THE HIGHLIGHTING OF FLUFFY-TYPE COLONIES FOR MOLDS

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RESEARCH ARTICLE

Abstract

Molds spread in nature through drought-resistant spores, which can remain viable for years. When such a spore lands on the surface of a growth medium favorable for development, with a sufficient amount of free water, the first stage involves water absorption and activation of enzymatic systems, followed by the germination of the spore cell and the formation of vegetative tubes called hyphae or mycelium. The hyphae extend across the surface of the medium, diversify, and perform certain specialized functions. The extension hyphae can develop along the medium, in the airspace, or within the medium itself, facilitating nutrient absorption and providing structural support. At a certain stage of development of the vegetative hyphae, reproductive hyphae are formed, which generate spores differentiated by gender and species. The totality of vegetative and reproductive hyphae constitutes the mycelium.

The fluffy colonies of mold are visible masses of spores and hyphae that develop on various surfaces, typically in damp and warm environments. These colonies can have different textures, ranging from fine and velvety to coarse and dense, and are known for their fluffy appearance, which may be colored and can vary depending on the type of mold. The colors of mold colonies can be white, green, blue, black, or even reddish, depending on the species and environmental conditions.

Keywords: molds, hyphae, colonies

INTRODUCTION

Molds are found in all natural habitats due to their remarkable ability to adapt to various environmental conditions. They possess a complex enzymatic system, allowing them to utilize macromolecular organic compounds for nutrition.

The genus *Aspergillus* is one of the most well-known molds with medical significance. Among the species impacting health are *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger*. These form velvety colonies in shades of green, gray, white, or black and are often present in the air, on food, in indoor dust, and on building materials.

Aspergillus colonies can cause aspergillosis, an infection that can affect the respiratory system and manifest as persistent cough, difficulty breathing, and, in severe cases, lung damage. Aspergillosis is more common in individuals with compromised immune systems, such as patients with HIV, those who have undergone organ transplants, or patients with chronic lung conditions.

Maple mold (*Penicillium*) produces green, blue, or gray colonies, often found on food and in damp spaces. Some species of *Penicillium* are used in the production of antibiotics (for example, penicillin); however, other species can cause allergic reactions and infections, especially in individuals with respiratory issues.

Penicillium marneffei, for instance, is known for its ability to cause severe infections, particularly in immunocompromised patients. This species produces blue-green colonies and can lead to fever, skin infections, and damage to internal organs.

Stachybotrys chartarum, also known as "toxic black mold," is a species that forms dense, black, mucous colonies growing on cellulose-containing materials (wood, cardboard, plaster) under conditions of high humidity. This species produces mycotoxins, which, when inhaled or touched, can cause severe symptoms, ranging from respiratory irritation, headaches, and cough to neurological problems and extreme fatigue.

Exposure to *Stachybotrys chartarum* can lead to toxic mold syndrome, a serious condition that includes symptoms such as confusion, memory loss, light sensitivity, and cognitive dysfunction. This mold is often present in buildings affected by flooding or those with chronic moisture problems.

Alternaria produces fluffy colonies that are dark brown or black and is commonly found outdoors as well as indoors, on walls and in bathrooms. This mold is a common allergen, and its spores can trigger allergic rhinitis, asthma, and other

respiratory conditions. Allergic individuals may experience symptoms such as sneezing, nasal congestion, itchy eyes, and cough. Additionally, this mold can cause skin infections and sinusitis in people with weakened immune systems.

The genus *Mucor* forms white or gray colonies with a fluffy texture and is found in damp environments, on spoiled food, and on decomposing organic materials. *Mucor* spores are opportunistic and can cause mucormycosis, a severe infection that affects the lungs, sinuses, and brain, especially in individuals with uncontrolled diabetes or weakened immune systems. Mucormycosis is an aggressive infection that can lead to severe tissue damage and requires immediate treatment. Symptoms include headaches, fever, facial swelling, and, in severe cases, may affect the central nervous system.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

In the identification of medically significant molds, the methods and materials used are essential for obtaining an accurate diagnosis and evaluating the associated health risks. The identification methods for potentially pathogenic molds involve complex laboratory analyses, and the materials include specialized equipment for sample collection, isolation, and analysis. The process can be divided into several steps: sample collection, isolation and cultivation, microscopic identification, molecular testing, and toxicological analysis.

Sample Collection

Sample collection is the initial and one of the most important stages, as it determines the quality of subsequent analyses. Samples can be taken from various surfaces (walls, furniture, food) or from the air, particularly in areas where humidity is high and mold is visible or suspected.

- **Instruments**: Sterile cotton swabs, microscope slides and cover slips, sterile bags, Petri dishes, or air sampling pumps.
- **Techniques**: Swabs are used to collect mold from solid surfaces, while air pumps are used to capture spores from the air by filtering it through special membranes.

1. **Isolation and Laboratory Cultivation** Isolation and cultivation help obtain a pure colony necessary for further identification. Samples are transferred to selective culture media that support mold growth, such as Sabouraud dextrose agar, malt agar, or Czapek agar.

- **Culture Media**: Specific media, such as Sabouraud agar, which stimulates fungal growth due to its high glucose content.
- **Incubation**: Petri dishes are incubated at controlled temperatures between 25-30°C for several days to weeks, depending on the species.

3. **Microscopic Identification** Once the colony has been isolated and grown, microscopic identification is essential for examining the morphology of the mold. Microscopy allows observation of the shape, size, structure of the hyphae, and appearance of the spores, which are unique characteristics for each species.

- **Equipment**: High-resolution optical microscope, electron microscope (for detailed analysis of spores).
- **Staining**: Lactophenol cotton blue or other stains that aid in visualizing structural details.

RESULTS AND DISCUSSIONS

Cultural observations revealed notable differences regarding the color, texture, and growth rate of mold colonies. **Aspergillus fumigatus** has colonies with a velvety texture, dense structure, and green or grayish-blue color. The growth rate is rapid, with colonies becoming visible within 48-72 hours on culture media such as Sabouraud dextrose agar. **Penicillium marneffei** forms bluish-green, powdery colonies with a characteristic texture that can become reddish upon maturation. This species is distinguished by producing a red pigment on culture media, an important marker for identification.

Stachybotrys chartarum presents black colonies with a slimy, almost rubbery texture. It grows slowly compared to other molds, reaching maturity in 7-10 days on Czapek agar. **Alternaria alternata** displays fluffy, woolly colonies of dark brown to black color. The growth rate is moderate, and the colonies have a distinct radial morphology. **Mucor spp.** presents white or gray colonies with

a fluffy texture. The growth rate is rapid, with colonies becoming visible within 24 hours, quickly expanding on culture media to cover the entire Petri dish within a few days. These cultural characteristics are useful for the rapid identification of mold species and contribute to preliminary laboratory diagnostics.

Biochemical tests were utilized to identify compounds produced by different mold species and the specific enzymatic activity of each. In particular, tests were used to detect extracellular enzymes, such as proteases and esterases, and to identify toxic metabolites. Biochemical tests for **Aspergillus fumigatus** indicated the production of fumagillin and gliotoxin, compounds with potential toxicity that affect the immune system. The enzymatic activity includes the presence of esterases and proteases, enzymes that contribute to the pathogenicity of the species. **Penicillium marneffei** secreted proteolytic enzymes and produced a characteristic red pigment. HPLC tests showed the presence of compounds with natural antifungal activity, as well as the ability to colonize human tissues in immunocompromised individuals. **Stachybotrys chartarum** produces dangerous mycotoxins, including satratoxin and roridin, both identifiable through toxicological testing. GC-MS analyses confirmed the presence of these toxins, known for their negative impact on the nervous and respiratory systems. **Alternaria alternata** produces alternariol toxin, a compound associated with respiratory irritations and dermatitis. The extracellular enzymes produced by Alternaria include oxidases, which contribute to the degradation of organic materials. **Mucor spp.** produces lipolytic and proteolytic enzymes but does not release significant toxic compounds. However, Mucor is an opportunistic pathogen, with the capacity to invade tissues in individuals with weakened immunity.

These biochemical characteristics contribute to understanding the pathogenic mechanisms of molds and allow for the assessment of infection and toxicity risks.

Morpho-staining analysis, which involves the use of microscopy and specific stains, allowed for the observation of the structural details of each mold species. **Aspergillus fumigatus**, following staining with lactophenol cotton blue, exhibited septate hyphae, straight conidiophores, and compact conidial structures characteristic of this genus.

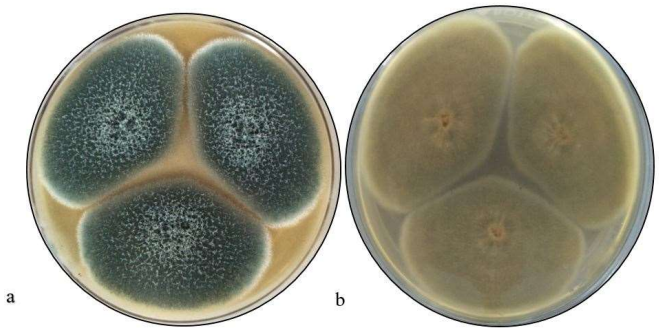

Fig.1. Colony growth of A. fumigatus in PDA in the (a) obverse and (b) reverse sides on the 7th day of incubation. (https://www.facesoffungi.org/aspergillus-fumigatus-facesoffungi-number-fof-10086/)

Penicillium marneffei exhibits branched, septate hyphae, with conidia developing in chains, giving a characteristic "brushed" appearance. These structures are easily observed after staining with lactophenol cotton blue.

Stachybotrys chartarum presents thick, septate hyphae, with conidia arranged on long, straight conidiophores. Staining with lactophenol highlighted a "black tube" appearance, characteristic of this species.

Alternaria alternata, from a microscopic perspective, includes septate hyphae and large, multicellular conidia arranged in short chains, giving a "stacked" appearance. The structures stain intensely with lactophenol cotton blue.

Mucor spp. features broad, coenocytic hyphae and large sporangia filled with spores. Staining revealed a "full sporangium" appearance, distinctive for this species.

Tab. 1. Cultural and Morphological Characteristics of Mold Species

Mold Species	Cultural Characteristics	Morphological Characteristics
Aspergillus fumigatus	Dense, velvety colonies in green or grayish-blue	Septate hyphae, straight conidiophores, compact conidia
Penicillium marneffei	Powdery, blue-green colonies with red pigment	Branched, septate hyphae; conidia develop in chains
Stachybotrys chartarum	Black, slimy colonies that grow slowly	Thick septate hyphae, conidia on long, straight conidiophores
Alternaria alternata	Fluffy, brownish-black colonies	Septate hyphae, large multicellular conidia, arranged in short chains
Mucor spp.	- White or gray, fluffy colonies that grow rapidly	Broad, coenocytic hyphae; large sporangia filled with spores

In the study "Identification of Aspergillus Using Morphological Characteristics," Aspergillus isolates were identified at the species level using differential culture media. A total of 205 Aspergillus isolates studied included: 153 (75%) environmental Aspergillus and 52 (25%) clinical isolates. Among the 11 identified species of Aspergillus, A. flavus (55%), A. niger (31.7%), and A. fumigatus (8.7%) were the most frequently isolated from the samples.

CONCLUSIONS

Aspergillus fumigatus has colonies with a velvety texture, dense, and green or gray-blue in color. Penicillium marneffei forms bluish-green colonies. Mucor species produce lipolytic and proteolytic enzymes but do not release significant toxic compounds. The results obtained through cultural, biochemical, and morphotintorial analysis allow for the differentiation and identification of medically significant mold species.

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