

Isolation of fungi in hyper saline Dead Sea water

T. I. Mbata.

T. I. Mbata, Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Nigeria

Abstract

Background: it has been established that some filamentous fungi can survive and grow in high concentration of salts and minerals.

Objective: To isolate and identify fungi in hyper saline Dead Sea water.

Methods: A total of 100 water samples were collected from Dead Sea and tested for the presence of fungi using the membrane filtration technique.

Results: Of a total of 100 water samples, 68 showed fungi contamination. Six (6) different genera of fungi were recovered. They included *Gymnascella* spp 1 (1.5%), *Eurotium* spp 6 (8.8%), *Chaetomium globosum* 14 (20.6%), *Aspergillus versicolor* 30 (44.1%), *Hortaea werneckii* 9 (13.2%), *Aureobasidium pullulans* 8 (11.8%). The most predominant species were *Aspergillus versicolor* and *Chaetomium globosum*.

Conclusion: The isolation of filamentous fungi from hyper saline environment (Dead sea) suggest that the sea could be considered a possible transmission route for filamentous fungi and may potential health hazard.

Introduction:

Fungi are native inhabitants of soil and water and some species behave as opportunistic pathogens in man. (1) They are ubiquitous and no geographical area or any group of people is spared by these organisms. They can contaminate rivers, lakes, ground waters, seas, and oceans. (1) They have been one of the problems facing society and the impact is acutely felt in all countries.

The world health organization (WHO) estimated that up to 80% of ill-health in developing countries is water and sanitation related and only 61% people in developing countries have access to a water supply source especially in rural communities while 36% have access to sanitation facilities especially in urban area. (2) These sources of

water supply are usually contaminated by microorganisms. (3)

Filamentous fungi and yeast are usually found wherever organic matter occurs being mostly parasitic and occasionally pathogenic. Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments. (1) The dead sea which is one of the extreme habitats for microorganisms on earth contain extremely high concentrations of salt (around 340 g/L total dissolved salts), and also its unique ionic composition make the lake a uniquely hostile environment even for halophilic and halotolerant microorganisms adapted to life at high concentrations of sodium chloride (NaCl).

Many of the fungi isolates recovered from dead sea water sample belong to non-

Original Article

halophilic terrestrial species, know for their cosmopolitan distribution examples are *Aspergillums niger*, *Cladosporium cladosporoides* and many others as been documented.(4)

The presence of filamentous fungi in such area as the Dead Sea water was investigated. The aims and objectives of the study were to isolate and identify such fungi found in this hyper saline environment.

Materials and Methods

Study Area

The Dead Sea (Ein Bokek) is the most phenomenal body of water on earth with high specific weight that one can float effortlessly with no fear of drowning. It is 400 meters (1300 ft) below sea level. It is the lowest spot on earth. Part of the 600km Great African Rift valley, 55km long by 17.5km at its widest point, with a depth of up to 400m, this inland sea is flanked by the Judean Hills to the West and by the mountain of Moab in Jordan to the east. Although rivers in Israel and Jordan entered the sea, as well as springs and winter floods, no water flows out of the Dead Sea. Instead it evaporates in the high temperatures twice the amount of water entering leaving one of the world's greatest concentrations of minerals.

Collection of Water Sample

A total of 100 samples of Dead Sea water was collected with sterile screw-capped bottles. The water was collected from three different parts of the site using sterile screw-capped bottles. During the collection, the bottle was dipped with its cap on, a few (about 30cm) below the water surface, the cap was removed with the other hand and water rushed inside the bottle until it was filled, and the bottle was recapped while it was still the water. Due to the distance between the site of collection and the laboratory, the water

was stored in the refrigerator until analysis was done.

Sample processing

Fungal culture

The presence of fungi in the water samples were investigated within one hour of collection using the membrane filtration technique. (5) A 100ml triplicate samples are filtered through sterile, white, grid-marked, 47mm diameter, millipore HA-type cellulose filters with pore size 0.45um. Samples were filtered using a vacuum/pressure pump, 115v, 60Hz filtration unit (Millipore, Bedford, U.K). The membrane filters were placed with the grid side upward on plates of Sabouraud's dextrose Agar (SDA) supplemented with chloramphenicol and on SDA with a chloramphenicol - actidione mixture. The plates were incubated at room temperature (28oc) for 7 days and examined daily for the presence of fungi. Fungal colonies that developed were subcultured onto fresh SDA for pure, single colony isolation and identification. The identification of filamentous fungi was based primarily on the macroscopic and microscopic morphology (slide culture) and the use of color atlas. (6, 7)

Results

Filamentous fungi were isolated from 68 of 100 (68%) water samples examined. a total of 6 genera of filamentous fungi were isolated. They included *Gymnascella* spp isolated from 1 (1.5%), *Eurotium* spp from 6 (8.85), *Chaetomium globosum* 14 (20.6%), *Aspergillus versicolor* 30 (44.1%), *Hortaea weneckii* 9 (13.2%) and *Aureobasidium pululans* from 8 (11.8%). The prevailing isolates were *Aspergillus versicolor* and *Chaetomium globosum*.

Table 1: Frequency of isolation of filamentous fungi in Dead Sea water

Filamentous fungi	Positive samples n	%
<i>Gymnascella</i> spp	1	(1.5)
<i>Eurotium</i> spp	6	(8.8)
<i>Chaetomium globosum</i>	14	(20.6)
<i>Aspergillus versicolor</i>	30	(44.1)
<i>Hortaea werneckii</i>	9	(13.2)
<i>Aureobasidium pullulans</i>	8	(11.8)
Total	68	(100)

Discussion

The study was initiated to isolate and identify the presence of filamentous fungi in the dead sea in order to obtain information on the possible ubiquitous nature of fungi species recovered from the lake.

The prevalence of filamentous fungi was found to be 68% in the examined water samples. The prevailing genera were *Aspergillus versicolor* from (44.1%) and *Chaetomium globosum* isolated from (20.6%) of all 100 samples.

Filamentous fungi are everywhere with world-wide occurrence but little attention has been given to their presence in aquatic environment and in high concentrations of salts and minerals. (1) In this study, the presence of filamentous fungi in the Dead Sea water is very significant because of the large quantities of salts and minerals deposits found in the sea. Two predominant, opportunistic moulds namely *Aspergillus versicolor* and *Chaetomium globosum* were found as the major contaminants of the environment (Dead Sea). (*Hortaea werneckii*, a dermatiaceous hypomycete which occur as halophilic saprophyte and could cause superficial infection on the skin mostly of the thick keratinized sites such as palmar surface of the hands and plantar surface of the feet was isolated. *Gymnascella* spp a non dermatophytic filamentous keratinophilic fungi known to cause skin infection was also recovered.

The presence of these fungi and others represents a potential risk to water borne/skin diseases to the local population and also for holiday makers who usually visit and swim in the sea and sometimes use the black sea mud which they claim to impart a relaxed feelings, nourishing the skin, activating the circulatory system and ease rheumatic discomfort.

In conclusion, the presence and survival of fungi in hyper saline environment like the dead sea have showed that fungi can occur everywhere and there is need to keep good hygiene practice by cleaning the body with good potable water immediately after swimming in the sea to avoid contracting fungal skin infections. This is because the sea could be considered a possible transmission route for filamentous fungi and may constitute a potential health hazard.

References

1. Arvanbitidou M, Kanellou K, Constantinides TC, Katsougannopoles V. The occurrence of fungi in hospital and community potable water. *Letters in Appl Microbiol* 1999; 29: 81-84.
2. WHO Water and sanitation. World health organization fact sheet No 112, 1996.
3. Orji MU, Ogbodo UJ, Mbata TI. Microbiological and consumer evaluation of NAFDAC registered polythene packaged (sachet) water in Abakaliki, Enugu, Awka and Onitsha, Nigeria. *J. Appl Sci* 2006; 9 (3):6685 - 6696.
4. Kis-papoi, Oren A, Wasser SP, Nevo E. Survival of Filamentous fungi in hyper saline Dead sea water. *Microb Ecol.* 2003; 45: 183 - 190.
5. American Public Health Association Standard Methods for the Examination of water and waste water. 19th edn, Eaton, AD Clesceri, LS and Greenberg, AE, 1995; 9:53 - 74. APHA, American water works Association (AWWA), Water Environment Federation (WEF) Washington D.C.
6. De Hoog GS, Guarro J. Atlas of clinical fungi Barcelona. CBS/Universita Rovivai Virgilli 1995.
7. Frey D, Old-field RJ, Bridger RCA. Colour atlas of pathogenic fungi London Wolfe Medical Publication Ltd Holland 1979.