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REVIEW ARTICLE

Fungal succession on carrion to determine the Post mortem interval: A Forensic Mycology

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ABSTRACT

Forensic mycology is a modern concept that has been used to explain the fungi succession and development of cadavers. It could lead to application in forensic medicine in determining presence of fungal strains which would, in turn, help in establishing the time of death. Since the corpse is an ample source of organic material, there has been an increase in research into the function of fungi in post-mortem decomposition. While some mentions of the participation of fungi in the post-mortem phase have been published in olde r literature and studies, they have rarely relied on the form or species of fungi that are present at each phase of decomposition. Apart from that, isolating fungal species in specified geographical areas will facilitate in the characterization and classification of region-specific microorganisms found on corpses under diverse growth condition. In this study, animal corpse and its specific organs were used to study the development of fungal strains developed in succession after specific intervals of time and stages of decomposition. The strains obtained were identified by lactophenol staining as *Rhizopus sps., Mucor, Aspergillus sps. and Alternaria sps.*

KEYWORDS | Forensic mycology, Fungi, postmortem, decomposition, staining

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INTRODUCTION

In medical terminology the word "thanatomicrobiome" refers to the array of germs present in different locations of decomposing bodies. An important outcome of these experiments is the potential to use forensic samples as microbial physical signs in medicolegal death investigations. Since the corpse is such a rich source of organic matter, there seems to be an uptick of study into the role of fungi in post-mortem decomposition, with an increasing number of laboratory accounts and case studies in forensic mycology. Any type of certain microorganisms have been established in these experiments, which offer useful hints for estimating the time of death.[1,2]

Thanatomicrobiome is a modern term for the study of microbes that colonise internal organs and orifices after death (thanatos, Greek for death). Recent discoveries in thanatomicrobiome have shown that obligate anaerobes, such as Clostridium spp., account for the overwhelming majority of microbes in the body and that the thanatomicrobiome inside internal organs grows across time. This knowledge can be used to predict the time of death when a human body decays.[3]

Many fungi, for example, are known

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to target insects and nematodes, and can play an important role in regulating their populations. Entomopathogens are fungi that infect flies, and they are classified as Ascomycota, Zygomycota, or Chytridiomycota. These fungi infect and ingest insects such as caterpillars and ants, and produce huge stromata that protrude significantly from the bodies of their victims. These fungi may also influence the insect's behaviour. Insect brains are riddled with Brazilian "zombieant" fungi, which cause the victim to rise up plants and bite into plant tissue in a "death grip".[4]

Biological (bacteria, fungi, arthropods, nematodes, etc.) and abiotic (weather, atmosphere, temperature, humidity, etc.) factors affect the complex mechanism of decomposition of human or other mammalian cadavers. Without the presence of bacteria, chemical decomposition can occur at a glacial rate, resulting in the formation of biochemical waste pools. The necrobiome, which is derived from the microbial populations that inhabited the live host as well as the surroundings where the cadaver collapsed, includes these cadaver-associated microbes.[5,6,7]

Fungi can colonise decomposed corpses during the dry phase of decomposition, causing distinctive mildew stains and eventually transforming them into rotting corpses. On heavily decomposed cadavers, especially those that are highly mummified, visible fungal growth is normal. Artificial cultivation allowed the parasites that purge cadavers to be classified morphologically.[8,9]

The current research investigated the function of fungi during three stages of decomposition: bloated, putrefaction, and skeletonization, based on these results. When cadaveric material is destined for anatomy testing, it is often screened for these pathogens prior to delivery; however, this is not a standard practise in all research centers and universities.

Forensic importance and justification

The current research will focus on fungal species that begin colonising cadavers. As a result, it will aid in detecting the various successions of fungal spores, and will aid in deciding the age of the cadaver and the time of death.

MATERIALS & METHODS

Preparation of Potato Dextrose Agar (PDA)

The microbiological medium potato dextrose agar, PDA, is made from potato infusion and dextrose (corn sugar). Potato dextrose agar is the most common medium for growing fungi and bacteria that attack live plants or decompose dead plant matter.

Fungus isolation from animal tissue

To obtain the growth of fungal strains, the tissues were split into smaller parts and held in different petriplates and incubated at different temperatures. Following that, the fungal growth was isolated on PDA and held for staining and detection.

Fungus identification

Lacto phenol cotton blue mounting of fungi is used for fungus detection.

Identification of yeast strains using biochemical tests

To distinguish yeast strains, biochemical tests such as the IMViC test, carbohydrate test, and starch hydrolysis were conducted.

Preparation of potato dextrose broth

Potato Dextrose Broth is recommended for yeast and mould isolation and enumeration.

Setup for the experiment

- PDA plates were designed for carefully positioning the animal tissues, which were freshly sliced, at room temperature (370C) and at 400C for various times or days (0 day, 2 day, 7 day, 10 day, and 12 day).
- 1 gm tissue was measured and then placed on media plates aseptically.
- For proper fungal growth, these plates were incubated for 48 hours.
- After growth and sporulation have been detected on the plates, staining is used to distinguish the cultures.

Results and discussion

The aim of this study was to better understand and analyse the nature and growth of fungi on animal corpse tissue after various time intervals and storage temperatures. Different tissues from freshly cut mammalian tissues were gathered, cut into 1cm by 1cm sections, and put in petriplates containing sterile potato dextrose agar for the sample.

All the experimentation was done in sterile

DAY	TEMPERATURE	STRAIN	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	4°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Green	Dark colored hyphae, Elongated beak like conidia, segmented conidia	Alternaria alternata.
2	4°C	1	Cream	Yeast cells, unicellular, oval to elongated cells, single-celled, cream coloured colony	Candida sps.
		2	White to greyish colony	Yeast cells, unicellular, elongated cells, single celled, white coloured colony	Candida sps.
7	4°C	1	Green	greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
		2	Green	Eurotium herbariorum is formed when Aspergillus glaucus develops massive cleistothecia. The ascopsores released by the cleistothecia have a central groove that makes them look like hamburgers.	Aspergillus glaucus
		3	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillus niger
10	4°C	1	Green	greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
12	4°C	1	Green	Colorless conidiophore, green spores, Uni/Biserate conidial head, Round /radial head	Aspergillus flavus

Table 1: Detail and Identification of Fungal Strains in Liver Tissue.

DAY	TEMPERATURE	STRAIN NO.	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	37°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Black	Dark colored hyphae, Elongated beak like conidia, segmented conidia	Alternaria alternata.
2	37°C	1	No growth	--	--.
7	37°C	1	Green	Greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
		2	Light greenish to colorless	Colorless conidia and hyphae, Biserate conidiophore	Aspergillus nidulans
10	37°C	1	Black	Dark colored hyphae, elongated beak like conidia segmented conidia	Alternaria alternate
		2	Green	Umbrella like conidias with green hyphae	Rhizopus sps.
12	37°C	1	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillus niger

Table 2: Detail and Identification of Fungal Strains in Liver Tissue at 37° C.

DAY	TEMPERATURE	STRAIN NO.	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	4°C	1	Green	Coinidiaphore umbrella shaped	Rhizopus sps
2	4°C	1	Whitish	Single-celled structures	Candida sps
7	4°C	1	Green	Colorless conidiophore, green spores, Uni/Biserate conidial head	Aspergillus flavus
10	4°C	1	Light greenish to colorless	Colorless conidia and hyphae, Biserate conidiophore segmented conidia	Aspergillus nidulans
		2	Green	Colorless conidiophore, green spores, Uni/Biserate conidial head Round/radial head	Aspergillus flavus
12	4°C	1	Green	Dark colored hyphae, elongated conidia, segmented conidia	Alternaria sps.

Table 3: Details & identification of Fungal strains found to populate Muscle tissue after different intervals of time at 4°C

DAY	TEMPERATURE	STRAIN NO.	COLOUR	MORPHOLOGY OF FUNGI	IDENTIFICATION
0	37°C	1	Brownish green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
2	37°C	1	Green	Single-celled oval to elongated cells, Whitish colony	Candida sps
		2	Green	Single celled oval to elongated cells, cream color	Candida sps.
7	37°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Black	Uniserate conidia, black spores in chains being released	Aspergillus terreus
		3	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillis niger
10	37°C	1	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus
		2	Green	greenish conidiophore, green spores, Uniserate conidial head, Round columnar head	Aspergillus fumigatus
		3	Green	Elongated club shaped conidiophore, uniserate	Aspergillus clavatus
12	370C	1	Black	Colorless conidiophore, Black spores, Biserate conidial head, Round /radial head	Aspergillus niger
		2	Green	Colorless conidiophore, green spores, Uni/ Biserate conidial head, Round /radial head	Aspergillus flavus

Table 4: Details & identification of Fungal strains found to populate Muscle tissue after different intervals of time at 37°C

S.NO.	TEST	Y1	Y2	Y3	Y4
1	Fructose	–	–	–	–
2	Dextrose	+	+	+	+
3	Maltose	+	–	–	+
4	Mannitol	+	+	+	+
5	Sorbitol	+	+	+	+
6	Xylose	+	+	+	–
7	Starch	+	+	+	+
8	Sucrose	+	+	+	+
9	Mannose	–	–	–	–
10	Lactose	+	+	+	+
11	Glycerol	+	+	+	+
12	Arabinose	+	+	+	+

Table 5: Test results of Carbohydrate for Yeast Characterization

condition.

The current study shows the presence of different fungal strains developing on different tissues at different times. The use of culture media, staining procedures, and other laboratory approaches to determine the existence of fungi is critical in forensic mycology science.

As is well established, (e.g. Aspergillus spp. most fungi reproduce asexually in nature, and many of their members are airborne strains that can thrive on almost any substrate (1988, Sharma). Alternaria alternata was found in liver tissue on 0 Day, similar to Sharma's findings in 1988.

According to table 1, 2, 3, 4 and 5 on 0 day

Liver and Muscle tissue, 7th day Muscle Tissue, 10th Day Muscle Tissue, and 12th Day Liver Tissue, Aspergillus flavus, a green filamentous fungus, was found. As a result, A. flavus appears to be a fungus that grows mostly on animal tissues. Aspergillus fumigatus was also observed to be prevalent in a 7-day-old liver tissue and a 10-day-old liver tissue preserved at both 40°C and 37°C. Aspergillus niger, a common black mould, was present in the liver and muscle tissue, but mainly in liver and muscle tissue stored at 37°.

However, very distinct and special filamentous fungi were found in the liver and muscle tissue. On the 7th day after being processed at 40°C, Aspergillus glaucus was discovered in liver tissue. On the 12th day of storage, Aspergillus clavatus with club-like conidiophore was found on Muscle tissue deposited at 370C.

De Hoog et al. discovered that there was a lot of Aspergillus spp. as well as Penicillium spp. Separation of standard soil plants, including hyphomycetes, was hampered under the conditions used, and hyphomycetes were not recovered even after the inhibitor cyclohexamide was added to the culture medium.

External collection sites were found to be better for fungal growth than internal collection sites for both airborne fungi and yeasts, particularly for Aspergillus and Candida, according to Collier (2005). Any of the observations on postmortem changes caused by fungi are similar to this one. On the scalp, yeasts sometimes grew faster than in the fur. This fact can be clarified by the after-death rupture of skin barriers and enhanced contact with mucous secretions, in addition to their potential inclusion in the skin microbiota.

Despite the recent discovery of fungi on the surface of bodies by forensic medical professionals, these species are not isolated on a routine basis.

Fungi need further analysis before they can be used as a forensic instrument since their description can also expose the site of death. (2006, Ishii).

The current study is just the beginning, and the findings are insufficient to show that fungi can be used as effective biological death markers. On the other hand, the isolation of fungi such *Aspergillus spp., Alternaria spp.,* and *Candida spp.* demonstrate that the fungi isolated during the corpse decomposition entomology process differ. These findings suggest that the existence of fungi on, in, and around cadavers can provide additional information that can help determine the exact time of death. As with forensic entomology, further study with larger samples and more detailed descriptions of conditions is required to validate the results reported here and to determine the true value of mycology as a forensic medicine tool.

Results and discussion

An investigation into the isolation and detection of fungal strains colonising animal corpses and internal organs after death revealed a succession of *Aspergillus* strains. Various strains of *Aspergillus* seem to colonise dead organs in different ways depending on the temperature. *Aspergillus flavus* grows first in both liver and muscle tissue, followed by filamentous fungi such as *Aspergillus fumigatus, Aspergillus nidulans,* and *Aspergillus niger* in both tissues. Some filamentous *Aspergillus* fungi may only be found on liver or muscle tissue, which may be used as a biomarker in forensic mycological studies. At a two-day cycle, yeast-like fungi are often found on all tissues, which is unusual and can be used as a particular biomarker.As a result, the use of fungal succession on corpses for identification and tracking may be precise, leading to accurate time of death and other forensic parameters. IJFMP

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Conflict of Interest:

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