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Original Research Article

Candida spp. are Largely Alien in Beach Mycosands

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Abstract: Only recently microbial impurity of beach sand has become an essential issue, particularly during the COVID-19 and in the post-Covid period when a large number of beachgoers are infected not only by the virus but also with other microbes. Besides the bacterial and viral microbes, fungal contamination of beach sand has also been studied in detail in the recent past. Although few *Candida* spp. have been isolated from the beach sand, the growths of common pathogenic *Candida* spp. like *Candida albicans* has not been studied so far. In this study, we used two species of pathogenic *Candida* – *Candida albicans* and *Candida parapsilosis* to observe their growth patterns in sea sand. Growths of both species were inhibited in sea sand. It eliminates the possibility of long survival of these species in sea sand and thus their existence mainly appears as surface contamination.

Keywords: beachgoers, COVID-19, *Candida* spp., *Candida parapsilosis*.

INTRODUCTION

Sandy beaches hold paramount significance for human recreation (Fig 1), tourism, and the establishment of coastal economic zones [1]. Globally, coastal areas featuring extensive, pristine sandy beaches have evolved into significant economic zones. Tourist spending on various services and goods such as accommodation, food and beverages, and entertainment in these regions surpasses US$1260 billion annually [2]. Nevertheless, sandy beaches are more than mere accumulations of sand; they host distinct micro-ecosystems. These environments receive substantial inputs of organic matter transported by seawater, including assimilates from phytobenthos, as well as products washed and leached from seaweeds, animal feces, and the remnants of plants and animals [3]. This establishes favorable conditions for the proliferation of a diverse population of organisms, including small invertebrates, bacteria, actinomycetes, fungi, yeast, viruses, algae, and diatoms, rendering beach sand a potential reservoir for etiological agents of diseases [4-6]. The development of significant urban centers in close proximity to the majority of sandy beaches has led to heightened anthropogenic impact on these natural resources. This impact stems from the discharge of sewage effluents and storm runoff, as well as direct pollution associated with recreational activities, such as fecal shedding from bathers [7]. Additionally, the microbiological quality of beach sand is positively linked to the microbiological quality of the beach water, influenced by tidal wave action [8]. Vulnerable populations, including children, the elderly, or individuals with compromised immune systems, face heightened susceptibility to potential long-term effects. From a public health standpoint, understanding the microbial communities in recreational sand beaches could facilitate the assessment of contaminant levels and trends. Additionally, it could contribute to evaluating the impact on public health following human contact with sand, thereby aiding in the assessment of potential health effects [9].

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Fig 1: Sea beach with a consortium of sand, water, wind, microbes, human beings and animals

The fungal kingdom establishes a extremely diverse collection of ubiquitous organisms characterized by heterotrophic metabolisms [10]. They derive energy from a spectrum of sources, ranging from simple sugars to complex compounds, such as cellulose, lignin, polycyclic hydrocarbons, and even human-made plastic materials [11]. Fungi play a crucial role in the decomposition of organic matter and require oxygen for this process. Consequently, they are typically found in water and aerated layers of soil across various geographical regions.

Certain genera and species of fungi, recognized as extremophiles, have been isolated from unconventional environments, including rocky and sandy deserts, salterns, and brine [12]. Extremophiles exhibit not only survival but also propagation capabilities in habitats characterized by harsh conditions, such as high salinity, low water activity, prolonged UV radiation, drought, and extreme temperatures [13, 14].

Notably, living conditions in diverse natural niches often fluctuate, leading to the selection of extremotolerant organisms. Beach sand represents one such habitat [15]. Similar to other soils, beach sand contains organic matter originating from microorganisms, plants, animals, human activities, and marine sources. The water activity in beach sand can frequently be low, exposing the sand to prolonged UV radiation and the presence of salts. The combination of these factors contributes to the overall microbial load in beach sand, providing a unique ecological niche for extremotolerant organisms [16, 17].

Understanding the adaptation of fungi to such environments is essential for comprehending microbial ecology and the role of fungi in nutrient cycling, decomposition, and ecosystem dynamics. This study aims to explore ecological adaptations of fungi in beach sand environments, shedding light on their functional roles and contributions to ecosystem resilience. Here, we employed an approach to monitor the growth or inhibition of the fungal strains like *Candida albicans* and *Candida parapsilosis* in extra pure sea sand collected from sea beach and processed appropriately to make it extra pure. The aims of this experiment are to show that sand sediments act as a possible reservoir or inhibitor of such microorganisms which helps them to either survive and thrive better or inhibit the growth of these fungal strains compared to their normal growth, in addition, evidence relationships between microorganism counts and environmental and human factors.

MATERIALS AND METHODS

PROCURATION OF SAND USED

The sand here used in the experiment is acquired from HiMedia (Ref: RM3062 Sea sand, Extra pure, Mol. Wt. :60.08, CAS No. :14808-60-7). When examined under microscope (40x) the specks were rough enough to hold microbes (Fig 2).

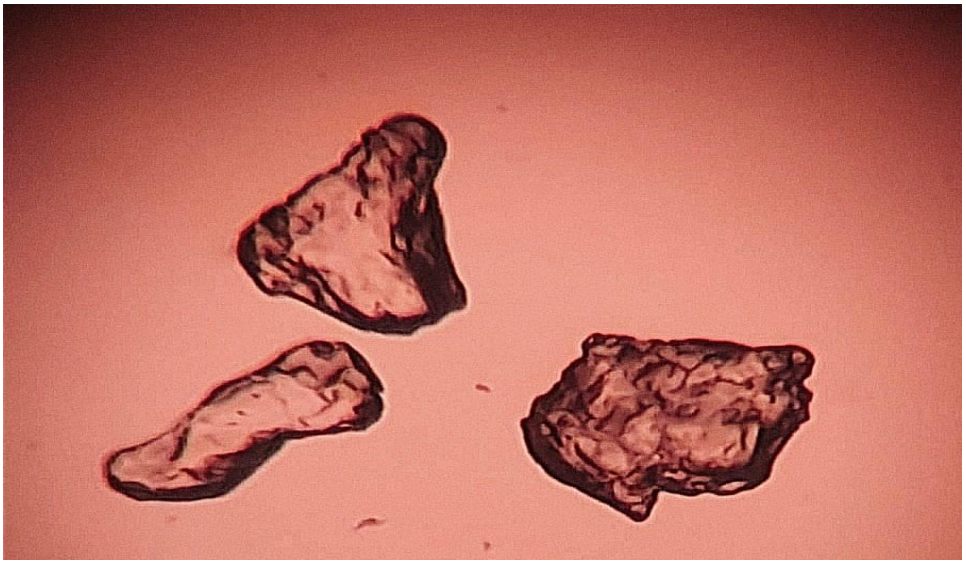

Fig 2: Sea Sand particles under microscope

MICROORGANISM UNDER STUDY

The experimental design involves three (with control) distinct setups, each cantered on specific strains of fungi: *Candida albicans* (Robin) Berkhout 10231 and *Candida parapsilosis* (Ashford) Langeron et Talice 22019. These fungal strains were sourced from Peeless Hospital, Pancha Sayar, Kolkata, West Bengal 700094. Comprehensive details regarding the fundamental characteristics of each fungal strain are meticulously presented in the text. This detailed information serves as a crucial reference for understanding and contextualizing the fungal specimens used in the study, thereby ensuring transparency and reproducibility of the experimental approach.

Preparation of sample for the experiment

Three test tubes for each of the fungal strain were taken, which were classified and was composed of:

SETUP 1 - Nutrient Broth + Fungi (either of *Candida albicans* or *Candida parapsilosis)*

In this investigation, a uniform volume of 2 mL of Nutrient Broth was dispensed into each of two distinct test tubes. Following this, the two test tubes underwent inoculation with unique fungal strains, yielding two distinct sets of growth suspensions for each fungal strain. Subsequently, the test tubes were subjected to an optimal incubation period in an incubator for 1-2 hours. Following this, the McFarland standard was maintained at 0.5.

It is noteworthy that McFarland standards play a pivotal role in calibrating the turbidity of microbial suspensions, ensuring a standardized microbial count for subsequent testing.

Physical parameter, DENSICHECK-

It is a device mainly used to measure the optical density of microbial suspension. In this study we used this device to adjust MacFarland standard.

SET UP 2 – (Control setup – Nutrient Broth + Sand)

In the control experiment, 1 gram of sand was introduced individually into designated test tubes. Following this, 1 mL of nutrient broth was added to each test tube, thereby establishing two distinct and uniform control setups for each fungal strain under investigation.

SET UP 3 – (Test set up – Sand + Nutrient broth+ Fungi)

In the initial phase, a precisely measured quantity of 1 gram of sand was introduced into each of the two designated test tubes. Following this, 1 mL of the culture suspension from Setup 1, specific to each respective fungal strain, was carefully pipetted into the corresponding test tubes. This systematic and sequential approach was employed to establish standardized test setups for the diverse fungal strains under investigation. The methodical execution of these steps was instrumental in ensuring a high degree of consistency and reliability in the preparation of test conditions, setting the foundation for subsequent analytical procedures. The rigorous implementation of this protocol contributes to the credibility and reproducibility of the experimental process, reinforcing the scientific integrity of the study.

The experiment involved the preparation of samples followed by a 24-hour incubation of test tubes in a controlled incubator environment. After the specified incubation period, suspensions were carefully extracted from the test tubes, and their optical density was quantified at a wavelength of 600nm using a spectrophotometer. To enhance comprehension and reliability, the experiment was conducted twice, yielding two distinct sets of observations presented below.

OBSERVATION

OBSERVATION 1				
MICRORGANISM	NUTRIENT BROTH + MICROBE	CONTROL (NUTRIENT BROTH + SAND)	TEST (SAND + NUTRIENT BROTH + MICROBE)	VARIATION (TEST – CONTROL)
Candida Albicans	0.1126	0.0544	0.1635	0.1091 (Inhibited)
Candida Parapsilosis	0.2407	0.0513	0.0789	0.0276 (Inhibited)

OBSERVATION 2				
MICROORGANISM	NUTRIENT BROTH + MICROBE	CONTROL (NUTRIENT BROTH + SAND)	TEST (SAND + NUTRIENT BROTH + MICROBE)	VARIATION (TEST – CONTROL)
Candida Albicans	0.0442	0.0442	0.0436	0.0006 (Inhibited)
Candida Parapsilosis	0.0442	0.0436	0.0491	0.0055 (Inhibited)

A pronounced decline in microbial growth is apparent in the test setup when contrasted with Setup 1. Noteworthy disparities in fungal growth are evident between the two configurations, emphasizing the impact of experimental conditions and identifying potential factors influencing microbial proliferation. The results presented herein hold significance in advancing our understanding of fungal behaviour within diverse experimental contexts, providing a foundation for future research endeavours aimed at elucidating the intricacies of fungal responses to varying environmental conditions.

DISCUSSION

The first published paper on beach microbes was on dermatophytes *Arthroderma insingulare*, isolated from supratidal sand [18], although later studies contradict the presence of these dermatophytes in sand [19]. In general, low nutrients and high temperatures generally explain fewer dermatophytes in beach sand. However, the existence of *Epidermophyton floccosum* [19] and other dermatophytes [20] was established later. Three types of dermatophytes explicitly anthropophilic, zoophilic and geophilic spread from human to human, animal to human, and soil to human respectively [21] and may contaminate sea sand. Candida albicans can survive in sand for at least one month [22]. *Histoplasma* spp., *Coccidioides* spp., *Paracoccidioides* spp., and *Blastomyces* spp. can endure in beach sand although there is no publication so far [23].

Fungal allergens may also affect beachgoers. Similarly, mycotoxins of *Aspergillus*, *Penicillium* that are common fungal species in the environment, may also exist in beach sands. Recently resistant, thermo- and halo-tolerant *Candida auris* has been found in beach environments [24-26] that may also be present in beach sand.

In studies on silicon-utilizing organisms moulds like *Aspergillus*, *Penicillium*, *Rhizopus* and dermatophytes were found to proliferate without difficulty in silicon-based media, but *Candida* spp. failed to display growth in the presence of silica [27-29]. It is important to note that *Candida* species grow like bacteria, not by the formation of hyphae, which may be a significant cause behind it.

Candida spp. is not as much of in coastal beach sands as in freshwater beach sands [30]. In a study in Brazil among 19 *Candida* spp. considered in coastal beach sands only *C. parapsilosis* and *C. catenulata* were isolated in substantial numbers while other *Candida* spp. were largely absent [31].

CONCLUSION

In conclusion, our study addresses the emerging concern of microbial impurity in beach sand, particularly in the context of the COVID-19 pandemic and the subsequent post-COVID period, where beachgoers may encounter various microbes. While bacterial and viral contaminants have been extensively studied, our focus on fungal contamination in beach sand reveals important insights. Although previous research has isolated a few *Candida* spp. from beach sand, our study uniquely investigates the growth patterns of common pathogenic *Candida* spp., namely *Candida albicans* and *Candida parapsilosis*, in sea sand. The inhibitory effects observed on the growth of both *Candida* species in sea sand suggest that their existence in these environments is primarily surface contamination, dispelling the possibility of long-term survival. The importance of sandy beaches in economic, recreational, and ecological aspects is undeniable. Microbial communities, including fungi, play a crucial role in these environments. Our study underscores the need to comprehensively understand the fungal ecology of beach sands, considering the potential risks associated with human exposure.

Fungi, known for their diverse metabolic capabilities, exhibit unique adaptations in extreme environments. Beach sand, characterized by fluctuating conditions, serves as a habitat for extreme-tolerant organisms, including certain fungi. Our exploration of the adaptation of *Candida* spp. in this context contributes to the broader understanding of fungal ecology in beach environments. The experimental setups involving

Candida albicans and *Candida parapsilosis*, along with the control conditions, provide valuable insights into the potential inhibitory or supportive roles of sea sand on these fungal strains. The findings contribute to the growing body of knowledge on the fungal dynamics in coastal areas, emphasizing the need for continued research to assess contaminant levels and potential health implications. The presence of *Candida auris*, a resistant and thermo-tolerant species, in beach environments adds a layer of complexity to the microbial ecology of sandy beaches. This highlights the importance of ongoing surveillance and monitoring to identify and understand emerging microbial threats in recreational areas.

Furthermore, our study touches upon the role of silicon in influencing the growth of fungi. While certain molds thrive in silicon-based media, *Candida* spp., which grow like bacteria without forming hyphae, face challenges in the presence of silica. This distinction contributes to the observed differences in *Candida* spp. prevalence between coastal and freshwater beach sands. In conclusion, our research provides valuable insights into the microbial ecology of beach sands, emphasizing the need for a nuanced understanding of fungal dynamics. The inhibitory effects on pathogenic *Candida* spp. in sea sand suggest a surface contamination rather than long-term survival, informing public health considerations for beachgoers. This work contributes to the broader scientific understanding of microbial interactions in coastal environments, urging continued research for the better management of these vital ecosystems and the protection of public health.

Conflict of Interest: The authors declare no conflict of interest.

Author's Contribution: Dr. Satadal Das designed the study procedure and procured the sea sand. Ms Nikita Parui and Mr. Arijit Halder carried out the experiment, analysed the data and wrote the manuscript. Dr. Satadal Das and other Authors reviewed and edited the manuscript.

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