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Preliminary Study to identify Filamentous Fungi in Sands of Three Beaches of the Caribbean

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Abstract:
The Caribbean has several white sand beaches visited by many tourists, especially during the summer. The beaches of Copacabana in Barbados Bridgetown, Flamenco Beach in Culebra Puerto Rico, and Maho Beach in St. Maarten were studied. The sand samples were taken in June 2016. The objective of the study was to know the diversity and quantity of filamentous fungi in the dry sand of the beaches and to determine if the fungi found are pathogenic to animals and humans. One gram of each beach sample was taken in triplicate and spread on a plate with rose bengal agar. The samples were incubated for 7 to 14 days at 25°C. The colonies were counted, then isolated in tubes with potato dextrose agar. Three genus of fungi were found; *Aspergillus, Penicillium,* and *Rhizopus*. The *Aspergillus* species resulted in higher identifications in the three beaches included in this preliminary study. The growth of filamentous fungi in the samples ranged from 6 CFU / g to 17 CFU / g. The species were: *A. niger, A. versicolor, A. flavus, A. oryzae, A. fumigatus, R. oligosporus, R. stolonifer, A. tamarii and P. wasksmanii*. Most of the identified filamentous fungi are pathogenic to humans and animals. Some of the species can cause diseases such as asthma, infections in the eyes, skin, and nails.

Keywords: Beaches, filamentous fungi, Pathogen, Sands.

INTRODUCTION

The sands of the beaches are exposed to diverse climatic changes, reason why it is a promising place for the development of the fungi that have particular characteristics and make them adaptable to the sandy ground. Microbiological contamination is more significant in the sand than in adjacent waters since sand acts as a passive port for cumulative contamination (Llewellyn *et al.,* 1996). The movement of water causes erosion, transport, and deposition of all the sediments of the beach, as well as the subsequent redistribution of microorganisms (Oliveira, 1992; Mendez, 1997). In the summer season the number of consultations related to skin infections caused by fungi increases from 20% to 25%. This occurs because in the summer the sufficient conditions are met such as the increase of temperature and humidity that facilitate the proliferation of these microorganisms (Pauw, 2011). Concern has been shown about actual and potential health risks due to exposure to beach sands (Nestor *et al.,* 1984; Mendes, 1997). Diseases caused by fungi of the skin, hair, and nails are common throughout the world, and their incidence continues to increase (Ameen, 1986). The investigations show the presence of fungi in the sands of the beaches. In Portugal, dermatophytes were found in 42% of the beaches analyzed. They were isolated from dry sandy areas with the presence of organic waste (Izquierdo *et al.,* 1986). On sands of Spanish Mediterranean beaches, the fungal density of 42 beaches reached several hundred thousand CFU/g samples (Llewellyn *et al.,* 1996). In the northern area of Puerto Rico, filamentous fungi were identified in the dry sand zone of the beaches of Vega Baja, Manatí, Barceloneta, and Arecibo. Recently, our group identified 129 fungal species, most of them were pathogenic (Echevarría, 2017).

Given the unknowns and previous studies, and considering that the microorganisms can proliferate in the environments, it was decided to study the white sands of tourist beaches in the Caribbean. The sands of the beaches studied were the Copacabana Beach in Barbados Bridgetown, Maho Beach in St. Maarten and Flamenco Beach in Culebra. The investigation sought to determine if there is a diversity of filamentous fungi in the sand of the three beaches, and if the fungi found are pathogenic to humans and animals.

The objectives of the study were to isolate filamentous fungi from the dry zone of the beaches, and to identify the genus and possible species of the filamentous fungi, using taxonomic keys. Furthermore, we estimated the number of filamentous fungi through the count of colony forming units (CFU).

MATERIALS AND METHODS

Sampling and culture media

The sands were obtained from the dry zone of the beaches, collected in three equidistant points and stored in sterile plastic bags. The agar used to culture the sand was Rose Bengal Agar (RBA). Potato Dextrose Agar (PDA) was used for further isolation of the colonies in tubes. To demonstrate that the medium can grow, the agar was inoculated on a plate with *Aspergillus niger* (positive control) and another plate without organism to guarantee the sterility of the medium (negative control), inoculated in both media (RBA and PDA). The growth of the pure culture was performed at 25 °C for 7 to 14 days in the incubator. Pure culture and isolation of colonies: 1 gram of the sand of each beach was weighed in triplicate, spread on a Petri dish with half RBA (Echevarría, 2017). Incubation was then performed at 25 °C for 7 to 14 days. After this period isolation of colonies of fungal filaments was made using PDA tubes (Fontalvo, 2012).

Counting and average of colony forming units (CFU)

The quality of the sands of Caribbean beaches was estimated through (CFU) colony forming units (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) (Brandao *et al.,* 2007) and the National Health Institute on Portugal (Pereira *et al.,* 2013). The values recommended by the institute (Table 1) were used to determine the quality of Caribbean beaches. Since beaches studied in Portugal have characteristics and an environment like the beaches in the Caribbean (Pereira *et al.,* 2013).

Macroscopic and microscopic identification

The identification of genus and species was achieved after a morphological study, both macroscopic and microscopic. For the macroscopic morphology, the color and appearance of the surface and the back of each sample were observed. To study the microscopic morphology, samples from the isolated colonies were transferred to a slide with lactophenol and observed in a Nikon Eclipse Ci microscope. The data obtained were compared using taxonomic keys.

RESULTS AND DISCUSSION

Species of filamentous fungi

At Maho Beach, in St. Maarten, fungi of the genus *Aspergillus* of five different species were identified. The species of filamentous fungi found in the white sands of Maho Beach were *A. oryzae, A. fumigatus, A. flavus, A. fumigatus*, and *A. versicolor.* The species of Copacabana Beach in Barbados Bridgetown were *A. niger*, A. *tamarii* and *Penicillum wasksmanii*. The species identified in the sand of Flamenco Beach, Culebra were *Rhizopus stolonifer* and *R. oligosporus*. The different species of filamentous fungi were identified using taxonomic keys. The number of species of filamentous fungi identified in the white sands of the beaches is diverse.

Counting colony forming units and sands quality

The count of colony forming units allowed us to estimate the number of total colonies to determine the quality of the sands of the beaches of the Caribbean. The average of the three courses was obtained, for each beach. According to the results, Maho beach in St. Maarten had 15CFU/g, Copacabana Beach in Barbados got 6 CFU/ g, and Flamenco Beach in Puerto Rico got 17 CFU/g. It can be determined that the Beach with the least number of colonies was Copacabana Beach in Barbados Bridgetown, followed by Maho Beach in St. Maarten and Flamenco Beach in Culebra. All three beaches demonstrated average quality sand following the INSA criteria (Table 1).

We identified fungi that are pathogenic to humans such as *Aspergillus* and *Penicillium* species that are a possible risk to public health. In previous research, an analysis of water and sand on Brazilian beaches found that the most common genus found was *Penicillium* and *Aspergillus* (Gomes *et al.,* 2008). In Puerto Rico an investigation in the sands of the beaches of the Northern area of Puerto Rico, six genus of filamentous fungi were identified: *Aspergillus, Penicillium, Rhizopus, Trichoderma, Hortaea* and *Fusarium* (Echevarría, 2017).

The colony count forming units was 15 CFU/g for Maho Beach in St. Maarten and Flamenco Beach in Culebra, P.R. 17 CFU/g, while Copacabana Beach in Barbados Bridgetown had a lower count of 6 CFU/ g. The count allows the determination of the quality of the sand, using as a preliminary study specification and from the result to know if the sands of the tourist beaches in June a warning for public health could be. In this case, the studied white sands of the three beaches are of average quality. According to other investigations, this preliminary study provides

vital information to carry out a broader study of the sands in a whole year. This is to determine effective cleaning techniques to minimize the growth of fungi in the sands, as well as the need to implement new measures to avoid the contamination of the sands due to the accumulation of solid waste or throwing garbage on the beaches. The measures are necessary since some of the fungi found in the sands are from the food that is thrown into the sands, and they manage to adapt to the sand. Table 2 shows the diseases that are caused by some of the fungi found in the study.

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	≤ MAV good quality
cfu / g = 85	cfu / g = 5	cfu / g = 5

Table 2. Isolated fungi and diseases they cause

Fungal species	Disease	Reference
Aspergillus versicolor, *Rhizopus oligosporus*	Onychomycosis	(Baran, 2006)
Rhizopus stolonifer	Allergic alveolitis	(Pontón, 2002)
Aspergillus flavus	Pulmonary infections	(Samson, 2014)
Penicillum waskmanii	Pulmonary, nasal and eye infections	(St- German, 2011)
Aspergillus fumigatus, *Aspergillus niger*	Aspergiloma	(St- German, 2011)
Aspergillus oryzae	Fermentation of food	(Samson, 2014)

CONCLUSION

Given to the results, it can be determined that the sands of the beaches would be classified as average quality. The growth of filamentous fungal colonies in the samples ranged from 6 CFU/g to 17 CFU/g. In the taxonomy analysis, three genus were found; *Aspergillus*, *Penicillium, Rhizopus*. The genus of fungus with more species identified in the three beaches in the analysis was *Aspergillus*. Most of the species in the study have been isolated from patients in nasal cultures and produce asthma. They also cause keratitis (inflammation of the cornea of the eye). Most use direct contact as a vehicle of transmission, and cause lung infections and sinusitis (Berger, 2015). Concerning the preliminary study in the future, a molecular analysis must be carried out to the sands of the three beaches of the Caribbean.

When visiting the beaches, a towel should be used to sit on the sand, as well as glasses to protect the eyes from contact with the spores. This works as a shield of protection for the spores that transports through the air and present in the sand. They are also acquired by direct contact (Gugnani, 2000), entering through skin and eye injuries. It is also recommended to start campaigns for cleaning and maintaining the sands of beaches.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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