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Antimicrobial and enzymatic activity of anemophilous fungi of a public university in Brazil

LAUREANA V. SOBRAL[1], KELLY N. MELO[1], CLECIANA M. SOUZA[1], SÍLVIO F. SILVA[1], GILVANIA L.R. SILVA[1], ANDRESSA L.F. SILVA[1], KATHARINE A.A. WANDERLEY[1], IDJANE S. OLIVEIRA[1] and ROBERTA CRUZ[2]

[1]Laboratório de Microbiologia e Imunologia, Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Rua Alto do Reservatório, s/n, Bela Vista, 55608-680 Vitória de Santo Antão, PE, Brazil
[2]Departamento de Micologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Avenida Professor Moraes Rego, 1235, Cidade Universitária, 50670-901 Recife, PE, Brazil

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ABSTRACT

To the fungal microbiota the UFPE and biotechnological potential enzymatic and antimicrobial production. Air conditioned environments were sampled using a passive sedimentation technique, the air I ratio and the presence of aflatoxigenic strains evaluated for ANVISA. Icelles were to determine the enzymatic activity of lipase, amylase and protease metabolic liquids to determine antimicrobial activity. Diversity was observed in all CAV environments, CFU/m^3 ranged from 14 to 290 and I/E ratio from 0.1 to 1.5. The of the fungal genera were: *Aspergillus* (50%), *Penicillium* (21%), *Talaromyces* (14%), *Curvularia* and *Paecilomyces* (7% each). *Aspergillus sydowii* (Bainier & Sartory) Thom & Church presented enzymatic activity and the *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga & Frisvad presented antibacterial activity against all bacteria that all environments present fungal species biodiversity no toxigenic or pathogenic fungi were found, according to ANVISA legislation for conditioned environments and airborne filamentous fungi present potential for enzymatic and antimicrobial activity.

Key words: Air quality, bacteria, enzymes, filamentous fungi, refrigerated environments.

INTRODUCTION

Fungi air, water, soil, animals and food. They colonize substrates, have an extensive geographic distribution and are able to grow under environmental conditions (Oliveira and Borges-Paluch 2015).

Fungal contamination is considered due to the production. Of the filamentous fungi under adequate conditions of oxygen, temperature and humidity, secondary metabolites (mycotoxins) both and animals, fungi of the genus *Aspergillus* and *Penicillium*. *A. flavus* Link and *A. parasiticus* Speare produce aflatoxins (Peluque 2014).

Therefore, monitoring air in artificially air-conditioned is according to Resolution RE No. 9 of January 16, 2003, the National Agency of Sanitary Surveillance (ANVISA), which defines reference standards cleaning and maintenance of the health and safety of individuals who attend

Correspondence to: Laureana de Vasconcelos Sobral
E-mail: laureanavsobral@gmail.com

public and collective places with conditioned environments. The recommended maximum acceptable contamination of ≤750 CFU/m^3 and ≤1.5 for the relation between internal and external air the presence of pathogenic and toxigenic fungi (Brazil 2003).

Biotechnically, microbial enzymes are by industries reduced production time, increased scale and purification, specificity and stabilization, as well as easy genetic manipulation (Nagarajan 2012) and high biochemical diversity (Silva et al. 2015) is related to the minimization production (Reinehr et al. 2014) and waste and electric consumption (Silva et al. 2015).

Dispersion and appearance of commercially available antimicrobial-resistant microbes, thus the search for sources of effective antimicrobial agents (Santos et al. 2014) researches with the purpose of intervening, preventing or intensifying the conventional bacterial resistance (Catão et al. 2014).

The antimicrobial using mycelia and liquid metabolites of anemophilous filamentous fungi this may be the first activity. The present study aimed to evaluate the microbiological quality of air, the presence of aflatoxigenic fungi in the climatic environments of the Federal University of Pernambuco/Vitória Academic Center and enzymatic production and antimicrobial activity.

MATERIALS AND METHODS

SAMPLING AND COLLECTION OF AIR

The study was conducted at the Federal University of Pernambuco/Vitória Academic Center (UFPE/CAV) and 82 artificially air-conditioned 3 board of directors, 9 library, 1 auditorium, 15 classrooms, 34 laboratories, 13 administrative rooms, 1 room of the Internal Accident Prevention Commission (CIPA), 5 student assistance centers, 1 teaching staff and an external area located in the courtyard of the main entrance of the CAV for fungi quantification. Non-air-conditioned environments were excluded from the study.

The samples were collected from the central area of the environments through the passive sedimentation technique (Lacaz et al. 1998) on solid culture medium using Petri dishes containing the culture (MEA) and *Aspergillus flavus* and *parasiticus* agar (AFPA) for total and aflatoxigenic fungi cultures, respectively. The plates were placed 1m the ground and opened for 15 min bioaerosols, containing microorganisms present in the atmospheric air. The plates were incubated at room temperature (28 ± 2ºC) for up to days.

MICROBIOLOGICAL ANALYSIS OF AIR

The colonies present in the respective culture media were quantified and the relation between internal and external air (I/E) was calculated to determine the internal air quality. ANVISA the recommended maximum acceptable microbiological contamination value of ≤750 CFU/m^3 and ≤1.5 for the relationship between internal and external air (Brazil 2003).

Sedimentation sampling does not directly determine the number of microorganisms present in a given volume of airit is possible to transform the results of this method (number of CFU/unit area) into CFU/volume unit number (Morais et al. 2010). Air sampling by sedimentation was using the following equation described by Friberg et al. (1999):

$$\text{No of CFU/m}^3 = \frac{\text{No of CFU on plate}}{\text{Petri dish area (m}^2\text{)}} \text{X} \frac{1}{23} \text{(SAR)}$$

The area of the petri dish 90 x 15 mm (our study 0.006m^2). SAR is the ratio between the in the air and on the surface of the culture medium. For environments with spontaneous sedimentation and without apparatus that forces the sedimentation of air, this ratio is 1:23 (Morais et al. 2010).

IDENTIFICATION OF AFLATOXIGENIC FUNGI

The presence of aflatoxigenic strains revealed by the orange reverse of the AFPA culture medium was evaluated. ANVISA the presence of pathogenic and toxigenic fungi in artificially climatized environments (Brazil 2003).

ISOLATION, IDENTIFICATION AND PRESERVATION OF FUNGI

After in the MEA solid culture medium, the fungal colonies were purified in the same culture medium. After the fungal species, the macroscopic (coloration, appearance and diameter of the colonies) and microscopic (somatic and reproductive microstructures) (Samson and Frisvad 2004) were observed through culture under coveralls (Riddell 1950) and preserved to the Castelani method distilled water and immersion of pure cultures mineral oil. The samples were collected test tubes containing MEA solid culture medium in order to each species identified in the Collection of Cultures Micoteca URM of the Federal University of Pernambuco.

DETERMINATION OF ENZYMATIC ACTIVITY

To determine the enzymatic activity of the fungi isolated from the air, 14 species were identified according to the number attributed to the isolates [*Paecilomyces variotti* Bainier (04), *Penicillium fellutanum* Biourge (06), *Aspergillus flavus* Link (09), *A. parasiticus* Speare (14, 20, 27 and 67), *A. sydowii* (Bainier & Sartory) Thom & Church (18), *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga & Frisvad (21 and 28), *Aspergillus japonicas* Saito (24), *Penicillium oxalicum* Currie & Thom (25), *Penicullium chrysogenum* Thom (26) and *Curvularia lunata* (Wakker) Boedijn (49)] for lipase, amylase and protease production, following the methodology Menezes and Hanlin (2004). Mycelial dis were from fungal colonies and peeled into the central area of the Petri dish containing culture media specific for each enzyme incubated for at room temperature. The tests were performed in triplicate for each culture medium.

The lipase reaction was by visualizing the presence of calcium salt crystals of lauric acid and/or the formation of a clear area around the fungal colony dis. To the production of amylase, 0.1N iodine solution was used, allowing the identification of the enzymatic reaction through a translucent halo around the colony. For protease, due to the chemical reaction it was possible to visualize the formation of translucent halo around the colony. After the development of the enzymatic reactions, a halo of enzymatic activity was measured to determine the production potential of the colonies, which is proportional to the halo diameter.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Of the species identified, 5 metabolic liquids from the species Paecilomyces *variotti* Bainier (04), *Aspergillus japonicas* Saito (24), *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga & Frisvad (28), *Curvularia lunata* (Wakker) Boedijn (49), *Aspergillus parasiticus* Speare (67) were randomly selected to perform antimicrobial activity and determine and purified fungal mycelia dis were cultured in 10 mL of liquid culture medium Sabouraud brothand incubated at room temperature for 20 days. The liquid metabolite was separated from the mycelium by filtration on sterile filter paper. The metabolic fluid was stored at 4°C to perform the antibacterial activity tests and the mycelium was frozen for subsequent extraction of metabolites.

The strains of Gram-negative bacteria: Escherichia coli (ATCC 25922), Shigella flexneri (ATCC 12022), Salmonella typhimurium (ATCC 14028), Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa and Gram-

positive: Staphylococcus aureus (ATCC 29213) and S. aureus ORSA. The bacterial suspensions were prepared in 0.85% saline and a concentration of 1.5 x 10^5 CFU/mL was obtained, from the dilution of the corresponding suspension to the 0.5 tube of the McFarland scale. Five metabolic liquids produced by filamentous fungi from atmospheric air were tested. All experiments were performed in triplicate. The antibacterial activity test was performed the broth dilution method of the ANVISAM7-A6 technical standard. Serial dilutions of metabolic liquids were performed. Positive control of the test was using chloramphenicol antibiotic and negative control of the test, in the absence of antibiotic, to confirm absences of contaminants in the metabolic liquid used in the test was added a column with the pure metabolic liquid. After distribution of the bacterial suspensions, the plates were incubated at 37°C for 24h. 20 µL 2,3,5-triphenyltetrazolium chloride (TTC) (2% v/v) diluted in water was added to all wells after 2h of incubation room temperature, bacterial growth. The wells stained in red indicate bacterial growth (Rahman et al. 2004).

The red coloration results from the TTC reaction with hydrogen ions constituted due to cellular respiration, generated by an insoluble reddish solution called formazan, indicating viable bacterial cells in the medium (Rahman et al. 2004).

The reading was performed through visual observation of the lowest concentration of metabolic liquids that inhibit bacterial growth. The test was performed, where was for Petri dishes containing Mueller-Hinton Agar, the plate incubated at 37°C for 24h. Bacterial growth, indicating bactericidal or bacteriostatic action of the fungal metabolic the wells not presentantimicrobial stained red.

The liquid metabolites used in the study were tested in their crude state after filtration. Therefore, the antimicrobial activity the inhibitory concentrations defined for the method in µL/mL: 500.00; 250.00; 125.00; 62.50; 31.25; 15.63; 7.81; 3.91; 1.95 and 0.97µL/mL.

RESULTS AND DISCUSSION

Growth was observed in all environments the lowest amount of CFU/m^3 in the classroom (14 CFU/m^3) and with the amount and diversity of the living pharmacy space, the CIPA room and the teaching office (Table I), all the evaluated environments of the CAV were within the ANVISA for microbiological air quality (≤750 CFU/m^3). Fungal colonies from environments observed in Figures 1 and 2 the colonies before and after purification of the fungal isolates, respectively.

The evaluated environments possibly the access flow of ventilation and passers-by in the environments, atmosphere is influenced by the climate, the interior atmosphere is influenced by building maintenance. Both environments influence human activities, humidity and little temperature, since many indoor fungi grow in a wide temperature range (Araújo and Cabral 2010).

The ratio of fungi quantification of I/E (internal/external) of the environments surveyed in the CAV (Table I) met the current ANVISA legislation for air quality in conditioned environments (≤1.5), this ratio 0.1 to 1.5, the minimum and maximum, respectively, since the external air quantification was 188 CFU/m^3.

The environments that presented an I/E statement in the maximum acceptable limit were: library entrance hall, living pharmacy space, CIPA room and laboratory 1 and teaching room. Fact that the entrance hall of the library by visitors, with human activity showing a direct influence on the live pharmacy laboratory handles plant and soil samples CIPA room is an environment used for meetings the room is relatively frequented by students. In these environments, the values of the air fungi quantification in CFU/m^3 were allowed by ANVISA (750), but the I/E ratio was the limit.

No environment presented aflatoxigenic fungi (Figure 3) in compliance with the aforementioned

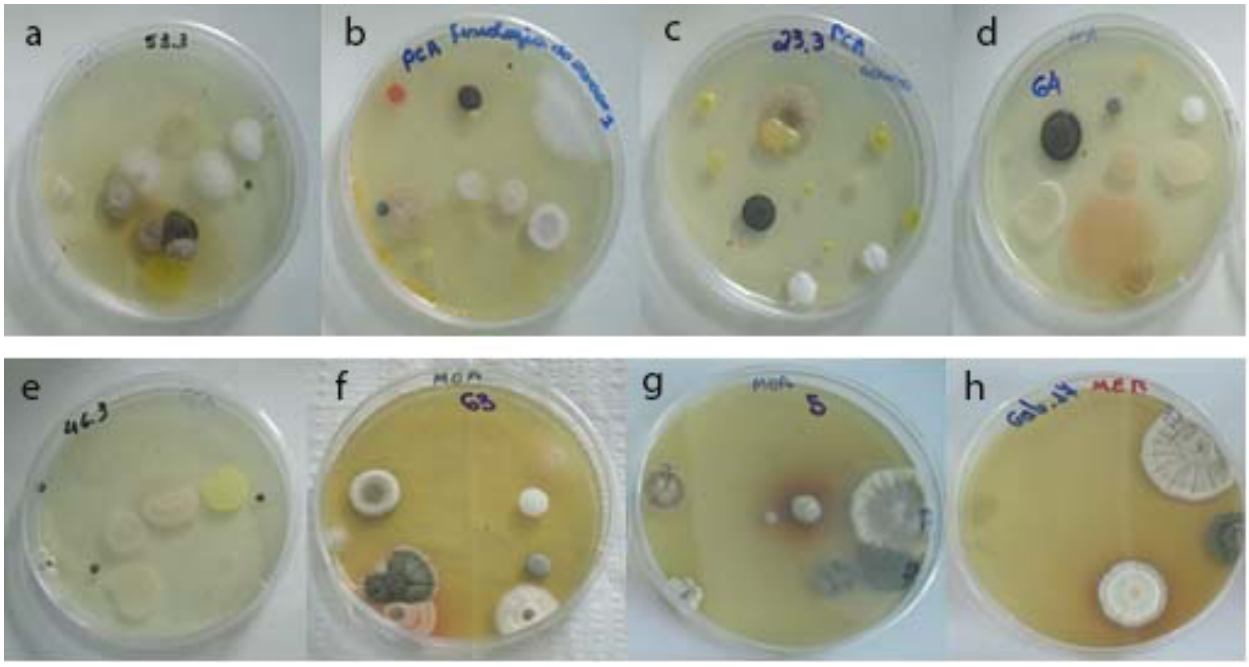

Figure 1 - Diversity of colonies of air bioaerosols of different environments of the Academic Center of Vitória - UFPE/CAV. Internal Commission for Accident Prevention - CIPA (**a**), Laboratory of Effort Physiology (**b**), Library - consultation collection room (**c**), Laboratory of Maternal and Child Nursing - LABEMI (**d**), Laboratory of Dietetic Technique (**e**), Laboratory of Semiology 2 and Clinical Evaluation (**f**), Nutrition Center (**g**), Library - study hall of the ground floor (**h**).

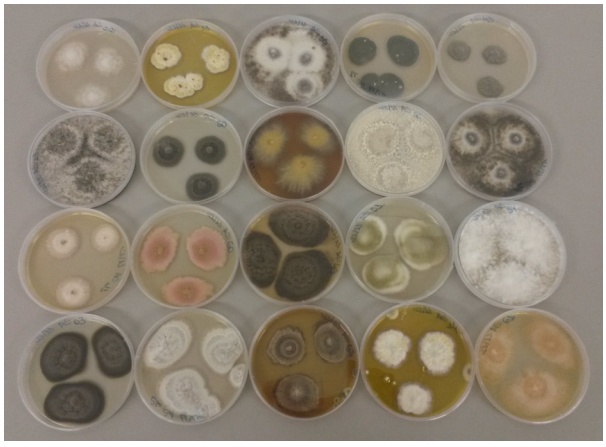

Figure 2 - Diversity of colonies after purification of fungi isolates from different environments of the Vitória Academic Center - UFPE/CAV.

legislation that establishes the absence of pathogenic and toxigenic fungi in indoor air.

Our study, Aboul-Nasr et al. (2013) found fungi isolated from unit intensive care unit hospital in India, 79 isolates were tested by thin layer chromatography (TLC) and at least one mycotoxin was these 79 isolates. Several mycotoxins are detected by TLC, the aflatoxins being zearalenone, gliotoxin, fumigiline, Mycotoxin production is a virulence factor.

The frequency of the fungal genera found in the analyzed environments, whose were: *Aspergillus* (50%), *Penicillium* (21%), *Talaromyces* (14%), *Curvularia* and *Paecilomyces* (7% each) as shown in Figure 4.

Anemophilous fungal infections in the medical literature and inhaled airborne spores have been implicated responsible for various allergic (Furtado and Ferraroni 1982). In addition to allergy, many opportunistic fungi such as those shown in Figure 4, are responsible for diseases otitis, mycotoxicosis, urinary infections, onychomycosis, eye infections fungemia. Since are dispersed abundantly in the environment (Grumach 2001). Therefore, monitoring the environment detect potential

TABLE I
Quantification of fungal colony forming units (CFU) from the air in the MEA and AFPA culture media and internal and external air ratio (I/E) of the Vitória Academic Center - UFPE/CAV.

Environments evaluated	MEA UFC/ m^3	AFPA	Ratio I/E	Environments evaluated	MEA UFC/ m^3	AFPA	Ratio I/E	Environments evaluated	MEA UFC/m^3	AFPA	Ratio I/E
Postgraduate studies	137	-	0,7	Room 03	123	-	0,6	Coordination of Laboratories	36	-	0,2
General Secretary of Courses	237	-	1,1	Security Center	29	-	0,1	Lab. Semiol./Aval. Clinic	138	-	0,7
CIOF	65	-	0,3	Room 04	188	-	1,0	Emergency Lab. and Enf. Surgical	94	-	0,5
Nucleus of Biological	115	-	0,6	Room 05	43	-	0,2	Emergency and Emergency Lab	181	-	1,0
Nutrition Core	58	-	0,3	Room 06	130	-	0,7	Laboratory of Semiology 1	72	-	0,4
Nucleus of Collective Health	29	-	0,2	Living room	123	-	0,6	LABEMI	152	-	0,8
Nucleus of Nursing	87	-	0,5	Anatomy Laboratory 1	29	-	0,1	Lab Physiology of Exercise 1	72	-	0,4
General Secretariat (Board)	72	-	0,4	Laboratory of Genetics	36	-	0,2	Lab Physiology of Exercise 2	152	-	0,8
Board of Directors	87	-	0,5	Anatomy Laboratory 2	167	-	0,9	Auditorium	44	-	0,2
Deputy director	51	-	0,3	Micro and Immunology Lab	51	-	0,3	NAEPS - Work Doctor	145	-	0,8
NATI	22	-	0,1	Living Pharmacy Space	290	-	1,5	NAEPS - Reception and Archive	51	-	0,3
Nucleus of Physical Education	51	-	0,3	Multifunctional Laboratory 1	51	-	0,3	NAEPS - Psychosocial Team	138	-	0,7
Schooling	108	-	0,6	Microscopy Laboratory 3	123	-	0,6	NAEPS - Atend. Individual	116	-	0,6
Computers room	22	-	0,1	Laboratory of Parasitology	65	-	0,3	Drivers Room	65	-	0,3

TABLE I (continuation)

Environments evaluated	MEA UFC/ m^3	AFPA	Ratio I/E	Environments evaluated	MEA UFC/ m^3	AFPA	Ratio I/E	Environments evaluated	MEA UFC/m^3	AFPA	Ratio I/E
Room 13	65	-	0,3	Multifunctional Laboratory 2	145	-	0,8	PET - Knowledge Connections	152	-	0,8
NEP	58	-	0,3	Lab of Dietetics	36	-	0,2	D. A. (Academic Directory)	65	-	0,3
Library - Ground floor	87	-	0,5	Lab. Bromatology	101	-	0,5	Lab. Food	101	-	0,5
Ground floor study / library	94	-	0,5	Lab. Microbiol. of food	138	-	0,7	NAFPF	65	-	0,3
Library / Collection	80	-	0,4	Lab. Tec. in Biomaterials	101	-	0,5	Biology Teaching Lab	87	-	0,5
Library / Study 1st floor	260	-	1,4	Rhythmic Room	80	-	0,4	Bioprocess Laboratory	130	-	0,7
Library / Study (group 1)	101	-	0,5	Judo / Karate Room	116	-	0,6	SIM	87	-	0,5
Library / Research Room	246	-	1,3	Bodybuilding Room	188	-	1,0	Research Lab Ens. of Biology	160	-	0,8
Library / Video Room	181	-	1,0	Physiology of Effort Lab	58	-	0,3	Room 8	94	-	0,5
Library / Collection query	65	-	0,3	Biodiversity Laboratory	80	-	0,4	Room 9	58	-	0,3
Library / Administration	65	-	0,3	Lab. Biotecnol. and drug	58	-	0,3	Room 10	51	-	0,3
Lab. Physiol. and Pharmacology	29	-	0,1	Microscopy Laboratory 1	275	-	1,5	Room 11	87	-	0,5
Room 01	80	-	0,4	Microscopy Laboratory 2	109	-	0,6	Office teacher	290	-	1,5
Room 02	14	-	0,07	CIPA	290	-	1,5				

CIOF: Coordination of Infrastructure, Budget and Finance, NATI: Nucleus of Support and Information Technology., NEP: Nucleus of Research and Extension, TECNOBIO: Laboratory of Technique in Biomaterials, CIPA: Internal Commission of Prevention of Accidents, NAEPS: Nucleus of Student Assistance and Psychosocial Support, SIM: Laboratory of Synthesis and Molecular Isolation, NAFPF: Laboratory Nutrition, Physical Activity and Phenotypic Plascity, LABEMI: Maternal-Infant Nursing Laboratory.

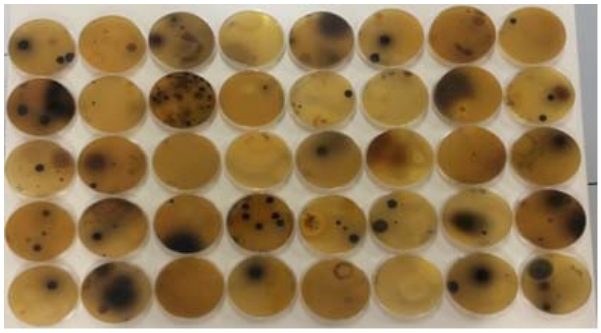

Figure 3 - Negative detection of aflatoxigenic fungi in CAV air in AFPA medium (absence of orange color on reverse of fungal colonies).

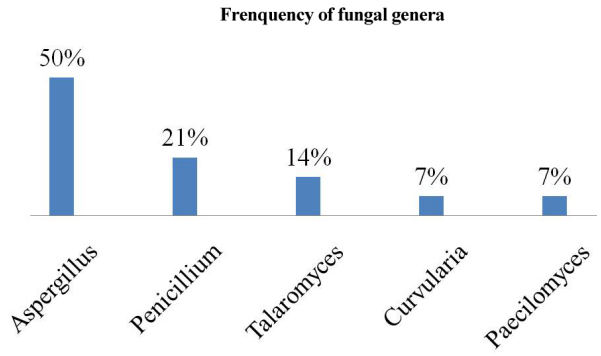

Figure 4 - Frequency of fungal genera found in the air of the analyzed environments of the CAV.

pathogenic prevent epidemics and protect public detect microorganisms (Zhoug et al. 2000).

Biologically, fungi are considered a promising source for new biomolecules with diverse biological activities for enzyme production and antimicrobial activity, due to chemical and structural diversity (Silva et al. 2015).

The majority of studies air quality were performed in hospital settings. Azimi et al. (2013) analyzed the quality of hospital air and found 70% of the samples fungi of *Penicillium* sp., 14% *Aspergillus* sp., 12% *Cladosporium* sp., *25% Alternaria* sp., indicating a risk factor for patients and professional these environments. Our study, since in addition to the analyzed environment being different, the most frequent was *Aspergillus*.

Library are similar with the study by Nascimento (2011) that when analyzing the internal air quality of the public library of the city of São Carlos/SP, did not find irregularities that would confront ANVISA legislation.

The CAV classrooms had values below 750 CFU/m^3, the results of this study differ from those presented by Morais (2010) who the microbiological quality of air in classrooms of a higher education institution, that 51% of the classrooms presented higher contamination than ANVISA.

Stryjakowska-Sekulska et al. (2007) studied nine environments at the University of Poznan in Poland. Chemistry laboratory, library, canteen, restrooms and board. All environments were evaluated morning and afternoon, 2 years. In the afternoon, possibly due to attending these environments throughout the day. They observed the following fungal genera in order of prevalence in the environments: *Cladospirium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Rhizopus* sp. and *Epicocum* sp. This result is in, since *Aspergillus* and *Penicillium* were also the most frequent genera the CAV according to Agarwal and Chakrabarti (2010) these genera as well as are the first colonizers of surfaces and interiors.

Pantoja et al. (2007) studied five sites (classroom, central library, warehouse, food court of the Campus of the State University of Ceará, Campus Itaperi. Among the 18 genera isolated from the evaluated environments, the most commonly found were *Aspergillus* sp. (78%),

Penicillium sp. (30%) and *Fusarium* sp. (22%). Classroom and central library indicating the importance of human activity as the main influencing factor of mycobiota contamination in these environments.

It is noteworthy that Pantoja et al. (2007) found fungal genera because the collection method, although it was passive sedimentation, stratified the air collection time generally recommended in the world literature, which is 15 min, and the samples were collected for 12h.

The concern to find the genus *Aspergillus* and *Penicillium* in indoor and air-conditioned environments is species these genera produce secondary metabolites with a toxic effect on health (Peluque 2014). Exposure to mycotoxins may lead to reactions including hemorrhages and necrosis, and in abundance such toxins have affinity for specific organs or tissues such as the liver, kidneys and nervous system (IARC 2002).

Kalwasinska et al. (2012) evaluated functional and nonfunctional environments of a university library in Torun, Poland. According to the authors, the internal air was according to the legislation, both in the functional (classroom, reading room of periodicals, collection conservation salt), in nonfunctional (bathroom, cafeteria) in which all sectors of the CAV library analyzed were below the limit established by ANVISA (Table I).

Boff (2011) environments with fungal agglomerates influenced by environmental factors such as temperature, humidity, airflow, available organic substrates, climatic conditions and seasonal variation, physical factors the shape, size and density of the particles, among other situations that corroborate the increase of conidia in the environment.

Ejdys et al. (2013) studied the fungal composition of air from school classrooms and monitored or years, classifying according to biosafety level. The authors isolated 151 species of fungi, of which 22 and 61 species of fungi were classified as class 1 of biological risk.

Hayleeyesus and Manaye (2014) studied university from Ethiopia and observed that the according to the legislation, with concentrations of fungi ranging from 367 to 2.595 CFU/m^3. *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp. and *Fusarium* sp. most frequently found found for the library and other environments of the CAV.

Many fungal species, including those present in the air, may be responsible for the production of metabolites with the most varied industrial and biological applications, enzymatic and antimicrobial activities (Viriato 2014).

The sources of extraction to study biological activities are soil, water, plants and more recently marine environments, exploring the secondary metabolism in order to perform tests using liquids and metabolic extracts or isolated metabolites by chromatography. The existing fungi in atmospheric air containing a range of pollutants still unexplored for the isolation of fungi some biological activity.

From the air, 67 specimens were initially isolated and then tested with 14 isolates after morphological identification to determine the enzymatic activity for the production of amylase, lipase and protease. The species of filamentous fungi identified in the air of the CAV were *Paecilomyces variotti* Bainier (04), *P. fellutanum* Biourge (06), *Aspergillus flavus* Link (09), *A. parasiticus* Speare (14, 20, 27 and 67), *A. sydowii* (Bainier & Sartory) Thom & Church (18),

Talaromyces purpureogenus Samson, Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga & Frisvad (21 and 28), *A. japonicas* Saito (24), *Penicillium oxalicum* Currie & Thom (25), *P. chrysogenum* Thom (26) and *Curvularia lunata* (Wakker) Boedijn (49) (Table II).

Among the species tested for enzymatic activity, *A. sydowii* presented better results for production of amylase (6 mm), lipase (14 mm) and protease (5 mm). Two other species produced the three enzymes (*A. parasiticus* Speare and *P. fellutanum* Biourge), but with less activity. Only three isolates belonging to three species were negative for the three enzymes simultaneously, namely*: P. variotii* Bainier (04), *A. parasiticus* Speare (20) and *C. lunata* (Wakker) Boedijn (49).

Not all isolates of a fungal species necessarily have the same enzyme the same intensity. Therefore our study that although the isolates 14, 27 and 67 belong to the same species *Aspergillus parasiticus* Speare, the same enzymatic reaction

was not observed among them. For, the isolate 27 only produced lipase and the isolates 14 and 67 produced all three enzymes, results the isolates of *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken,

Spierenburg, Seifert, Peterson, Varga & Frisvad. The fungi species that presented the best amiolitic activities were *Penicillium fellutanum* Biourge (7 mm) and *P. Oxalicum* Currie & Thom (7 mm) and lipase activity were *Aspergillus parasiticus* Speare (10 mm) and *A. sydowii* (Bainier & Sartory) Thom & Church (14 mm). The production of protease, although the one of lower intensity for the producing species, had the isolate 18 of *A. sydowii* (Bainier & Sartory) Thom & Church as the best producer (5 mm) (Table III). The intensity of the in solid substrate can be directly influenced the solubility, diffusibility, enzyme, other factors.

The results presented in this study show that amiolytic activity was the most common among the species, corroborating with the finding of Wenzel et al. (2013) evaluated endophytic fungi of soybean and revealed a greater quantitative enzymatic activity for the amylase (83.3% 20).

The amylase reaction halos in our study varied between 4 and 7 mm the results found by Firmino and Furtado (2014) the fungus *Ceracystis* sp., whereas Tavares et al. (2012) 29 mm halos evaluating *Aspergillus* isolated from noni (*Morinda citrifolia* L.).

A study by Soares et al. (2010) for amylase production, suggest that fungi under stress conditions interfere with enzymatic production. Firmino and Furtado (2014) also evaluated lipase activity with isolates of the fungus *Ceracystis* sp. did not observe any activity for this enzyme. The authors also tested the protease and halos did not exceed 1 mm.

The averages of enzymatic activities of our study differ from the results presented by Griebeler et al. (2015) who found higher values halos above 7.08 mm for amylase, lipase and protease. These

TABLE II
Identification of the species of the fungi isolated in the air of the CAV/UFPE.

Identification of species of filamentous fungi	Identification code of the fungal isolate
Paecilomyces variotti	(04)
Penicillium fellutanum	(06)
Aspergillus flavus	(09)
Aspergillus parasiticus	(14), (20), (27) and (67)
Aspergillus sydowii	(18)
Talaromyces purpurogenus	(21) and (28)
Aspergillus japonicus	*(24)*
Penicillium oxalicum	*(25)*
Penicullium chrysogenum	*(26)*
Curvularia lunata	*(49)*

authors worked with isolated fungi from different sources, such as soil, olive and soybean oil, cheeses, tomato extract, cream of milk, meat, soybean meal and contaminated culture media.

A study evaluated fungi isolated Baru seeds, bark and pulp (*Dipteryx alata* Vog.) and observed that none of the fungi had proteolytic activity, but all of them were able to present some amiolytic and lipolytic activity (Molina et al. 2012). The evaluation performed by Bezerra et al. (2012) with isolates of a Caatinga plant (*Opuntia ficus-Mil*) revealed that a strain of *A. japonicas* Saito did not produce proteolytic activity, corroborating with our study. Sunitha et al. (2013) enzymatic production differs among fungi and is related to the habitat where it is found.

Aboul-Nasr et al. (2013) tested 110 fungal isolates from intensive care unit air and hospital operating room in India and found that among the 110 isolates tested, 73% produced protease, lipase and urease, and this was the only the literature testing the enzymatic and mycotoxin production potential of anemophilous fungi. Among the lipase and protease isolates were *Aspergillus* sp., *Cladosporium* sp., *Myrothecium* sp. and *Fusarium* sp. According to the authors, these results are important since proteases and lipases play a role

TABLE III
Identification of the species and metabolic product of fungi isolated in the air of the CAV/UFPE and halo of enzymatic activity (in mm).

Species and metabolic		Halo and enzymatic activity (in mm)		
Code	product	A	L	P
04	*Paecilomyces variotti*	-	-	-
06	*Penicillium fellutanum*	+ (7 mm)	+ (5 mm)	+ (1 mm)
09	*Aspergillus flavus*	+ (6 mm)	-	-
14	*Aspergillus parasiticus*	+ (5 mm)	+ (10 mm)	+ (2 mm)
18	*Aspergillus sydowii*	+ (6 mm)	+ (14 mm)	+ (5 mm)
20	*Aspergillus parasiticus*	-	-	-
21	*Talaromyces purpurogenus*	+ (4 mm)	+ (6 mm)	-
24	*Aspergillus japonicus*	-	+ (2 mm)	-
25	*Penicillium oxalicum*	+ (7 mm)	-	+ (1 mm)
26	*Penicullium chrysogenum*	+ (6 mm)	-	+ (4 mm)
27	*Aspergillus parasiticus*	-	+ (3 mm)	-
28	*Talaromyces purpurogenus*	+ (7 mm)	-	-
49	*Curvularia lunata*	-	-	-
67	*Aspergillus parasiticus*	+ (5 mm)	+ (4 mm)	+ (2 mm)

(Code) Identification code of fungal isolate, (mm) Millimeter, (A) Amylase, (L) Lipase, (P) Protease, (+) Produced enzymatic activity, (-) It did not produce enzymatic activity.

during microbial infection and suggest these results are important since the extracellular lipases play an important role during microbial infection, since they digest lipids to obtain nutrients by pathogenic microbes, allowing the growth of microbes where lipids are the only source of carbon and proteolytic degradation of lung tissues has been identified as a key phenomenon in the pathophysiology of *Aspergillus fumigatus*, several species of *Aspergillus* secrete protease.

In relation to the tests of antibacterial activity of the fungi isolated from the air of the CAV, three metabolic liquids presented activity against all bacteria tested, represented by the species *Paecilomyces variotii* Bainier (04), *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga & Frisvad (28) and *Aspergillus parasiticus* Speare (67). The metabolic liquid of *Curvularia lunata* (Wakker) Boedijn (49) no antibacterial activity against the bacteria tested (Table IV).

The metabolic liquid of the species *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenburg, Seifert, Peterson, Varga & Frisvad (isolate 28) presented the best antibacterial activity against all the bacteria tested, reaching the of 125 µL/mL, none of the metabolic liquids tested presented bactericidal action, only bacteriostatic.

Duarte (2006) reports that no consensus what is acceptable the level of inhibition for antimicrobial activity related to natural products versus standard antibioticssome authors consider only antibiotic-like results, although others understand that levels inhibition good potential.

The study by Paraginskia et al. (2014) analyzed all bacterial strains evaluated in our study, but used different concentrations of triazenes compounds for antimicrobial activity and obtained bacteriostatic action in at least one of the six evaluated compounds.

CONCLUSIONS

It was concluded that the environments analyzed in the UFPE/CAV presented great diversity of fungi species they met the requirements of the ANVISA resolution. It was also verified that not present fungi potentially harmful to human health in the environments. Our study demonstrated that some species of anemophilous filamentous fungi are capable of producing the amylase, lipase and

TABLE IV
Minimum inhibitory Concentration of the antibacterial activity of the isolated fungi species in the CAV/UFPE air.

Species and Metabolic Product	Metabolic Fungus Product Lower Inhibitory Minimum Concentration (μL / mL)							
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**
Paecilomyces	+	+	+	+	+	+	+	+
variotti (04)	(500)	(500)	(500)	(250)	(500)	(500)	(500)	(500)
Aspergillus	+	+	+	+	-	+	+	-
japonicus (24)	(500)	(500)	(500)	(250)		(500)	(500)	
Talaromyces	+	+	+	+	+	+	+	+
purpurogenus (28)	(250)	(125)	(125)	(125)	(250)	(125)	(125)	(250)
Curvularia lunata	-	-	-	-	-	-	-	-
*(*49)								
Aspergillus	+	+	+	+	+	+	+	+
parasiticus (67)	(500)	(500)	(500)	(250)	(500)	(500)	(250)	(500)

protease enzymes the fungi *Aspergillus parasiticus* Speare and *A. sydowii* (Bainier & Sartory) Thom & Church better amiolytic potential among the other species and enzymes under the conditions of this experiment. The antimicrobial activity of the species *Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenburg, Seifert, Peterson, Varga & Frisvad was the one that presented the best result however, showed low bactericidal potential.

REFERENCES

ABOUL-NASR MB, ABOUL-NASER AZ AND AMER EM. 2013. Enzymatic and toxigenic ability of opportunistic fungi contaminating intensive care units and operation rooms at Assiut University Hospitals, Egypt. SpringerPlus 2(347).

AGARWAL R AND CHAKRABARTI A. 2010. Epidemiology of allergic bronchopulmonary Aspergillosis. In: Pasqualotto AC (Ed), Aspergillosis: from diagnosis to prevention. New York: Springer Science. Biomed Life Sci, p. 671-688.

ARAÚJO R AND CABRAL JP. 2010. Fungal air quality in medical protected environments. In Kumar A (Ed). Air Quality, p. 357-382.

AZIMI F, NADDAFI K, NABIZADEH R, HASSANVAND MS, ALIMOHAMMADI M, AFHAMI S AND MUSAVI SN. 2013. Fungal air quality in hospital rooms: a case study in Tehran, Iran. J Environ Health Sci Eng 11(30): 1-4.

BEZERRA JDP, SANTOS MGS, SVEDESE VM, LIMA DMM, FERNANDES MJS, PAIVA LM AND SOUZA-MOTTA CM. 2012. Richness of endophytic fungi isolated from *Opuntia ficus- indica* Mill. (Cactaceae) and preliminary screening for enzyme production. World J Microb Biot 28(5): 1989-1995.

BOFF C. 2011. Monitoring fungi in the air of intensive care units. 61p. Dissertation (Master's Degree in Pneumological Sciences) - Faculty of Medicine. Federal University of Rio Grande do Sul, Porto Alegre. (Unpublished).

BRAZIL. 2003. Ministry of Health. National Agency of Sanitary Surveillance (ANVISA). Methodology of Sensitivity Tests to Dilution Antimicrobial Agents for Aerobic Growth Bacteria. Approved Standard M7-A6, 6th ed. 23(2): 1-81.

CATÃO RMR, NUNES LE, VIANA APP, ROCHA WRV AND MEDEIROS ACD. 2014. Antibacterial activity and interactive *in vitro* effect of a cranberry product on *Escherichia coli*. Rev Ciênc Basic Farm Apl 35(4): 723-729.

DUARTE MCT. 2006. Antimicrobial activity of medicinal and aromatic plants used in Brazil. Rev Multiciência 7: 1-16.

EJDYS E, DYNOWSKA M, BIEDUNKIEWICZ A AND SUCHARZEWSKA E. 2013. An Overview of the Species of Fungi Occurring in School Rooms – a Four-Year Study. Pol J Environ Stud 22(6): 1691-1700.

FIRMINO AC AND FURTADO EL. 2014. Production of extracellular enzymes by *Ceratocystis* spp. Summa Phytopathol 40(4): 371-374.

FRIBERG B ET AL. 1999. Correlation between surface and air count of particles carrying bacteria in operating rooms with turbulent ventilation. J Hosp Infect 42: 61-68.

FURTADO MSS AND FERRARONI JJ. 1982. Anemophilous fungi in hospital environments of the city of Manaus, Amazonas, Brazil. Sci Cult 34(12): 1642-1647.

GRIEBELER NE, BORTOLI V, ASTOLFI AL, DARONCH NA, SCHUMANN AC, SALAZAR LN, CANSIAN RL, BACKES GT AND ZENI J. 2015. Selection of filamentous fungi producing amylases, proteases, cellulases and pectinases. Rev Acad Ciênc Anim 13: 15-24.

GRUMACH AS. 2001. Allergy and immunology in childhood and adolescence. São Paulo: Atheneu, p. 16-21.

HAYLEEYESUS SF AND MANAYE AM. 2014. Microbiological Quality of Indoor Air in University Libraries. Asian Pac J Trop Biomed 4(1): S312-S317.

IARC - INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. 2002. Some traditional herbal medicine, some mycotoxins, naphthalene and styrene. IARC Monograph Eval. Carcinog. Risks Human 82: 1-556.

KALWASIŃSKA A, BURKOWSKA A AND WILK I. 2012. Microbial air contamination in indoor environment of a university library. Ann Agric Environ Med 19(1): 25-29.

LACAZ CS ET AL. 1998. Guide for identification of fungus actinomycetes and algae of medical interest, 8th ed., São Paulo: Sarvier, 445 p.

MENEZES M AND HANLIN SMP. 2004. Practical guide to phytopathogenic fungi. 2nd ed., Recife, PE. University Press, UFRPE.

MOLINA G, PIMENTEL MR, BERTUCCI TCP AND PASTORE GM. 2012. Application of fungal endophytes in biotechnological processes. Chem Eng Trans 27: 289-294.

MORAIS GR, SILVA MA, CARVALHO MV, SANTOS JGS, DOLINGER EJOV AND BRITO DVD. 2010. Indoor air quality in a Brazilian higher education institution. Biosci J 26: 305-310.

NAGARAJAN S. 2012. New tools for exploring old friends-microbial lípases. Appl Biochem Biotechnol 168(5): 1163-1196.

NASCIMENTO GC. 2011. Evaluation of air quality in indoor environments: public library. 170 p. Dissertation (Master in Hydraulics and Sanitation) - University of São Paulo, School of Engineering of São Carlos, São Carlos. (Unpublished).

OLIVEIRA LDC AND BORGES PALUCHA LR. 2015. Respiratory allergies: a review of the main anemophilous fungi and triggering factors. RBSP 39(2): 426-441.

PANTOJA LDM, COUTO MS AND PAIXÃO GC. 2007. Diversity of bioaerosols present in urbanized and preserved environments of a university campus. Biological, São Paulo 69(1): 41- 47.

PARAGINSKIA GL, BERTICELLIA CR, ZAMBIAZIA PJ, PARAGINSKIA VTK, HÖRNERA M, SANTOS AJRWA AND HÖRNERC R. 2014. *In vitro* antibacterial activity and toxicity to *Artemia saline* Leach. of some triazenes compounds. Chem New 37(7): 1138-1144.

PELUQUE E. 2014. Isolation, molecular identification and toxigenic potential of fungi and occurrence of mycotoxins in mixtures of cereals marketed in Brazil. 80 p. Dissertation (Master in Food Engineering) - Faculty of Zooctenia and Food Engineering, University of São Paulo, São Paulo. (Unpublished).

RAHMAN M, KÜNHN I, RAHMAN M, OLSSON-LILJEQUIST B AND MÖLLBY R. 2004. Evaluation of a scanner-assisted colorimetric MIC method for susceptibility testing of gram- negative fermentative bacteria. Appl Environ Microbiol 70(4): 2398-2403.

REINEHR CO, RIZZARDI J, SILVA MF, OLIVEIRA D, TREICHEL H AND COLLA LM. 2014. Production of lipases of *Aspergillus niger* and *Aspergillus fumigatus* through solid state fermentation, evaluation of substrate specificity and its use in esterification and alcohololysis reactions. Chem New 37(3): 454-460.

RIDDELL RW. 1950. Permanent staired mycological preparation sobtained by slide culture. Mycology 42: 265-270.

SAMSON RA AND FRISVAD JC. 2004. *Penicillium* subgenus *Penicillium*: new Taxonomics Schemes, Mycotoxins and Other Extrolites. Stud Mycol 49: 257.

SANTOS LA, MENEZES JS, RUFINO LRA, OLIVEIRA NMS AND FIORINI JE. 2014. Determination of the antimicrobial activity of the hydroalcoholic extract of the plant *Plectranthus ornatus* codd (Bilberry Chinese). Rev Univ Vale do Rio Verde 12(1): 119-129.

SILVA EB, OHARA A, FERNANDES CM, BARBOSA PPM AND MACEDO GA. 2015. Screening of filamentous fungi from Brazilian rainforests for enzyme production. Afr J Microbiol Res 9(5): 332-342.

SOARES IA ET AL. 2010. Identification of the amylolytic potential of mutant strains of the filamentous fungus *Aspergillus nidulans*. Ciênc Tecnol Food 30(3): 700-705.

STRYJAKOWSKA-SEKULSKA M, PIOTRASZEWSKA-PAJAK A, SZYSZKA A, NOWICKI M AND FILIPIAK M. 2007. Microbiological Quality of Indoor Air in University Rooms. Polish J Environ Stud 16(4): 623-632.

SUNITHA VH, DEVI NIRMALA D AND SRINIVAS C. 2013. Extracelullar enzymatic activity of endophytic fungal strains isolated from medicinal plants. World J Agric Sc 9(1): 1-9.

TAVARES ACD, FONSECA JS, FONSECA TRB, BARRONCAS JF, SOUZA RAT, SILVA TA AND

TEIXEIRA MFS. 2012. Extracellular enzymes of anamorphic fungi isolated from *Morinda Citrifolia* L. Biochem Biotecnol Rep 1(2): 1-6.

VIRIATO A. 2014. Terpenoids with antifungal activity for Candida Berkhout, causing nosocomial infections. World Health 38(1): 40-50.

WENZEL JB ET AL. 2013. Enzymatic and antimicrobial activity of endophytic fungi isolated from soybean. Pesq. Online: Biol and Health 9(3): 1-15.

ZHOUG G, WHONG WZ, ONG T AND CHEN B. 2000. Development of a fungus-specific PCR assay for detecting low-level fungi in a indoor environment. Mol Cell Probes 14: 339-348.