

 Open Access

Article Information

Received: February 6, 2026

Accepted: April 28, 2026

Published: May 12, 2026

Authors' Contribution

LE designed the study, performed the experiments. LE and MNI wrote and revised the paper. The authors read and approved the final manuscript.

Citation

Echevarría, L., Iqbal, M.N., 2026. Study of the Fungal Community in the Sand of Bocagrande Beach, Cartagena, Colombia. PSM Microbiol., 11(1): 68-75.

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Study of the Fungal Community in the Sand of Bocagrande Beach, Cartagena, Colombia

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Abstract:

Colombia is the fifth most attractive tourist destination in South America. As such, it is highly visited by tourists year-round, but especially during the summer. Many tourists tend to get sick, which can be due to exposure to wind carrying sand particles or direct contact. This study collected samples of dry sand at three equidistant points along the beach. The samples were collected on January 3, 2024. The main objective of this research was to identify the colony-forming unit (CFU) and species of filamentous fungi and *Candida* species present in the sand of Bocagrande beach in Cartagena, Colombia. The aim was to determine if the fungi and yeasts found are pathogenic to public health and to assess the quality of the sand. Positive and negative controls were prepared, and then one gram of sand was transferred to each of the following culture media: Mycosel, SDA, RBA, and HardyCHROM™ *Candida*. The samples were prepared in triplicate and incubated for 7 to 14 days at 25°C. Hardy CHROM™ *Candida* samples were incubated at 35°C for 48 hours. Colony counts (CFU) were performed, the average was calculated, and the species were isolated in tubes of each medium and incubated at the same temperature. To identify the fungi, a small amount of sample was transferred to a slide stained with lactophenol cotton blue for microscopic observation. Four genus of filamentous fungi two species of yeast from the genus *Candida* were isolated from the beach sand samples. The identified fungal genus were: *Aspergillus*, *Candida*, *Rhizopus*, *Trichoderma*, and *Ascomycetes*. The genus *Aspergillus* showed the highest percentage of growth. The isolated filamentous fungal species were: *A. niger*, *A. flavus*, *A. glaucus*, and *A. terreus*. However, *A. niger* was the predominant species. The *Candida* species identified were *Candida albicans* and *Candida tropicalis*. The average filamentous fungi count (including *Candida*) in the samples was 40 CFU. This indicates that the sand is classified as being of average quality based on the results. Most of the fungi identified are pathogenic to humans. In particular, *Aspergillus*, in high concentrations, can cause aspergillosis, which leads to lung problems such as infections.

Keywords: Sand, filamentous fungi, pathogens, beach, yeasts, *Candida*.



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INTRODUCTION

Boca Grande Beach is located in the city of Cartagena, Colombia, in the Caribbean region, known for its warm climate and impressive historical and cultural heritage (Ruiz-Merchán *et al.*, 2025). Cartagena, an iconic city, is situated on Colombia's northern coast and is a renowned tourist destination, both for its historic center and its beautiful beaches. Boca Grande, in particular, is one of the city's most famous and popular beaches, stretching along the coastline of Cartagena's modern district, near numerous hotels, shopping centers, and restaurants. The beach is characterized by its fine, golden sand and warm, clear waters, making it an ideal place to enjoy the sun and sea. Its proximity to Cartagena's historic center and easy access from various points in the city make it a key tourist destination (Cabrera and Botero, 2018; Moreno-Egel *et al.*, 2006). Ecologically, Boca Grande Beach is situated within a coastal ecosystem characteristic of the Caribbean region. Beaches like Boca Grande are areas of high biodiversity, not only for marine species but also for microorganisms, including bacteria, algae, and fungi (Díaz and Acero, 2003; Manjarrez-Paba *et al.*, 2017; Paba and Ávila, 2022). However, although research on marine microbiology has been conducted in coastal areas of Colombia, specific studies on fungi at Boca Grande beach are limited. In general, fungi inhabiting marine or coastal environments include diverse species that play crucial roles in the decomposition of organic matter and in the biogeochemical cycles of these ecosystems. Previous research has documented the presence of fungi in the marine waters and sediments of various beaches in the Colombian Caribbean, such as *Penicillium* and *Aspergillus*, which are known for their ability to decompose organic matter and, in some cases, for being pathogens of marine plants or animals (Londoño *et al.*, 2018; Vélez Moreno, 2025). These studies, although limited in their specific focus on Boca Grande beach, provide information on the behavior and ecology of fungi in coastal areas of the Caribbean, highlighting their importance in ecological balance. Interest in

fungi in these environments is not only related to their ecological role but also to the potential identification of pathogenic species that may pose a risk to public health, especially in areas with high tourist traffic like Boca Grande. Furthermore, the study of these microorganisms on urban and tourist beaches like this one is fundamental to understanding the environmental health of these ecosystems and their interaction with human activities (Acevedo-Barrios, 2017; Echevarría, 2022; Montiel-Mora and Gómez-Ramirez, 2023). This study aimed to explore the mycological environment of Boca Grande beach, focusing on the diversity and role of fungi present in the sand. Through this research, we intend to provide relevant information on the mycological ecology of one of the most visited beaches in Cartagena and contribute to the knowledge of environmental mycology in the Colombian Caribbean region. The objectives of this study were to isolate filamentous fungi from the sand of Bocagrande beach in Cartagena, Colombia, and to identify the genus and possible species of the filamentous fungi using taxonomic keys. Estimate the number of fungi by counting colony-forming units (CFU). Finally, examine whether the fungi found are pathogenic to humans.

MATERIALS AND METHODS

Samples were collected in January 2024 from the beach sand at Bocagrande in Cartagena, Colombia (Figure 1). Sand samples were obtained from the dry zone at three equidistant points.



Fig. 1. Bocagrande Beach in Cartagena, Colombia.

Approximately 100 grams of sand were placed in a sterile bag. The culture media used were Rose Bengal Agar (RBA), Sabouraud Dextrose Agar (SDA), Mycosel, and Hardy Chrom™ Candida. One gram of sand was weighed and then spread in triplicate in a Petri dish containing RBA, SDA, and Mycosel. The plates were incubated at 25°C for approximately 7 to 14 days. The Hardy CHROM™ Candida plates were incubated at 35°C for 48 h. As part of the analysis, positive and negative controls were performed (Echevarría, 2017; Echevarría, 2019). For the positive control, each plate containing the Mycosel/SDA/RBA medium was inoculated with *A. niger* to demonstrate the medium's capacity for growth. For Hardy Chrom™ Candida, the same procedure was followed, except that the yeast *Candida albicans* was used. A negative control was also included without inoculation to ensure the sterility of the medium. These were incubated together with the samples. Colony-forming units (CFU) were then counted in triplicate on the plates, and the average was calculated (Echevarría, 2022). The different colonies were selected and isolated on tubes containing SDA, Mycosel and RBA agars and

incubated for 7 to 14 days at 25°C. The Hardy CHROM™ Candida tubes were incubated at 35°C for 48 h. Genus and species identification was achieved after macroscopic and microscopic analysis. For the macroscopic study, the morphology, color, and surface appearance of the back of each sample were observed. For samples in Hardy CHROM™ Candida, identification was based on the color change of the medium (Echevarría, 2022; Scharmann *et al.*, 2020). For the microscopic study, samples of the isolated colonies were transferred to slides stained with lactophenol cotton blue. The data obtained were compared with taxonomic keys. To determine sand quality, the average colony-forming unit (CFU) was estimated (Forbes, 2009). Based on the total number of colonies, sand quality was determined using the maximum values recommended by the Ricardo Jorge National Institute of Health (INSA) Standard (Brandão *et al.*, 2011). The recommended values presented in Table 1 of the institute helped us determine the sand quality of Bocagrande beach in Cartagena (Pereira *et al.*, 2013).

Table 1. Recommended maximum values of filamentous fungi in beach sand.

Recommended maximum values for filamentous fungi in beach sand		
>MVA	>MRV	≤ MAV
Poor quality CFU/g=85	Average Quality CFU/g = 5	Good Quality CFU/g=5

Values recommended by the Instituto Nacional de Saúde Ricardo and National Institute of Health in Portugal (Pereira *et al.*, 2013), Maximum recommended values: Instituto Nacional de Saúde Ricardo Jorge INSA – National Health Institute on Portugal (Brandão *et al.*, 2011).

RESULTS

Four genus of filamentous fungi and two species of yeast from the genus *Candida* were isolated from the beach sand samples. The identified fungal genus were: *Aspergillus*, *Candida*, *Rhizopus*, *Trichoderma*, and *Ascomycetes*. The genus *Aspergillus* showed the highest percentage of growth. The isolated filamentous fungal species were: *A. niger*, *A. flavus*, *A. glaucus*, and *A. terreus*. However, *A. niger* was the predominant species. Yeasts were identified by the color of the colonies on the agar. The

Candida species identified were *Candida albicans* and *Candida tropicalis* (Table 2).

The positive control showed growth in each culture medium, while the negative control showed no growth in any of the media. The growth of filamentous fungal colonies in the samples ranged from 29 CFU/g to 52 CFU/g, with average colony-forming units 40 CFU/g (Table 3). Based on the averages for the sand sample, the sand quality, according to the parameters in Table 1, was classified as average quality (>5CFU/g).

Table 2. The Classification of fungal isolates from beach sand.

Family	Genus	Species
Aspergillaceae	Aspergillus	<i>niger</i>
		<i>flavus</i>
		<i>glaucus</i>
		<i>terreus</i>
Debaryomycetaceae	Candida	<i>albicans</i> <i>tropicalis</i>
Mucoraceae	Rhizopus	sp.
Hypocreaceae	Trichoderma	sp.
	Ascomycetes	sp.

Table 3. Average growth of filamentous fungal colonies (CFU) in different culture media.

Culture medium	CFU/g
SDA	29
Mycosel	36
RBA	45
Hardy CHROM Candida ^{IM}	52
Total average	40

DISCUSSION

The results of this study on the fungal community in the sand of Bocagrande beach in Cartagena, Colombia, reveal a diversity of filamentous fungi and yeasts that may have ecological and public health implications. Four genus of filamentous fungi were isolated: *Aspergillus*, *Trichoderma*, *Ascomycetes*, and *Rhizopus*, and two species of yeast from the genus *Candida* (*C. albicans* and *C. tropicalis*). Among the fungal species, *Aspergillus niger* had the highest number of colony-forming units (CFU), reflecting its broad tolerance to dry, sandy environments and its ability to compete in nutrient-poor substrates.

The average values of 40 CFU/g obtained in this study classify the sand from Bocagrande as being of average quality according to the criteria used (INSA, Instituto Nacional de Saúde Ricardo Jorge, Portugal) for filamentous fungi in beach sands. This figure is lower than the median reported by the Mycosands initiative, which included 91 sampling sites and established a reference value of 89 CFU/g in coastal and

freshwater beach sediments (Brandão *et al.*, 2021). This suggests that, although fungal abundance in Bocagrande is moderate, it is not without a significant presence of fungi.

The presence of *Aspergillus* species in beach sands is consistent with global reports of fungal communities in coastal environments. Previous studies have identified *Aspergillus*, *Candida*, *Fusarium*, *Cryptococcus*, and other genus in sand samples from various beaches, reflecting the ubiquity of these fungi in terrestrial and coastal substrates and their association with both organic matter and human activity (Brandão *et al.*, 2021; Echevarría, 2019; Echevarría, 2022; Gangneux *et al.*, 2024). The detection of *Candida albicans* and *C. tropicalis* in Bocagrande is relevant, given that *Candida* species are considered indicators of fecal contamination and are opportunistic in humans, particularly in immunocompromised individuals or those with underlying health conditions (King and Leonard, 2023).

From a public health perspective, the reviewed literature indicates that certain fungal genus isolated in this study (*Aspergillus* and *Candida*) are capable of causing respiratory, cutaneous, and systemic illnesses in humans exposed to large quantities of spores or prolonged direct contact with contaminated substrates. *Aspergillus* spores can induce respiratory aspergillosis and allergic reactions, while *Candida* can cause mucocutaneous or systemic mycoses in susceptible individuals. In urban beach environments like Bocagrande, exposure can occur through recreational activities that stir up sand and release spores into the air, or through direct skin contact while playing and resting on the sand (Andrade *et al.*, 2025; Vieira *et al.*, 2012).

Another important dimension of these findings involves coastal fungal ecology. Sand acts as a passive and accumulating reservoir of microorganisms, associated with both environmental and anthropogenic agents (Ashraf and Iqbal, 2021; Ashraf and Iqbal, 2022; Echevarría and Iqbal, 2021; Iqbal *et al.*, 2019; Moazeni *et al.*, 2022). Sand acts as a passive

port for cumulative contamination and a reservoir for microorganisms (Oliveira and Mendes, 1992). Most of these fungi cause infections, bronchitis, and sinusitis. Several articles indicate that transmission occurs through direct contact (King and Leonard, 2023; Moazeni *et al.*, 2022; Selvarajan *et al.*, 2024).

Studies in other regions have shown that the fungal community of marine sand varies according to factors such as human presence, season, and geography, although in many cases fungi are more abundant and diverse in the sand than in seawater itself (Brandão *et al.*, 2021; Echevarría, 2022; Echevarría and Iqbal, 2021; Montás-Bravo *et al.*, 2026). This also has implications for environmental monitoring. However, unlike the bacterial indicators commonly used to assess beach quality, fungal parameters are not systematically integrated into health surveillance programs, despite their potential health risks (Leonard and Eaton, 2021; WHO, 2022).

In summary, the data obtained in Bocagrande are consistent with findings from other international studies that highlight the presence of opportunistic fungi in beach sands and suggest the need to consider fungi as an integral part of microbiological assessments of recreational environments. Classifying the sand as “average quality” in terms of CFU/g does not imply an absence of risk, given the presence of potentially pathogenic species. Future studies incorporating molecular analyses and sequencing methodologies could broaden our understanding of fungal diversity and assess its relationship with environmental, climatic, and human-induced factors, as well as correlate the presence of specific species with potential health effects on beach users (Brandão *et al.*, 2011; Li *et al.*, 2026; Mataragka, 2026; Owen *et al.*, 2026; Whitman *et al.*, 2014). The presence of fungi in sand has been studied in various parts of the world, leading to the conclusion that it should be monitored to establish preventative measures.

CONCLUSION

Our study demonstrated the isolation of four genus of filamentous fungi two species of yeast from the genus *Candida* from the beach sand samples. The filamentous fungi isolated were: *Aspergillus*, *Trichoderma*, *Ascomycetes*, and *Rhizopus*. The most abundant genus was *Aspergillus*. The isolated filamentous fungal species were: *A. niger*, *A. flavus*, *A. terreus*, and *A. glaucus*. The isolated *Candida* species were: *Candida tropicalis* and *Candida albicans*. The growth of filamentous fungal colonies in the samples ranged from 29 CFU/g to 52 CFU/g, with an average of 40 CFU/g. Some of the identified species can cause respiratory illnesses. Therefore, people with compromised immune systems should exercise caution when visiting the beach. *Aspergillus* has been found in sand on various beaches around the world.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to PUCPR- Arecibo for Sciences laboratory for supporting this work.

CONFLICT OF INTEREST

The authors declare this article content has no conflict of interest.

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