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Variety of Filamentous Fungi and Yeast Species Found on the White Sands of Crystal Beach in the Bahamas: Swimming with the Pigs

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Abstract:

Research on filamentous fungi in sand in tropical ecosystems is very limited. Every year many tourists visit the beach, especially in summer. Therefore, we want to continue learning about the variety of species of fungi and yeasts that live in the sand on the beach. Especially this beach that has pigs that swim with the people. The quality of the sand was analyzed. The following culture media were used: SDA, RBA, Mycosel and HardyCHROM™ Candida. One gram of sand was weighed in triplicate and spread over the different media. The samples with the SDA, RBA and Mycosel media were incubated at 25 °C for 7 to 14 days. As for the samples with HardyCHROM™ Candida, they were incubated at 35 °C for 48 hours. The colonies were isolated and counted. Macroscopic and microscopic analysis was used. Four genus were identified: *Aspergillus* sp., *Penicillium* sp., *Candida* sp., and *Curvularia* sp. The isolated yeast species were: *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata*. The filamentous fungal species were: *Penicillium* sp., *Aspergillus* sp., *A. flavus*, *A. parasiticus* and *A. glaucus*. The *Candida* genus was the most isolated. The average number of fungal and yeast colonies was 24 CFU/g. The quality of the sand was classified as average quality. Most of the isolates are fungi or yeasts of clinical interest. Many of these identified species are pathogenic, causing respiratory and skin diseases. Other species cause skin infections, respiratory allergies and risk of infection in immunosuppressed people. It is recommended to take measures to prevent these fungi from continuing to develop and thus avoid contamination of the sand.

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INTRODUCTION

Fungal species that inhabit sand and sea water have been identified as dangerous to human health. For this reason, it is extremely important to know the quantity, species and genus that inhabit there. The number of medical consultations related to various fungal infections on the skin can increase to 25%. It has been documented that this is due to the fact that in places with hot conditions (tropics), in the summer season, which are mostly the months of June and July, favorable conditions such as temperature and humidity meet, which favor the growth of these fungi and yeasts (Van den Nest *et al.*, 2021; Salmon and Fuller, 2013). Crystal Beach in the Bahamas, in addition to having white sand and crystal-clear waters, is characterized by the visit of many tourists annually. This is because they have pigs living there. Pigs have become a tourist attraction, so they live with people. Those animals have captured the attention of many tourists, to have a unique experience in the world (Hanes, 2023).

The islands are a meeting point in the months of June, July and August for those tourists who want to enjoy the sun, the beach and take photos in the water and sand with the pigs. Tourism accounts for more than 60% of the island's economy (Basquiat, 2018). These animals feed on fruits. Once they enter the water, they usually leave their excrement in the water and sand (Figure 1).

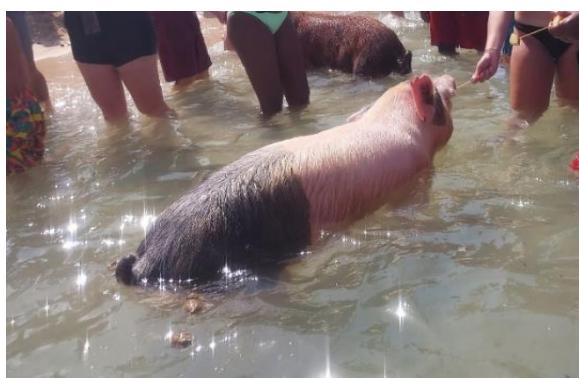


Fig. 1. Pigs at the Crystal beach, Bahamas.

Identifying some articles analyzing fungi in sand in various countries, we can observe the different isolated species. We start with a tropical country such as Puerto Rico. In the study, Echevarría (2017) showed that the dry season was the season of greatest growth of fungal species. 129 species were identified, most of them pathogens. Regarding the quality of the sand, it was classified as average quality. Another investigation by Echevarría (2019a), fungi were isolated in the sand of three beaches in the Caribbean; Puerto Rico, Barbados and Saint Martin respectively. The sand quality analysis resulted in being of average quality. The species isolated in this study were: *A. niger*, *R. stolonifer*, and *P. waksmanii*. Echevarría and Iqbal (2021) managed to identify, in the sand of the pyramids of Egypt, the following species: *Aspergillus*, *Penicillium*, *Rhizopus*, *Candida*, *Alternaria* and *Hortea*. In this study, the sand quality was classified as average quality. Both the geographical location and the distribution of these fungi are variable. As shown by INSA (National Institute of Health Dr. Ricardo Jorge, Portugal), filamentous fungi are the largest group of pathogenic and allergic fungi for humans. Among the most frequently mentioned are the species of *Penicillium* sp., *A. fumigatus*, *A. niger*, *Aspergillus* sp., *Cladosporium* sp., *Paecylomices* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp., *Geotrichum* sp., *Acremonium* sp. (Daoud and Gulati, 2023; Van den Nest *et al.*, 2021; Merad *et al.*, 2021; White *et al.*, 2021; Iturrieta *et al.*, 2020; Brandão *et al.*, 2007). As an important fact, yeasts are an important part of the biodiversity of different tropical ecosystems. They are characterized by being unicellular and microscopic, and are distributed throughout the world. One of their main functions is the recycling of plant and animal remains. They can be found in marine environments, such as lagoons, estuaries, and are related to the geographical and hydrological conditions of the ecosystem. Yeasts can be found in these environments due to the large amount of nutrients accumulated, either naturally or through human pollution (Caetano *et al.*, 2023; Sharma and Chakrabarti, 2023; Segal-Kischinevzky *et al.*, 2022; García and Giraudo,

2014). The main *Candida* species common to humans include: *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. Krusei*, *Meyerozyma guilliermondii* (anamorph *Candida guilliermondii*) and *C. dubliniensis* (Govrins and Lass-Flörl, 2024; Duggan and Usher, 2023; Soliman, 2023; Dos Santos and Ishida, 2023; Branco *et al.*, 2023; Richardson, 2022; Jamiu *et al.*, 2021; Hassan *et al.*, 2021; Gomez-Gaviria and Mora-Montes, 2020; Chowdhary *et al.*, 2017; Lockehart *et al.*, 2017). Solo-Gabriele, (2016), in their study isolated fungi from samples of marine environments (seawater, sand) and compared them with the isolates from clinical samples. They evaluated the fungal species that were found in the three samples. Some species mentioned in this study were; *Candida*, *Microsporum*, *Trichophyton*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Alternaria*. These species were found in common in clinical samples, sand samples and seawater samples. Therefore, this study shows us that there is convincing scientific evidence that beaches, through their sand and water, contribute significantly to the pathogen load to which beach users are exposed. The objectives of this research are to isolate filamentous fungi and yeasts from the dry sand of Crystal Beach in the Bahamas. To document what species and genus of filamentous fungi and yeasts were found in the beach sand. To work on the identification of the species, several taxonomic keys were used. Use of the microscope and different specific selective culture media to grow environmental fungi and yeasts. On the other hand, to know the average number of colonies (CFU) of fungi and yeasts from sample isolations. In order to identify the quality of the sand on this beach. To know the fungi and yeasts found in the sand and to perform an analysis of which ones are pathogenic to humans.

MATERIALS AND METHODS

The samples were taken on July 5, 2023, at Crystal Beach on the island of Bahamas. A well-known beach that receives thousands of visitors

in summer to bathe with the pigs in the sea (Figure 2).

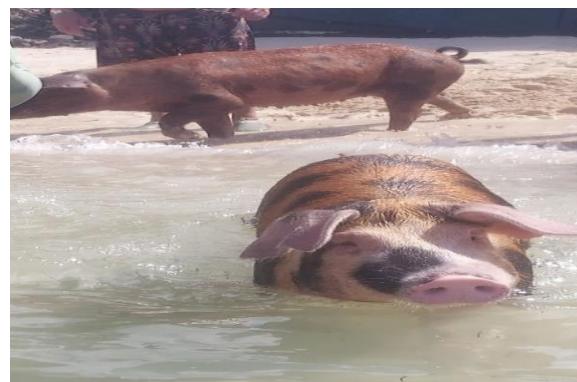


Fig. 2. Pigs in sea water and sand.

The samples were taken once the activity was concluded (Interacting with the pigs in the water and sand of the beach). Sand samples were taken in the dry area of the beach. The beach was divided into three equidistant points from the front of the beach shore. Approximately 100 grams of sand was taken, using a sterilized bag spatula (Echevarría, 2022). The depth of taking the sample was approximately 2 inches (Frenkel *et al.*, 2022) deep from the area where the wave ends, and the dry area bathers are located (Figure 3).



Fig. 3. Sampling location at Crystal Beach Bahamas.

One (1) gram of the sand sample was weighed (Echevarría, 2019a). It was carefully poured by spreading it throughout the agar in the plates containing each of the culture media, in triplicate. The culture media used were Sabouraud dextrose agar (SDA), Rose Bengal agar (RBA), Mycosel agar and HardyCHROM™ Candida (García *et al.*, 1998). The incubation temperature was 25°C for a period of 7 to 14 days, in the case of RBA, SDA and Mycosel media (Echevarría, 2022). The HardyCHROM™ Candida culture medium is incubated at 35°C for 48 hours (Hardy Diagnostics, 2022). Positive and negative controls were performed. For the positive control of filamentous fungi, plates containing the culture media (SDA, RBA and Mycosel) were inoculated with the fungus *A. fumigatus*. The objective of the positive control is to demonstrate that the medium has the capacity to grow the fungal species in the sample. The same process is applied to the HardyCHROM™ Candida, with the difference that it was inoculated with the yeast *Candida albicans*. On the other hand, we have the negative control. This was done by taking a plate of each culture medium, without inoculating. The objective is to ensure that the culture medium is free of microorganisms (sterile). These controls are incubated at the same temperature and time as the samples of each culture medium. Once the time has passed, it is verified if there was growth in the controls. The positive controls should obtain growth and the negative controls should not have growth. The colony forming units (CFU) of each sample was counted. Since the samples are in triplicate, the average of each sample in the different culture media is calculated. The species that grew in the different media were then isolated by transferring them to tubes with the same culture medium (Cavalcante *et al.*, 2021; Meyer *et al.*, 2021; Black, 2020; Scharmann *et al.*, 2020). To perform the calculation, the average of each culture medium was used using the colony forming units (CFU). Then, the general average of all fungi and yeasts is taken (Forbes, 2009). Depending on the total number (average) of colonies, the quality of the sand is determined. We used Table 1 as a reference to determine the quality

of beach sand and the maximum values recommended by the Ricardo Jorge National Health Institute (INSA) (Brandão *et al.*, 2011) and the National Health Institute of Portugal (Pereira *et al.*, 2013). Microscopic and macroscopic studies were carried out to identify the genus and species of filamentous fungi. For the macroscopic study, the color and appearance of the surface and back of each fungi sample were identified. Likewise, for the microscopic study, the samples were placed on a slide together with the Lactophenol reagent. The Nikon Eclipse Ci microscope was used to observe them. The species identified in the microscope were compared with of taxonomic keys. The HardyCHROM™ Candida samples were identified by the color change of the sample in the culture medium (Cavalcante *et al.*, 2021; Mulet *et al.*, 2020; Tapia and Correa, 2015; Scharmann *et al.*, 2020).

RESULTS

Both the negative and positive controls for each sample obtained the expected results. The positive controls of fungi and yeasts showed growth of colonies of *A. fumigatus* and *Candida albicans* in their respective culture media. The negative controls of each medium did not obtain growth. From the isolated samples, four (4) genus of fungi were identified. This genus was *Aspergillus*, *Penicillium*, *Candida* and *Curvularia*. Of the species of filamentous fungi and yeasts found are: *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus glaucus*, *Aspergillus* sp., *Penicillium* sp., *Curvularia*, *Candida parapsilosis*, *C. tropicalis*, *C. krusei*, *C. albicans* and *C. glabrata*. In figure 4 you can see the average (CFU) of the samples of fungi and yeasts. The average number of filamentous fungal colonies for each medium was: 3 CFU/g in RBA, 29 CFU/g in SDA and 43 CFU/g in Mycosel. The average number of yeast colonies (*Candida*) was: 22 CFU in HardyCHROM™ Candida. The *Candida* genus was the most abundant species. The overall average (CFU) of fungi and yeasts in the sand samples was 24 CFU/g. With this data,

it indicates that the quality of the sand at Crystal Beach, according to the parameters in Table 1, is classified as average quality ($>\text{CFU/g}=5$). This

is compatible with other studies conducted on other beaches with tropical conditions and in the months of June and July.

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	> MRV average quality	\leq MAV good quality
cfu / g = 85	cfu / g = 5	cfu / g = 5

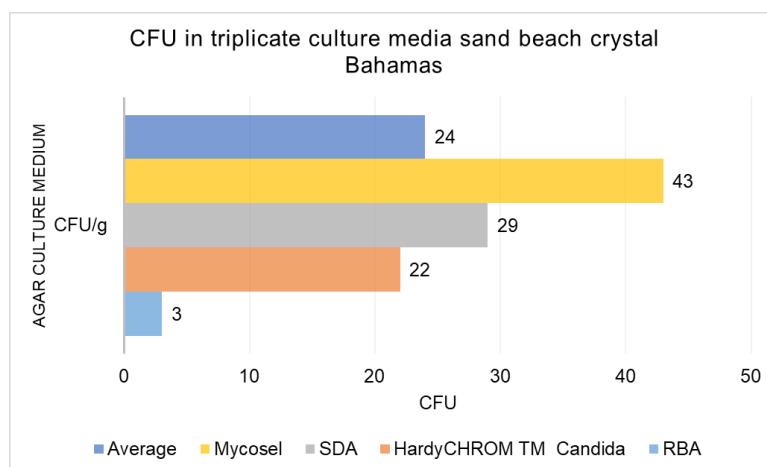


Fig. 4. Yeast and molds isolated from sand samples.

DISCUSSION

The species of fungi and yeasts isolated in this study coincide with the results of other investigations (Echevarría, 2017; Echevarría, 2019a; Echevarría, 2019b; Echevarría and Iqbal, 2021; Echevarría, 2022; De Araújo Pinto *et al.*, 2018). Solo-Gabriele *et al.* (2016) confirm that sand can be a host for fungi and yeasts, which can use it as a vehicle to pass or transmit various diseases. The variety of species identified in this research uses direct contact with the air, contact with sand and seawater as a transmission route. Table 2 shows the fungi found in this research, showing their level of pathogenicity. Many of the species mentioned here have been found in patient samples and

may cause asthma (Behera and Das, 2023; Walker and Robicheau, 2021; Echevarría and Iqbal, 2021; Hu *et al.*, 2017; Echevarría, 2017; St-Germain and Summerbell, 2011). Research indicates that it is necessary to continue performing and documenting these analyses, since in recent years there has been a notable increase in cases of candidiasis, with *Candida albicans* being the main agent (Govrins and Lass-Flörl, 2024; Behera and Das, 2023; Duggan and Usher, 2023; Soliman, 2023; Branco *et al.*, 2023; Richardson, 2022; Jamiu *et al.*, 2021; Walker and Robicheau, 2021; Hassan *et al.*, 2021; Van den Nest *et al.*, 2021; Gomez-Gaviria and Mora-Montes, 2020; Chowdhary *et al.*, 2017; Lockehart *et al.*, 2017).

Table 2. Summary of fungal species found and their pathogenicity.

Species of fungi pathogenic to humans		
Species	Pathogenicity	References
<i>Aspergillus flavus</i>	Opportunistic pathogen that causes invasive aspergillosis.	(Shittu <i>et al.</i> , 2022)
<i>Aspergillus parasiticus</i>	Recognized as major contaminants of organic and nonorganic items, produces the polyketide mycotoxin aflatoxin, one of the most mutagenic and carcinogenic natural compounds described to date.	(Shabeer <i>et al.</i> , 2022)
<i>Aspergillus glaucus</i>	Is known for producing a variety of secondary metabolites, including mycotoxins. They can have detrimental effects on human health. Aflatoxins, for example, are potent carcinogens and can cause liver cancer.	(Qiu <i>et al.</i> , 2020)
<i>Aspergillus sp.</i>	It can cause a significant group of superficial and cutaneous mycoses, including distal lateral subungual onychomycosis, proximal subungual onychomycosis, cutaneous aspergillosis and otomycosis.	(Merad <i>et al.</i> , 2021; White <i>et al.</i> , 2021)
<i>Penicillium sp.</i>	A very important genus of fungi due to its widespread presence in the environment and the role of many species in food spoilage and the production of mycotoxins.	(Toghneuo and Boyom, 2020)
<i>Curvularia sp.</i>	This pathogen is found mainly in plants. We can also identify it in clinical cases such as sinusitis and keratitis. To date, it is the cause of fungal keratitis.	(Daoud and Gulati, 2023; Iturrieta <i>et al.</i> , 2020)
<i>Candida albicans</i>	Opportunistic pathogen in humans, causes candidiasis.	(Richardson, 2022)
<i>C. tropicalis</i>	This species is the most virulent and is resistant to antifungals.	(Dos Santos and Ishida, 2023)
<i>C. krusei</i>	This species has the characteristic of being resistant to different medicines. It causes very dangerous infections in patients with compromised immune systems.	(Jamui <i>et al.</i> , 2021; Gomez-Gaviria and Mora-Montes, 2020)
<i>C. glabrata</i>	This yeast causes candidiasis. It is a difficult disease to treat because it is resistant to antifungal therapies for most mycoses.	(Hassan <i>et al.</i> , 2021; Duggan and Usher, 2023)
<i>C. parapsilosis</i>	It causes invasive candidiasis infection, especially in people with weak immune systems.	(Branco <i>et al.</i> , 2023; Govrins and Lass-Flörl, 2024)
<i>Penicillium sp.</i>	This fungus is responsible for causing food spoilage by creating mycotoxins. It is widely found in all ecosystems.	(Toghneuo and Boyom, 2020)
Yeast	Is used to cause fermentation and leavening, some yeast are mild to dangerous pathogens of humans, especially on patients with weakened immune systems.	(Caetano <i>et al.</i> , 2023; Sharma and Chakrabarti, 2023)

Other studies have shown that 8% of *C. tropicalis* and between 3% and 6% of *C. glabrata* and *C. Krusei* show greater resistance to the imidazole fluconazole (Dawoud *et al.*, 2024; Barantsevich and Barantsevich, 2022; Hassan *et al.*, 2021; Jamui *et al.*, 2021; Mendoza, 2005). Further research is needed to be able to work with environmental assessment plans in countries that have this beautiful natural resource. Therefore, it is important that people who go on vacation to the beach consider being

aware of the fungi that inhabit the sand and their possible impact on health. Several articles indicate that we need to continue researching and documenting the findings of each investigation. The possibility of considering the implementation of sand quality programs on beaches in each country should be evaluated. With the purpose of protecting the public health of beach users, even more so on beaches with a high level of tourism like this one, and the ecosystem.

CONCLUSION

1. The genus *Aspergillus* and *Candida* are the fastest growing in the samples.
2. Five species of *Candida* were identified in the samples: *C. tropicalis*, *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata*.
3. The species of filamentous fungi found were; *Aspergillus glaucus*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus* sp., *Penicillium* sp., and *Curvularia* sp.
4. Sand samples are classified as average quality, 24 CFU/g (>CFU/g=5).
5. Many of the fungi identified in this research use direct contact and air as a vehicle of transmission.
6. The isolates found in this investigation are mostly pathogenic to humans. We must continue to document the findings from all beaches to create a profile and monitor growth and species so that consideration can be given to implementing sand quality procedures on all beaches with high levels of tourists.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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