Djilali Bounaama University of Khemis Miliana. **First semester/2025-2026**

FNLSES

Department of Biology

Module Manager: Prof. Guetarni H.

Cycle: Microbiology degree

DW1: Characterization of fungi

Fungal characterization plays a key role in various fields such as clinical, food safety, and scientific research. This process combines phenotypic and genotypic techniques, allowing for accurate identification, strain classification, and reliable assessment of associated risks.

1. Phenotypic characterization

This first phase is crucial for quickly guiding the diagnosis. It is essential for differentiating the main taxonomic groups.

1.1. Macroscopic observations and use of differential media

The fungi are grown on selective media, such as Sabouraud dextrose agar, and incubated at different temperatures. This approach allows for the assessment of their growth capacity and morphological transformations.

	Observations and details
Textures	- Woolly, characterized by aerial mycelium. - Powdery due to the abundance of spores. - Creamy, typical of yeast colonies. - Examination of the back of the Petri dish reveals the presence of diffusible pigments.

	Observations and details
Dimorphism	The transition of the organism from its filamentous form to its yeast morphology occurs under specific conditions, particularly when it is cultivated in nutrient-rich media such as agar or Brain Heart Infusion (BHI) broth.
Chromogenic Media	This method is specifically used for the identification of yeasts, particularly those of the genus *Candida*. It relies on enzymatic substrates that undergo hydrolysis, producing distinct colors specific to each species. For example, in *Candida albicans*, a green coloration is generally observed.

1.2. Microscopic and Functional Analysis

Methods such as lactophenol cotton blue or India ink staining are used during detailed microscopic examination. They allow the capsule structures to be highlighted. This crucial approach serves to accurately identify and confirm the genus and species of a microorganism.

	Characteristic and Details
Conidiophores	- *Aspergillus*, characterized by its vesicular structure. -*Penicillium*, known for its complex branched brush pattern The specific architecture and arrangement of the sporulating organ are the most important criteria for taxonomic classification and

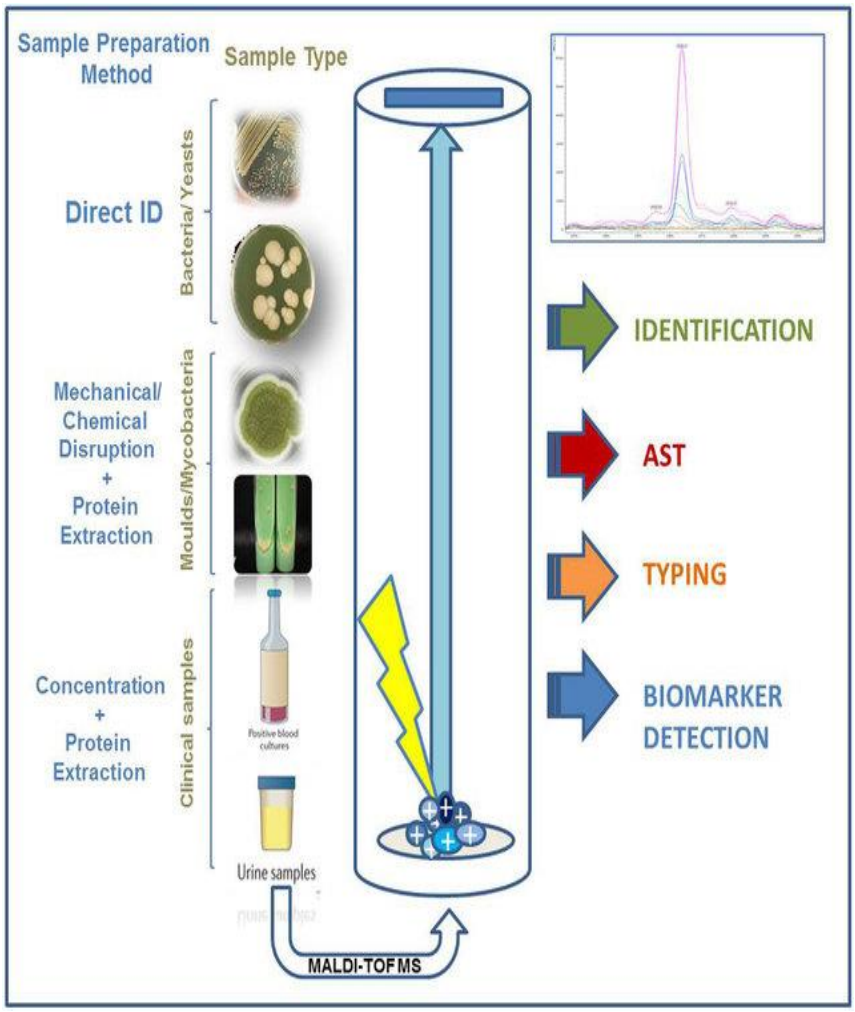
	Characteristic and Details
	differentiation of different mold species.
Macroconidia	Macroconidia are large multicellular spores frequently found in dermatophytes. They are classified according to their shape (club-shaped or spindle-shaped) and the texture of their walls, which may be smooth or echinulate.
Germ Tube Test	The ability of the yeast species *Candida albicans* to transform into a germ tube or pseudohypha is a remarkable characteristic, particularly when incubated in serum at 37°C.
Enzyme Tests	The urease test is used to identify *Cryptococcus*, while the phenol oxidase test, performed on birdseed agar, detects melanin production.

2. Molecular and Instrumental Reference Methods

With their advanced resolution, these innovative techniques enable an exceptional level of precision in identifying microorganisms, as well as in the in-depth typing of various strains, particularly in clinical diagnostics where accuracy and speed are paramount.

2.1. Mass Spectrometry (MALDI-TOF MS)

The principle behind this technique involves utilizing an instrument designed to measure the time of flight for ionized ribosomal proteins following laser irradiation. Through this process, mass spectra are generated for each species along with its corresponding fungal spectrum. In the identification phase, the spectral profile obtained is compared against an extensive reference library, enabling rapid and precise identification of the species.



2.2. DNA Sequencing and Molecular Typing

The internal transcribed spacer (ITS) region of ribosomal DNA is amplified using PCR techniques. Subsequently, the resulting sequence undergoes analysis and comparison against public databases, including GenBank and UNITE, utilizing the BLAST algorithm. Whole genome sequencing (WGS), recognized as the most efficient analytical tool, provides superior resolution by exploiting single nucleotide polymorphisms (SNPs). This methodology is especially advantageous for conducting intricate epidemiological tracing and investigating mechanisms of antimicrobial resistance.

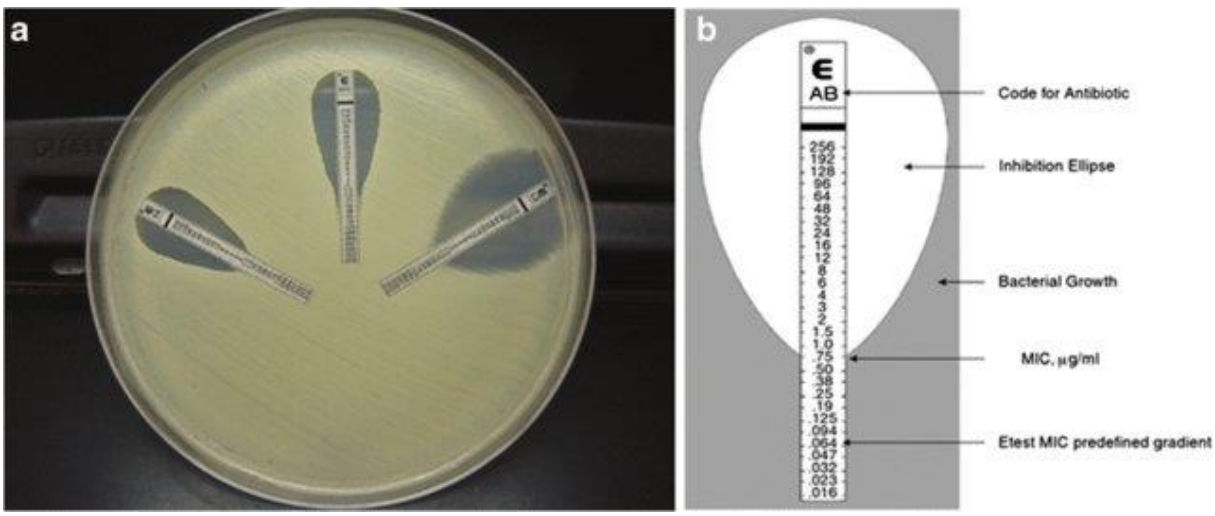
2.3. Advanced Microscopy Techniques

Transmission Electron Microscopy (TEM) serves as a powerful tool for generating high-resolution images capable of revealing cellular ultrastructures such as cell wall layers, septa, and organelles. This level of detail is particularly valuable for taxonomic validation and classification. Conversely, Confocal Laser Scanning Microscopy (CLSM) enables three-dimensional visualization of biofilm architecture and spatial arrangement. Such insights are essential for elucidating the role of biofilms in the pathogenesis of chronic infections.

3. Functional Characterization and Risk Assessment

3.1. Antifungal Susceptibility Testing (AST)

The E-test, also known as Gradient Diffusion, involves the application of a strip impregnated with a gradient concentration of an antifungal agent onto an agar plate. This method facilitates the identification of the Minimum Inhibitory Concentration (MIC) by examining the elliptical zone of inhibition. The Microdilution Method serves as the reference protocol established by CLSI and EUCAST for the precise quantitative determination of MIC values in broth medium. It provides a standardized approach to measure antifungal activity. Molecular resistance detection employs techniques such as PCR or sequencing to identify genetic mutations—commonly within specific genes of pathogens like Aspergillus—that are associated with antifungal resistance. This method enables the detection of mechanistic changes at the molecular level driving resistance.



3.2. Virulence and Toxicological Analysis

Virulence Factors involve the assessment of key characteristics such as the production of hydrolytic enzymes, including proteases and phospholipases, alongside the evaluation of thermotolerance, which examines the organism's ability to thrive at elevated temperatures ranging between 42 °C and 45 °C. These measures are crucial for understanding the pathogenic potential and adaptability of fungi to hostile environments. For Mycotoxin Quantification, the advanced analytical approach of Liquid Chromatography tandem Mass Spectrometry (LC-MS) is recognized as the preferred technique. This method offers exceptional sensitivity and precision, enabling the simultaneous detection and accurate quantification of multiple mycotoxins, such as aflatoxins or ochratoxins, present in fungal extracts. The use of LC-MS plays a significant role in maintaining food safety standards by ensuring compliance with rigorous regulatory requirements concerning toxin levels.

Tasks to complete

- Develop a concise summary, limited to no more than 10 lines, outlining the various methods and techniques employed in this practical session to characterize fungi.

First name :……..... 2025-2026

Last name :……

Group :……

Cycle :……..

Title of DW :……

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