

Filamentous fungi isolated from sand and water of “Bairro Novo” and “Casa Caiada” beaches, Olinda, Pernambuco, Brazil

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Abstract

Fungi comprehend a heterogeneous group of heterotrophic microorganisms that act as saprobes or parasites or, less frequently, as symbionts living in association with other organisms. They are cosmopolitan and important components of ecosystems. Considering the small number of Brazilian papers on the filamentous mycota in marine environments, and the need to improve knowledge of the diversity of these microfungi in “Casa Caiada” and “Bairro Novo” beaches, Olinda, PE, the aim of this work was to isolate and identify the fungi from sand and water samples of these ecosystems. Thirty two samplings of sand (surface and 20 cm deep) and water (surface and 1 m deep) were carried out during the dry and rainy seasons, in low and high tide. From each sand sample, a suspension was made with 50 g of sand diluted in 90 mL of sterilized distilled water. From each sand suspension and water sample, 0.5 mL was spread, in triplicate, onto Petri dishes containing Sabouraud Agar added of chloramphenicol. The plates were incubated at 28 °C (± 2 °C). Fifty seven species were isolated, identified, and classified in 20 genera. *Aspergillus* and *Penicillium* were the most frequent genera in both sand and water, with a total of 11 and 19 species, respectively.

Keywords: taxonomy, filamentous fungi, marine environments.

Fungos filamentosos isolados do solo e da água nas praias de Bairro Novo e Casa Caiada, Olinda, Pernambuco, Brasil

Resumo

Os fungos compreendem um grupo heterogêneo de microorganismos heterotróficos, atuando como sapróbios ou parasitas, ou menos frequentemente como simbioses, vivendo em associação com outros organismos. São cosmopolitas e componentes importantes dos ecossistemas. Considerando-se a escassez de trabalhos no Brasil que tratam da micota filamentosa em ambientes marinhos, e ainda a necessidade do conhecimento da diversidade desses microfungos nas praias de Casa Caiada e Bairro Novo, Olinda, PE, este trabalho teve como objetivo o isolamento e identificação de fungos, em amostras de solo e de água, desses ecossistemas. Foram realizadas 32 coletas do solo (em superfície e a 20 cm de profundidade) e da água (em superfície e a 1 m de profundidade), nos períodos de estiagem e chuvoso, na baixa-mar e preamar. De cada amostra de solo, foi feita uma suspensão de 50 g de solo em 90 mL de água destilada esterilizada, e de cada amostra de solo e água foi retirado 0,5 mL para o semeio em triplicata em placas de Petri contendo ágar Sabouraud acrescido de cloranfenicol e incubadas sob temperatura ambiente (28 ± 2 °C). Foram isoladas e identificadas 57 espécies correspondentes a 20 gêneros. *Aspergillus* e *Penicillium* dominaram tanto no solo quanto na água, com um total de 11 e 19 espécies, respectivamente.

Palavras-chave: taxonomia, fungos filamentosos, ambiente marinho.

1. Introduction

“Bairro Novo” beach is approximately 2 km long, with 50 m long coast protection walls built perpendicularly to the beach at intervals of 50 m. There are also man-made reef rocks along the sand for breaking the waves. There are many environmental problems like marine erosion, pluvial water and domestic waste disposal on the beach, causing the water to be inappropriate for bathing and contact sports in some areas. “Casa Caiada” is an urban beach with calm waters and artificial reefs. It is approximately 4.5 km long, with a hotel on the shore. The beach is used for bathing, soccer and contact sports, as well as fishing and mollusk catching. Its main environmental problems are disposal of pluvial waters, domestic waste and solid residues (Beltrão et al., 1995). Fungi are important components of ecosystems as they are cosmopolitan and usually isolated from tropical, subtropical and temperate countries (Smith and Berry, 1975). They are considered the most active microorganisms in the decomposition of organic compounds both in sand and water (Harley, 1971; Moore-Landecker, 1996). When compared to the thousands of fungal species known from terrestrial environments, only 500 have been described for oceans and estuaries which comprehend the largest part of the Earth’s surface (Kohlmeyer and Kohlmeyer, 1979; Kohlmeyer and Volkmann-Kohlmeyer, 1991). The described species are mainly the anamorphous of Ascomycota and Basidiomycota, including some marine yeasts. Some of these marine filamentous fungi are parasites of marine algae or marine angiosperms, or they grow symbiotically with brown algae (Kingham and Evans, 1986). Most of the papers referring to filamentous fungi in marine environments are centered on Europe and North America (Dabrowa et al., 1964; Kishimoto and Baker, 1969; Berner and Wagner-Merner, 1977; Kirk, 1983; Udagawa and Ueda, 1985; Tan, 1985). In Brazil, studies such as Faraco and Faraco (1974), Mattede et al. (1986), Purchio et al. (1988), Pinto et al. (1992) and Sarquis and Oliveira (1996) may be highlighted. “Casa Caiada” and “Bairro Novo” are the main beaches in Olinda. However, they present serious environmental problems as they are intensively visited by tourists and locals. The mycota of these beaches is practically unknown, especially with respect to human pathogens. Therefore, considering the lack of studies on the diversity of filamentous fungi in Olinda, the aim of this work was to isolate and identify filamentous fungi from the beach sand and water samples from “Casa Caiada” and “Bairro Novo”, in the dry and rainy seasons.

2. Material and Methods

2.1. Study area

Olinda city is approximately 40.83 km² located between the parallels 7° 57' 30" S and 8° 02' 30" S and the meridians 39° 49' 41" W and 39° 55' 00" W. It is delimited by the city of Paulista to the North, the Atlantic Ocean to the East, and the city of Recife to the South. The original vegetation covering this area was composed

of the Atlantic Forest and its related ecosystems: mangroves and “restinga”. According to Köppen’s general classification, Olinda’s weather is of the type As’, hot and humid, with a rainy season during autumn and winter, lasting from March to August, with the coldest temperature of 18 °C and the mean temperature of 27 °C. The months with the greatest amount of rain are May (224.4 mm) and July (453.3 mm), and the annual precipitation varies from 1,000 to 2,000 mm with the annual average ranging from 74 to 94%. The beaches of “Casa Caiada” and “Bairro Novo” are the main beaches in Olinda. As urban beaches, they are intensively visited by tourists and locals, and present serious environmental conflicts (Beltrão et al., 1995). The sampling points were chosen in the regions with the greatest access of bathers and release of domestic sewage.

2.2. Sand and water samplings

Sand and water samplings were concomitantly collected in “Bairro Novo” and “Casa Caiada” beaches, in December/2000 and February/2001 (dry season) and June and July/2001 (rainy season). Thirty two sand samples and 32 water samples were collected in syzygy’s low-tide and high-tide, based on the harbor of Recife tide tables (Brasil, 2000). The sand sampling in each site was carried out using a garden shovel, in mid-seashore at 1 m from the tideline, on the surface and at 20 cm deep. The samples were placed in labeled plastic bags. Surface and 1 m deep water samples were taken in each sampling site using sterile labeled glass tubes. The sand and water samples were kept at room temperature and transferred to the laboratory at the Dept. Mycology (Universidade Federal de Pernambuco).

2.3. Isolation, purification and identification of filamentous fungi

Each sand sample (50 g), was diluted in 90 mL of sterilized distilled water; 0.5 mL of this suspension was spread, in triplicate, onto Petri dishes containing Sabouraud Agar (SA) added of chloramphenicol (500 mg.L⁻¹). Each water sample (0.5 mL, undiluted) was also spread onto Petri dishes as above. The plates were incubated at 28 °C (±2 °C). As soon as the first colonies were developed, they were transferred to test tubes containing SA. After the purity of the colonies was confirmed, they were subcultured onto Potato Dextrose Agar, Malt Agar or Czapeck, in glass tubes. Fungal identification was carried out by macroscopic and microscopic observation of colonies and when needed, by microculture on a microscope glass slide (Riddell, 1950). Raper and Thom (1949), Booth (1971), Ellis (1971), Ellis (1976), Raper and Fennell (1977), Domsch et al. (1980), Sutton (1980) and Pitt (1985) were mainly used for species identification.

2.4. Sand and water abiotic data

Sand and water pH and temperature were measured with a digital pH-Meter and a digital thermometer (Hanna), respectively.

Species frequency. The occurrence of fungal species was calculated according to Dajoz (1983), using the formula $Fo = Ta.100/TA$, where Ta = number of samples in which a taxon has occurred, TA = total number of samples. Values were considered according to the following classification: $<10\%$ = Rare, $10 \leq 25\%$ = Low frequency, $25 < 35\%$ = Frequent, $35 < 50\%$ = Abundant, and $> 50\%$ = Very abundant.

3. Results and Discussion

3.1. Sand and water abiotic data for "Bairro Novo" and "Casa Caiada" beaches

Factors such as water salinity, temperature and pH may influence in the activity, abundance and distribution of marine fungi (Dix and Webster, 1995). The water temperature of "Bairro Novo" and "Casa Caiada" was between 24.3 °C and 29.4 °C in the dry season (December and February). In the rainy season (June and July), the minimum water temperature was 25.3 °C while the maximum temperature was 28.2 °C. The sand temperature in the dry season reached its minimum at 25.7 °C and maximum at 29.4 °C, and in the rainy season, it was between 24.4 and 28.8 °C. In general, marine fungi need high temperatures (usually between 25 and 30 °C), to reproduce (Griffin, 1981). All water and sand samples had a slightly alkaline pH, varying from 7.6 to 8.2. In the "Bairro Novo", the water and sand salinity was of 20 and 40‰ in the rainy and dry seasons, respectively. Water and sand salinity in "Casa Caiada" was of 24 and 39‰ in the dry and rainy seasons, respectively (Table 1). According to Gambale et al. (1977), salinity has a great influence in the microbiota of the estuaries. However, very little is known about the distribution of Ascomycetes and anamorphous fungi related to water temperature. Some observations made by Borut and Johnson (1962) on the fungi isolated from estuary sediments on North Carolina, EUA, showed that the germination and development of the fungi was influenced by salinity. *Aspergillus wentii* Wehmer and *Penicillium janthinellum*

Biourge showed lower germination in filtered and autoclaved sea water, and good germination in water with high concentration of NaCl (3.5%). These species were isolated from "Casa Caiada" and "Bairro Novo" in conditions of high salinity (7.5 to 8.2). The pH registered in "Casa Caiada" and "Bairro Novo" beach sand and water was alkaline. Some species that were isolated from these beaches, such as *Aspergillus flavus* Link, *A. terreus* Thom, *A. niger* van Tieghem, *Curvularia palescens* Boedijn, *Fusarium oxysporum* Schltdl., *Cladosporium cladosporioides* (Fresen.) de Vries, *C. sphaerospermum* Pens., *Paecilomyces variotti* Bainier and *Penicillium corylophilum* Dierckx were also mentioned for the same pH range in England (Pugh and Mathison, 1962), Egypt (Abdel-Fattah et al., 1977), Saudi Arabia (Abdel-Hafez, 1982) and Brazil (Pinto et al., 1992).

Isolation and identification of filamentous fungi from sand and water, during the dry and rainy seasons, on "Bairro Novo" and "Casa Caiada" beaches. Thirty six species of filamentous fungi were isolated and identified from the sand and water samples taken from "Casa Caiada" during the dry and rainy seasons. Most of the species were of anamorphous fungi. *Penicillium* and *Aspergillus* were represented by 11 and 8 species, respectively, followed by *Cladosporium* (3), *Emericella* (2), *Acremonium*, *Chaetomium*, *Cunninghamella*, *Curvularia*, *Eurotium*, *Fusarium*, *Lasiodiplodia*, *Monilia*, *Paecilomyces*, *Phoma*, *Tilachlidium* and *Trichoderma* (1) (Figure 1). In the "Bairro Novo", during the dry and rainy seasons, 44 species of filamentous fungi, mostly anamorphous, were isolated and identified. These were mainly represented by *Penicillium* and *Aspergillus*, with 14 and 9 species, respectively, *Fusarium* and *Trichoderma* (4), *Cladosporium* (3), *Absidia*, *Acremonium*, *Colletotrichum*, *Curvularia*, *Emericella*, *Eurotium*, *Paecilomyces*, *Phoma*, *Spegazzinia* and *Stilbella* (1) (Figure 2). Some species were common for both beaches, like *Aspergillus flavus* Link, *A. janus* Raper and Thom, *A. japonicus* Saito, *A. niger* van Tieghem, *A. sydowii* (Bain. and Sart.) Thom and Church, *A. terreus* Thom, *Cladosporium cladosporioides* (Fresen.) de Vries, *C. oxysporum* Berk.

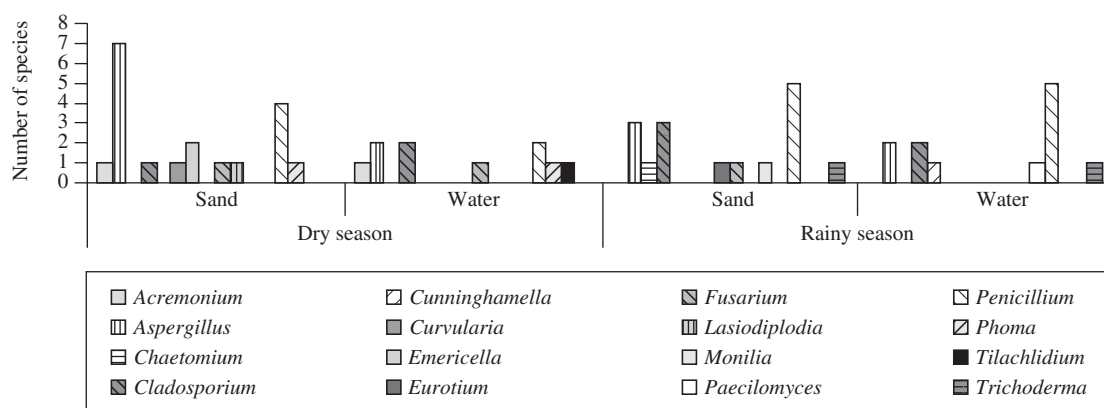


Figure 1. Filamentous fungi isolated from "Casa Caiada" beach during the dry and rainy seasons.

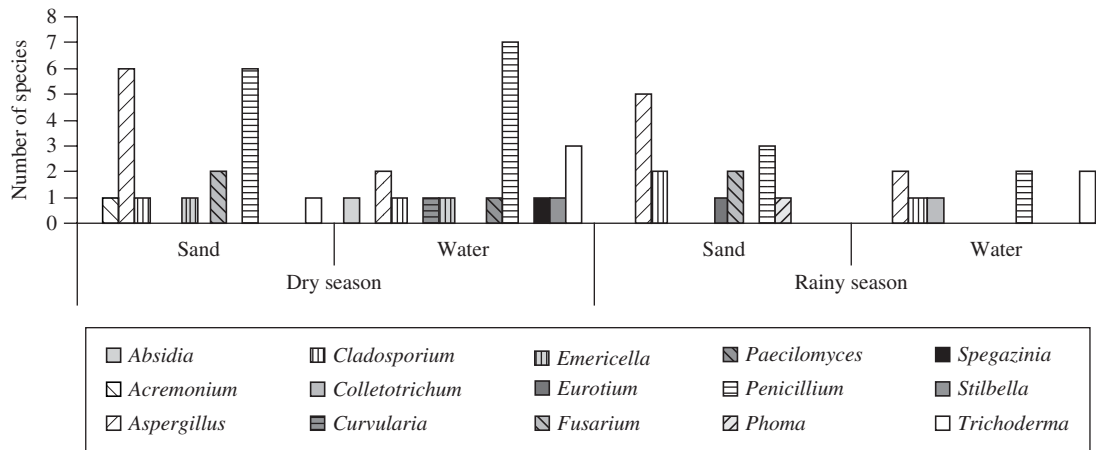


Figure 2. Filamentous fungi isolated from “Bairro Novo” beach during the dry and rainy seasons.

Table 1. Temperature, pH and salinity of the water and sand from “Bairro Novo” and “Casa Caiada” beaches.

Sampling Date	Casa Caiada						Bairro Novo					
	Water			Sand			Water			Sand		
	pH	T (°C)	S (‰)	pH	T (°C)	S (‰)	pH	T (°C)	S (‰)	pH	T (°C)	S (‰)
12/11/00	8.16	24.3	38	7.72	28.4	35.0	8.12	24.3	40	7.70	28.8	38
12/29/00	8.07	24.3	35	7.80	26.5	36.0	7.88	24.3	36	7.72	25.7	37
02/09/01	8.15	29.4	37	7.60	28.9	24.0	8.22	28.0	37	7.85	28.3	37
02/16/01	8.12	29.0	37	7.60	29.4	38.0	7.98	28.2	25	7.82	28.0	37
06/06/01	7.94	28.0	36	7.82	28.0	26.0	7.94	26.9	35	7.85	27.3	36
06/15/01	7.84	26.3	33	7.83	25.9	32.0	7.97	26.0	35	7.83	25.1	20
07/13/01	7.88	25.5	31	7.80	24.5	29.5	7.94	25.3	28	7.85	24.4	30
07/23/01	7.87	28.2	37	7.85	28.8	39.0	7.78	27.1	38	7.82	28.6	15

and M.A. Curtis, *C. sphaerospermum* Pens., *Curvularia palescens* Boedijn, *Eurotium chevalieri* Mangim, *Emericella nivea* Willey and Simmons, *Fusarium solani* (Mart.) Sacc., *Paecilomyces variotti* Bainier, *Penicillium corylophilum* Dierckx, *P. glabrum* (Wehmer) Westling, *P. minioluteum* Dierckx, *P. restrictum* Gilman and Abbott, *P. varians* Smith, *P. waksmanii* Zaleski, *Phoma putaminum* Speg. and *Trichoderma pseudokoningii* Rifai. *Spegazzinia therssathra* (Berk and Curt) Sacc. has already been mentioned as present in estuary sediment in Neuse-Newport, North Carolina, EUA, by Borut and Johnson (1962) and *Stilbella clavisporea* Seifert is mentioned as present in a marine environment, probably for the first time. *Tilachlidium brachiatum* Petch is mentioned for the second time in a marine environment; the first occurrence was registered on Boa Viagem beach, Recife, Pernambuco, by Pinto et al. (1992). The main pathogenic fungi to men and animals can be found within the anamorphic fungi. These fungi are saprophytic and occasionally pathogenic, and can be isolated from water, soil, animals and humans. Species of *Aspergillus*,

Cladosporium and *Penicillium*, found in these beaches, can be a source of infection for superficial and deep mycosis (Sidrim and Moreira, 1999). Most of the filamentous fungi isolated from the beaches of “Casa Caiada” and “Bairro Novo” were anamorphous. The same was observed in researches carried out in beaches of California (Dabrowa et al., 1964), Hawaii (Kishimoto and Baker, 1969), Florida (Bergen and Wagner-Merner, 1977), and Brazil, in Boa Viagem (Pinto et al., 1992) and Ipanema (Sarquis and Oliveira, 1996). The number of genera in “Casa Caiada” was higher than in “Bairro Novo”. The reason for this may be the fact that “Casa Caiada” is more frequently visited by tourists and local bathers than “Bairro Novo”. This certainly contributes to increasing the pollution. Similar facts were observed in research carried out by Kishimoto and Baker (1969), Bergen and Wagner – Merner (1977) and Purchio et al. (1988), who obtained a higher incidence of fungal genera in the beaches of Oahu (Hawaii), Tampa (EUA), and São Vicente and Bertioiga (São Paulo). In the studies carried out by Mattede et al. (1986), the opposite was found.

The authors evaluated dry and wet sand samples from polluted and non-polluted beaches of the city of Vitória, Espírito Santo. The incidence of fungal genera was greater in non-polluted beaches (55%) than in polluted ones (45%). The frequency and incidence of fungi varied and pollution factors inhibited some species. According to Gambale et al. (1983), the differences of fungal genera found in this study may be related to the sampling methods, to the geographical location and to the method of data analysis.

Frequency of occurrence of filamentous fungi isolated from “Casa Caiada” and “Bairro Novo” beaches. The frequency of occurrence of species isolated from “Casa Caiada” and “Bairro Novo” during the dry and rainy seasons showed *Aspergillus niger* van Tieghem (Group 1 = 75%) and *Penicillium corylophilum* Dierckx (Group 2 = 68.75%) as the very abundant species; *Aspergillus flavus* Link and *Penicillium waksmanii* Zaleski (Group 3 = 37.5%), as abundant; *Acremonium rutilum* W. Gams, *Aspergillus janus* Raper and Thom, *Cladosporium oxysporum* Berk. and M.A. Curtis (Group 4 = 31.25%) and *Aspergillus sydowii* (Bain. and Sart.) Thom and Church, *Cladosporium cladosporioides* (Fresen.) de Vries, *Penicillium decumbens* Thom, *P. restrictum* Gilman and Abbott (Group 5 = 25%) as frequent; *Aspergillus japonicus* Saito, *A. terreus* Thom, *Cladosporium sphaerospermum* Pens., *Emericella nivea* Willey and Simmons, *Fusarium solani* (Mart.) Sacc., *Penicillium minioluteum* Dierckx, *P. varians* Smith, *Stillbella clavispora* Seifert, *Trichoderma aureoviride* Rifai, *T. pseudokoningii* Rifai, *Phoma putaminum* Speg. (Group 6 = 18.75%) and *Aspergillus ochraceus* Wilhelm, *A. wentii* Wehmer, *Curvularia palescens* Boedijn, *Eurotium chevalieri* Mangim, *Paecilomyces variotii* Bainier, *Penicillium glabrum* (Wehmer) Westling, *Trichoderma harzianum* Rifai (Group 7 = 12.5%) of low frequency. *Absidia cylindrospora* Hagem, *Aspergillus caespitosus* Raper and Thom, *A. niveus* Blochw., *A. ustus* (Bain.) Thom and Church, *Chaetomium globosum* Kunze, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Cunninghamella elegans* Lendner, *Emericella nidulans* (Eidam) Winter, *Fusarium dimerum* Penzig., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdl., *Lasiodiplodia theobromae* (Pat.) Grif. Maubl., *Monilia sitophila* (Mont.) Sacc., *Penicillium chrysogenum* Thom, *P. citreonigrum* Dierckx, *P. commune* Thom, *P. griseofulvum* Dierckx, *P. herquei* Bainier and Sartory, *P. janthinelum* Biourge, *P. implicatum* Biourge, *P. miczynski* Zaleski, *P. pinophilum* Hedgecock, *P. purpurogenum* Stoll, *P. rugulosum* Thom, *P. solitum* Westling, *Spegazzinia thersatra* (Berk and Curt) Sacc., *Tilachlidium brachiatum* Petch, *Trichoderma virens* (Miller et al.) von Arx. (Group 8 = 6.25%) were species of rare occurrence (Figure 3). Studying the diversity of filamentous fungi on Ipanema beach, Sarquis and Oliveira (1996) isolated and identified 34 genera and 170 species. The genera with the most frequent species were: *Aspergillus* (30.4%) and *Penicillium* (16.2%). Similarly to this study, Tauk-Tornisielo (2005), isolat-

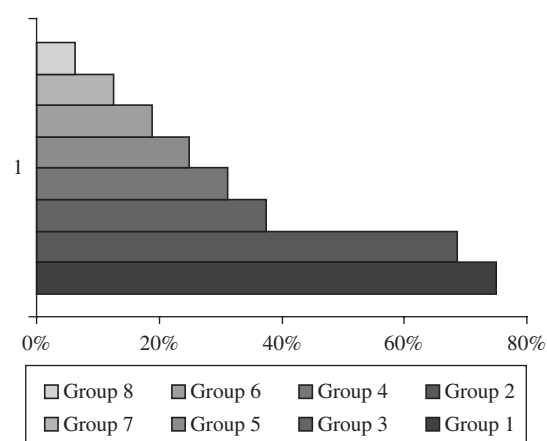


Figure 3. Frequency of occurrence of filamentous fungi isolated from “Bairro Novo” and “Casa Caiada” beaches.

ing soil filamentous fungi from the Ecological Park of Juréia-Itatins, found that *Aspergillus* and *Penicillium* were the genera with the highest diversity of species. *Aspergillus niger* van Tieghem and *Penicillium corylophilum* Dierckx were considered the most frequent species. Studying the incidence of anemophilous fungi isolated from Praia do Laranjal, Pelotas, RS, Bernardi and Nascimento (2005) identified 18 genera. *Cladosporium* (18.22%), *Alternaria* (13.84%), *Penicillium* (10.20%), *Curvularia* (7.47%) and *Aspergillus* (3.28%) were the genera with the species most frequently found.

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