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White fungal biotechnology: prospecting anemophilic fungi for bioindustrial applications

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INFO

Keywords
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ABSTRACT

White biotechnology focuses on industrial processes, being an interesting field for the application of fungi. In this sense, microorganisms were prospected both in the external and internal environment of a university. The passive sedimentation technique was conducted, using sterile 50 mm Petri dishes. The collections were in 10 environments (5 open and 5 closed), being carried out in replicate. The Tukey statistical test was used.. In open locations, a greater quantity of microorganisms was quantified, in addition to having a more vigorous maximum specific growth rate. Through the ratio between microorganisms in the Internal/External environments of 0.23, it was found that the study environment is healthy, despite having a microbial presence. Rhizopus sp, Aspergillus sp, Cladosporium sp, Penicillium sp and Scedosporium sp were identified, which could be applied in white biotechnological processes such as bioremediation, enzyme production, nanoparticle synthesis and others. In this way, microorganisms with real potential for industrial applications were prospected.

Palavras-chaves
biotecnologia
fungos filamentosos
processos industriais

RESUMO

Biotecnologia branca foca nos processos industriais, sendo um campo interessante para aplicação de fungos.
Neste sentido, fez-se uma prospecção de microrganismos tanto em ambiente externo quanto interno de uma universidade. Foi conduzida a técnica de sedimentação passiva, utilizando placas de Petri estéreis de 50 mm. As coletas foram em 10 ambientes (5 abertos e 5 fechados), sendo realizadas em triplicata. Foi utilizado o teste estatístico de Tukey. Nos locais abertos, observou-se maior quantidade de microrganismos, além de terem velocidade específica máxima de crescimento mais vigorosa. Por meio da razão entre microrganismos dos ambientes Interno/Externo de 0,23, constatou-se que o ambiente de estudo é saudável, embora tenha presença microbiana. Foram identificados *Rhizopus sp, Aspergillus sp, Cladosporium sp, Penicillium sp* e *Scedosporium sp*, os quais, poderão ser aplicados em processos biotecnológicos brancos como biorremediação, produção de enzimas, síntese de nanopartículas e outros. Deste modo, prospectou-se microrganismos com potencial real de aplicações industriais.

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INTRODUCTION

The atmosphere consists of the gaseous layer that surrounds planet Earth, and is also a complex ecosystem. In addition to atmospheric gases, the atmosphere is home to a wide variety of microorganisms, including bacteria, viruses and, in particular, fungi (Šantl-Temkiv et al., 2022). Anemophilic filamentous fungi are characterized by the production of spores in the reproduction process, enabling their dispersion through the air. The spore is released from the original organism and begins the development of a second organism, a structure that aims to guarantee the survival of the species in adverse conditions (Tortora et al., 2016).

There is a wide variety of anemophilic fungi, many of which have the ability to cause allergic reactions, especially those that colonize the human body, such as those belonging to the *Deuteromycetes* class, specifically *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium* and yeasts, promoting or aggravating diseases respiratory (Oliveira, et al., 2007, Nageen et al., 2021).

At the same time, these microorganisms play crucial roles within the scope of White Biotechnology, also known as Industrial Biotechnology, in which microorganisms (or products derived from them) are used to improve industrial processes, generating a wide variety of applications (Heux, et al., 2015). Some uses are, for example, in the manufacture of enzymes with commercial applications, in the execution of bioremediation and biocontrol processes, in improving soil fertility, in food production and in several other industrial procedures (Elizei et al., 2014; Meyer et al., 2020).

An advantage of applying fungi in industrial processes, compared to other organisms such as plants, is being able to cultivate them in large bioreactors in the factory environment, being effective for scaling up (Hyde et al., 2019). Furthermore, organic acids and other metabolic compounds produced by filamentous fungi play a fundamental role in the industrial production of enzymes for biomass conversion. This preference for filamentous fungi is based on their greater enzymatic productivity compared to yeasts and bacteria, being widely used in the production of valuable chemicals, such as the organic acids, citric fumaric and gluconic acid (Troiano, 2020).

Knowing this, understanding microbial diversity in varied environments with an eye on industrial processes plays a fundamental role today, in view of the global demands for structuring sustainable bioindustries based on the conversion of renewable bioinputs into a wide range of high value-added products and services, from biofuels, fertilizers, food, cosmetics and medicines, to the biodegradation of plastics (Chinchilla e Rua, 2018). Therefore, the present work aimed to initially prospect for anemophilic filamentous fungi for future application in biotechnological and industrial processes, such as the production of enzymes, antibiotics, and organic acids. Additionally, an analysis of the microbiological quality of the collection environments was carried out, comparing open and closed environments.

MATERIALS AND METHODS

Sample collection was carried out using the passive sedimentation technique (Lacaz et al., 1998), which consists of the gravitational method of exposing Petri dishes with appropriate solid media, in pre-selected locations, for 20 min. The plates were positioned 1 m high from the ground during collection. The temperature and humidity of the locations were also measured. Subsequently, the incubation, identification and analysis of the samples took place.

Selection of locations

To prospect for filamentous fungi, a university environment was selected because it is composed of both buildings and wooded areas, in addition to being an environment with a lot of human traffic. Given the variety of locations, it was decided to study open and closed environments, including study, leisure and sports areas. In total, samples were collected from 10 pre-selected locations (5 open environments and 5 closed environments), with 3 samples from each location. Sample points

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are indicated in Figure 1.

Environments	Latitude	Longitude
Open environments		
Lake	-22.41148	-45.45144
Road	-22.41134	-45.44997
Square	-22.41221	-45.44922
Block	-22.41471	-45.44742
Athletics track	-22.41374	-45.44598
Closed environments		
Meeting room	-22.41305	-45.45248
Library	-22.41287	-45.44895
Classroom	-22.41186	-45.44960

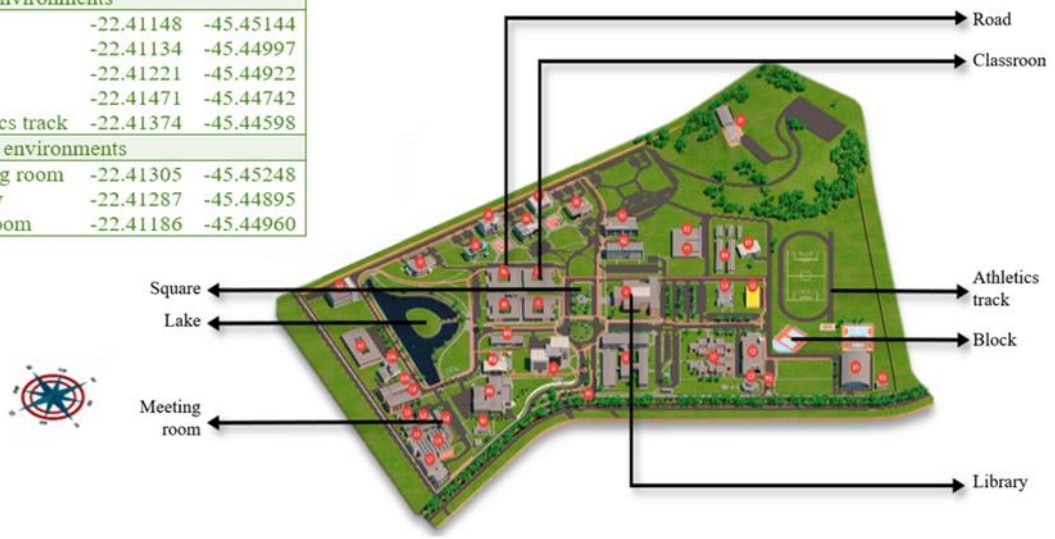

Figure 1 – University environment and places where air samples were collected for fungal prospecting. Source: Adapted from Unifei (2023).

Culture medium

For sampling, sterile (previously autoclaved) 90 mm glass Petri dishes were used. The culture medium was 4% Sabouraud Dextrose agar, described in the standard Resolution RE No. 9 of the National Health Surveillance Agency (Brazil, 2003). To isolate the fungi, the same medium was used in sterile 50 mm Petri dishes. It is noteworthy that this medium was chosen because it is suitable for the quantitative growth of filamentous and yeast-like fungi (Miranda et al., 2023).

Incubation

The 90 mm diameter Petri dishes, after sampling, were incubated for 7 days at an approximate temperature of 25°C, according to Technical Standard 001 of Resolution RE No. 9 of the National Health Surveillance Agency (Brazil, 2003). The colonies with the most expressive and macroscopically distinct growth were isolated with the aid of a properly flamed platinum loop, and inoculated into new 50 mm Petri dishes. The isolated microorganisms were incubated under the same conditions for 15 days.

Data analysis

During incubation, colonies present in each Petri dish were counted, and the results obtained were expressed as the number of colony forming units (CFU) of anemophilic fungi per dish. Collections were carried out in triplicate, at equidistant triangulated points, in order to obtain a mean and standard deviation that covered the entire study environment. The data were statistically analyzed using the Tuckey test with a 95% confidence level. A digital thermohygrometer (Incoterm) was used to collect temperature and humidity data at the time of collection.

Microbiological identification

Identification was carried out under a microscope (Zeiss Primo Star) with Cotton Blue dye for contrast. For identification, the morphology of the hyphae and conidia were evaluated, in addition to macroscopic analyses of the fungi.

RESULTS AND DISCUSSIONS

Microbiological air quality

Before prospecting and fungal identification for future industrial applications, an

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assessment of the microbiological quality of the air in different open and closed environments was carried out. For this, colonies present in the samples were counted throughout the incubation time. From the fifth day onwards, it was no longer possible to count colonies from external environments, as overlaps occurred. Thus, microbial growth was monitored for up to 4 days, which is shown in Figure 2. Initially, it is highlighted that the majority of colonies were filamentous fungi, indicating little or no presence of yeast-like fungi in the samples from the study sites. Therefore, all colonies were considered to be filamentous fungi.

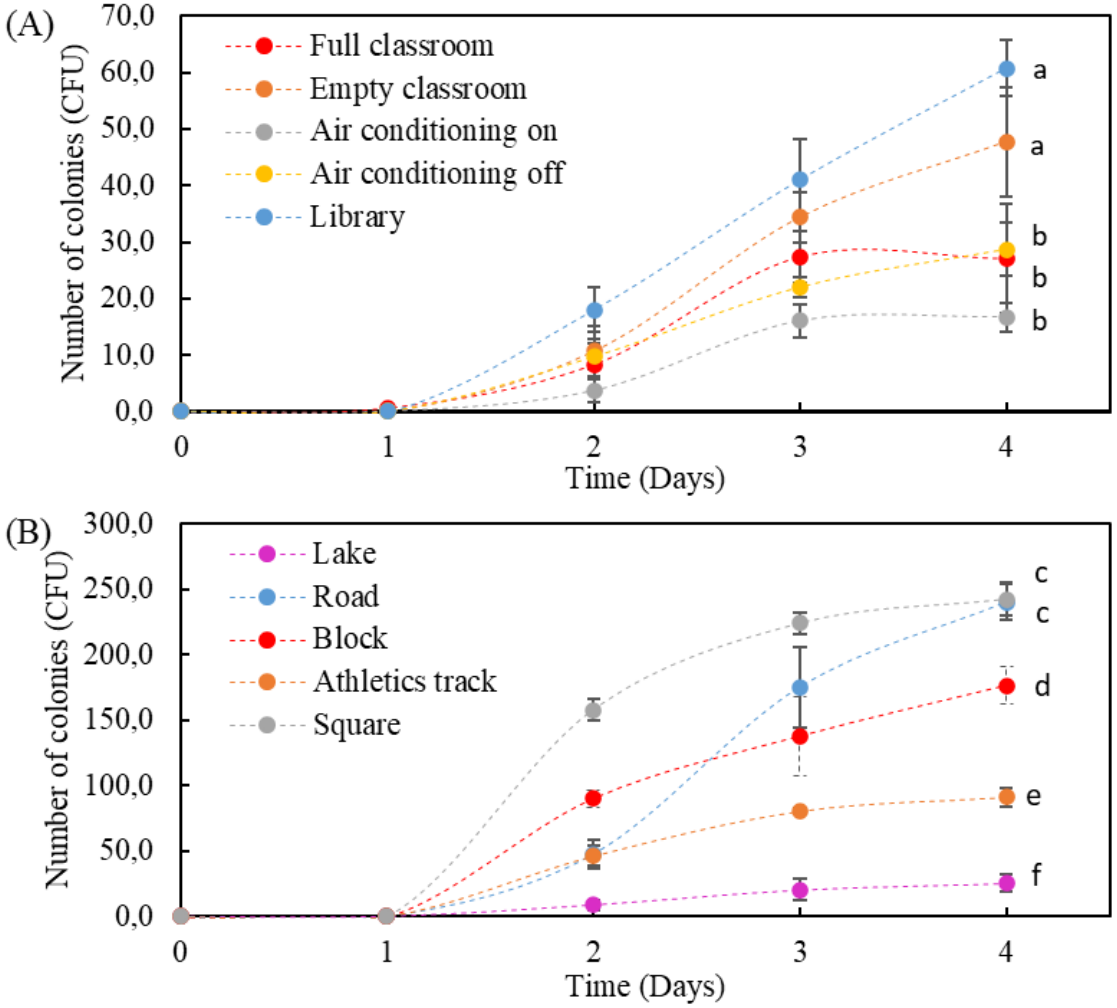

Figure 2 – Quantification of colonies over time in closed (A) and open (B) environments. Equal letters indicate that the means do not differ significantly according to the Tukey test with a 95% confidence level.

When comparing the order of magnitude between open and closed environments, it was noticed that external areas had a greater number of microorganisms than confined spaces. In a recent study, Abrego et al. (2020) reported that urbanization has reduced the fungal microbiota, pointing out that urban environments have a lower presence of aerial fungi than natural environments, corroborating the findings of the present study that compared indoor environments that are entirely made of concrete and open environments that have, generally, afforestation and with less influence from buildings. Thus, the quantification of fungi can be considered a way of identifying anthropogenic stressors, as they are sensitive to this reality.

Additionally, environmental aspects can influence the natural microbiota of the location. Thus, some environmental characteristics of

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the studied locations were shown in Table 1. Using Tuckey's statistical test, it was not possible to relate temperature and humidity variations to the number of colonies. The non-correlation between temperature values must have occurred because the range of values was narrow, while humidity tends to have more influence with yeast-like fungi. Contrary to this, Bernardi et al. (2007) and Spilak et al. (2015) noted that with increasing temperature and decreasing humidity, the number of anemophilic fungi was reduced when studying the microbiota of a beach.

On the other hand, a climatic aspect that interfered with microbial transport was wind speed, a parameter that is more visible in open places. According to the Beaufort Scale, the wind that was observed at the lake and on the street was classified at force level 6 (very fresh wind/Strong breeze), when the branches move, when it is possible to hear the wind buzzing and when it is difficult to use umbrella. The index for other external environments was classified as moderate wind/moderate breeze (force index 4). Therefore, these atmospheric displacements may have been responsible for the greater quantity of spores suspended in the air in outdoor locations, as they are light and small (Tortora et al., 2016). Another aspect that may have favored these results is the fact that closed environments undergo periodic cleaning, removing a large part of the local microbiota. Thus, given the objective of prospecting fungi for later application in production processes, the external environment is more likely to present anemophilic microbial diversity.

Table 1 – Environmental information of the evaluated environments.

Local	CFU/Dish	Temperature (ºC)	Moisture (%)	Wind speed (km/h)
Open environments				
Lake	25.7 ± 7.0	$26.5\pm1.1^{a.b.c}$	54.3 ± 1.5^{A}	44.4
Street	240.3 ± 13.3	$25.7\pm0.4^{a.b}$	65.0 ± 2.0^{B}	44.4
Square	242.3 ± 12.5	25.6 ± 0.1^{b}	71.0 ± 0.0^{C}	20.4
Court	176.7 ± 14.5	27.7 ± 0.1^{d}	65.0 ± 1.0^{B}	20.4
Athletics track	91.3 ± 7.4	$27.2\pm0.5^{c.d}$	65.7 ± 2.9^{B}	20.4
Average	155.3	-	-	-
Closed environments				
Meeting room with air on	16.7 ± 2.5	24.1 ± 0.4^{e}	41.3 ± 1.5^{D}	-
Meeting room with air off	28.7 ± 4.7	$25.8\pm0.1^{a.b}$	75.7 ± 2.5^{E}	-
Library	60.7 ± 4.9	$25.6\pm0.1^{a.b}$	$72.7\pm0.6^{C.E}$	-
Full classroom	27.0 ± 12.5	29.3 ± 0.1^{f}	45.3 ± 0.6^{D}	-
Empty classroom	47.7 ± 9.7	29.3 ± 0.1^{f}	42.3 ± 0.6^{D}	-
Average	36.1	-	-	-

Although open spaces are microbiologically quite populated, such values are within normal limits. This can be observed when compared with the literature reported in Table 2.

Table 2 – Comparative data from other studies that quantified anemophilic fungi in different internal (I) and external (E) environments.

Local	I	E	I/E Ratio	Reference
Museum/ Scientific collection	0.4 – 16.8 CFU/m^3	300 CFU/m^3*	0.001 – 0.056	Costa et al., 2011
Hospital	0 – 127 CFU/Placa	9 – 91 CFU/Placa	0.2 – 1.5	Sales et al., 2011
Agro-industrial company	-	-	0.05 – 4.15	Cunha et al., 2013
University	13 – 58 CFU/m^3	73 – 93 CFU/m^3	0.17 – 0.70	Neto et al., 2013
School	0 – 538 CFU/m^3	4 – 516 CFU/m^3	0.02 –	Caixeta et al.,

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			21.43	2016
Library	14 - 68 CFU/Placa	12 -37 CFU/m^3	0.81 – 7.75	Campos et al., 2017
Cemetery	506.2 – 668.4 CFU/Placa	795.5 – 1127.4 CFU/Placa	0.63	Siebra et al., 2021
Library/Special Collections Section	15 – 35 CFU/Placa	27 CFU/Placa	0.5 – 1.3	Agertt et al., 2022
University	16.7 – 60.7 CFU/Placa	25.7 – 242.4 CFU/Placa	0.23	Present study

* Value arbitrated by the authors

Additionally, as all study sites are part of a single university, to identify whether this university environment poses a risk to human health, averages were calculated between the quantities of Colony Forming Units in the internal (I) and external (E) locations. , which are in Table 1. With these values, the I/E ratio was calculated, obtaining a value of 0.23, which is lower than the maximum allowed by current legislation. Resolution RE No. 9 of the National Health Surveillance Agency establishes that the I/E ratio must be less than or equal to 1.5, being the recommended limit value that separates the conditions of absence and presence of the risk of harm to human health. (Brazil, 2003). In the literature, other authors observed reasons within and outside the standard, as shown in Table 2.

Then, the environments were compared to each other with the help of the Tuckey Test. Regarding closed environments (Figure 2a), when analyzing classrooms, the absence of people in the environment provided a greater quantity of microorganisms, that is, better air quality. This may have occurred because the presence of people in the room caused less air flow, making it more stagnant and reducing microbiological transport.

Additionally, it was noted that the number of colonies obtained in the library was statistically similar to those in the empty classroom. This can be attributed to the absence of people, at the time of collection, at the two study points. It is worth noting that libraries must be monitored in terms of microbiology for security in relation to the collection, and some other authors have carried out analyzes of libraries and found values close to the present study (Table 1; Costa et al., 2011; Campos et al., 2017; Agertt et al., 2022).

When comparing the same meeting room with the air conditioning turned on and off (Figure 2), statistical similarity was noted, indicating that filter changes and environmental cleaning are being carried out periodically and correctly. Studies have reported a lot of fungal presence in areas conditioned with incorrect hygiene or have compared disinfection methods, making this analysis important on a constant basis (Gołofit-Szymczak et al., 2019; Liu et al., 2021).

Moving on to the comparison between the unconfined environments, the following order was followed: Street = Square > Court > Athletics track > Lake. The equality between square and court may be due to both having similar flow of people and similar winds. The wind from these environments is channeled through the side buildings, resulting in greater microbiological movement. The square and the court have intermediate movement, as there is a lot of wind, but it is not carried by side buildings, reducing the final movement. And the lake has a reduction in winds due to the high trees that prevent air flow.

Microbial growth kinetics

One of the advantages of fungal biotechnology is the possibility of scaling its production in bioreactors (Hyde et al., 2019). Therefore, for future industrial application of anemophilic fungi, it is important to obtain the maximum specific growth rate (μmax), as it is a necessary parameter in the bioreactor design equations. This constant is characteristic of the exponential phase, when microorganisms reach their maximum growth (Doran, 1995). Although each location studied has a great diversity of anemophilic fungi, the microbial growth curve shown in Figure 3 was plotted to

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get an idea of the average growth rate of microorganisms in each environment. From the plotted curves, the exponential growth periods were delimited and the specific velocities for each of the environments were calculated (Table 3).

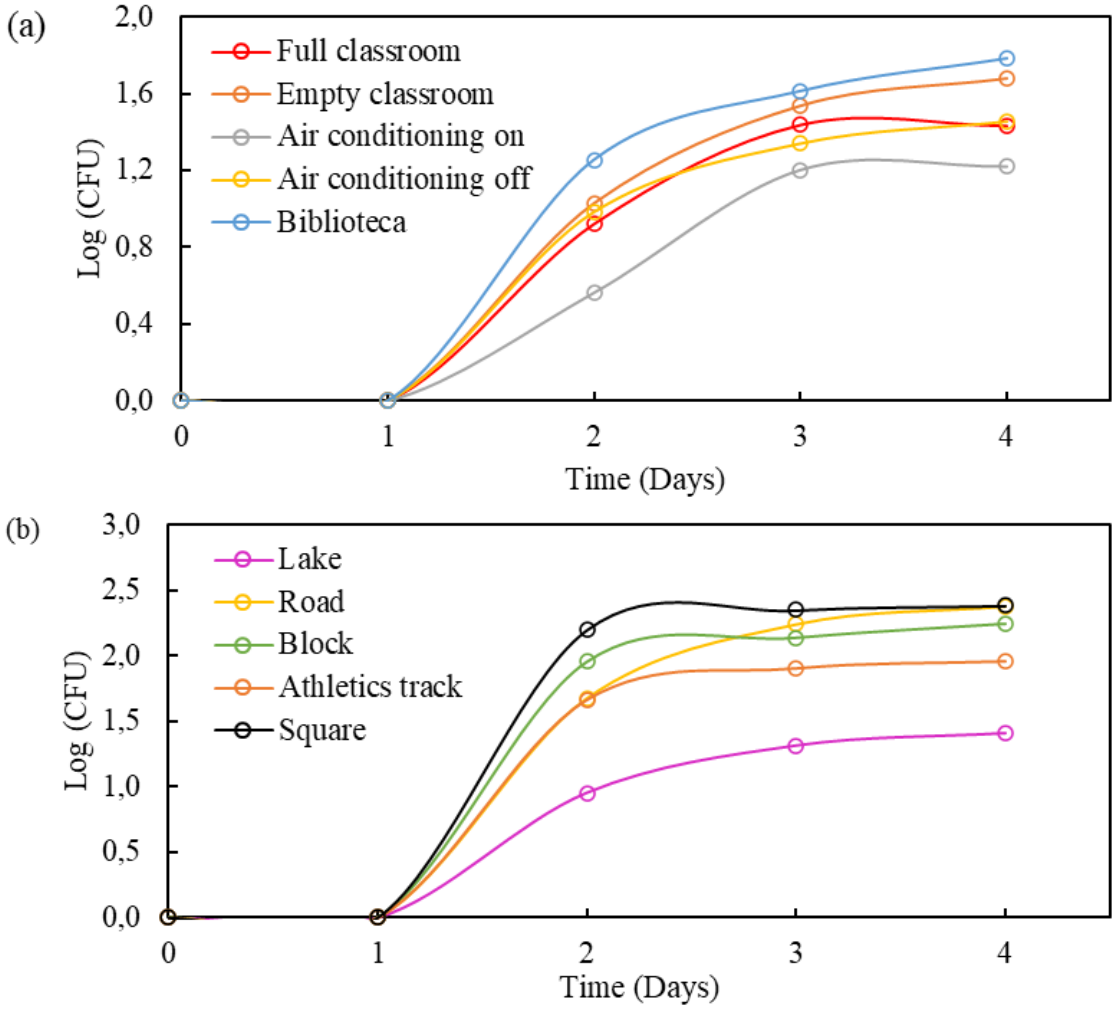

Figure 3 – Growth curves of microorganisms present in closed (a) and open (b) environments.

Table 3 - Maximum specific speed of cell growth (μmax) of anemophilic fungi in different environments.

Local	$\mu_{máx}$ (day^{-1})	Exponential period (day)	R^2
Open environments			
Lake	0.6576	1-3	0.9365
Street	1.1219	1-3	0.9243
Square	2.1987	1-2	1.0000
Court	1.9558	1-2	1.0000
Athletics track	1.6659	1-2	1.0000
Average	1.5200	-	0.9722
Closed environments			
Meeting room with air on	0.6021	1-3	0.9987
Meeting room with air off	0.6712	1-3	0.9320
Library	0.5706	1-2	1.0000
Full classroom	0.7183	1-3	0.9742
Empty classroom	0.7679	1-3	0.9631
Average	0.7992	-	0.9736

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When comparing the values, it was noted that the highest growth speed was obtained in external environments, corroborating that such places presented microorganisms with greater multiplication vigor. Therefore, they are more interesting when the objective is to scale a production process.

Identification of microorganisms

Based on the macromorphological characteristics of anemophilic microorganisms, various filamentous fungi were isolated so that their identification could be carried out. Given the micro and macromorphological characteristics, *Rhizopus sp, Aspergillus sp, Cladosporium sp, Penicillium sp* and *Scedosporium sp* were found, as shown in Figure 4.

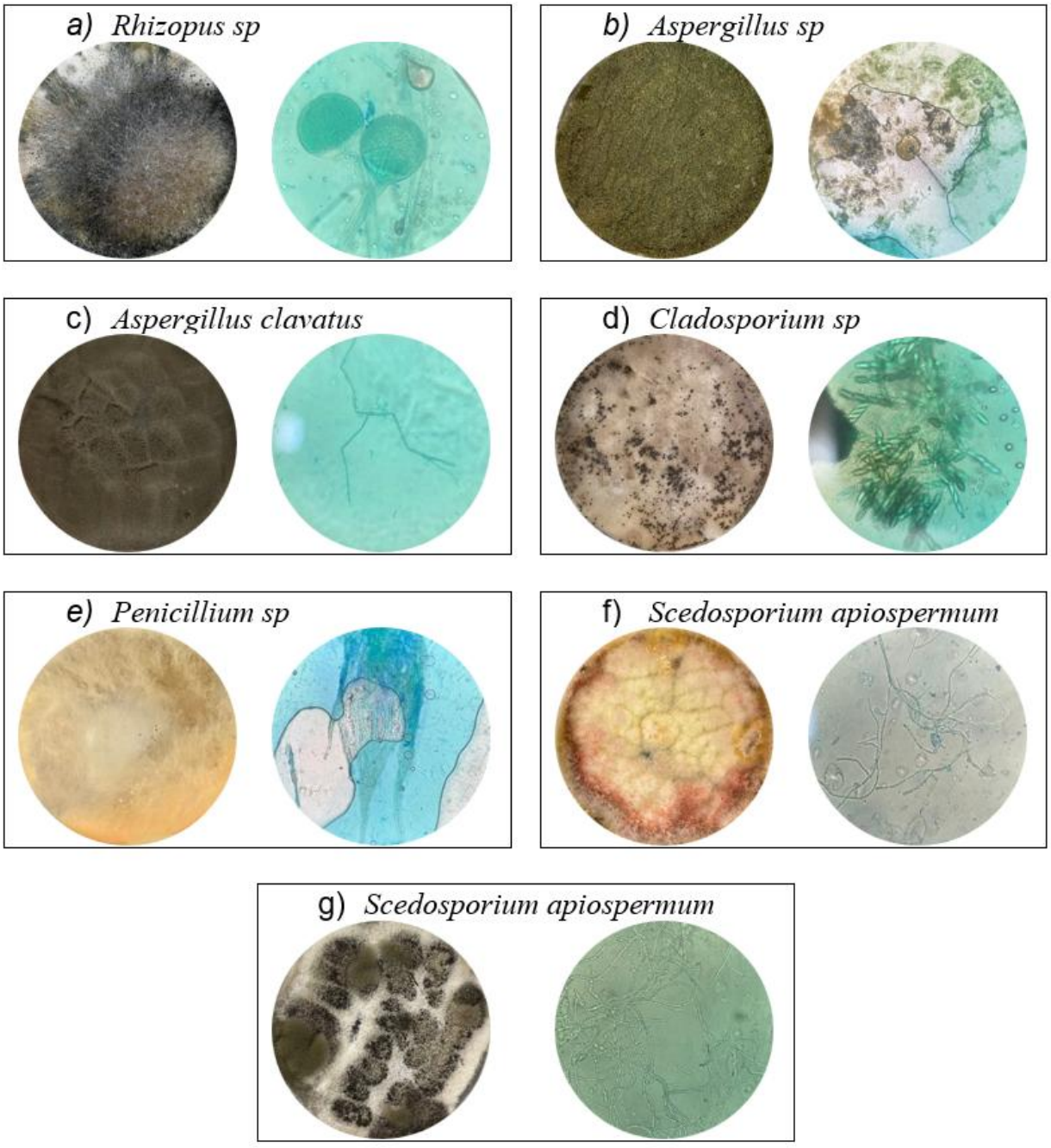

Figure 4 – Macroscopy and microscopy (400x magnification) of fungi isolated in open and closed environments.

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Applications in white biotechnology

Filamentous fungi have great biotechnological potential, which can be seen, in general, in Table 4. Thus, many studies have reported important advances in the application of these microorganisms in industrial processes. Given the possibilities, the filamentous fungi identified in this work could be studied in future work by the research group. Thus, a survey of real applications for such microorganisms was carried out.

Table 4 – Possible large-scale applications of filamentous fungi.

Aplication	References
Production of pigments/dyes	Velmurugan et al., 2010; Venil et al., 2020; Suciatmih, 2020
Enzyme production	Hosten, 2019;Saini et al., 2022; Wang e al., 2019
Biofuel production	Raven et al., 2019; Saye et al., 2021
Nanoparticle synthesis	Moghaddam et al., 2017; Ganesan et al., 2020;
Degradation of pollutants	Quintella et al., 2019; Juárez-Hernández et al. 2021; Cowan et al., 2022

As already reviewed in the literature, the genus *Rhizopus* can be used to produce lipases, which are a very versatile enzyme. This biocatalyst acts in the hydrolysis of fats at the water/lipid interface and also in synthesis reactions in solvent-free or non-aqueous media, such as transesterification, interesterification and esterification (López-Fernández et al., 2020; Valério et al., 2022). Additionally, there is research related to the production of protease, amylase, cellulase, xylanase, lignin peroxidase, polyphenol oxidase and laccase by this filamentous fungus (Benabda et al., 2019; Hasanin et al., 2020). There are also reports that this microorganism can be applied in the synthesis of nanoparticles. Hassan et al. (2021), for example, reported that the metabolites produced by *Rhizopus oryaze* were used in the green synthesis of magnesium oxide nanoparticles, showing good results as an antimicrobial agent against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*, in addition of its larvicidal and repellent activity against *Culex pipiens*. Another application of this type is in bioremediation. Kjoku et al., (2020) used *Rhizopus stolonifer* to decontaminate a synthetic solution containing lead, cadmium and nickel, indicating that they are useful for removing heavy metals from the environment. Chromium is another metal that can also be bioremediated by *Rhizopus sp*., as done by Espinoza-Sánchez et al. (2019),

Aspergillus sp. it also has a wide spectrum of applications. It has already been proven that *Aspergillus flavus* is efficient in the biodegradation of microplastic polyethylene particles (Zhang et al., 2020) and that a microbial consortium of different species of *Aspergillus* is capable of biodegrading low-density polyethylene (DSouza et al., 2021). Copper and silver oxide nanoparticles were synthesized with *Aspergillus terreus* and showed antibacterial, antifungal and antitumor activities (Mani et al., 2021; Lotfy et al., 2021). Silver nanoparticles have also been synthesized with *Aspergillus sydowii* (Wang et al., 2021), while Aspergillus niger has been used in the biosynthesis of zinc oxide nanoparticles (Es-Haghi et al., 2021). It is also known that *Aspergillus sp*. are capable of producing different chemicals such as butenolides, alkaloids, terpenoids, cytochalasins, phenalenones, ρ-terphenyls, xanthones, sterols, diphenyl ether and anthraquinone derivatives with diverse biological activities (El-Hawary et al., 2020). Enzyme production has also been reported in the literature as alpha-amylase, cellulases, β-glucosidases, hemicellulases, laccases, lipases, proteases, β-galactosidases, tannases, keratinase, cutinases and aryl alcohol oxidase. (Asemoloye et al., 2020; Kumar, 2020; Melnichuk et al., 2020)

Cladosporium sp. is also another filamentous fungus that has been applied in some industrial processes. Liu et al. (2023), for example, analyzed the biodegradation of polyurethane polyester with this microorganism. Still on the environmental theme, there is a report on the use of this fungus in the bioleaching of fly ash (Seddiek et al., 2021). *Cladosporium cladosporioides* can also be used to produce cellulase, xylanase and alpha amylase (Mushimiyimana et al., 2019). Ge et al. (2020) studied a new glucose oxidase from *Cladosporium neopsychotolerans*, aiming for application in baking. Hamed et al. (2021) carried out a study on the production of L-asparaginase by *Cladosporium tenuissimum*. Additionally, although it is a pathogenic fungus (Solairaj et al., 2022), there is a report that *Cladosporium* is capable of protecting plants against biotic and abiotic stresses (Raut et al., 2021), being capable of generating fungicide against *Botrytis cinerea* (Pan et al., 2023).

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Penicillium sp. it was the first microorganism used for the industrial production of a bioproduct on a large scale, being marked by the generation of penicillin in the Second World War (Pereira and Pita, 2018). Currently, new applications are being investigated. For example, it can be applied to the production of hemicellulases (Méndez-Líter et al., 2021). Additionally, it can be used to obtain natural dye (Morales-Oyervides et al., 2020). Interestingly, Nobre et al. (2019) developed a process for producing probiotic fructooligosaccharides with *Penicillium citreonigrum*. Coelho et al. (2020), in turn, carried out the bioremediation of water contaminated with uranium, using *Penicillium pistarium*.

Scedosporium is still little applied in biotechnological processes. Acevedo et al. (2020) reported the use of *Scedosporium apiospermum* for bioregeneration of activated carbon by degrading phenolic compounds. Atakpa et al. (2023), in turn, obtained interesting results on the biodegradation of crude oil by co-culture of *Scedosporium sp*. and *Acinetobacter sp*. Dyes have also been degraded with *Scedosporium apiospermum* (Kumaravel et al., 2022).

Given the reports published in the literature, the biotechnological potential of anemophilic fungi found in open and closed environments became evident. Therefore, genetic identification can be carried out and studies will be initiated with different processes using such microorganisms..

CONCLUSIONS

Knowing that anemophilic fungi are of great importance today, prospecting for microorganisms in open and closed environments was carried out. It was noted that external environments have a greater fungal microbiota, but that the university environment, in general, is healthy as it has an I/E ratio of 0.23. When studying the microbial growth of fungi, greater growth vigor was observed in fungi in the open environment, with a higher maximum specific growth rate. Next, *Rhizopus sp, Aspergillus sp, Cladosporium sp, Penicillium sp* and *Scedosporium sp* were identified in open and closed environments. In future work, they could be applied to white biotechnological processes such as bioremediation, enzyme production, nanoparticle synthesis and others. Thus, it was possible to prospect microorganisms with real potential for industrial applications.

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