

news digest #026

Fungal identification in pharmaceutical laboratories

Author: Olaf Degen, Director Industry Microbiology, Bruker Microbiology & Infection Diagnostics

Fungi, including molds and yeasts, pose significant contamination risks to pharmaceutical products and manufacturing environments, potentially causing production delays, product recalls, or serious health concerns. Effective identification and control of fungal contamination is therefore crucial, but challenging, as most molds are filamentous, featuring aerial dispersal via spores. Instruments like the MALDI Biotyper® can help to improve fungal identification, starting from colony material, during the manufacturing process.

Looking into the literature, certain fungi species are commonly found in pharmaceutical environments.[1,2] Fungal spores remain a persistent contamination challenge in the biopharma and cell and gene therapy industries. As emerging industry sectors continue to grow, shifts in contamination types and risks are anticipated, but the fungal identification challenge will remain. Accelerating the time required to identify microorganisms in cleanroom environments can streamline CAPA investigations, allowing more time to focus on production activities.[3]

The process of identifying fungi following cultivation on agar plates involves several steps. Environmental samples are collected from various areas within the pharmaceutical facility, including air, surfaces, and water.

These samples are then inoculated onto agar plates, providing nutrient-rich environments conducive to fungal growth. Commonly used agar media for fungal cultivation include Sabouraud Dextrose Agar (SDA, glucose-peptone agar), Malt Extract Agar (MEA), and ID Fungi Plates (IDFP).

Traditional microbiological education included the identification of fungi through colony morphology and microscopic examination. This approach involves analyzing the fungi's morphological features, such as color, texture, size, and structures like hyphae, spores, and reproductive organs. The next advancement in fungal identification introduced biochemical tests, such as carbohydrate assimilation assays, which offered additional precision.

Modern molecular methods have since revolutionized fungal identification. Techniques like MALDI-TOF MS for protein pattern detection and PCR or DNA sequencing for genetic analysis enable highly accurate and detailed identification of fungi. Bruker recently improved the workflow for MALDI-TOF MS-based fungi identification, which can now start with the isolation of early front mycelium from an agar plate. This saves time and makes more tedious workflows unnecessary.

Let's have a look at the most prominent fungal groups encountered in the pharmaceutical environment:

Aspergillus species are common contaminants in pharmaceutical environments, causing possible allergic reactions or infections with the end-user, and are notorious for producing mycotoxins. Among them, *Aspergillus brasiliensis* and *Aspergillus niger* are of particular interest, the latter also playing a significant role in the biotechnology and fermentation industries, especially for the production of industrial enzymes.

Candida albicans is closely monitored as a potential contaminant in sterile injectable products. In humans, *Candida* infections, particularly those caused by some specific strains, have garnered increasing attention over the past years. This highlights the importance of rapid identification tools, such as MALDI-TOF MS, for early and accurate detection of such contaminations.[4]

Penicillium species are known to contaminate products, leading to spoilage. *Fusarium* species, commonly found in soil and plant materials, can produce mycotoxins, posing a significant risk to pharmaceutical products. *Exserohilum rostratum*, frequently identified during environmental monitoring, is one of over 30 *Exserohilum* species occasionally implicated as opportunistic pathogens in humans, causing conditions such as skin lesions, sinusitis, and meningoencephalitis.

Stachybotrys chartarum (synonymous with *Stachybotrys atra*) and *Stachybotrys chlorohalonata*, collectively referred to as black mold, are often linked to poor indoor air quality due to fungal growth on water-damaged building materials. *Chaetomium*, a genus in the Chaetomiaceae family, is typically found in soil, air, cellulose, and plant debris. Its mycelia form dense, rope-like masses, and the mold is a significant contributor to the sick building syndrome phenomenon.[5] *Rhizopus* and *Cladosporium* species are common environmental contaminants, the latter is known for causing allergic reactions and infections.

Advancements in microbiological tools continue to enhance fungal research. Innovative methods now support optimal fungal growth and identification. Modern ID plates for fungi simplify the identification of filamentous molds, enabling accurate routine classification using MALDI-TOF MS with a rapid-extraction protocol.[6]

To further advance fungal testing, Bruker has developed the Mycelium Transfer (MyT) procedure, streamlining sample preparation. Inspired by the extended Direct Transfer (eDT) technique, this method offers a user-friendly approach without compromising accuracy. The MyT procedure is ideal for handling visible front mycelium, facilitating a process similar to the eDT method used for yeast identification. Coupled with the MBT HT Filamentous Fungi Module, this innovative technique enables rapid and precise identification of filamentous fungi at the species level.

References
1 Tony Cundell. Mold contamination in pharmaceutical drug products and medical devices, published on December 15, 2013. https://www.europeanpharmaceuticalreview.com/article/23351/microbiology-mould-contamination-pharmaceutical-drug-products-medical-devices/
2 Tony Cundell. Mold Monitoring and Control in Pharmaceutical Manufacturing Areas, published on July 30, 2016. https://www.americanpharmaceuticalreview.com/Featured-Articles/190686-Mold-Monitoring-and-Control-in-Pharmaceutical-Manufacturing-Areas/
3 Sahil Parikh et al. A Risk Assessment Approach to Address Fungal Spore Contamination in a Cell and Gene Therapy Cleanroom and Modern Methods for Control. Published: February 1, 2023. https://www.americanpharmaceuticalreview.com/Featured-Articles/594394-A-Risk-Assessment-Approach-to-Address-Fungal-Spore-Contamination-in-a-Cell-and-Gene-Therapy-Cleanroom-and-Modern-Methods-for-Control/
4 Evren E et al. Medically important Candida spp. identification: an era beyond traditional methods. In: Turk J Med Sci. 2022. https://doi.org/10.55730/1300-0144.5380
5 DC. Straus: The possible role of fungal contamination in sick building syndrome. In: Front. Biosci. (Elite Ed) 2011. https://doi.org/10.2741/e270
6 Robert MG at. El. Evaluation of ID Fungi Plates Medium for Identification of Molds by MALDI Biotyper. In: J Clin Microbiol. 2020. https://doi.org/10.1128/jcm.01687-19

Online information
bruker.com/microbiology

Bruker Daltonics GmbH and Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

info.md@bruker.com

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660