Advanced Techniques in Biology & Medicine

Cecilia and Noemí, Adv Tech Biol Med 2017, 5:2
DOI: 10.4172/2379-1764.1000226

Mini Review Open Access

The Mycology as Forensics Tool

Tranchida María Cecilia* and Cabello Marta Noemí

Botanical Institute C. Spegazzini, National University of La Plata, CONICET- CCT- La Plata, Argentina

Abstract

In a murder case it is very common to find a corpse in a grave followed by the human decomposition. In a criminal act, the facts in a legal investigation are not clear enough to help clarify unnatural causes of death by suicide or homicide. Estimating the post-mortem interval (PMI), and mainly in cases where there are no witnesses, is crucial to the investigation process. However, the today study of certain species of fungi found and collected from soil in contact with a rotting human body; contribute to obtain important data useful to estimate the PMI of the victim in crime scene investigation. *Dichotomomyces cejpii*, *Talaromyces trachyspermus*, *Talaromyces flavus* and *Talaromyces udagawae*, teleomorphic Ascomycota fungal are the mycobiota currently found and clearly differs to associated mycobiota in control sample and from previously described species Buenos Aires Province, Argentina. Furthermore, additional tests are needed to finally rely on the mycology as a forensic tool.

The main focus of the forensic taphonomy is the study of environmental conditions influencing the decomposition process to estimate the postmortem interval and determine the cause and manner of death. The study is part of a specific branch of the forensic science that makes use of a broad aspect of methodologies taken from different areas of expertise such as botany, archeology, soil microbiology and entomology, all used for uncovering and examining clandestine graves allowing to succeed in the investigation process. Therefore, the "Forensic Mycology" emerges as a new science term meaning the study of the coexistence of fungal species nearby human cadavers as well as those fungal groups potentially useful in establishing a time of death [1,2].

When the researchers specialized in the criminal sciences find clandestine burials, they employ a wide variety of different techniques to study the ground changes where cadaver decomposition happens. Our goal was to isolate and identify the presence of soil mycobiota beneath human cadavers in decomposition based on evaluating the interaction between those under current conditions.

After the investigators or police department considered the death "suspicious in nature" but the cause and manner of death have not yet been determined, the officer authorized to take samples of soil for being studies. Our tools consisted of a set of sterile spoons. Randomly, we collected small portions of soil beneath the corpse and 15 m away of it, transferring the samples in hermetic lab bags to the laboratory. The mycobiota of the collected soil was analyzed by the techniques of: serial dilutions, wet chamber and soil washing, according to Tranchida et al. [3].

For the identification of fungal species, culture media as Corn Meal Yeast Agar (CMYA) (BBLTM, USA), Malt Extract Agar (MEA) (Difco™, USA) and Potato Dextrose Agar (PDA) (Britania SA, Argentina) were used to study the morphology of the colonies. On the other hand, under optical microscopy using staining with cotton-blue lactophenol, the morphological structure of the fungal isolates obtained was observed. The Ultrastructure of fungal species was also observed in the Electronic Microscopes Service of the Museum of Natural Sciences in La Plata, using the scanning electron microscope Jeol JSM-6390 (JSM-6390LV, Jeol, Akishima, Tokyo, Japan), and the molecular identification was performed according to Stenglein et al. [4]. Original taxonomic documents and specific works such as Sotolk and Samson [5] and Domsch et al. [6] were used for the identification of sporulating fungi.

The mycobiota found at the present study area clearly differs to mycobiota identified in control sample and from previously described species for other areas of Buenos Aires Province, Argentina. The most representative fungal species were *Dichotomomyces cejpii* (Milko) Scott, *Talaromyces trachyspermus* (Shear) Sotolk and Samson [5], *Talaromyces flavus* (Klocker) Sotolk and Samson [5] and *Talaromyces udagawae* Sotolk and Samson [5]. These species are pioneers in the colonization of soils where mycobiotic had been modified due to the decomposition of cadaver contributing to the growing of fungi ammonia group. Four representative species were kept under accession numbers LPSC 1160, 1161, 1162 and 1163, in the collection of the *C. spegazzini* Botanical Institute of the National University of La Plata, being *Dichotomomyces cejpii*, *T. trachyspermus*, *T. flavus* and *T. udagawae*, respectively.

Talaromyces udagawae (teleomorphic, Ascomycota) is the most relevant species found in this work, as it's a first record for Argentina and is the first time found in relation to human cadavers, pointing it as a tool to identify soils belonging to the human body in decomposition [7]. The anamorphic state of the specie was not observed. This fungus presents asci with 8 ornamented and ellipsoidal ascospores. The colonies are bright yellow color and very restricted growth in different culture media. It was identified through the use of molecular genetic techniques and in GenBank its DNA sequence was registered under accession number KF723838.

In recent decades, a number of field experiments and cases studies in mycology provided useful data for detecting sites where cadaver decomposition was in place. The data showed that certain chemoecologic groups of fungi can act as aboveground grave markers [1,2,8,9]. According to Carter and Tibbett [2], varieties of fungi that can potentially act as clandestine grave markers are known as ammonia and post-putrefaction fungi and can serve as a tool for the estimation of the post-burial interval as well. These early fungi can bear fruiting bodies from 1 to 10 months after soil fertilization with nitrogenous compounds and consist in the ascomycetes (anamorphs and teleomorphs) along

***Corresponding author:** Tranchida María Cecilia, Botanical Institute C. Spegazzini, National University of La Plata, CONICET-CCT-La Plata, Argentina, Tel: +542214219845; E-mail: ctranchida@conicet.gov.ar

Received May 16, 2017; **Accepted** May 22, 2017; **Published** May 29, 2017

Citation: Cecilia TM, Noemí CM (2017) The Mycology as Forensics Tool. Adv Tech Biol Med 5: 226. doi: 10.4172/2379-1764.1000226

with the saprotrophic basidiomycetes [10,11]. According to Sagara [10] and Fukiharu and Hongo [11] although their work dealt with forest soils *D. cejpii*, *T. trachyspermus*, *T. flavus* and *T. udagawae*, of the Ascomycota phylum, would correspond to early phase fungi: thus in our case, these structures were found 25 days after the individual's death. The contribution of nitrogen compounds including amino acids from the proteins of the decomposing remains could have had a significant influence on the occurrence of these fungal species under the cadaver.

A systematic survey of fungal fruiting structures on grassland soil could be used to designate potential grave sites, thereby reducing the amount of time required to screen a large area. Such surveys would be appropriate where burial over months or years was suspected as a cadaver-related fruiting would not occur immediately after burial.

Although, in order to be used as an effective forensic tools, the fungal communities found in soil under decaying corpses need of additional studies, the results obtained here shows a very important starting point. The work aimed at the knowledge of fungal communities and the succession of these experiences when the death human body goes through the process of decomposition in the grave or on the ground. In Argentina, these studies represent the beginning of a new line of mycological investigation.

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