Fungal pathogens were detected in both private bathroom settings, and were characterized through a combination of genetic and physical examination [5]. Fungi that thrive in warm temperatures and alkaline environments colonized bathrooms and contaminated residential water systems, including appliances [5, 6].

Species of Exophiala, including *Exophiala jeanselmei*, *E. moniliae*, and *E. dermatitidis*, were found in public bathhouses, bathwater, and bathroom drainpipe sludge, indicating that these environments may serve as a primary habitat for Exophiala fungi [6].

This has left man with the only option of isolating, sub culturing and to identify and characterize these fungi in order to create awareness of the colonization of such spores on bathroom floor bathroom sludge effluents which are not channeled to a waste water pit and exposed to the environment.

MATERIALS AND METHODS

Sample Collection

Samples were collected from bathroom sludge effluents using a sterile universal bottle located from Ten (10) female student's hostel, Enugu State University of Science and Technology Agbani, and transported to the Department of Microbiology. Analysis was carried out at Microbiology Department Laboratory

Preparation of Media

Potato dextrose agar and Sabroud dextrose agar was prepared according to the manufacturer's instructions. The media and glass wares were sterilized in an autoclave at a temperature of 121ºC for 15 minutes. The ager powder of 5.6g of the Pd ager was dissolved in 150 ml of distilled water. The agar media were supplemented with 0.25 mg/ml chloramphenicol to inhibit the growth of bacteria and algae and enhance the growth of fungi.

Inoculation of Fungi

A wire loop was used to collect samples of the bathroom sludge which was streaked on the amended potato dextrose agar supplemented with antibiotics into petri dishes. The incubation of the agar was done at 28 ± 2ºC (room temperature) for 4 – 7 days [7].

Isolation of Pure Culture of Fungi

After incubation, appearance of discrete well separated colonies in culture plates were examined for suspected fungal species base on their colonial morphology. Each distinct colony of fungi were sub–cultured into separate potato dextrose agar plate by picking minute number of hyphae or spores onto a non-inoculated solidified agar plate, much rather at the centre of the agar plate so as to support best colonial growth and spore formation of the fungi. The incubation of the plates was done at room temperature (28 ± 2°C). Fungal growth of pure culture was seen from three (3) days after incubation [8].

Identification of Fungi

The sub-cultured fungi were identified based on their cultural and morphological characteristics such as mycelia, spore type and other fruiting bodies by viewing a lactophenol cotton blue wet mount at x40 objective lens of a compound microscope and comparing the structures to a fungal atlas. *C.albicans* was identified by carrying out germ tube test [9, 10].

Procedure of Germ Tube Test

Put 0.5 ml of serum into a small tube. Using a pipette, touch a colony of yeast and gently emulsify it in the serum. Incubate the tube at 37°C for 2 to 4 hours. Transfer a drop of the serum to a slide for examination. Cover slip and examine microscopically under low and high power objectives. Positive Test: A short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin. Germ tube is half the width and 3 to 4 times the length of the yeast cell and there is no presence of nucleus [9].

RESULT

Table 1: Colonial and Microscopic characteristics of *Candida albicans*

Fungus	Colonial Characteristics on PD Agar	Microscopic Characteristics
Candida albcans	Smooth Creamy Colonies	Oval in shape, taking a yeast form

Table 2: Germ tube test for the confirmation of *Candida albicans*

Fungus	Positive Test	Confirmation
Candida albicans	A short hyphal extension arising laterally from a yeast cell	*Candida albicans* confirmed based on microscopic characteristics.

Table 3: Colonial and Microscopic characteristics of *Aspergilus nidulans*

Fugus	Colonial Characteristics on PD Agar	Microscopic Characteristics
Aspergilus nidulan	Initially white, but turns black/ greenish black with conidal production. Circular in plates	Brown in age, smooth walled surface, conidia surface is smooth, slightly rough in shape.

Table 4: Colonial and Microscopic characteristics of *Aspergilus niger*

Fungus	Colonial Characteristics on PD Agar	Microscopic Characteristics
Aspergilus niger	Compact white or yellow basal felt with a dense layer of dark brown.	Slightly brown smooth walled, glubose in shape and condia surface is very rough irregular.

Table 5: Colonial and Microscopic Characteristics of *Aspergilus flavus*

Fungus	Colonial Characteristics on PD Agar	Microscopic Characteristics
Aspergilus flavus	Consist of dense felt of yellow – green conidiophores.	Conidiophores are hyaline and coarse vesicles are glubose.

Table 6: Colonial and Microscopic characteristics of *Mucor Mucedo*

Fungus	Colonial Characterictics on PD Agar	Microscopic Characteristics
Mucor mucedo	White and beige colour that becomes grey to brown as they age.	Nonseptate, broad hyphae, spore and sporangiosphores is visualized.

Table 7: Proliferation/ Growth Rate of the Isolated Fungi

Fungi	Proliferation Rate in 4 Days	Proliferation Rate in 7 Days
Candida albicans	++	++++
Aspergilus nidulans	++	++++
Aspergilus niger	++	++++
Aspergilus flavus	++	++++
Mucor mucedo	+	+
Key: +: Scanty growth, ++: Moderate growth, +++: Heavy growth		

DISCUSSION

The result showed the colonial and microscopic characteristic of colonial and microscopic characteristics of *Candida albicans* isolated from bathroom sludge (Table 1). The presence of *C. albicans* might be a result of indiscriminate urination on bathroom floor which are passed out as bathroom sludge by condict pipes as researched by Gajdacs *et al*., [11]. This is in agreement with Jabber *et al*., [12], which isolated *C.albicans* in high quantity and percentage from Thi-Qar Government School female bathrooms. The positive germ test tube result to further identify *C.albicans* and differentiate it from other species of candidiasis (Table 2). This is in agreement with the study of Sagar [9], which differentiated *C.albicans* with a positive result from other species of Candidiasis.

Species of Aspergillus was isolated from effluents of bathroom sludge and identified as *Aspergillus nidulans, Aspergillus niger* and *Aspergillus flavus* and identified by colonial and microscopic characteristics using catalogs of fungi (Table 3-Table 5). This result is in agreement with Diba [13], where Aspergillus isolates were identified in the level of species using the differential culture media. A total of 205 Aspergillus isolates studied included: 153(75%) environmental Aspergilli and 52 (25%) clinical isolates. Within 11 Aspergillus species identified, *A.flavus* (55%), *A.niger* (31.7%) and *A.fumigatus* (8.7%) were the most common Aspergillus isolates from all of the specimens.

Mucur mucedo was identified by colonial and microscopic characteristic and we're stated to further differentiate *M.mucedo* from other species of mucor. The fungi as isolated from samples of bathroom sludge effluents from female hostel (Table 6). This result is in agreement with Ozoaduche and Idemudia [14], which showed fungal isolates species *M.mucedo* was only isolated and identified after washing of the bathrooms. There was a significant reduction of the fungal loads on the first week ($p = 0.049$), second week ($p = 0.030$) and third week ($p = 0.048$) in the hostel, after cleaning the bathrooms. Cleaning with

detergent and disinfectant only decrease the number of the microbial load but didn't eliminate the organism totally.

The proliferation and growth rate was determined in day four and day seven respectively by the colonial mass appearance on potato dextrose again plate. *C.ablicans, A.nidulans, A.niger* and *A.flavus* all reordered medium growth in day four of incubation and heavy growth on day seven. *M.mucedo* had scanty growth both in day four and day seven of incubation (Table 7) *M.mucedo* scanty growth was contributed because the fungus require low temperature of about 15°C to grow, while all identified organism in was incubated at 30°C, hence the reason of the scanty growth of *M.mucedo*.

CONCLUSION

Fungi found in bathroom sludge effluents not channeled to a soak away pit was studied for their identification. All identified fungi were isolated from bathrooms sludge effluent and was identified by their colonial and microscopic characteristics. These fungi when inhaled by human or in contact with human can asthmatic attacks, allergies, skin infection and other diseases. In this regard strict practice of hygiene should be observed by humans living close to these bathroom sludge effluents. Identifying this fungi from bathroom sludge effluent exposed to the environment is a step to prevent their infection and disease. Government should ensure that modern building and structure of bathroom sludge should be channeled to a soak away pit, rather than exposed to the environment. Regular cleaning should be enforced and monitored by the Enugu State Waste Management Authority (ESWAMA) where such bathroom sludge is found to avoid proliferation. Cleaning of bathroom with detergent and disinfectant should be encouraged, although it only reduces the microbial load of the fungi but does not eliminate it.

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