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Inventory of Filamentous Fungi and Yeasts Found in the Sea Water and Sand of the Beach of Pier in Arecibo Puerto Rico

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Abstract:

In recent years, an increase in filamentous fungal and yeast infections has been observed. The water and sand of the beach were studied, for three weeks beginning in February, and culminating in march 2021. This study was conducted to determine the diversity of filamentous fungi and yeasts in the sea water and sand of the beach, to determine if they are pathogenic to human and to know the mycological quality of the sand. Samples were taken at three points equidistant from the water and the sand in sterile bags. The culture media were used: SDA, RBA, Hardy CHROM and CHROM agar. For sea water analysis, 100mL were filtered in triplicate and placed on each plate with the different culture media. For sand analysis, 1 gram was weighed in triplicate and spread on the plates with each culture medium. They were incubated for 7 to 14 days at 25°C. Colonies were counted and then isolated. Macroscopic and microscopic analysis was performed. The sea water analysis determined the presence of three genus: *Aspergillus sp., Rhizopus sp.* and *Penicillium sp.* Average yeast ranged from 21 CFU on CHROMagar and 12 CFU on HardyCHROM. The yeast species identified were *Candida albicans* and *C. tropicalis*. The average of filamentous fungi was 12 CFU in RBA and 15 CFU in SDA. The fungal species identified in the water were *A. ochraceus, A. flavus, A. parasiticus, A. niger, A. versicolor P. citrinum, P. chrysogenum, R. oligosporus* and *R. stolonifer*. Two genus were identified in the sand: *Aspergillus sp.* and *Penicillium sp*. The genus *Aspergillus* was the one with the highest identification. Yeast average was: 19 CFU on CHROM agar, 20 CFU on Hardy CHROM. The two yeasts identified were: *C. albicans* and *C. tropicalis*. The average of filamentous fungi in each culture medium was 30 CFU in RBA and 38 CFU in SDA. The filamentous fungi species identified in the sand were *A. terreus, A. versicolor, A. niger, A. flavus, A. oryzae, A. fumigatus*, *P. monoverticillate* and *A. aculeatus*. The quality of the sand was classified as average. There is a connection between filamentous fungi and yeasts identified in sea water and sand with clinical samples. Most of the fungi identified are human pathogens causing infections in different parts of your body.

INTRODUCTION

Numerous studies have been conducted documenting the existence of pathogenic microorganisms in beach sand, providing evidence that sand is a potential reservoir of etiologic disease agents (Whiman *et al.,* 2014, Sabino *et al.,* 2014). Sand and sea water, due to their changing environment, favor the development of fungi. The quantitative and qualitative composition of communities depends on a variety of chemical and biological factors: chemical composition, pH, surrounding vegetation, presence of large and small animals, litter, and climatic factors (Abril *et al.,* 1991). Fungal diagnoses of the skin, respiratory system, hair, and nails are common throughout the world and their incidence continues to increase (Forbes, 2009). Filamentous fungi are identified as potentially dangerous for human health and are found in sand and sea water, so it is important to know their diversity and quantity. In the summer season (June and July) the number of consultations related to fungal skin infections increases from 20% to 25%. This is because in the summer season or in tropical countries there are adequate conditions, such as increased temperature and humidity, which facilitate the growth of these microorganisms (Salmon, 2013).

In Puerto Rico, filamentous fungi were found in the dry area of the northern beaches. 129 fungi were identified, mostly pathogens, in the dry season it was the one with the highest growth (Echevarría, 2017). In another study, fungi were identified on three Caribbean beaches (Puerto Rico, Barbados, and St. Martin), they found three genus: *Aspergillus, Penicillium* and *Rhizopus*. The quality of the sand was identified as average quality. The following species were isolated; *A. niger*, *R. stolonifer*, *P. wasksmanii* (Echevarría, 2019a). In Egypt, Saudi Arabia, they studied fungi in sandstorms, to measure air quality. *Fusarium* (21%), *Cladosporium* (15.8%), *Aspergillus* (10.9%) and *Alternaria* (8.6%) were predominantly identified. The study highlights the need for preventive safety measures to protect the public from exposure to dust (Rajendran, 2017). Likewise, in Egypt, 6 genus of fungi were identified in the sand of the pyramids: *Aspergillus Penicillium*, *Rhizopus*, *Candida*, *Alternaria* and *Hortea*. The sand was classified as average quality (Echevarría, 2021). The geographic location and distribution of fungi is variable. The epidemiology of fungal diseases has been evolving because of migration, lifestyle, pharmacological treatments, and socioeconomic conditions (Mahrean, 2010).

The INSA (National Institute of Health Dr. Ricardo Jorge, in Portugal) indicates that filamentous fungi are the largest group of pathogenic and allergic fungi for humans; *Penicillium sp*., *A. fumigatus*, *A. niger*, *Aspergillus sp*., *Cladosporium sp*., *Paecylomices sp.*, *Curvularia sp*., *Fusarium sp*., *Mucor sp*., *Geotrichum sp*., *Acremonium sp*. (Brandão *et al.,* 2007). Most of the literature reports that the main source of contagion is sand, very few studies in sea water. The presence of fungi in the sand has been studied in various parts of the world, from which they conclude that it should be monitored for the establishment of prevention procedures (Nestor *et al.,* 1984; Méndez, 1997).

On the other hand, yeasts are unicellular, microscopic fungi. These are part of the biodiversity of natural environments and are distributed throughout the world. Its main function is recycling plant and animal remains (García, 2014). The distribution of yeasts in the marine environment is related to geographic and hydrological conditions. They can be found in these places because of the high amount of nutrients. In general, the density and diversity of yeasts begins to decrease towards the open sea and towards the depths of the sea (Latisnere, 2006).

The main common pathogenic *Candida* species for humans include *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis complex*, *Candida Krusei*, *Meyerozyma guilliermondii* and *Candida dubliniensis*. The recently detected pathogenic species, *Candida auris*, whose ecological niche is unknown, is of special interest as it shows high levels of resistance to currently available antifungal drugs (Chowdhary *et al.,* 2017; Lockehart *et al.,* 2017).

The study by (Solo-Gabriele, 2016) indicates that colonies of fungal genus were identified in environmental samples (sea water, sand) and in clinical samples. When comparing the identified species, the interaction of fungal and yeast species found in the three samples (sea water, sand, and clinical samples) is observed. Some species mentioned in the study are *Candida*, *Microsporum*, *Trichophyton, Aspergillus*, *Fusarium, Mucor*, *Penicillium, Alternaria*, these species were found in clinical samples, sand samples and sea water samples. Therefore, the study concluded that there is convincing scientific evidence that beaches, through their sand, contribute significantly to the pathogen load to which beach users are exposed.

So, the objectives of this study were to isolate filamentous fungi and yeasts from beach sand and seawater. Identify the genus and possible species of filamentous fungi and yeasts, using taxonomic keys. Estimate the number of colonies (CFU) of fungi and yeasts. Analyze the ecological quality of the sand. Check if the fungi found are pathogenic for humans.

MATERIALS AND METHODS

The samples were taken from February to March 2021 (three weeks) on the beach at the pier in the town of Arecibo, P.R. The sand samples were obtained from the dry zone of the beach. Approximately 100 grams were taken in sterile bags at three equidistant points and the water sample was taken at three equidistant points from the shore of the beach in sterile bags. The culture media used were RBA, SDA, CHROM agar and Hardy CHROM agar (García *et al.,* 1998). To perform the analysis of the sand sample, 1 gram was weighed. This is spread over a plate containing each of the media; RBA, SDA, CHROM agar and Hardy CHROM in triplicate. They were incubated at 25°C for 7 to 14 days (Echevarría, 2019b). For the water sample, 100 ml are filtered. The membrane was placed in a dish containing each medium; RBA, SDA, Hardy CHROM, CHROM agar in triplicate. They were incubated at 25°C for 7 to 14 days.

As part of the analysis, we perform positive and negative controls. To perform the positive control, one plate is inoculated with each medium (SDA/RBA) with the fungal species A. fumigatus. The objective is to demonstrate that the medium has growth capacity. The same procedure applies for Hardy CHROM and CHROM agar used for Candida albicans yeast. Then we have the negative control that is taken from an uninoculated plate to ensure the sterility of the medium. They are incubated together with the sample. Controls are checked. The average number of colonies forming units (CFU) on each plate is counted and tabulated. Colonies of filamentous fungi and yeast were then isolated in tubes with medium RBA, SDA, CHROM agar and Hardy ChROM agar. To determine the quality of the sand, it was estimated using colony-forming units (CFU) (Forbes, 2009). According to the total number of colonies, the quality of the sands was determined using the maximum values recommended by the National Institute of Saúde Ricardo Jorge (INSA) (Brandão *et al.,* 2011) and the National Institute of Health of Portugal (Pereira *et al.,* 2013). The values recommended by table 1 of the institute, this was used to determine the quality of the beach sands (Pereira *et al.,* 2013). The identification of genus and species was achieved after a macroscopic and microscopic morphological study. For the macroscopic morphology, the color and appearance of the surface and the back of each sample were observed. To study the microscopic morphology, the samples of the isolated colonies were transferred to a slide with the Lactophenol reagent. They were observed under the Nikon Eclipse Ci microscope. The data obtained were compared using taxonomic keys.

Table 1. Values recommended by the National Institute of Saúde Ricardo Jorge and the National Health Institute on Portugal (Pereira *et al.,* 2013).

Values to Determine the Quality of Sands		
> MVA poor quality cfu / g = 85	> MRV average quality cfu / g = 5	≤ MAV good quality cfu / g = 5

RESULTS AND DISCUSSION

After the incubation period, colonies of *A. fumigatus* and *Candida albicans* were observed to grow on the positive controls. The negative control did not grow. The results agree with other studies (De Araújo Pinto et al. 1992). In the sand samples, 2 genus of filamentous fungi and 8 species were found. The genus found in sand samples were *Aspergillus* and *Penicillium*. The species of filamentous fungi in the sand identified were *A. terreus*, *A. versicolor, A. niger, A. flavus, A. oryzae, A. fumigatus, P. monoverticillate* and *A. aculeatus*. One yeast genus and 2 species were found in sand samples. The yeast genus in the sand was *Candida*. The yeast species identified were *C. tropicalis* and *C. albicans*. In the sea water samples, 3 genus of filamentous fungi and 9 species were found. The genus found in the sea water were *Aspergillus, Rhizopus* and *Penicillium*.

Fungal species identified in the water samples were *A. ochraceus*, *A. flavus*, *A. parasiticus, A. niger, A. versicolor, P. citrinum, P. chrysogenum, R. oligosporus*, and *R. stolonifer.* 1 yeast genus and 2 species were also found. The 2 yeasts identified in sea water samples were: *C. albicans* and *C. tropicalis*. In table 2, a summary of the species isolated from the sand and water of the beach was made. The common species of sea water and sand are *A. versicolor, A. niger, A. flavus, C. tropicalis,* and *C. albicans*. The study by Papadakis et al. (1997) found isolates of *Penicillium sp, Aspergillus sp, Mucor, Rhizopus* among many more species.

Table 2. Yeast and molds isolated from water and sand samples.

Organisms	Water	Sands
A. terrus	-	+
A. versicolor	+	+
A. niger	+	+
A. flavus	+	+
A. oryzae	-	+
A. fumigatus	-	+
A. aculeatus	-	+
A. ochraceus	+	-
A. parasiticus	+	-
C. tropicalis	+	+
C. albicans	+	+
P. monoverticillate	-	+
P. citrinum	+	-
P. chrysogenum	+	-
R. oligosporus	+	-
R. stolonifer	+	-

The average (CFU) of fungi in the sea water sample was 15 CFU/ml (RBA and SDA) and in yeast 17 CFU/ml (Chrom agar and Hardy CHROM). The average (CFU) of fungi from sand samples was 34 CFU/g and of yeasts 20 CFU/g. According to the averages of the sand sample, it indicates that the sand quality according to the parameters of table 1 was classified as average quality (>CFU/g=5) (Brandão *et al.,* 2007). The results of fungi and yeasts found in the sand and sea water agree with the results of the study by (Papadakis, 1997), where it is indicated that they found *Candida* in the sea water and sand.

The study by Solo-Gabriele et al. (2016) indicates that, like other studies, there is evidence that sand can serve as a reservoir for microorganisms and fungi, which can be

vehicles for the transmission of diseases on beaches. This study shows a comparison of organisms isolated from sea water, sand, and clinical samples. Most of the species identified in this study use direct contact through air and sea water as a vehicle of transmission. Table 3 shows a summary of the species identified in this study, showing their pathogenicity or ecology. This fungal species causes keratitis (eye infections). Most species have been isolated from patients in nasal cultures and cause asthma (Echevarría, 2017; Echevarría, 2021; St-Germain, 2011; Hu, 2017; Mitchell, 2014; Reza, 2011).

Table 3. Summary of fungal species found and their pathogenicity or ecology (Echevarría 2017, Echevarría 2021, St-Germain 2011, Myung 2020, Hu 2017, Mitchell 2014, Reza 2011).

Species	Pathogenicity or ecology
A. terreus	Pulmonary aspergillosis.
A. versicolor	Onychomycosis
A. niger	Frequent agent of aspergilloma. Causes skin conditions and respiratory infections.
A. flavus	Occasional agent pulmonary infections. Sinusitis and onychomycosis.
A. oryzae	Probiotic filamentous fungus, used in fermentation processes.
A. fumigatus	Most frequently isolated agent of aspergillosis. Causes diseases in the lung, nasal, eye.
A. aculeatus	Plant pathogen
A. ochraceus	Food and beverage contamination.
A. parasiticus	Aflatoxin's food and agricultural commodities.
P. monoverticillate	Sea sand
P. citrinum	Produces mycotoxins. They cause fungal pneumonia and pericardial tamponade.
P. chrysogenum	Can be an allergen, produce skin reactivity, and colonize the airways of patients with respiratory allergies.
R. oligosporus	It causes respiratory diseases, nosocomial infection, and necrosis.
R. Stolonifer	Exposure to high concentrations causes dry socket, skin reactivity has also been observed.
Cladosporium	Infections in the skin and nails.
C. albicans	Superficial skin and nail infection, oropharyngitis, vaginitis.
C. tropicalis	A cause of bloodstream infection is wound infection following major surgery and disseminated infection. Mostly seen in patients with impaired immunity.

The incidence in the last 20 to 30 years has increased the number of cases of *candidiasis*, with *Candida albicans* as the main causal agent in the first place. An investigation found that 8% of *C. tropicalis* and between 3% and 6% of *C. glabrata* and *C. Krusei* tend to show greater resistance to the imidazole fluconazole (Mendoza, 2005). Sand samples are classified as average quality (Pereira et al. 2013). Many of these fungi cause lung and sinus infections (Berger 2015, St-Germain 2011). Most of these fungi use direct contact and airborne transmission as a vehicle of transmission. They cause lung infections and sinusitis (Berger 2015).

Current policies in our country consider the impact of sands on the health of beach users. Several investigations continue to document data and conclude that sand quality testing is recommended to be considered in beach quality programs to protect the public health of beach users.

CONCLUSION

1. The genus *Aspergillus* and *Penicillium* are common in both samples (sea water and sand).

2. The species in common in both samples are *A. niger, A. flavus, A. versicolor, C. tropicalis* and *C. albicans*.

3. Two species of yeasts were identified in the sea water and sand samples: *C. tropicalis* and *C. albicans*.

4. Most of these fungi use direct contact and airborne transmission.

5. Most species of fungi and yeasts are pathogenic to humans.

6. This research showed that the filamentous fungi isolated from the samples are mostly pathogenic and with the reference of other studies that indicates that we must begin to monitor and create controls so that it does not become a public health problem.

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CONFLICT OF INTEREST

The author declares that this article content has no conflict of interest.

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