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The Dead Sea Water Harbors No Keratinophilic Fungi

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Many species of filamentous fungi are known to survive in the Dead Sea, but the presence or absence of keratinophilic fungi in this hypersaline lake has not been investigated. In this work, 10 water samples were collected from different locations in the Dead Sea. All samples were studied for the detection of keratinophilic fungi using two procedures in which three different keratin sources and various incubation temperatures were employed. Dead Sea water samples inoculated with dermatophytes were included as positive controls. Results indicated the absence of these fungi from all Dead Sea water samples studied regardless of the keratin source used or the incubation temperature employed. Results indicated that fears of acquiring skin fungal infections "particularly dermatophytoses" as a result of swimming in the Dead Sea are not justified.

Key Words: Dead Sea water, Keratinophilic fungi, Isolation methods, Health hazards.

The Dead Sea is a land locked lake, located approximately 420 meters below sea level in the Jordan rift valley. At present, the Dead Sea is probably the most hypersaline lake in the world with a salinity of 340 gram / liter. This lake differs from others as it contains higher concentration of divalent cations Calcium ang Magnesium[1].

However, plants and animals can not thrive in the Dead Sea environment; many types of microorganisms were isolated. Studies in the past have shown that the most important inhabitants of the Dead Sea water were the unicellular green alga *Dunaliella* and red halophilic *Archaea* of the family Halobacteriaceae[2]. Recent studies have shown that green algae are no more existent; whereas halophilic *Archaea* are still recovered[3, 4]. The first report on the occurrence of filamentous fungi in the Dead Sea was published by Buchalo *et al*[5] and ever since, several other species of filamentous fungi were also isolated[6]. Kis-Papo et al [6] found that the isolated fungi were capable of survival in the hypersaline water of the Dead Sea for a long time. No specific attempt was made to isolate keratinophilic fungi.

Mbata[7] drew attention to the possible health hazard which may be encountered due to the presence of filamentous fungi in the Dead Sea. This alarm is justified as tourists to this attractive site as well as locals, spend long time floating on the sea water. If the water contains potential pathogenic fungi such as dermatophytes, then users of this lake might be exposed to health risks. In fact, swimming in water contaminated with dermatophytes resulted in an out break of dermatophytoses to users[8] and many investigators have raised the possibility of acquiring such

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infections while using recreational water containing keratinophilic fungi[9, 10, 11]. The aim of this investigation was to establish the presence or absence of this group of fungi from Dead Sea water using different keratinaceous materials and different incubation temperatures.

MATERIALS AND METHODS

A total of 10 samples of surface water were collected from the eastern beaches of the Dead Sea. Five samples were obtained from the vicinity of hotels and equal number of samples from public recreational areas. All samples were collected in sterile bottles and were processed in the laboratory within 2 days of collection. The water was divided into portions, centrifuged at 800 rpm for 15 minutes and three fourth of the supernatant was discarded. The remaining portions were pooled and then warmed to 40 ºC before being tested as described below. This pooled sample is referred to hereafter as Pooled Sea Water (PSW) which was used for the isolation of keratinophiles. These samples were presumed to represent four times the strength of Dead Sea water in terms of microbial content.

Isolation technique

Two techniques were used for the isolation of keratinophilic fungi from water as follows:

Method number 1

Autoclave sterilized and dried soil (25 gram) was added to a sterile Petri dish, moistened evenly with 15 ml of PSW before sprinkling approximately 50 grams of keratinaceous material on the surface as baits for the keratinophilic fungi. Three types of keratin were used; baby hair, sheep hair and chicken feather. All were cut into 1-2 centimeters fragments and then sterilized by autoclaving at 121 ºC for 20 minutes before being used in the experiments. Sets of triplicate plates were prepared for each keratinaceous substrate and then incubated at 25 ºC, 30 ºC and 35 ºC for as long as 8 weeks. Plates were inspected at weekly intervals visually and microscopically for the detection of fungal elements (spores and/ or Hyphae) using 10 % KOH wet mount. Results obtained were further confirmed by culture on Sabouraud Dextrose agar (Difco- USA) supplemented with chloramphenicol (50 mg/l) and cycloheximide (500 mg/l).

Method number 2

A 60 ml aliquots of PSW sample was added to a flask containing 100 ml of autoclave sterilized molten mineral salts agar prepared using Venkatesan *et al*.[12] medium supplemented with 3 % Bacto Agar (Difco- USA). The resultant content of each flask was mixed gently then poured into sterile Petri dishes. Three sets of 3 plates were prepared and each set was used for one of the three baits employed in first method. After solidification, fragments of autoclave sterilized keratinaceous material were sprinkled on the surface of the medium and pressed to ensure contact. A moist filter paper was placed on the inner side of the led of each plate and kept wet to prevent drying of the medium over the incubation period which lasted for 8 weeks. Incubation temperatures and verification of the presence or absence of keratinophilic fungi was typically the same as described for method one

Validation of the isolation technique

The ability of both techniques used to isolate keratinophilic fungi was confirmed by using *Microsporum gypseum* and *Trichophyton mentagrophytes* (isolated from soil by Abu Shaqra *et al*.[13]), Each fungus was separately grown on SDA (supplemented with antibiotics and cycloheximide) for 10 days before colonies were harvested with sterile distilled water. The resultant suspension was homogenized and then adjusted with sterile distilled water to give a density of 0.7 at 450 nm. This density was equivalent to a concentration of 10^5. colony forming unit/ ml as determined by spread plate count. When count was not as required, the original suspension was either diluted or concentrated by centrifugation till the right count was obtained. This fungal suspension was prepared for the two soil dermatophytes and each was used separately to inoculate molten Venkatesan agar medium and 25 g of soil so that the final concentration of the respective test organism was approximately 1×10^3 colony forming unit / ml of medium or g of soil. In both cases baby hair fragments were added and plates were then incubated at 25 ºC for 8 weeks.

Determination of fungal colonization of the baits

This was achieved visually and by light microscopy as well as stereomicroscopy. Negative growth was recorded when fungal colonization was not established by any of the verification means,

whereas one plus was assigned when light microscopy revealed the presence of fungal elements while the other two means could not detect fungal colonization which was marked by the presence of whitish fungal growth on the baits. Plus 2 were given when by using stereomicroscopy it was possible to see the whitish growth but when this became visible by the naked eye, plus 3 was assigned.

RESULTS AND DISCUSSIONS

None of the 10 collected Dead Sea water samples gave any sign of positive presence of keratinophilic fungi on plates baited with different keratinaceous materials and incubated under different temperatures. Different baits were used because keratinophilic fungi may exhibit substrate specificity and this is evident from the variation in the rates of keratin degradation by these fungi[14]. Various temperatures was also used in the recovery experiment in order to eliminate the possibility of missing some keratinophiles that may have become adapted to elevated temperatures prevalent in the Dead Sea region. Therefore, the inability to isolate keratinophiles from the Dead Sea water could not have been affected by the type of keratin used in the experiment or the incubation temperatures employed. It was definitely due to the actual absence of these organisms from the Dead Sea water tested.

The first method used for the isolation of keratinophiles from the Dead Sea water was a modification of the hair bait technique commonly used for the isolation of these fungi from soil. The only difference was the substitution of soil by mineral salts medium. The second method involved the addition of centrifuged Dead Sea water to autoclave sterilized soil. In this case, had keratinophilic fungi been present in the water, they would have been distributed in the soil and consequently hair baits colonization could have been detected.

In fact other techniques could have been used in this study. The most commonly employed methods for the isolation of keratinophilic fungi from water are direct plating and filtration techniques[9,15]. Technical difficulties are often encountered in the filtration process particularly if the water sample contains un-dissolved substances and crystallized salts[7]. This is a tedious procedure and might result in the under estimation of the microbial communities present as many organisms might become adherent to filters used

Fig. 1. a photograph which demonstrates the visual colonization of hair baits by *M. gypseum* as obtained from the sterilized soil technique after 8 weeks of incubation

in the preliminary filtration for clearing purposes. In the techniques described in this communication, filtration was avoided and was replaced by centrifugation. Although 4 strength Dead Sea water was used, any other strength could have been prepared simply by discarding as much as required from the supernatant of the spun water sample and thus concentration of keratinophilic fungi could have been achieved. Direct plating method requires additional work to distinguish between keratinophiles and other fungal population which might be present.

Validation studies were mainly directed to establish the ability of the procedures employed to isolate the keratinophiles if present in the water. Fig. 1 is a photograph which demonstrates the visual colonization of hair baits by *M. gypseum* as obtained from the sterilized soil technique after 8 weeks of incubation. Tables 1 and 2 demonstrate that baby hair fragments were colonized by both *M. gypseum* and *T. mentagrophytes* when these organisms were used to inoculate the PSW used in both experiments. Thus, both techniques were effective in the recovery of keratinophiles from water.

The rate of colonization was faster when the mineral salts medium was used instead of soil. This could be explained on the basis of the chemical composition of the soil as compared to that of the mineral salts medium. The salts medium probably was more nutritional than the soil in meeting the growth requirements of the dermatophytes studied and hence faster recovery was noted.

Hair colonization was faster in case of *M. gypseum* as compared to *T. mentagrophytes* (Table 1 & 2). These observations were initially made by light microscopy and were confirmed using stereomicroscopy as well as visual means by noting the appearance of whitish growth on the hair baits. Colonization was recorded for *M. gypseum* microscopically after 3 weeks of incubation on mineral salts medium whereas using the same medium 5 weeks were needed for the detection of growth on the baits by *T. mentagrophytes*. These results demonstrated that the affinity of *M. gypseum* for hair attack was higher than that of *T. mentagrophytes* and this is in agreement with the findings reported by Sharma *et al*.[14] who found that *M. gypseum* was a more potent degrader of hair than *T. mentagrophytes*.

Oren3 considered fungi as halophilic if they were able to grow above 100 g / l salts, even if their salinity optimum was lower. Whereas, Gunde-Cimerman *et al* [16,17] considered fungi as halophilic if they could be isolated in high frequency on selective saline media from environments at

Table 1. Time required for fungal colonization on baby hair used on mineral salts agar medium inoculated with either *M. gypseum* or *T. mentagrophytes*

Dermatophyte	Incubation time in weeks							
	1	2	3	4	5	6	7	8
M. gypseum	-	-	+	+	+ +	+ +	+ + +	+ + +
T. mentagrophytes	-	-	-	-	+	+	+ +	+ + +

Table 2. Time required for fungal colonization on baby hair used on soil mixed with Dead Sea water containing either *M. gypseum* or *T. mentagrophytes*

Dermatophyte	Incubation time in weeks							
	1	2	3	4	5	6	7	8
M. gypseum	-	-	-	+	+	+ +	+ +	+ + +
T. mentagrophytes	-	-	-	-	-	+	+	+ +

salinities above 10% and able to grow *in vitro* with 17% NaCl. The inability to isolate any keratinophilic fungi from Dead Sea water indicated that either this group of organisms does not include halophilic species or they have never been introduced into this environment. The former indication implies that these organisms were at occasions introduced into the sea but were not able to survive due to its high salinity. This is a prudent assumption as users of the sea water who might be infected with dermatophytes; may shed these organisms into it. Thus, had these mycota been capable of surviving in this harsh environment, they would have been isolated using the techniques employed in this work.

The salinity of Dead Sea water is 340 g / liter with high concentrations of divalent cations Ca^{2+} and Mg^{2+} 1. Okafor and Ngwogu18 demonstrated that $CaCl_2$ at a concentration between 0.01M and 1.0 M showed only 50% inhibitory effect against dermatophytes while $MgCl_2$ at a concentration between 0.1M and 1.0M showed less than 50% inhibitory effect on the same group of fungi. These observations may further support our findings as they provide evidence to the inability of dermatophytes to withstand high salts concentrations such as that available in the Dead Sea water.

In conclusion, this communication has clearly demonstrated the absence of keratinophilic fungi from Dead Sea water and established the safe use of the lake for swimming and other recreational purposes without the fear of acquiring skin fungal infections particularly dermatophytoses.

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