Mucorales from the semiarid of Pernambuco, Brazil

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Submitted: August 8, 2011; Approved: July 2, 2012.

Abstract

Nineteen taxa of Mucorales, belonging to Absidia, Apophysomyces, Cunninghamella,
Fennellomyces, Lichtheimia, Mucor, Mycotypha, Rhizopus and Syncephalastrum were isolated from
36 composite soil samples in three semiarid areas in the State of Pernambuco (Triunfo, Cabrobó and
Belém de São Francisco), Northeast Brazil, which are characterized by Caatinga vegetation. Triunfo
is preserved, whereas Cabroró and Belém de São Francisco are experiencing low and severe deserti-
fication processes, respectively. Mucorales were isolated in Petri dishes in triplicate from 5 mg sam-
ples of soil placed on the surface of wheat germ agar plus chloramphenicol and Cercobin [Dimethyl
4,49-(103 phenylene) bis (3-thioallophanate)] medium. The plates were left on a bench at room tem-
perature (28 ± 2 °C) for 72 h of alternating dark and light periods. Absidia cylindrospora
presented the highest amount of CFU/g of soil, followed by L. hyalospora, C. phaeospora and C. echinulata
var. echinulata. The latter, and R. microsporus var. microsporus, presented the highest frequencies of
occurrence. Soils from Triunfo showed higher diversity of Mucorales than the samples from the other
areas, although without differing statistically in relation to species richness. The communities of
Mucorales from the degraded areas were more similar, while that from the preserved area was quite
different. Most of the identified specimens have been commonly isolated from soil in other Brazilian
regions, which indicates that they are not endemic of the semiarid. Eleven taxa are registered for the
first time in this ecosystem, while F. heterothallicus is reported for the first time in Brazil.

Key words: biodiversity, Caatinga, Mucoromycotina, soil degradation, taxonomy.

Introduction

The Mucorales is the largest order of the traditional Zygomycota (a phylum not accepted in the new classifica-
tion since it is polyphyletic), and today is classified among the Mucoromycotina (Hibbett et al., 2007), a subphylum of
fungi with 325 known species (Kirk et al., 2008), from which 90 are registered in Brazil (Santiago, 2012). These
fungi are characterized by the production, during sexual re-
production, of zygospores, which are thick-walled pig-
mented spores, in general resistant to stressful conditions. More often these fungi reproduce asexually, forming small,
hyaline, sporangiospores which are also responsible for
species dissemination. The Mucorales can be easily iso-
lated from soil, dung, water, stored grains, plants, and even
from other fungi, including zygomycetes (Benny, 2009).
Although living as saprobes in most ecosystems,
Mucorales parasites have also been described. Some spe-
cies have been reported as agents of systemic infections in
humans, especially in immunocompromised patients
(Ribes et al., 2000) and others cause diseases in plants and
seeds (Riccardi and Bashore, 2003) and decay of fruit,
stored grains and other cereals (Hesseltine and Fennel,
1995). Beneficial species are traditionally used in the production of fermented foods in Asia (Nout and Kiers, 2005). Species of Cunninghamamella, Mucor and Rhizopus are able to produce metabolites such as amylase, lipase, inulinase, pectinase, renin and protease (Alves et al., 2002; Santiago and Souza-Motta, 2006) as well as citric, linolenic, arachidonic, oxalic and lactic acids (Yin et al., 1998; Zhou et al., 1999; Magnuson and Lasure, 2004). Studies of the role of Mucorales in the bioremediation of heavy metals (Zafar et al., 2007), and in biologic control (Wekesa et al., 2007), have been also carried out.

Knowledge of the diversity of Mucorales is precarious and fragmented, especially in mega-diverse countries such as Brazil, where rapid environmental changes make biota recognition a more urgent issue. In Brazil, this group of fungi has mainly been studied in the States of Bahia, Maranhão, Pernambuco and São Paulo (Upadhyay, 1969; Lira, 1971; Viriato and Trufem, 1985; Alves et al., 2002; Santiago and Souza-Motta, 2006), and only 13 species were reported in the semiarid regions of Brazil (Santiago, 2012). This does not reflect the real richness of these regions, which are practically unexplored as regards fungi.

Desertification is defined by the United Nations Convention on Combat Desertification (UNCCD) as “land degradation in arid, semiarid and dry sub-humid areas, resulting from various factors, including climatic variations and human activities”. A series of data is required to characterize the desertification process (Sampaio et al., 2003) and in the Northeast of Brazil, although data are still scarce, the Ministry of Environment recognizes four nuclei of desertification, one of which is located in the State of Pernambuco and comprises the Municipalities of Cabrobó, Floresta and Belém de São Francisco. Knowledge of the mycota in these areas is important since these microorganisms have been used as indicators of environmental changes and are key components of the microbiota, contributing for the maintenance of ecosystems (Turco et al., 1994).

Considering the ecological importance and the insufficient data about the Mucorales, the aims of this study were: to study the diversity and distribution of the group in three semiarid areas (preserved, under low and severe desertification processes); to determine the similarity of species composition among the studied areas; to evaluate the communities of Mucorales using quantitative and qualitative population data (frequency of occurrence and relative abundance) and through ecological indexes (richness and diversity), and to discuss the influence of the desertification process on the community of Mucorales in the studied areas.

Materials and Methods

Studied areas

Soil samples were collected in Cabrobó, Triunfo and Belém de São Francisco during dry (November) and wet (March) periods, as follows: [November/2008: Cabrobó (11/24/2008 = 0.0 mm precipitation during all month); Belém de São Francisco (11/25/2008 = 0.0 mm precipitation during all month); and Triunfo (11/26/2008 = 6.8 mm during all month and 4.8 mm precipitation during the seven days that preceded the collection)], [March/2009: Cabrobó (03/19/2009 = 108.5 mm precipitation during all month and 62.8 mm during the seven days that preceded the collection); Belém de São Francisco (03/22/2009 = 35 mm precipitation during all month and 19.6 mm during the seven days before collection); and Triunfo (03/20/2009 = 134.5 mm during all month and 32.9 mm during the seven days before collection)]; [November/2009: Cabrobó (11/11/2009 = 0.0 mm precipitation during all month); Belém de São Francisco (11/12/2009 = 8.2 mm precipitation during all month and 1.4 mm during the seven days before collection); Triunfo (11/13/2009 = 0.0 mm precipitation during all month)] (www.inmet.com.br). Soil samples taken in November/2008 and November/2009 were dry, and the weather was hot, with clear sky, and no rain on the day of collection; soil samples collected in March/2009 from Cabrobó and Triunfo were moist, and the leaves on the trees were green, although it was not raining during the collection day; samples collected in Belém de São Francisco were dry, and the leaves on the trees were dry or absent.
Each area was divided in two sectors of 500 m² and in each sector ten collection points were chosen. The first one was chosen randomly and the other points were located at least 10 m apart from the previous one, maintaining a random design. At each point, using sterilized spatulas, three subsamples of 15 g portions of soil were collected to 10 cm depth, mixed and placed in new, clean, plastic bags, forming one composite sample stored in polystyrene boxes containing ice bags. From the 180 subsamples, 36 composite samples (108 Petri plates) were made by combining two sets of five subsamples each, from each of the two areas of the three sites, for each of the three collections. Thus at each area ten samples were collected per sector, or twenty from each area (total of 180 soil samples, considering the three collections at the three Caatinga sampling areas).

Isolation of the Mucorales

For each one of the 36 samples, three 5 mg subsamples of soil particles were placed on the surface of wheat germ agar (Benny, 2008) plus chloramphenicol (80 mg/L) and Cercobin [Dimethyl 4,49-(O-phenylene) bis (3-thiolallophanate)] (4 mg/L) in Petri dishes, according to Ho et al. (2002), modified. The Petri dishes were left on a bench at room temperature (28 ± 2 °C) for 72 h of alternating light and dark periods and observed in a stereomicroscope (Carl Zeiss Axioscope 40) and a light microscope (Leika EZ4). Fragments of colonies were transferred to the surface of M agar (malt extract 20 g, glucose 20 g, peptone 1 g, agar 15 g, distilled water 1000 mL, pH = 6.0) (O’Donnell, 1979) plus chloramphenicol (80 mg/L). After the isolation and purification of the fungi were identified based on macroscopic (color, aspect and diameter of the colonies) and microscopic (microstructures) characters according to Schipper (1978), Misra and Lata (1979), Schipper and Samson (1994), Hesseltine and Fennel (1995), Zheng and Chen (2001), Zheng et al. (2007) and Alastruey-Izquierdo et al. (2010). Mucorales communities were evaluated qualitatively and quantitatively using data from the population (frequency of occurrence and relative abundance), and its structure analyzed by ecological indices (richness and diversity). Frequency of occurrence (Fi) was calculated according to the following equation: Fi = Ji/i, where Fi = frequency of occurrence of the species i; Ji = number of samples in which the species i has occurred; i = total number of soil samples (Brower et al., 1990).

Relative abundance of each species within the three studied areas was evaluated according to the following equation: Ra = (Ni/N) x 100, where Ra = relative abundance of the species i; Ni = number of CFU of the species i; N = total number of CFU of fungi in all samples, in each area). According to this formula, each taxon can be classified as: Ra < 0.5% = rare; 0.5 ≤ Ra < 1.5% = occasional; 1.5 ≤ Ra < 3.0% = common; Ra > 3.0% = abundant (Schnittler and Stephenson, 2000, modified).

The Shannon-Wiener index was used to estimate the diversity of Mucorales in the areas studied, according to Peet (1974). Chao1 and Jacknife1 richness estimators as available in Primer 5.2.4 (Clarke and Warwick, 1994) were applied to the data in order to evaluate the completeness of the survey. The similarity of the species between the sampling sites was estimated by the Sorensen index, using the equation: S = (2c /a + b) x 100, where c = number of species common to both areas; a = number of species from area 1; b = number of species from area 2 (Sorensen, 1978).

Statistical analysis

Differences in the communities of Mucorales species among different areas were analyzed using Analysis of Similarity (ANOSIM Primer 5.2.4) (Clarke and Warwick, 1994). Differences in species richness among the three areas were evaluated by the ANOVA like non-parametric Kruskal-Wallis test (Zar, 1996).

Results

Altogether, 17 species and six varieties of Mucorales (9.3 x 10^2 CFU/g of soil) were isolated from the soil samples; ten taxa distributed in six genera were isolated from the soil of Belém de São Francisco; 11 taxa where obtained from samples collected in Cabrobó, and eight taxa were isolated from Triunfo’s soil (Table 1).

Absidia cylindrospora var. cylindrospora presented the highest number of CFU/g of soil (3.16 x 10^5), followed by Lichtheimia hyalospora (1.36 x 10^5), C. phaeospora and C. echinulata var. echinulata (1.12 x 10^5). More colonies of Mucorales (6.84 x 10^6) were registered in the soil of the preserved area than in that of the degraded areas (Belém de São Francisco = 1.64 x 10^6 and Cabrobó = 8.2 x 10^5) (Table 1). Only L. hyalospora (Di = 3.46%) can be considered abundant, while C. echinulata var. echinulata and C. phaeospora (2.77%) were quite common. Absidia cylindrospora var. cylindrospora (1.04%), R. arrhizus var. arrhizus (0.89%) and and R. microsporus var. microsporus (0.57%) were occasional. All other taxa were rare. Rhizopus microsporus var. microsporus (10.19%) and C. echinulata var. echinulata (9.26%) were the most frequent in the three areas, followed by A. cylindrospora and R. arrhizus var. arrhizus (7.41%). The other taxa showed a low (< 5%) frequency of occurrence (Table 2).

Chao1 richness estimator indicates an expected richness of 21.7 (± 3.5) and the Jacknife1 method estimates 27.9 taxa for the combined data of the sampling area. The diversity of Mucorales was higher in Triunfo (H1 = 2.85), followed by Cabrobó (H1 = 2.73) and Belém de São Francisco (H1 = 2.13). The differences in species richness among the three areas were not significant [(p) Kruskal-Wallis = 0.854; H = 0.3157; G.L = 2]. Species similarity was higher between samples collected in Belém de São Francisco and Cabrobó (66.7%), which differs from what was
observed between Belém de São Francisco and Triunfo (31.58%) and Cabrobó and Triunfo (20%). The ANOSIM “one-way” showed significant differences on composition of Mucorales between samples from Triunfo and Belém de São Francisco (R_{global} = 0.75; p = 0.029; number of permutations = 35); Triunfo and Cabrobó (R_{global} = 0.651; p = 0.029; number of permutations = 35). However, the differences were not significant when comparing soils from Cabrobó and Belém de São Francisco (R_{global} = 0.266; p = 0.057; number of permutations = 35).

### Discussion

The results of this manuscript increase the knowledge about diversity of Mucorales in soils of Caatinga. To date, there are no references regarding ecological studies of Mucorales from soils of semi-arid regions outside Brazil, and in this country, only 13 taxa of Mucorales have been reported from Caatinga soil (Santiago, 2012). *Absidia cylindrospora*, *C. elegans*, *L. hyalospora* (as *A. blakesleeanum*), *R. arrhizus* var. *arrhizus* (as *R. oryzae*) and *S. racemosum* were cited from the Caatinga soil of Jaguari - Bahia (Santiago and Souza-Motta, 2006); *Rhizopus microsporus* var. *microsporus* and *R. microsporus* var. *chinensis* were reported in Serra Talhada and Canindé de São Francisco - Pernambuco (Cavalcanti et al., 2006; Trufem et al., 2006). Therefore, the other 11 taxa reported here are first records for Caatinga. More recently, a new parasitic zygomycetes (Zoopagales), *Syncephalis aggregata* A.L. Santiago & Benny was isolated from soil in Triunfo-PE (Santiago et al., 2011a), contributing to the knowledge of zygomycetes distribution in this ecosystem. Most of the genera and species identified in this study were isolated from soil in different ecosystems, both within Brazil and abroad. In Brazil, *A. cylindrospora* was isolated from soil in São Paulo, Bahia and Maranhão (Lira, 1971; Schoenlein-Crusius et al., 1996; de Souza et al., 2008); *R. arrhizus* var. *arrhizus* was reported (some as *R. oryzae*) in Canindé de São Francisco (SE), Olho d’água do Casado (AL), Recife and São Paulo (Trufem, 1981a; Cavalcanti et al., 2006; de Souza et al., 2008; Santiago et al., 2011b); *C. elegans* was isolated from soil in Canindé de São Francisco (SE), Olho d’água do Casado, Piranhas (AL) (Cavalcanti et al., 2006) and in São Paulo (Trufem, 1981b), and *C. phaeospora* was cited in Maranhão, São Paulo and Pernambuco (Upadhyay, 1970; de Souza et al., 2008; Santiago et al., 2011b). *Mucor luteus* (as *M. hiemalis* f. *luteus*) and *M. prayagensis* were only reported in São Paulo (Schoenlein-Crusius et al., 2006; de Souza et al., 2008). *Apophysomyces elegans* and *Mycotypha microspora* were reported as new to Brazil (Santiago and Maia, 2010).
Among all taxa, only *L. hyalospora* was abundant, while *C. echinulata var. echinulata* and *C. phaeospora* were common. The majority of Mucorales showed rare distribution. With the exception of *F. heterothallicus*, all of these species have been previously reported in soil samples. Curiously, the four known species of *Fennellomyces* [*F. linderi* (Hesselt. & Fennell) Benny & R.K. Benj., *F. heterothallicus*, *F. verticillatus* J.H. Mirza, S.M. Khan, S. Begum & Shagutta and *F. gigacellaris* J.H. Mirza, S.M. Khan, S. Begum & Shagutta], were isolated only from animal dung, particularly rodents (Benny and Benjamin, 1975). Thus, this represents the first record of the genus in soil. Considering that dung is commonly in contact to soil, it is possible that the fungus had been isolated as a soil contaminant. Specimens of *Cunninghamella*, *Mucor* and *Rhizopus* are common soil inhabitants (Domsch et al., 1993).

*Absidia cylindrospora var. cylindrospora*, *C. echinulata var. echinulata*, *R. arrhizus var. arrhizus* and *R. microsporus var. microsporus* were the most frequently found in all three areas. Generally, in a community, very few taxa have high frequencies of occurrence, while the majority present low frequencies (Richardson, 2001; Nyberg and Persson, 2002). These observations were based on studies with coprophilous fungi, which display different behavior in relation to soil fungi. Generally, herbivorous dung is very rich in nutrients, minerals and red blood cells from the rumen, making it rich and favorable for fungal growth, including the Mucorales (Dix and Webster, 1995). However, the same was observed in this work, considering that only four species presented high frequency of occurrence (> 7%). Statistical analysis showed no differences in species richness between the three areas, which indicates that apparently desertification, climate and altitude are not affecting the number of Mucorales species. However, the community structure of Mucorales was different when comparing Triunfo to the other two areas which did not differ between them. Corroborating these results, the similarity studies highlighted a greater similarity between the Mucorales from soil from Belém de São Francisco and that of Cabrobó. This was expected since the area of Triunfo is quite different from the other two in terms of altitude and climate (Ministério das Minas e Energia, 2005a, 2005b, 2005c). Thus, these differences make it difficult to determine whether the variations in the structure of the Mucorales community between Triunfo and the other areas reflect or not the impact of desertification. Apparently, in Triunfo the general conditions are more suitable for development of some Mucorales species. According to Bills et al. (2004), the heterogeneity of micro-habitats, competition within and between specific types of organic matter present in the substrate, temporal changes in climate and vegetation and the physiology of the fungi are all important factors to consider. It is possible that with more data the statistical analysis would present other values for richness and diversity.

This manuscript reports the occurrence of 17 species and six varieties of Mucorales from semiarid areas of Northeast Brazil. Eleven taxa are registered for the first time in this ecosystem, while *F. heterothallicus* is reported for the first time in Brazil.

Acknowledgments

The authors are grateful to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for providing a post-doc fellowship to A.Santiago and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNpq - Protax Proc. 562.330/2010-0; INCT-Herbário Virtual Proc. 573.883/2008-4; Sisbiota Proc. 563.342/2010-2; PQ Proc.302.416/2010-2) for a research fellowship to L.C.Maia and research grants.

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