Electronic Journal of Biotechnology ISSN: 0717-3458
Electron. J. Biotechnol.
http://www.ejbiotechnology.info
DOI: 10.2225/vol15-issue4-fulltext-6 *RESEARCH ARTICLE*

Culturable fungi associated with urban stone surfaces in Mexico City

Leandro Páramo-Aguilera[1] · Benjamín Otto Ortega-Morales[2] · José Alberto Narváez-Zapata[1] ✉

1 Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Col. Narciso Mendoza, Reynosa, Tamaulipas, México

2 Universidad Autónoma de Campeche, Departamento de Microbiología Ambiental y Biotecnología, Col. Buenavista, Campeche, México

✉ Corresponding author: jnarvaez@ipn.mx
Received October 19, 2011 / Accepted June 1, 2012
Published online: July 15, 2012
© 2012 by Pontificia Universidad Católica de Valparaíso, Chile

Abstract

Background: Urban surface stones in Mexico City are exposed to a temperate climate and a range of atmospheric conditions ranging from mildly impacted to heavily polluted areas. In this study, we focused on the characterization of the cultivable fungal component of selected biological patinas in the surrounding area of Chapultepec castle, a historic monument in Mexico City. Thirty four representative fungal isolates selected based on distinctive differential macroscopic characteristics out of a total of 300 fungi, were characterized using morphological and molecular approaches. **Results:** This identification strategy based on the combination of phenotypic- and molecular-based methodologies allowed us to discriminate the fungal community in some cases down to the species level. **Conclusions:** The characterization of this mycoflora revealed the presence of a complex fungal community mainly represented by filamentous fungi belonging to the genera *Fusarium, Trichoderma, Aspergillus, Cladosporium, Alternaria, Mucor, Penicillium*, *Pestalotiopsis*, and the dimorphic fungus *Aureobasidium*, along with the yeast *Rhodotorula*. A specific distribution of fungi could be observed based on the type of biological patina analyzed.

Keywords: aeroterrestrial habitat, fungi, stone surfaces, urban buildings

INTRODUCTION

The stone ecosystem is subjected to harsh environmental changes, especially temperature and moisture, which exerts extreme selective pressure on any developing microbial community (May, 2003). The microbial colonization of stones depends on environmental factors such as water availability, pH, climatic exposure, nutrient sources, and on petrologic parameters, such as mineral composition porosity and permeability of the rock material (Warscheid and Braams, 2000). In addition, at the monument or building level, different micro-niches also occur considering outdoor or indoor environments and different expositions. In these spatial conditions, different groups of organisms can settle and spread on and into the rock material. Fungi are an important component of microbial communities colonizing different types of building stone in environments ranging from temperate to tropical settings (Gorbushina, 2007; Scheerer et al. 2009). Studies aiming at describing the diversity and ecological significance of fungi in urban environments have mainly centred on their impact on human health (O'Gorman and Fuller, 2008), but have also been reported on stone surfaces, particularly important in historic and cultural buildings, where they often exert biodeterioration effects (Gorbushina et al. 2002; Cappitelli et al. 2007; Gadd, 2007). Microbial community establishment on clean surfaces usually starts with phototrophic organisms (algae, cyanobacteria) which use CO_2 from the atmosphere and sunlight as their carbon and energy source. Heterotrophic organisms (most

bacteria and all fungi) need some organic source for their growth, and this is provided by the metabolites of phototrophic organisms or by air-borne deposition. It has been shown that the very low nutrient requirements of some rock inhabiting heterotrophic microorganisms may be fulfilled by remains of polluted air and rain or animal remains and secretion (Suihko et al. 2007). Bare rock surfaces are harsh environments that are commonly inhabited by diverse fungal communities that exhibit metabolic traits that warranty their survival (Gorbushina and Broughton, 2009). Little is known about the microbial composition of outdoor fungal communities in urban environments in Mexico. In this study, by using a combined approach of morphological and molecular analyses we characterized the heterotrophic composition of the cultivable fungal community of stone surfaces in the surrounding area of Chapultepec castle at Mexico City. This sampling site is allocated in a highly polluted atmospheric region with many airborne organic sources which are the main requirement for survival of these fungal communities (Elliott et al. 2000). The identification of the cultivable fungal community settled on this stones is the first step of an overall diagnostic study in this class of urban environment in Mexico City for both health and cultural heritage issues.

MATERIALS AND METHODS

Site description and sampling strategy

Mexico City is located in an endorheic lake basin surrounded by mountain chains. The city is bounded by the coordinates 19º 03' to 19º 36'N and 98º 57' to 99º 22'W. Its location in an interior valley at 2240 masl, with elevation increasing from north to south, gives it a tropical climate tempered by altitude (Jáuregui, 2000). The northeast of the city tends to be dryer, with 400 to 500 mm annual precipitation, while the centre and south, especially at the base of the mountains, receive 700 to 1200 mm precipitation annually (Jáuregui, 2000). In fact, the climate in this city is heterogeneous depending of the altitude and land use (Estrada et al. 2009). Average monthly concentration of pollutants include 300-650 ppb for $S0_2$, 200 ppbv for O_3 and an average of 40 μg/m^3 for PM 10 and PM 2.5 (Elliott et al. 2000). In May 2009, loose patina material from three sites (Figure 1a-c) were aseptically sampled using a scalpel from the outer stones surrounding of the Chapultepec Castle (sites A and B) and a monument located in the courtyard of the building (site C). Outer surfaces are fully exposed to the atmosphere and are badly degraded presumably by microbial activity as evidenced by extensive epilithic microbial growth. Biological patina A (Figure 1a) seems be more complex due to significant number of different biological colonization patterns. We found light green, white and dark grey patinas. Incrustation B (Figure 1b) exhibits peeling process and incrustation on external stones, for this reason a compact, hard, mineral outer layer adhering to the stone is evidenced. The surface morphology and colours found were usually different from those of the underlying stone and some appears to be calcareous incrustation. Patina C (Figure 1c) localized on a monument with deterioration symptoms presents a black crust as results of accumulation of exogenic material of variable thickness with a common alteration and advanced decay. The chemical and physical characteristics of the rocks analyzed were not recorded in this study, as only scrapped material for microbiological analysis was conducted. However, there is a report that suggested that the rocks of this region, sampled here as crust A and patina B, might have a volcanic origin (Flores-Román et al. 1996).

Fungal isolation strategy

Scraped samples were suspended in 10 mL of sterile V-8 vegetable juice (Campbell Soup Co.) and TGY (Tryptone 5 g, glucose 5 g and yeast extract 5 g) and incubated in shaker at 30ºC for eight days. In addition, other samples were streak-inoculated in PDA plates (Potato Dextrose Agar, Difco, Becton Dickinson & Co., USA) and TGY plates (Tryptone 5 g, glucose 5 g and yeast extract 5 g) added with agar at 15 g/L. Aliquots of 0.1 mL from V-8 and TGY incubated broths were also streak-inoculated onto potato dextrose agar (PDA; Difco, Becton Dickinson & Co., USA) and incubated at 30ºC for 7 days. Appearing colonies were picked and then re-isolated using Petri dishes containing the same solid media. The isolates were maintained in 20% v/v glycerol, prepared by harvesting biomass at the mid exponential growth phase (~5 to 7 days), grown on yeast extract-peptone-dextrose broth (YPD; BD Bioxon, Mexico) and mixing the appropriate broth volume with sterile 86% v/v glycerol, storing them at -70ºC. Thirty four fungal isolates were selected from a collection of ~300 isolates, based on a preliminary morphological identification. Growth rate and colony morphology were considered to select isolates of different genus with the aim to cover the mayor fungal diversity.

DOI: 10.2225/vol15-issue4-fulltext-6

Fungal identification

The collection selected of 28 filamentous fungi, 1 dimorphic fungus and 5 yeasts was characterized using morphological, biochemical and molecular methodologies. All 28 filamentous fungi were characterized with respect to 12 parameters recorded on PDA plates. An Image-Pro Express software package (Media Cybernetics, Silver Spring, MD, USA) was used for phenotypic (qualitative) assessment and morphometric (quantitative) evaluation (Larralde-Corona et al. 2008). The parameters included the size and shape of the spore, the time of initiation of spore production, colony colour, colony margin and the radial growth rate at 29ºC, and grown at pHs 3.6, 5.6, 7.6, and 9.6. The remaining 6 isolates were identified by using the API 20 C AUX system (bioMérieux, Inc., Durham, NC) according to the manufacturer instructions. The experiments were performed in duplicate.

DNA extraction procedure

Fungal isolates were grown in 100 mL of Yeast Peptone Dextrose (YPD; Difco, Becton Dickinson & Co., USA) during three days at room temperature (28ºC ± 2ºC) using a rotary shaker at 250 rpm. Cells were harvested by filtration through a piece of filter paper and were washed with distilled water. Fresh biomass (500 mg) were homogenized in liquid nitrogen and transferred to a 1.5 ml tube containing 1 mL of TEN buffer (100 mM Tris-HCl, 50 mM EDTA, 500 mM NaCl, pH 8.0) and vortexed for 1 min. After centrifugation for 10 min at 10,000 g at room temperature, the pellet was resuspended in 1 mL of TEN buffer and transferred to a clean 1.5 mL tube to continue the silica based-protocol as described in Rojas-Herrera et al. (2008).

PCR amplification

The identification of fungal isolates was supported by the alignment of the D1/D2 region of the 28S rDNA. Primers NL1/NL4 (described by O'Donnell, 1993) were used to amplify the desired fragment of rDNA. Amplification of the D1/D2 region rDNA region were performed in 50 µL of PCR reaction mix containing 5 µL of reaction buffer, 5 units of Taq polymerase (Gibco-BRL, Rockville, MD), 1.5 mM $MgCl_2$, 50 pmol of each primer, 120 µM dNTPs and 50 µg of template cDNA. Amplification was done with an initial denaturation of 5 min at 94ºC, followed by 30 cycles of 1 min at 94ºC, 45 sec at 58ºC and 1 min at 72ºC with a final extension of 5 min at 72ºC. Amplicons were checked by electrophoresis on 1% agarose gel in TBE buffer (89 mM TrisHCl, 89 mM boric acid, 2 mM EDTA, pH 8.0). The PCR products were gel purified with a GeneClean II kit (Bio 101, Vista, CA, USA) and eluted in sterilized distilled water and reamplified using the conditions of initial amplification. PCR products were sequenced (ABI 377 DNA sequencer, Applied Biosystems, USA) by using the BigDye terminator cycle sequencing ready reaction kit according to the instructions of the manufacturer (Applied Biosystems, USA).

Fungal rDNA sequence analysis

The nucleotide sequences of fungal rDNAs were aligned and compared with those in the GenBank database. In addition, nucleotide sequences obtained in this study were deposited in the GenBank database and summarized in Table 1. The dendrograms were constructed by used the MEGA 5.1 program (Tamura et al. 2011) using the neighbour-joining methods. The reliability of the clusters was evaluated by bootstrapping with 1000 replicates. All positions containing gaps and missing data were eliminated. High classification levels (Phylum and Order) were revised of according to Hibbett et al. (2007). Classification to family levels follows Entrez Taxonomy database (NCBI). On the clades where fungi were identified to species level some type strains were incorporated from the StrainInfo database (http://www.straininfo.net). It is worth noticing that not all isolates had a type strain in this database.

Statistical analysis

Relatedness of microbial communities was determined using similarity coefficients for genera and families that were common for both sites. Our working definition was that some genera or families are common if they occur on the both patinas. Particular species were not included in this analysis due to the fact that some isolates were not fully identified. Family classification level for *Rhodotorula* and *Fusarium* isolates was considered as no rank groups named mitosporic Sporidiobolales and mitosporic Hypocreales, respectively, according to Entrez Taxonomy database. The total number of different

genera or families was determined for the samples being compared, then each sample was scored based on the presence or absence of each genera or families in the different communities being analyzed. Sørensen coefficient (Cs) of similarity was used to make pairwise calculations of genera or families shared between samples as described Soni et al. (2010). Genera considered for this analysis included *Trichoderma*, *Alternaria*, *Mucor*, *Fusarium, Cladosporium*, *Aspergillus*, *Leptosphaerulina*, *Penicillium, Phoma*, *Pestalotiopsis*, *Aureobasidium*, and *Rhodotorula*. Families considered were Hypocreaceae, Pleosporaceae, Mucoraceae, Davidiellaceae, Trichocomaceae, Didymellaceae, Amphisphaeriaceae, Dothioraceae, and the *Rhodotorula* and *Fusarium* isolates of the orders Sporidiobolales and Hypocreales.

RESULTS AND DISCUSSION

Phenotypic and molecular identification

A total of 28 filamentous fungi were preliminary identified down to the genus level using different parameters recorded on PDA plates (Table 2). This characterization allowed us to identify ten genera of filamentous fungi including *Fusarium*, *Trichoderma*, *Aspergillus*, *Epicoccum*, *Mucor*, *Penicillium*, *Phoma*, *Cladosporium*, *Alternaria* and *Pestalotiopsis.* The most variable phenotypic traits were conidial colour, growth at 72 hrs, and time of the first conidia observation. Conidial colour varied from dark green (isolates II2, II8, III5, T21, III10 and III6), yellow (isolates I2 and III9) and white (isolates III16, II4, T11, T23, III1, I10, T12, II1, I13, I7, III2 and III3). Other fungal isolates were gray (I9 and I1) and black (T22). Most of the isolates were able to thrive in a wide pH range (pH 3.6 - 9.6). A pH value of 7.6 was the most suitable to support the mycelia growth. On the other hand, preliminary dimorphic fungus and yeast identification based on biochemical tests (data no shown), allowed us to determine 1 dimorphic fungus (*Aureobasidum*) and 5 yeasts belonging to the genus *Rhodotorula*. Recent developments in molecular classification based on rDNA regions have allow to clarify the biological interaction in complex communities (Begerow et al. 2010). In this study most fungal species were identified based on morphological and rDNA sequencing (Table 1 and Table 2). BLAST analyses of the D1/D2-28S rDNA region (a single product of 700-900 bp) allowed us to identify twelve genera (Table 1). All identities were compared with the phenotypic data (Table 2). In general, the isolates used in this study showed high homology (95%-100%) to reported sequences in public databases. The most abundant group was the Phylum Ascomycota with 25 isolated. This Phylum is represented by 6 orders; Hypocreales (*Trichoderma, Fusarium*), Pleosporales (*Alternaria*, *Phoma* and *Leptosphaerulina*), Capnodiales (*Cladosporium*), Eurotiales (*Penicillium* and *Aspergillus*), Xylariales (*Pestalotiopsis*) and Dothideales (*Aureobasidum*) (Figure 2). The isolate T21 presents a high identity (100%) with *Trichoderma harzianum* while isolates II2 and II8 exhibited a high identity (99 to 100%) with *T. viride* and *T. atroviride* respectively, but differed from each other by 3 nucleotides. The classification of *Trichoderma* genus based on morphological characterization might allow relatively easy identification of the genus, but the species concept is difficult to interpret and delimit, especially for isolates with biotechnological application (Larralde-Corona et al. 2008). According to the above, in this study, a combined molecular- and phenotypic-based classification was mandatory. *Trichoderma* isolates could be successfully classified down to the species level as *Trichoderma viride* (GenBank accession no. EF417482), *Trichoderma atroviride* (GenBank accession no. EF591763) and *Trichoderma harzianum* (GenBank accession no. AF399236).

Some genera such as *Alternaria, Cladosporium*, *Aspergillus* and *Fusarium* were well represented in most of samples. The isolates I1 and III10 showed a high identity (98%) with *Alternaria* genus, even though only the isolate III10 can be classified as *A. tenuissima* with 98% of identity (Table 1, Figure 2). The isolates III2, III3 and III5 exhibited high identity with *Cladosporium* spp. (99%), but differed among them by 10, 6 and 12 nucleotides, respectively. Isolates such as III11 and T22 exhibited high identity with *Aspergillus fumigatus* and with *A. niger* with 98 and 100%, respectively, but differed among them by 56 nucleotides. In this study, we suggest that these isolates can be considered as *A. fumigatus* and as *A. niger* according to phylogenetic analysis. This last result was supported by the incorporation in this analysis of the reference type strains of *A. fumigatus* (ATCC 16903) and of *A. niger* (NRRL 348), respectively.

One dimorphic fungus (isolate 11) was also classified within of this Phylum. This isolate presents a high identity (~100%) with *Aureobasidium pullulans* isolates previously reported. Only the isolates III9 and I11 produced unclear results when morphological and molecular methods were used for their identification, and were only reported as *Leptosphaerulina* sp. and *Phoma* sp., respectively. It is

important to mention that both isolates are phylogenetically related and belong to the same family Didymellaceae (Figure 2).

The isolates II4, T11, I13, III1, I10, T12, II1, I2, I7 and I9 exhibited high identity with *Fusarium* spp. with values ranged among 98 to 99% (Table 1, Figure 2), but differed among them in different nucleotide positions (data no shown). Only isolate T23 was identified as *Fusarium solani* with 99% of homology (Table 1, Figure 2). This identification was supported by the incorporation in the phylogenetic analysis of a type strain of the *F. solani* complex, the strain CBS 115.40 characterized as *F. lichenicola* (Summerbell and Schroers, 2002).

Most fungi related with *Fusarium* sp. were grouped in an only operative taxonomic unit (OTU) although it presents differences in its D1/D2-28S rDNA nucleotide-base composition and in its phenotypical traits (Table 2). All fungi belonging to this branch were only classified as *Fusarium* sp. (Figure 2). *Fusarium* species have traditionally been identified by morphology although in recent years, their identification has been supplanted by sequencing of the translation elongation factor 1-alpha (TEF1) gene (Geiser et al. 2004) or by sequencing of the cytochrome oxidase 1 gene (Gilmore et al. 2009), although a universal DNA barcoding system for the identifying of this species remains to be accepted (Begerow et al. 2010). Our results confirm the presence of different *Fusarium* isolates both by morphological and by ribosomal sequencing descriptors, however more effort must be done to clarify the reliable identity of this abundant genus in these biological patinas.

Concerning the Subphylum Mucoromycotina, three isolates were obtained. The isolates III12, II6 and III13, which exhibited high identity with *Mucor hiemalis* f. *hiemalis* with 97 to 100% (Table 1, Figure 3), but differed among them by 3, 4 and 26 nucleotides, respectively. Phylogenetic analysis was conducted with other closest related species as *Mucor mucedo*, *Mucor fuscus* and *Mucor racemosus* to better discriminate the specie (*Mucor hiemalis* f. *hiemalis*) in the analysis. In addition, two type strains of *M. hiemalis* f. *hiemalis* codes CBS 201.65 and NRRL3624 were incorporated, to support the identification of these isolates. Our results suggest that these isolates (III12, II6 and III13) are *Mucor hiemalis* f. *hiemalis.*

Finally, yeast isolates were grouped within of the Phylum Basidiomycota (order Sporidiobolales). Yeast isolates 15, 16 and 17 exhibited high identity with *Rhodotorula dairinensis* with 99 and 100%, respectively, but differed among them in around 15 nucleotides; while isolates 18 and 19 showed high identity with *Rhodotorula mucilaginosa* with 99 and 95%, respectively, but differed among them in 73 nucleotides. Identity of the isolates 15 to 17, and 18 was supported by the type strains *R. dairinensis* (JCM 3774) and *R. mucilaginosa* (CBS 316), respectively. Clade distances were low (~0.02) but enough to discriminated *R. dairinensis* or *R. mucilaginosa* of other closest related species as *R. paludigenum* and *R. glutinis.*

Finally, the isolate 19 showed high identity (99%) with *Rhodotorula* sp. (Figure 4). We suggest that isolates 15 to 17 should be considered as *R. dairinensis* since it constitutes a different clade in the Basidiomycota tree even when all isolates are closely related. However, a polyphasic taxonomy, as proposed Gadanho and Sampaio (2002) including other fingerprinter analysis might be considered in further studies to confirm this identity.

Assessment of the culturable fungal community

Studies aiming at describing the diversity and ecological significance of fungi in urban environments have mainly centred on their impact on human health (O'Gorman and Fuller, 2008). However, the settled of this community on urban stone surfaces also might give useful insight in the development of procedures aimed in the preservation of historic buildings. In this sense, fungal community on stone surfaces consists mainly of filamentous and microcolonial fungi (Sterflinger, 2000; Urzi et al. 2000; Gorbushina et al. 2002; Gorbushina et al. 2003). In this study, 34 different filamentous fungi, 1 dimorphic fungus and some yeast were characterized. In general, when are considered the different genera associated to different patinas, all the fungal communities were different with a Sørensen coefficient of similarity (Cs) lower to 0.7 (Table 3). This suggests that each these patina harbours a different fungal community. Only the Patina B and C shared some common genera reached 0.66 of Cs. As expected, when this analysis of similarity is done again considering only the classification to level of family more relations occurs between different patinas reached a Cs of 0.75 between A and C

samples. Minor differences among fungal communities to level of families is a consequence of that some representative genera as *Aspergillus* and *Penicillium* belongs to same family.

Particularly, patina A harboured most *Fusarium* sp. isolates (seven), all the recovered yeasts and some other genera such as *Phoma* and *Alternaria*. The total number of isolates found in this patina was the most numerous with 44% of the total fungal collection. However, when it was taken into account only the filamentous fungi, the patina B was diverse. Some genera as *Trichoderma* were only found in this type of patina and the total number of isolates corresponded to 24% of the total. Finally, the patina C although only presenting 32% of the of isolates was that exhibits the most number of species with several genera as *Aspergillus, Fusarium, Mucor* and *Alternaria*, *Penicillium, Pestalotiopsis, Cladosporium* and *Leptosphaerulina*. The occurrence of these fungi can explain, at least in part, the observed decay features. *Cladosporium* and *Penicillium* isolates were the most common genera detected in soapstone and quartzite walls of churches in the Brazilian state of Minas Gerais (Resende et al. 1996). Gorbushina et al. (2002) detected mainly deuteromycetes, such as *Alternaria*, *Cladosporium* and *Trichoderma*, on historic marble monuments in St. Petersburg and Moscow. In our study, different isolates of *Cladosporium* and *Penicillium*, as well as few isolates of *Aspergillus*, *Leptosphaerulina*, *Fusarium* and *Pestalotiopsis* occurred on the black patina, a location with biodeterioration symptoms (Figure 1c). Sterflinger (2000) suggested that *Aspergillus niger*, *Penicillium simplissimum* and *Scopulariopsis brevicaulis* were important deteriogenic fungi attacking siliceous stone. Various metabolic substances excreted by fungi are coloured, leading to significant aesthetic alterations, and physical stress (Urzi and Krumbein, 1994; Sterflinger, 2000; Burford et al. 2003).

The presence of a significantly rich fungal community on these urban stone surfaces might be explained as a consequence of the abundance of microbial biomass, organic metabolizable substrates, bird droppings and the occurrence of some pollutants such as hydrocarbons in these urban habitats (Suihko et al. 2007; Newbound et al. 2010). In this sense, it is important to mention that although climatic conditions in Mexico City are milder, this climate changes extremely due to fluctuating and permanent exposure to high pollution levels, surrounding vegetation and water availability event at local levels within Mexico City basin (Elliott et al. 2000; Raga et al. 2001; Estrada et al. 2009). In this respect, the heterotrophic fungi together with the heterotrophic bacteria could be expected as the primary colonizers of rocks substrata instead of phototrophs, especially when airborne organic sources are abundant (Viles and Gorbushina, 2003; Suihko et al. 2007) or when the main requirement for survival is high tolerance to environmental stresses (Staley et al. 1982). Diverse authors have suggested that these fungi are fast growing and therefore become more abundant in more northerly parts of Europe and in climatic areas considerably less polluted (Sterflinger, 2002; Scheerer et al. 2009). The hyphomycetes have also be found in (sub) tropical climates (Resende et al. 1996). These filamentous hyphomycetes remain metabolically active even in low nutrient conditions and have high resistance to desiccation, UV radiation, and osmotic stress (Urzi et al. 2000), thus being well adapted to growth on external stones as the sites sampled in this study. Concerning with the yeast isolates in this study, it is important mention that some *Rhodotorula* yeasts have been isolated from high-altitude lakes exposed to high UVB, thus suggesting physiological adaptation among these organisms (Libkind et al. 2009). This adaptation is related to the occurrence of several compounds as mycosporines, ubiquinone Q_{10} and carotenoid pigments have been proposed as involved with the UV resistance (Yurkov et al. 2008; Libkind et al. 2009). More efforts should be considered in a future to quantify these compounds in these fungal communities in order to increase the knowledge on UV resistance in this class of environments. In addition, the fungi found in the course of this study may play also other functional roles in this environment beyond deterioration. Fungal biomineralization was probably occurring in patina B suggesting a protective role for the underlying substratum. Most of the fungi of this study were isolated of this biological patina (Figure 1b). *Trichoderma* sp*., Mucor hiemalis* f. *hiemalis, Fusarium* sp*., Aspergillus niger,* and *Phoma* sp. were the isolates detected in this sampling site (Table 1). Biodeterioration and biomineralization capacities have been highlighted among fungi in rock-inhabiting microbial communities (Burford et al. 2003; Fomina et al. 2005; Burford et al. 2006). Many of the genera reported in this study as *Alternaria*, *Cladosporium*, *Penicillum Aspergillus*, *Fusarium*, *Epicoccum* and *Trichoderma* and have been previously reported in soils, air and surfaces of urban areas (Rosas et al. 1993; O'Gorman and Fuller, 2008; Mafernina et al. 2011; Shirakawa et al. 2011). It is important to note that certain airborne fungi, in particular *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium* are also considered as allergenic for humans (Kurup et al. 2000). *Penicillium* conidia have also been shown to be present in low concentrations in the atmosphere of Mexico City (Rosas et al. 1993), and were not considered as important outdoor airborne allergens in this city. Fungal counts may vary significantly in different seasonal and spatial (air or soils surfaces) sampled (O'Gorman and Fuller, 2008; Mafernina et al. 2011). Therefore, further research including the spore count quantification of these communities should be considered previously to discuss their potential impact on human

health. The possible roles of this microbial community as reservoirs of human health-threatening agents, biodeterioration and mineral formation agents remain to be further investigated using a more sophisticated cultural approach along with culture independent methods in field and experimental trials with appropriate surface analysis.

In conclusion, a specific distribution of fungi across the different sampled patinas was observed. High levels of richness were evidenced at this small scale level analysis, indicating the high heterogeneity of occurring mycoflora. The combination of phenotypic- and molecular-based methodologies allowed a better identification of heterotrophic fungal community extant at this study site. The fungal communities detected comprise 12 genera that have been previously related to other important fungi traits.

ACKNOWLEDGMENTS

We thank E. Quintana and R. Trujillo Montez for their support in the fieldwork, and to E. de La Cruz for their laboratory support.

Financial support: The authors gratefully acknowledge the financial support by the Technological and Science Institute of DF (ICyTDF) (Grant SIP/DF/2007/056) and by the Instituto Politécnico Nacional (Grant GPI-20100700).

REFERENCES

BEGEROW, D.; NILSSON, H.; UNTERSEHER, M. and MAIER, W. (2010). Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology*, vol. 87, no. 1, p. 99-108. **[CrossRef]**

BURFORD, E.P.; FOMINA, M. and GADD, G.M. (2003). Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineralogical Magazine*, vol. 67, no. 6, p. 1127-1155. **[CrossRef]**

BURFORD, E.P.; HILLIER, S. and GADD, G.M. (2006). Biomineralization of fungal hyphae with calcite ($CaCO_3$) and calcium oxalate mono- and dihydrate in Carboniferous limestone microcosms. *Geomicrobiology Journal*, vol. 23, no. 8, p. 599-611. **[CrossRef]**

CAPPITELLI, F.; NOSANCHUK, J.D.; CASADEVALL, A.; TONIOLO, L.; BRUSETTI, L.; FLORIO, S.; PRINCIPI, P.; BORIN, S. and SORLINI, C. (2007). Synthetic consolidants attacked by melanin-producing fungi: Case study of the biodeterioration of Milan (Italy) cathedral marble treated with acrylics. *Applied Environmental Microbiology*, vol. 73, no. 1, p. 271-277. **[CrossRef]**

ELLIOTT, S.; SIMPSON, I.J.; BLAKE, D.R.; BOSSERT, J.E.; CHOW, J.; COLINA, J.A.; DUBEY, M.K.; DUCE, R.A.; EDGERTON, S.; GAFFNEY, J.; GUPTA, M.; GUZMAN, F.; MATSON, P.A.; MCNAIR, L.A.; ORTIZ, E.; RILEY, W.; ROWLAND, F.S.; RUIZ, M.E.; RUSSELL, A.G.; SMITH, F.A.; SOSA, G.; STREIT, G. and WATSON, J. (2000). Mexico City and the biogeochemistry of global urbanization. *Environmental Science & Policy*, vol. 3, no. 2-3, p. 145-156. **[CrossRef]**

ESTRADA, F.; MARTÍNEZ-ARROYO, A.; FERNÁNDEZ-EGUIARTE, A.; LUYANDO, E. and GAY, C. (2009). Defining climate zones in Mexico City using multivariate analysis. *Atmósfera*, vol. 22, no. 2, p. 175-193.

FLORES-ROMÁN, D.; ALCALÁ-MARTÍNEZ, J.R.; GONZÁLEZ-VELAZQUEZ, A. and GAMA-CASTRO, J.E. (1996). Duripans in subtropical and temperate sub humid climate of the trans-Mexican volcanic belt. *Revista Mexicana de Ciencias Geológicas*, vol. 13, no. 2, p. 228-239.

FOMINA, M.; HILLIER, S.; CHARNOCK, J.M.; MELVILLE, K.; ALEXANDER, I.J. and GADD, G.M. (2005). Role of oxalic acid overexcretion in transformations of toxic metal minerals by *Beauveria caledonica*. *Applied Environmental Microbiology*, vol. 71, no. 1, p. 371-381. **[CrossRef]**

GADANHO, M. and SAMPAIO, J.P. (2002). Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodotorula*: *Rh. glutinis* sensu strict and *Rh. dairenensis* comb. nov. *FEMS Yeast Research*, vol. 2, no. 1, p. 47-58. **[CrossRef]**

GADD, G.M. (2007). Geomycology: Biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research*, vol. 111, no. 1, p. 3-49. **[CrossRef]**

GEISER, D.M.; JIMENEZ-GASCO, M.M.; KANG, S.; MAKALOWSKA, I.; VEERARAGHAVAN, N.; WARD, T.J.; ZHANG, N.; KULDAU, G.A. and O'DONNELL, K. (2004). *Fusarium*-ID v.1.0: A DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology*, vol. 110, no. 5-6, p. 473-479. **[CrossRef]**

GILMORE, S.R.; GRÄFENHAN, T.; LOUIS-SEIZE, G. and SEIFERT, K.A. (2009). Multiple copies of cytochrome oxidase 1 in species of the fungal genus *Fusarium*. *Molecular Ecology Resources*, vol. 9, no. s1, p. 90-98. **[CrossRef]**

GORBUSHINA, A.A.; LYALIKOVA, N.N.; VLASOV, D.Y. and KHIZHNYAK, T.V. (2002). Microbial communities on the monuments of Moscow and St. Petersburg: Biodiversity and trophic relations. *Microbiology*, vol. 71, no. 3, p. 350-356. **[CrossRef]**

GORBUSHINA, A.A.; WHITEHEAD, K.; DORNIEDEN, T.; NIESSE, A.; SCHULTE, E.A. and HEDGES, J.L. (2003). Black fungal colonies as units for survival: Hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. *Canadian Journal of Botany*, vol. 81, no. 2, p. 131-138. [CrossRef]

GORBUSHINA, A. (2007). Life in the rock. *Environmental Microbiology*, vol. 9, no. 7, p. 1613-1631. [CrossRef]

GORBUSHINA, A.A. and BROUGHTON, W.J. (2009). Microbiology of the atmosphere-rock interface: How biological interactions and physical stresses modulate a sophisticated microbial ecosystem. *Annual Review of Microbiology*, vol. 63, p. 431-450. [CrossRef]

HIBBETT, D.S.; BINDER, M.; BISCHOFF, J.F.; BLACKWELL, M.; CANNON, P.F.; ERIKSSON, O.E.; HUHNDORF, S.; JAMES, T.; KIRK, P.M.; LÜCKING, R.; LUMBSCH, H.T.; LUTZONI, F.; MATHENY, P.B.; MCLAUGHLIN, D.J.; POWELL, M.J.; REDHEAD, S.; SCHOCH, C.L.; SPATAFORA, J.W.; STALPERS, J.A.; VILGALYS, R.; AIME, M.C.; APTROOT, A.; BAUER, R.; BEGEROW, D.; BENNY, G.L.; CASTLEBURY, L.A.; CROUS, P.W.; DAI, Y.-C.; GAMS, W.; GEISER, D.M.; GRIFFITH, G.W.; GUEIDAN, C.; HAWKSWORTH, D.L.; HESTMARK, G.; HOSAKA, K.; HUMBER, R.A.; HYDE, K.D.; IRONSIDE, J.E.; KÕLJALG, U.; KURTZMAN, C.P.; LARSSON, K.H.; LICHTWARDT, R.; LONGCORE, J.; MIĄDLIKOWSKA, J.; MILLER, A.; MONCALVO, J.M.; MOZLEY-STANDRIDGE, S.; OBERWINKLER, F.; PARMASTO, E.; REEB, V.; ROGERS, J.D.; ROUX, C.; RYVARDEN, L.; SAMPAIO, J.P.; SCHÜßLER, A.; SUGIYAMA, J.; THORN, R.G.; TIBELL, L.; UNTEREINER, W.A.; WALKER, C.; WANG, Z.; WEIR, A.; WEISS, M.; WHITE, M.M.; WINKA, K.; YAO, Y.-J. and ZHANG, N. (2007). A higher-level phylogenetic classification of the *Fungi*. *Mycological Research*, vol. 111, no. 5, p. 509-547. [CrossRef]

JÁUREGUI, E. (2000). *El clima de la ciudad de México*. Instituto de Geografía, UNAM- Plaza y Valdés. México, 131 p. ISBN 968-36-8090-9.

KURUP, V.P.; SHEN, H.D. and BANERJEE, B. (2000). Respiratory fungal allergy. *Microbes and Infection*, vol. 2, no. 9, p. 1101-1110. [CrossRef]

LARRALDE-CORONA, C.P.; SANTIAGO-MENA, R.; SIFUENTES-RINCÓN, A.M.; RODRÍGUEZ-LUNA, I.C.; RODRÍGUEZ-PÉREZ, M.A.; SHIRAI, K. and NARVÁEZ-ZAPATA, J.A. (2008). Biocontrol potential and polyphasic characterization of novel native *Trichoderma* isolates against *Macrophomina phaseolina* isolated from sorghum and common bean. *Applied Microbiology and Biotechnology*, vol. 80, no. 1, p. 167-177. [CrossRef]

LIBKIND, D.; MOLINÉ, M.; SAMPAIO, J.P. and VAN BROOCK, M. (2009). Yeasts from high-altitude lakes: Influence of UV radiation. *FEMS Microbiology Ecology,* vol. 69*,* no. 3, p. 353-362. [CrossRef]

MARFENINA, O.E.; MAKAROVA, N.V. and IVANOVA A.E. (2011). Opportunistic fungi in soils and surface air of a megalopolis (for the Tushino Region, Moscow). *Microbiology*, vol. 80, no. 6, p. 870-876. [CrossRef]

MAY, E. (2003). Microbes on building stone - for good or ill? *Culture*, vol. 24, no. 2, p. 5-8.

NEWBOUND, M.; MCCARTHY, M.A. and LEBEL, T. (2010). Fungi and the urban environment: A review. *Landscape and Urban Planning*, vol. 96, no. 3, p. 138-145. [CrossRef]

O'DONNELL, K. (1993). Fusarium and its near relatives. In: REYNOLDS, D.R. and TAYLOR, J.W. eds. *The fungal holomorph: Mitotic, meiotic and pleomorphic speciation in fungal systematic*. Wallingford, UK. CAB International. p. 225-233.

O'GORMAN, C.M. and FULLER, H.T. (2008). Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. *Atmospheric Environment*, vol. 42, no. 18, p. 4355-4368. [CrossRef]

RAGA, G.B.; BAUMGARDNER, D.; CASTRO T.; MARTÍNEZ-ARROYO, A. and NAVARRO-GONZÁLEZ, R. (2001). Mexico City air quality: A qualitative review of gas and aerosol measurements (1960-2000). *Atmospheric Environment*, vol. 35, no. 23, p. 4041-4058. [CrossRef]

RESENDE, M.A.; REZENDE, G.C.; VIANA, E.V.; BECKER, T.W. and WARSCHEID, T. (1996). Acid production by fungi isolated from historic monuments in the Brazilian state of Minas Gerais. In: GAYLARDE, C.C.; DE SÁ, E.L.S. and GAYLARDE, P.M. eds. *Biodegradation & Biodeterioration in Latin America,* Porto Alegre: *Mircen/UNEP/UNESCO/ICRO-FEPAGRO/UFRGS*. p. 65-67.

ROJAS-HERRERA, R.; NARVÁEZ-ZAPATA, J.A.; ZAMUDIO-MAYA, M. and MENA-MARTÍNEZ, M.E. (2008). A simple silica-based method for metagenomic DNA extraction from soil and sediments. *Molecular Biotechnology*, vol. 40, no. 1, p. 13-17. [CrossRef]

ROSAS, I.; CALDERÓN, C.; ULLOA, M. and LACEY, J. (1993). Abundance of airborne *Penicillium* CFU in relation to urbanization in Mexico City. *Applied and Environmental Microbiology*, vol. 59, no. 8, p. 2648-2652.

SCHEERER, S.; ORTEGA-MORALES, O.B. and GAYLARDE, C. (2009). Chapter 5 microbial deterioration of stone monuments-an updated overview. *Advances in Applied Microbiology*, vol. 66, p. 97-139. [CrossRef]

SHIRAKAWA, M.A.; LOH, K.; JOHN, V.M.; SILVA, M.E.S. and GAYLARDE, C.C. (2011). Biodeterioration of painted mortar surfaces in tropical urban and coastal situations: Comparison of four paint formulations. *International Biodeterioration & Biodegradation,* vol. 65, no. 5, p. 669-674. [CrossRef]

SONI, R.; SALUJA, B. and GOEL, R. (2010). Bacterial community analysis using temporal temperature gradient gel electrophoresis (TTGE) of 16S rDNA PCR products of soil metagenome. *Ekologija*, vol. 56, no. 3-4, p. 94-98. [CrossRef]

STALEY, J.T.; PALMER, F. and ADAMS, J.B. (1982). Microcolonial fungi: Common inhabitants on desert rocks? *Science*, vol. 215, no. 4536, p. 1093-1095. [CrossRef]

STERFLINGER, K. (2000). Fungi as geologic agents. *Geomicrobiology Journal*, vol. 17, no. 2, p. 97-124. [CrossRef]

STERFLINGER, K. (2002). Fungi on stone monuments: State of the art. *Coalition*, no. 4, p. 3-5.

SUIHKO, L.M.; ALAKOMI, L.H.; GORBUSHINA, A.A.; FORTUNE, I.; MARQUARDT, J. and SAARELA, M. (2007). Characterization of aerobic bacterial and fungal microbiota on surfaces of historic Scottish monuments. *Systematic and Applied Microbiology*, vol. 30, no. 6, p. 494-508. [CrossRef]

SUMMERBELL, R.C. and SCHROERS, H.J. (2002). Analysis of phylogenetic relationship of *Cylindrocarpon lichenicola* and *Acremonium falciforme* to the *Fusarium solani* species complex and a review of similarities in

the spectrum of opportunistic infections caused by these fungi. *Journal of Clinical Microbiology*, vol. 40, no. 8, p. 2866-2875. **[CrossRef]**

TAMURA, K.; PETERSON, D.; PETERSON, N.; STECHER, G.; NEI, M. and KUMAR, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, vol. 28, no. 10, p. 2731-2739. **[CrossRef]**

URZI, C. and KRUMBEIN, W.E. (1994). Microbiological impacts on cultural heritage. In: KRUMBEIN, W.E. ed. *Durability and change. The science, responsibility, and cost of sustaining cultural heritage.* NJ. Wiley, John & Sons, Incorporated. p. 343-368.

URZI, C.; DE LEO, F.; DE HOW, S. and STERFLINGER, K. (2000). Recent advances in the molecular biology and ecophysiology of meristematic stone-inhabiting fungi. In: CIFERRI, O.; TIANO, P. and MASTROMEI G. eds. *Of microbes and art. The role of microbial communities in the degradation and protection of cultural heritage.* NY. Kluwer Academic/Plenum Publisher. p. 3-21.

VILES, H.A. and GORBUSHINA, A.A. (2003). Soiling and microbial colonisation on urban roadside limestone: A three year study in Oxford, England. *Building and Environment*, vol. 38, no. 9-10, p. 1217-1224. **[CrossRef]**

WARSCHEID, T. and BRAAMS, J. (2000). Biodeterioration of stone: A review. *International Biodeterioration & Biodegradation*, vol. 46, no. 4, p. 343-368. **[CrossRef]**

YURKOV, A.M.; VUSTIN, M.M.; TYAGLOV, B.V.; MAKSIMOVA, I.A. and SINEOKIY, S.P. (2008). Pigmented basidiomycetous yeasts are a promising source of carotenoids and ubiquinone Q10. *Microbiology*, vol. 77, no. 1, p. 1-6. **[CrossRef]**

How to reference this article:

PÁRAMO-AGUILERA, L.; ORTEGA-MORALES, B.O. and NARVÁEZ-ZAPATA, J.A. (2012). Culturable fungi associated with urban stone surfaces in Mexico City. *Electronic Journal of Biotechnology*, vol. 15, no. 4. http://dx.doi.org/10.2225/vol15-issue4-fulltext-6

Note: Electronic Journal of Biotechnology is not responsible if on-line references cited on manuscripts are not available any more after the date of publication. Supported by UNESCO / MIRCEN network.

FIGURES

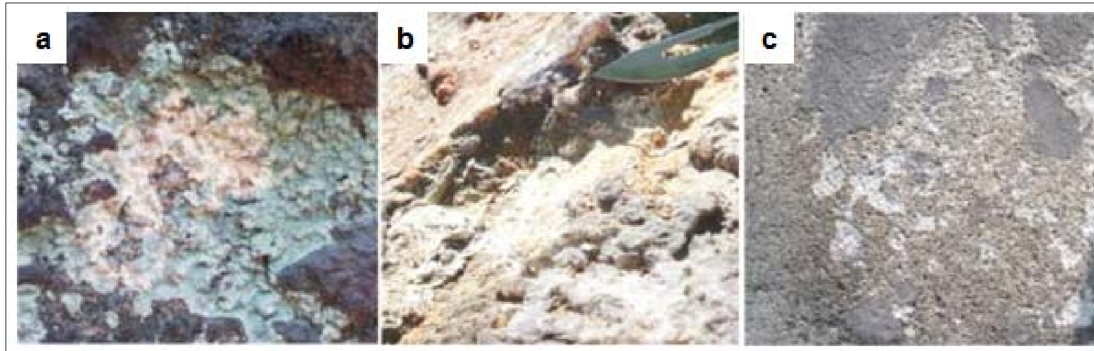

Fig. 1 Biological patinas colonizing urban stone surfaces in Mexico City. Patina a exhibits a colourful crust (a); patina B exhibits an incrustation with evident mineralization features (b); while patina c occurs as thin black crust on surfaces probably under microbiological attack (c).

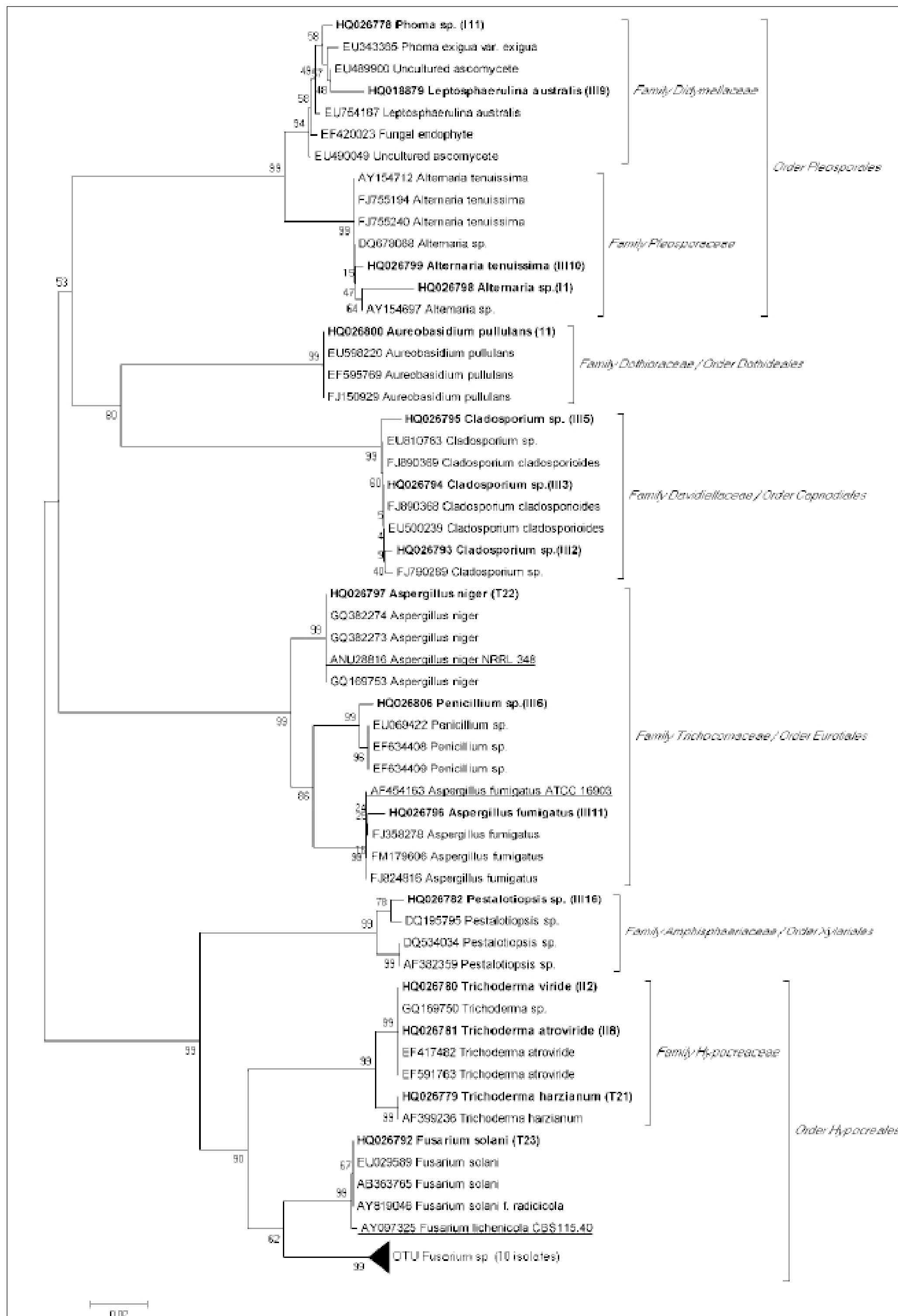

Fig. 2 Phylogenetic relationship from fungal isolates of the Phylum Ascomycota with the Neighbour-Joining method. Numbers over the branches are bootstrap values (over 1000 repetitions). OTU *Fusarium* sp. group was resumed. Fungal isolates of this study are represented in bold. Type strains are underlined. Brackets indicated the family and/or order phylogenetic classification, which was revised of according to Entrez Taxonomy database and Hibbet et al. (2007), respectively.

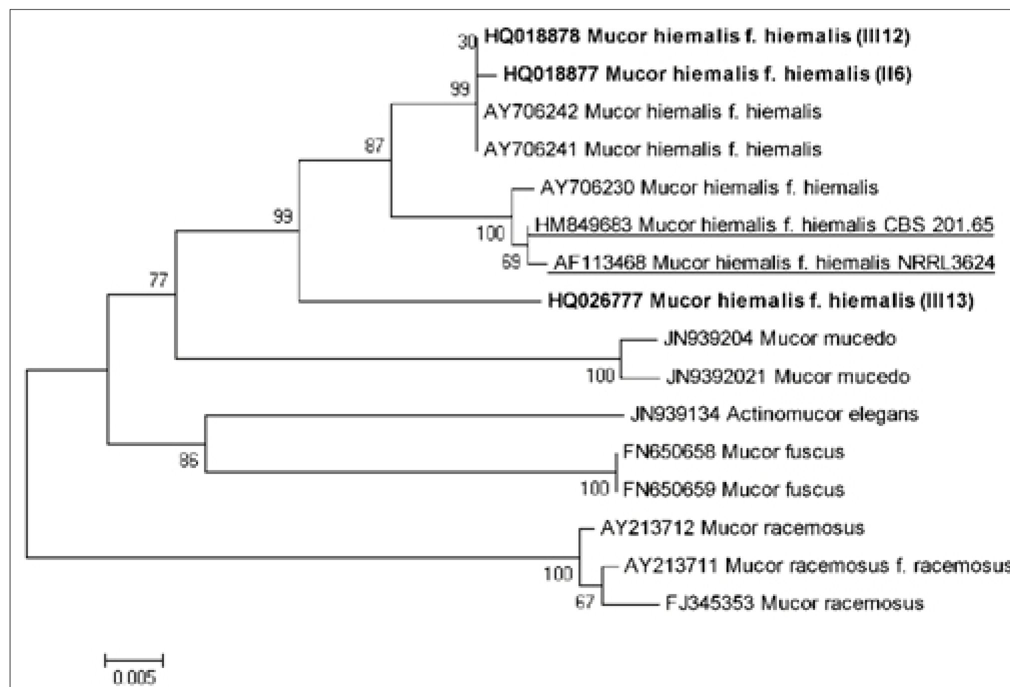

Fig. 3 Phylogenetic relationship from fungal isolates of the Subphylum Mucoromycotina with the Neighbour-Joining method. Numbers over the branches are bootstrap values (over 1000 repetitions). Fungal isolates of this study are represented in bold. Type strains are underlined.

DOI: 10.2225/vol15-issue4-fulltext-6

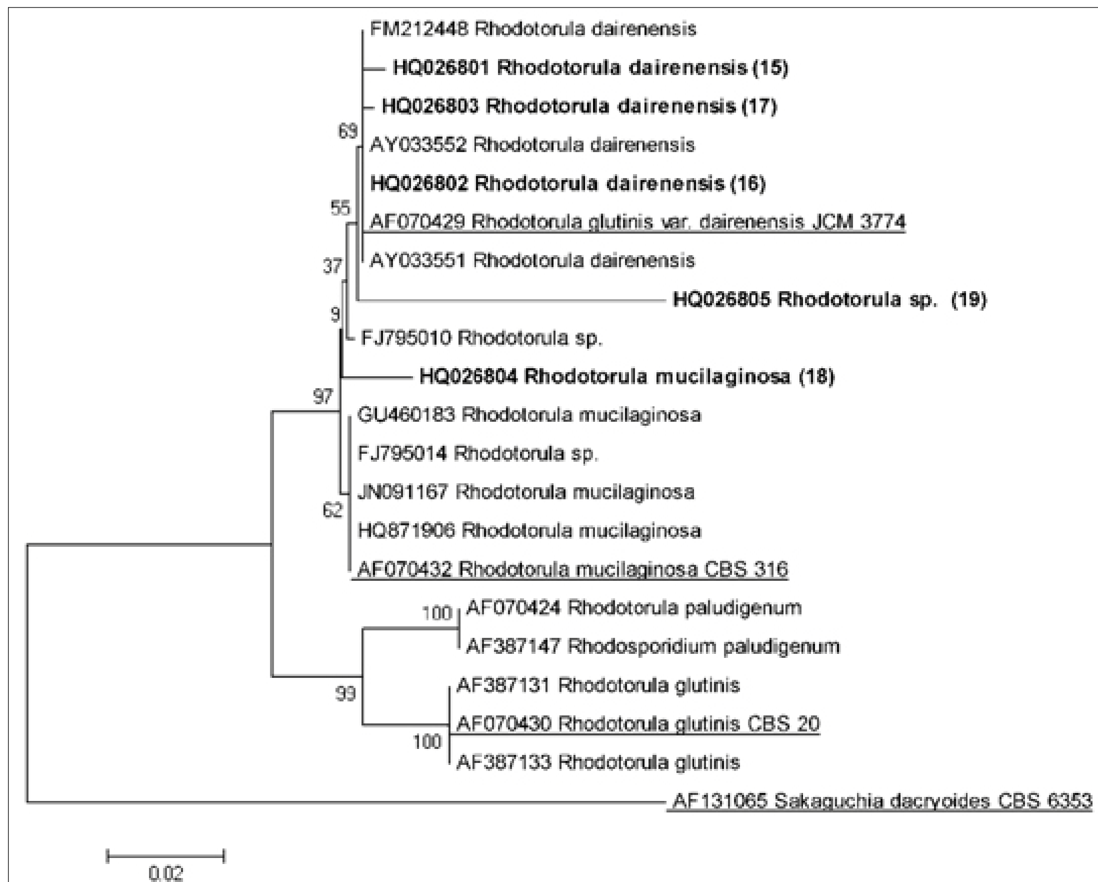

Fig. 4 Phylogenetic relationship from fungal isolates of the Phylum Basidiomycota with the Neighbour-Joining method. Numbers over the branches are bootstrap values (over 1000 repetitions). Fungal isolates of this study are represented in bold. Type strains are underlined. *Sakaguchia decryoides* (type strain CBS 6353) was used as out-group.

TABLES

Table 1. Identification of fungal isolates isolated from biological patinas of this study.

Code (Accession number)	Morphological identity[a]	Nearest neighbour (Accession number)	Blast	Site of recovery[b]		
			Identity (%)	A	B	C
II2 (HQ026780)	*Trichoderma* sp.	*Trichoderma viride* (EF417482)	99		X	
II8 (HQ026781)	*Trichoderma* sp.	*Trichoderma atroviride* (EF591763)	100		X	
T21(HQ026779)	*Trichoderma* sp.	*Trichoderma harzianum* (AF399236)	100		X	
I1(HQ026798)	*Alternaria* sp.	*Alternaria* sp.(AY154697)	98	X		
III10(HQ026799)	*Alternaria* sp.	*Alternaria tenuissima* (AY154712)	98			X
III12(HQ018878)	*Mucor* sp.	*Mucor hiemalis f. hiemalis* (AY706241)	100			X
II6(HQ018877)	*Mucor* sp.	*Mucor hiemalis f. hiemalis* (AY706242)	99		X	
III13(HQ026777)	*Mucor* sp.	*Mucor hiemalis f. hiemalis* (AY706241)	97			X
II4(HQ026785)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	98		X	
T11(HQ026807)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99	X		
T23(HQ026792)	*Fusarium* sp.	*Fusarium solani* (EU029589)	99		X	
III1(HQ026787)	*Fusarium* sp.	*Fusarium* sp.(FJ240316)	99			X
I10(HQ026791)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99	X		
T12(HQ026788)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99	X		
II1(HQ026783)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99		X	
I2(HQ026784)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99	X		
I13(HQ026790)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99	X		
I7(HQ026789)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	99	X		
I9(HQ026786)	*Fusarium* sp.	*Fusarium* sp. (FJ240316)	98	X		
III2(HQ026793)	*Cladosporium* sp.	*Cladosporium* sp. (EU810763)	99			X
III3(HQ026794)	*Cladosporium* sp.	*Cladosporium* sp. (FJ890368)	99			X
III5(HQ026795)	*Cladosporium* sp.	*Cladosporium* sp. (EU810763)	99			X
III11(HQ026796)	*Aspergillus* sp.	*Aspergillus fumigatus* (FJ824816)	98			X
T22(HQ026797)	*Aspergillus* sp.	*Aspergillus niger* (GQ382273)	100		X	
III9(HQ018879)	*Epicoccum* sp.	*Leptosphaerulina australis* (EU754167)	96			X
III6(HQ026806)	*Penicillium* sp.	*Penicillium* sp. (EF634408)	99			X
I11(HQ026778)	*Phomopsis* sp.	*Phomas exigua* (EU343365)	97	X		
III16(HQ026782)	*Pestalotiopsis* sp.	*Pestalotiopsis* sp.(DQ195795)	99			X
11(HQ026800)	*Aureobasidium pullulans*	*Aureobasidium pullulans* (FJ150929)	100	X		
15(HQ026801)	*Rhodotorula glutinis var dairenensis*	*Rhodotorula dairenensis* (FM212448)	99	X		
16(HQ026802)	*Rhodotorula glutinis var dairenensis*	*Rhodotorula dairenensis* (FM212448)	100	X		
17(HQ026803)	*Rhodotorula glutinis var dairenensis*	*Rhodotorula* sp. (AY437842)	99	X		
18(HQ026804)	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa* (EU642632)	99	X		
19(HQ026805)	*Rhodotorula mucilaginosa*	*Rhodotorula mucilaginosa* (EF644445)	95	X		

[a]According to Table 2.

[b]As it was showed in Figure 1.

DOI: 10.2225/vol15-issue4-fulltext-6

Table 2. Description of phenotypic characteristics used for phenotypic-based identification.

Isolates	Length of spore (μm)	Width of spore (μm)	Conidial shape	Growth at 72 hrs (mm)[a]	Colony margin at 48 hrs (mm)*	Colony reverse colour *	Conidia first observed (hrs) at 29°C*	Colour of spores	Maximum growth at: (mm)			
									pH 3.6	pH 5.6	pH 7.6	pH 9.6
II2	4.55 ± 0.37	4.55 ± 0.37	Spherical	21.00	11.00	Beige	96	Dark green	Full growth	Full growth	33.5 ± 00	Full growth
II8	4.24 ± 0.36	4.29 ± 0.39	Spherical	Full growth	16.75	Beige	96	Dark green	Full growth	Full growth	Full growth	Full growth
T21	4.38 ± 0.48	4.38 ± 0.48	Spherical	Full growth	34.00	Beige	72	Light green	Full growth	Full growth	Full growth	Full growth
I1	37.82 ± 13.94	11.10 ± 1.96	Ellipsoid	14.00	9.00	Dark gray	168	Gray	18 ± 00	20 ± 00	19.5 ± 00	17.75 ± 0.61
III10	33.03 ± 6.39	11.77 ± 1.43	Ellipsoid	13.00	7.75	Dark gray	96	Dark green	13.5 ± 00	19 ± 0.40	20.25 ± 0.61	18.5 ± 00
III12	9.29 ± 0.32	5.30 ± 0.55	Ellipsoid	Full growth	22.25	Light green	96	Dark gray	Full growth	Full growth	Full growth	Full growth
II6	9.87 ± 0.82	5.29 ± 0.27	Ellipsoid	32.50	21.75	Light green	96	Dark gray	Full growth	Full growth	Full growth	Full growth
III13	10.56 ± 1.03	5.26 ± 0.34	Ellipsoid	30.25	21.25	Light green	96	Dark gray	Full growth	Full growth	Full growth	Full growth
II4	17.46 ± 2.38	4.20 ± 0.46	Ellipsoid	23.25	13.25	Beige	120	White	21 ± 00	31.75 ± 0.61	31.5 ± 0.40	31.5 ± 0.40
T11	21.02 ± 3.18	5.19 ± 0.54	Ellipsoid	21.50	11.00	Beige	120	White	17 ± 00	29 ± 0.40	29.5 ± 00	29.5 ± 00
T23	29.95 ± 4.93	7.32 ± 0.79	Ellipsoid	9.75	4.75	Beige	120	White	11 ± 00	15 ± 0.81	15.5 ± 0.40	15 ± 00
III1	19.02 ± 2.79	5.78 ± 0.89	Ellipsoid	13.50	7.50	Beige	120	White	17.75 ± 0.61	22.25 ± 1.02	22.25 ± 1.02	22.25 ± 1.02
I10	34.72 ± 3.24	7.11 ± 2.05	Ellipsoid	16.50	8.00	Beige	144	White	19.5 ± 00	21.5 ± 0.40	21.5 ± 0.40	22.25 ± 0.65

DOI: 10.2225/vol15-issue4-fulltext-6

T12	23.12 ± 1.64	6.79 ± 0.56	Ellipsoid	21.00	11.00	Beige	120	White	18.5 ± 00	30.25 ± 1.42	31.5 ± 0.40	30.5 ± 0.40
II1	20.02 ± 6.72	5.89 ± 1.25	Ellipsoid	20.00	10.75	Beige	144	White	Full growth	Full growth	33.5 ± 00	Full growth
I2	19.43 ± 2.23	4.75 ± 0.46	Ellipsoid	22.00	13.25	White	96	Yellow	Full growth	Full growth	Full growth	Full growth
I13	25.21 ± 4.65	7.35 ± 1.49	Ellipsoid	6	3.25	Beige	168	White	Full growth	Full growth	Full growth	Full growth
I7	22.36 ± 2.03	4.74 ± 1.30	Ellipsoid	14	8.00	Beige	192	White	18 ± 00	20 ± 00	19.5 ± 00	17.75 ± 0.61
I9	20.58 ± 4.34	5.84 ± 0.62	Ellipsoid	7	3.75	Beige	144	Gray	13.5 ± 00	19 ± 0.40	20.25 ± 0.61	18.5 ± 00
III2	6.178 ± 0.78	3.72 ± 0.67	Ellipsoid	4.25	2.00	Dark green	120	White	Full growth	Full growth	Full growth	Full growth
III3	8.091 ± 3.25	4.08 ± 0.57	Ellipsoid	3.75	2.00	Dark green	120	White	Full growth	Full growth	Full growth	Full growth
III5	8.389 ± 1.18	4.64 ± 0.99	Ellipsoid	3.50	1.75	Dark green	120	green	Full growth	Full growth	Full growth	Full growth
III11	2.87 ± 0.24	2.87 ± 0.24	Spherical	17	9.50	White	120	Translucent	21 ± 00	31.75 ± 0.61	31.5± 0.40	31.5 ± 0.40
T22	5.44 ± 0.60	5.44 ± 0.60	Spherical	Full growth	12.25	Light green	96	Black	17 ± 00	29 ± 0.40	29.5 ± 00	29.5 ± 00
III9	5.11 ± 1.08	5.11 ± 1.08	Spherical	7.50	5.00	Dark red	284	Yellow	11 ± 00	15 ± 0.81	15.5 ± 0.40	15 ± 00
III6	3.41 ± 0.33	3.41 ± 0.33	Spherical	5.00	2.75	Light green	96	Dark green	17.75 ± 0.61	22.25 ± 1.02	22.25 ± 1.02	22.25 ± 1.02
I11	9.35 ± 2.97	2.92 ± 0.70	Ellipsoid	3.50	2.50	Beige	240	Gray	19.5 ± 00	21.5 ± 0.40	21.5 ± 0.40	22.25 ± 0.65
III16	19.60 ± 1.12	4.33 ± 0.20	Ellipsoid	10.50	6.00	Beige	168	White	18.5 ± 00	30.25 ± 1.42	31.5 ± 0.40	30.5 ± 0.40

[a]Grown on PDA at 29ºC, pH 5.6.

DOI: 10.2225/vol15-issue4-fulltext-6

Table 3. Similarity coefficient (*Cs) of heterotrophic fungal community colonizing urban stone surfaces in Mexico City.

Samples compared by genera[a]	Site of recovery		
	A	**B**	**C**
A	1		
B	0.22	1	
C	0.44	0.66	1
Samples compared by families	**A**	**B**	**C**
A	1		
B	0.22	1	
C	0.75	0.54	1

*Cs = 2 (No of genera or families shared) / total of genera or families.

DOI: 10.2225/vol15-issue4-fulltext-6