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Mycobiota associated with human cadavers: First record in Argentina

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ABSTRACT

Cadavers are an abundant source of organic matter. During their decomposition, a variety of organisms – insects, bacteria, and fungi – can feed on them. Within the ambit of forensic science, fungi have thus far received little attention. Nevertheless, the current study found that forensic mycology can be developed as a tool that provides useful evidence for case resolution. The fungal biota found growing on the surface of two cadavers with different post-mortem intervals (PMI) was examined and identified. The fungal samples were cultured and identified by morphology and molecular genetics. Fungal species such as *Arthrinium arundinis*, *Aspergillus niger*, *Aspergillus terreus*, *Candida guillermondii*, *Candida lypolitica*, *Cladosporium cladosporioides*, *Chrysosporium merdarium*, and *Scopulariopsis brevicaulis* were registered. These findings are the first contributions to forensic mycology from Argentine research. In combination with the joint investigations of forensic researchers worldwide, these results should contribute in the discussion of the use of mycology as a valid forensic tool in which fungi can provide evidence in complex cases.

KEYWORDS

Forensic mycology; fungi; cadaver decomposition; forensic Ascomycetes; forensic Eurotiomycetes

RÉSUMÉ

Les cadavres sont une source abondante de matière organique. Au cours de leur décomposition, divers organismes - insectes, bactéries et champignons - peuvent s'en nourrir. Dans le domaine de la science médico-légale, les champignons ont jusqu'ici reçu peu d'attention. Néanmoins, la présente étude a révélé que la mycologie médico-légale peut être développée comme un outil pouvant fournir des preuves utiles pour la résolution des cas. Le biote fongique trouvé à la surface de deux cadavres avec des intervalles post-mortem différents (PMI) a été examiné et identifié. Les échantillons fongiques ont été cultivés et identifiés par morphologie et génétique moléculaire. Des espèces fongiques telles que: *Arthrinium arundinis, Aspergillus niger, Aspergillus terreus,*

MOTS-CLÉS

mycologie médico-légale; champignons; décomposition de cadavre; Ascomycètes médico-légals; Eurotiomycètes médico-légals

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Candida guillermondii, Candida lypolitica, Cladosporium cladosporioides, Chrysosporium merdarium et Scopulariopsis brevicaulis ont été identifiées. Ces résultats sont les premières contributions à la mycologie médico-légale d'une recherche argentine. Ces résultats, en complément aux enquêtes conjointes de chercheurs en médecine légale du monde entier, devraient contribuer à la discussion sur l'utilisation de la mycologie en tant qu'outil médico-légal valide dans lequel les champignons peuvent fournir des preuves dans des cas complexes.

Introduction

Forensic mycology is a term describing the study of the species of fungi associated with cadavers. Although the presence of fungi on the surface of corpses has been recognized for some time by forensic pathologists, that association has still not received much attention by researchers, since studies of the fungal biota present on decomposing bodies, along with the relevant literature, have been extremely scarce in the past. Only quite recently have forensic scientists begun to consider the distinct possibility that fungi can be used as a tool in legal medicine since the different groups of fungi present on a cadaver can be useful in estimating the time of death [1].

In addition, the number of studies on the relevancy of the role of fungi in post-mortem decomposition has been increasing lately, as evidenced by a rising number of experimental descriptions and case studies in forensic mycology [2–5] as the corpse is an abundant source of organic material [6]. Moreover, considering the variation in the species that could come in contact with corpses under different growth conditions, the isolation of certain fungal species in specific geographical areas as an aid in the characterization and classification of the typical regional microorganisms could provide information on the location of death [7, 8]. In 2003, Carter and Tibbett [4] demonstrated how and why field mycology might provide a further tool for the investigation of crime scenes in forest ecosystems.

Forensic mycology has been applied several times under different circumstances in the last three decades in Belgium [2], the United States [1, 3], Japan [4, 6], and Brazil [9], and more recently in Argentina [10, 11]. In 2011, Hawksworth and Wiltshire [8] published a substantial review about its development in Europe. The use of this discipline of forensic sciences is thus seen to be increasing worldwide, as certain groups of fungi have generated a substantial amount of data concerning the decomposition of cadavers and the related environment. The aim of this work was to isolate and identify the fungi present in two human bodies with different post-mortem intervals (PMIs).

Materials and methods

Two human bodies were examined in the Gonnet Hospital Morgue after the autopsies were performed.

(1) Cadaver 1 was found on the concrete floor of a room in a house in Gonnet (Buenos Aires, Argentina) (–34° 55'41.1816", –58° 0'27.0156"), by the police of the province

of Buenos Aires. This human body was found in a supine position in a decomposed state with remarkable growth of fungi, and had been four months in the room at 8–17°C (environmental temperature recorded during autumn–winter seasons of 2012). The result of the autopsy carried out in the Gonnet Hospital Morgue concluded that death was due to natural causes by cardiorespiratory arrest.

(2) Cadaver 2 was found approximately 20 hours after the victim was reported missing. The body was located in a supine position on the floor of a building under construction in Gonnet (Buenos Aires, Argentina) (–34° 50' 29.0267", –58° 0' 16.0051") and transferred to the Gonnet Hospital Morgue where it was examined. The average temperature was 12°C (June 2012), and the cause of death was determined to be skull fracture.

The fungal samples were taken by standard microbiological techniques using sterile loops, tweezers, scalpels, and swabs. Each of the tools used was considered to be in optimum sterilization conditions. The fungal samples were inoculated on to potato-dextrose agar (PDA, Britania S.A Argentina) and malt-yeast extract agar (MYA, Difco™, USA) as culture media, containing streptomycin (100 μg/mL) and chloramphenicol (50 μg/mL) to inhibit bacterial growth.

From Cadaver 1, samples were taken from each of the colonies that were visible on the body's surface and were individually placed in 9 cm diameter Petri dishes containing sterile growth media as described above. With Cadaver 2, where no fungal growth was visible, the mouth, eyes, ears, and anus were swabbed, as well as under the nails and skin surfaces at different parts of the body, selected randomly.

From both bodies hair samples with length greater than 1 cm were taken from the scalp and pubic area and placed directly in Petri dishes with culture medium.

Six samples from the morgue environment were collected in order to determine what potential body-colonizing species were present at the sampling site. Three of these samples were placed in PDA and three in MYA medium.

The collected samples were incubated at 25°C for up to 25 days with daily inspection until fungal growth was detected. The resulting mycelium was then transferred to a new dish with medium, in order to maintain the axenicity of the isolate. The new cultures were maintained at 25°C in the dark.

The fungal morphologic structures were observed by optical microscopy after lactophenol–cotton-blue staining [12]. Primary taxonomic publications and specific literature citations were used for identifying the sporulating fungi. The yeast isolates were identified by the Mycology Service of the Malbran Institute, (Buenos Aires, Argentina) using biochemical techniques and molecular-genetic characterization of DNA, according to Mondelli et al. [13], Pincus et al. [14], and Rodrigues et al. [15].

For the *Aspergillus* species, the taxonomic criteria of Raper and Fennell [16] were used. The strains of *Aspergillus niger* van Tieghem and *Aspergillus terreus* Thom obtained were compared with original strains described at the Germoplasm-Bank–Culture Collection of the Spegazzini Institute, La Plata, Buenos Aires, Argentina (LPSC 51 and 54, respectively). For the other isolated species, the molecular-genetic taxonomic identification was performed for the confirmation of the species initially indicated by their morphologic characteristics according to Stenglein and Balatti [17].

The ribosomal-DNA–internal-transcribed–spacer region (rDNA-ITS) was amplified by the polymerase-chain reaction (PCR) in an XP thermal cycler (Bioer Technology Co, Hangzhou, China) using the primer pairs ITS5 (5′–GGA AGT AAA AGT CGT AAC AAG G–3′) and ITS4 (5′–TCC TCC GCT TAT TGA TAT GC–3′) [18]. The success of the amplification was confirmed by electrophoresis in 1.5% (w/v) agarose gels containing GelRed™ (Biotium Inc., CA, USA) at 90 V in 1X Tris-borate-EDTA buffer at room temperature. The PCR product was purified with PureLink™ PCR-purification kit (Invitrogen, Löhne, Germany) and then sequenced from both the sense and anti-sense ends by the Big Dye Terminator version 3.1 Cycle-Sequencing Ready-Reaction Kit (Applied Biosystems, CA, USA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyser 3130). The obtained sequences were compared with previously published sequences using BLASTn software [19] in the National Center for Biotechnology Information web page. The sequences were submitted to the GenBank (accession numbers: KX463059-KX463062).

Results

From the environmental samples taken, only nine colonies of *Rhodotorula* sp. – a genus of unicellular pigmented yeasts, member of the fungal phylum Basidiomycota – were cultured, isolated, and identified. This genus is readily recognizable from the distinctive red-orange colonies when grown on the two culture media used (i.e. PDA and MYA).

From Cadaver 1, colonies of *Chrysosporium merdarium* Link (Hyphomycetes; Onygenaceae), *Cladosporium cladosporioides* (Fresenius) de Vries (Dothideomycetes; Davidiellaceae), *Arthrinium arundinis* (Corda) Dyko and Sutton (Sordariomycetes; Apiosporaceae), *Scopulariopsis brevicaulis* (Saccardo) Bainier (Sordariomycetes; Microascacea), *Asperillus niger* (Eurotiomycetes; Trichomaceae), *Aspergillus terreus* (Eurotiomycetes; Trichomaceae), *Candida guillermondii* (Castellani) Langeron and Guerra (Saccharomycetes; Saccharomicetaceae), and *Candida lypolitica* (Harrison) Diddens and Lodder (Saccharomycetes; Saccharomicetaceae) were isolated and identified; whereas in Cadaver 2 only *A. terreus*, *A. niger*, and *C. guillermondii* were present.

Table 1 lists the species found, the body areas from which each sample was collected and the number of colonies identified. The morphologic aspects and colony characteristics of the filamentous fungi were recorded and analyzed for the purpose of taxonomic identification, according to standard taxonomic keys and the appropriate literature – e.g. Barron [20], Dykon and Sutton [21], Domsch et al. [22], Matsushima [23] (see Table 2).

The sequences of the ribosomal DNA of *C. cladosporioides*, *A. arundinis*, *C. merdarium*, and *S. brevicaulis*, were submitted in GenBank under accession numbers KX463059, KX463060, KX463061, and KX463062, respectively.

Discussion

Most fungi found on decomposing human cadavers correspond to opportunistic species that are unspecialized and unable to invade living tissue. The Hyphomycete *S. brevicaulis* are known to be human pathogens that, due to their keratinolytic activity, can infect fingernails and feet [24]. No information is currently available about the particular role of enzymatic and metabolic activities of species obtained from cadavers [25]. Soil fungi are

Table 1. Site of the body from which samples were taken for analysis. Indicated are the number of observed colonies of myceliar fungi species.

	Cadaver 1		Cadaver 2	
Fungus species	Number of colonies	Body site (visible presence)	Body site (by swabbing)	
Chrysosporium merdarium	5	legs	*NF	
	2	chest		
Cladosporium cladosporioides	3	abdomen	NF	
	2	cheekbone		
	1	chin		
Scopulariopsis brevicaulis	2	fingers	NF	
	1	toes		
	1	ocular orbits		
	1	pubic area		
	1	chin		
Arthrinium arundinis	3	legs	NF	
	2	fingers		
	1	arms		
	1	scalp		
Aspergillus niger	2	abdomen	*UC	mouth
	1	mouth	UC	nasal pit
	1	chin		
	1	forehead		
	1	neck		
Aspergillus terreus	3	chest	UC	arms
	2	abdomen	UC	mouth
	1	arms		
Candida lipolytica	UC	pubic area	NF	
	UC	abdomen		
	UC	mouth		
Candida guillermondii	UC	forehead	UC	mouth
	UC	chest	UC	nasal pit
	UC	abdomen	UC	eyes
	UC	pubic area		

*NF, not found
*UC, unposted colonies

known to be capable of invading the surface of a lifeless body due to the lack of the immunologic barriers of a living, healthy human body [26, 27]. In contrast, the fungal species that are pathogenic to humans – from dermatophyte fungi to those that cause lung infections and thrush – can tolerate mammalian body temperatures and overcome immune resistances. Moreover, in immunosuppressed individuals, a variety of less specialized fungi can also invade living tissue [28]. Similar to the aforementioned circumstance, the results reflected the presence of unspecialized fungal species, except for the two species of *Candida* that are common in human corpses and, according Sidrim et al. [9], are more abundant in putrefied state tissue.

The current study contributes to previous findings in forensic mycology, marking the first isolation of the species *C. merdarium*, *C. cladosporioides*, *A. arundinis*, and *C. lypolitica* in association with human cadavers, whereas the species *S. brevicaulis*, *A. niger*, *A. terreus*, and *C. guillermondii* – also documented here – have already been cited in previously reported cases [2, 5, 6, 29]. According to Hawksworth et al. [8], to determine the PMI, it is necessary to know the fungal succession, but due to the fact that the fungal samples were collected at one time during the succession process, it would be risky to determine the PMI of cadaver 1, it being necessary to have more data. Nevertheless, according to Domsh et al. [22] the species present, *S. brevicaulis*, *A. niger*, *A. terreus*, *C. cladosporoides*, *C. merdarium, A. arundinis*, are able to grow in a range of temperature between 10–15°C,

Table 2. Morphological characteristics of the fungal species recorded.

Species	Culture characteristics	Conidiophore	Conidia	Ontogeny
Chrysosporium merdarium	Flat, powdery-granular white-beige	None	Hialine pyriform-clavate 6–7 × 3.5–4 μm	Holoblastic
Cladosporium cladosporiodes	Effuse, velvety olive green-olivaceous brown	Macro-micronematous 350 μm smooth- verruculose	Pale olivaceous- brown ellipsoidal-limoniform 3–11 × 2–5 μm smooth-verruculose	Holoblastic
Arthriniun arundinis	Flat, branched. Surface iron-grey with patches of off-white, reverse iron-grey	Macronematous, erect, simple, flexuous.	Brown, smooth, globose. 6–7 × 3–4 μm. Equatorial slit	Holoblastic
Scopulariopsis brevicaulis	Smooth-rough light brown	Micronematous one or twice verticillately branched	Hyaline-brown globose to pyriform 7.5–13.5 × 3.3–6.5 μm	Holoblastic
Aspergillus niger	Velvety, powdery black	Macronematous, 1.5–3.0 mm. Metulae present.	Brown, globose, irregularly roughened, 4.0–5.0 μm.	Phialidic
Aspergillus terreus	Velvety, powdery Cinnamon-brown	Macronematous 100–250 μm Metulae present	Cinnamon to orange brown, globose-slightly ellipsoidal, smooth-walled. 1.8–2.4 μm	Phialidic
Candida guillermondii	Smooth, glabrous white-cream	None	Globose-subspherical 2–4 × 3–6.5 μm	Buddgin
Candida lypolitica	Smooth, cerebriform, convoluted white-cream	None	Globose-ellipsoidal, 3–5 × 3–15 μm	Buddgin

so, due to the measured temperatures during the time of decomposition being 8–12°C, it can be said that this group of fungi can be found on human cadavers when the death happened four months earlier, during fall–winter in the province of Buenos Aires, Argentina. The PMI of cadaver 2 can be estimated at less than 72 hours according to Hawksworth et al. [8], who mentioned that the fungi generally colonize the body in 3–7 days, and are not generally found before this time.

In the current work, *A. niger*, *A. terreus*, and *C. lypolitica*, were isolated from both bodies despite the difference in the post-mortem intervals between them (e.g., Cadaver 1, four months; Cadaver 2, 20 hours), thus demonstrating that species of the genus *Aspergillus*, can be the first to settle on a body and are still able to be present at further stages of decomposition.

The *Aspergillus* species belong to the Tricomaceae family of the class Eurotiomycetes. Genera of the Tricomaceae family, such as *Aspergillus* and *Penicillium*, have been isolated and identified in all the investigations reported so far; thus suggesting that the family is widespread and their species can be isolated from cadavers at different stages of decomposition from around the world. For example, in Belgium in 1982, de Voorde and van Dijck [2] identified *Penicillium chrysogenum* Thom on a body that had been dead for 18 days; in Japan in 2006, Ishii et al. [6] isolated *Aspergillus chevalieri* (Mangin) Tom and Church, *Aspergillus repens* (Corda) Saccardo, and *Aspergillus rubrum* (König) Thom & Church from one corpse 6 months after death and from another 10 months post-mortem, where the latter had even become mummified; also in Japan in that same year, Hitosugi reported a cadaver found after significant time under water, from which they could sample and identify *A. terreus* and *Penicillium* sp.; in Scotland in 2009, a case was reported [8] in

which *Penicillium brevicompactum* Dierckx and *Penicillium citrinum* Thom were obtained and characterized from a body that had been dead for 5 days; and finally in South America, data were provided by Sidrim et al. in 2010 [9], who conducted a comprehensive survey of fungi associated with corpses in various states of decomposition, who found species belonging to the genera *Aspergillus* and *Penicillium*.

On the other hand, *C. lypolitica*, is a non-filamentous Ascomycete (i.e. yeast) that, when present in the fungal biota of a living body, can rapidly increase the number of colonies if not confronted with the immunologic barriers of a live organism. Sidrim et al. [9] mentioned species of *Candida* in a bloated and putrefied state and isolated *C. guillermondii* from those corpses, consistent with our findings with Cadaver 1.

Records about the potential of fungi to be a tool to establish the time and even the place of death are still preliminary and, thus far, studied mainly in isolated individual cases, and the results were not acquired by systematic experimentation. Nevertheless, they clearly provide useful data, and for this reason the fungal communities could be proposed as a forensic tool. The reduced amount of available data in the field results from the lack of forensic investigators who employ the relevant techniques as well as the lack of general awareness that fungi can contribute significantly to that research. A complete characterization of the fungal isolates at a species level in each forensic case is critical for obtaining the necessary information about the fungal colonies isolated – such as the nutritional requirements for growth, optimal temperature, and enzyme activities – that will allow researchers to determine the specific time and place of death. The acquisition of such data, however, requires personnel trained in the area of mycology with an emphasis on the forensic sciences. With the continuing efforts in providing additional information to this type of research, forensic mycology would become a solid and well-established tool that could assist in resolving complex cases worldwide.

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Disclosure statement

No potential conflict of interest was reported by the authors

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