



## **Basic microscopy: principles of light microscopy, types of microscopes, and specimen preparation.**

➤ **Gram stain**

➤ **Fungus stain**



# Microscope

- **Microscope** – an instrument that **produces an enlarged image of an object.**
- Biologists use **microscopes** to study cells, cell parts, and organisms that are **too small** to be seen with the naked eye.
- Microscopes **magnify** and **show details** of the image.
- Types of microscopes:
  - Light Microscope** – **light** passes through **one or more lenses** to produce an enlarged image of a specimen
  - Electron Microscope** – forms image of a specimen using a **beam of electrons** rather than light



# Types of microscopes

## 1. Optical Microscope:

The optical microscope has one or two lenses that work to enlarge and enhance images, placed between the lower-most lens and the light source. It was the first device ever created.

### a. Simple Optical Microscope:

It was used by Anton Van Leeuwenhoek during the late-sixteen and early-seventeenth centuries, around the time that the microscope was invented. It has one lens, the convex lens, in the magnifying process.

### a. Compound Optical Microscope:

It has two lenses, work to minimize both chromatic and spherical aberrations so that the view is unobstructed and uncorrupted. The compound light microscope consists of three sets of lenses:-

- Condenser contains lenses that collect and focuses the light to upward directing through any object on the stage. The amount of light is controlled by shutter, or iris diaphragm, which adjust the amount of light admitted.
- The objectives include three or four lenses that provide a magnified and inverted image of the specimen
- The eyepiece adds further magnification

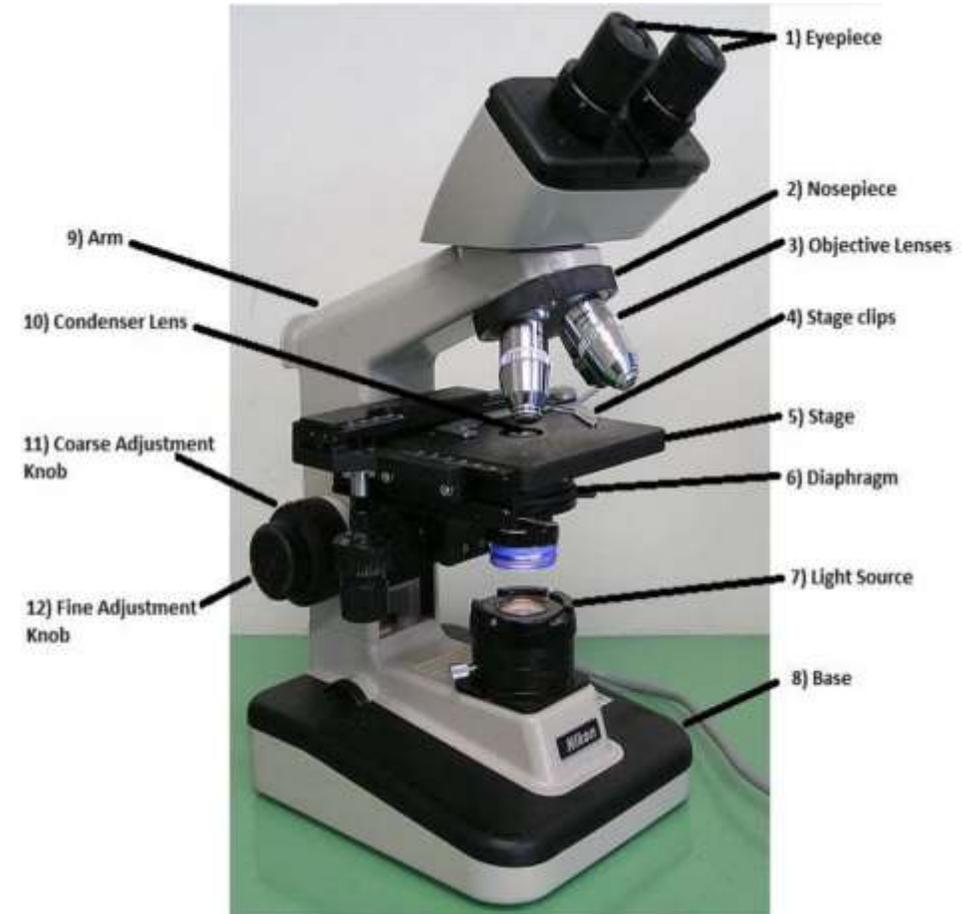


# Microscope parts and its functions

1. **Eyeiece:** contains the ocular lens, which provides a magnification power of 10x to 15x, usually. This is where you look through.
2. **Nosepiece:** holds the objective lenses and can be rotated easily to change magnification.
3. **Objective lenses:** usually, there are three or four objective lenses on a microscope, consisting of 4x, 10x, 40x and 100x magnification powers. In order to obtain the total magnification of an image, you need to multiply the eyepiece lens power by the objective lens power.

So, if you couple a 10x eyepiece lens with a 40x objective lens, the total magnification is of  $10 \times 40 = 400$  times.

4. **Stage clips:** hold the slide in place.
5. **Stage:** it is a flat platform that supports the slide being analyzed.





# Microscope parts and its functions

**6. Diaphragm:** it controls the intensity and size of the cone light projected on the specimen. As a rule of thumb, the more transparent the specimen, less light is required.

**7. Light source:** it projects light upwards through the diaphragm, slide and lenses.

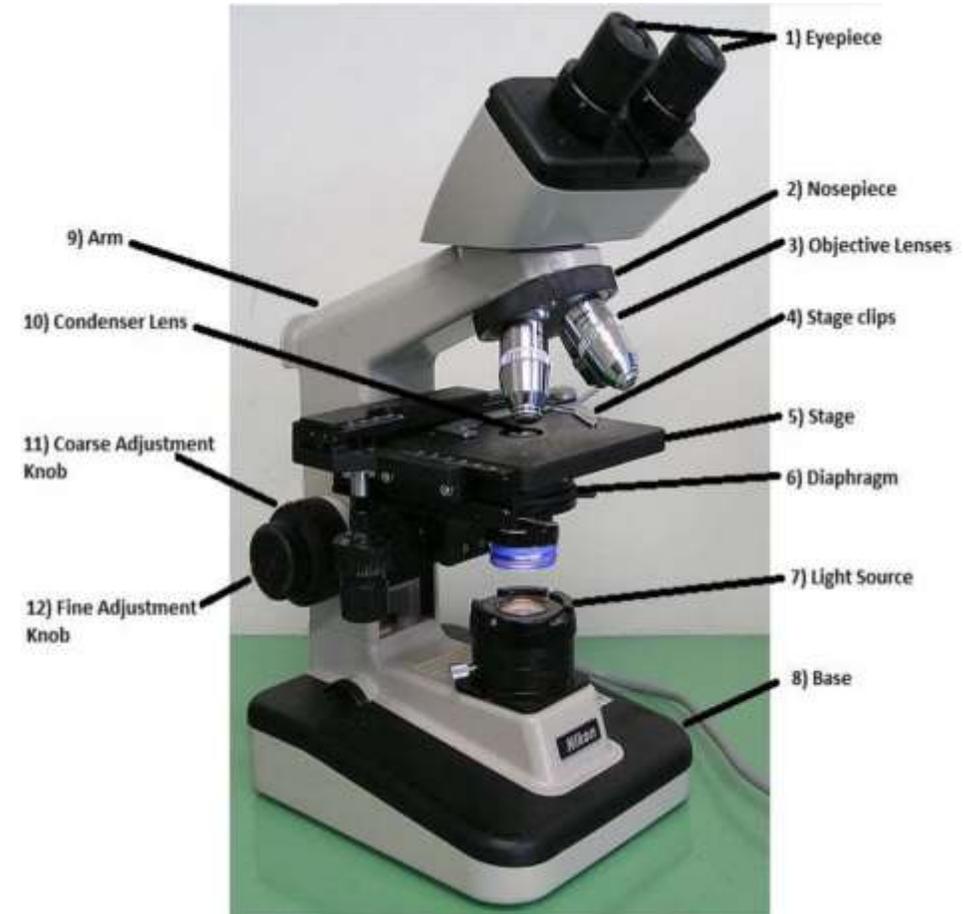
**8. Base:** supports the microscope.

**9. Condenser lens:** it helps to focus the light onto the sample analyzed. They are particularly helpful when coupled with the highest objective lens.

**10. Coarse adjustment knob:** when the knob is turned, the stage moves up or down, in order to coarse adjust the focus.

**11. Fine adjustment knob:** used fine adjust the focus.

**12. Arm:** supports the microscope when carried.

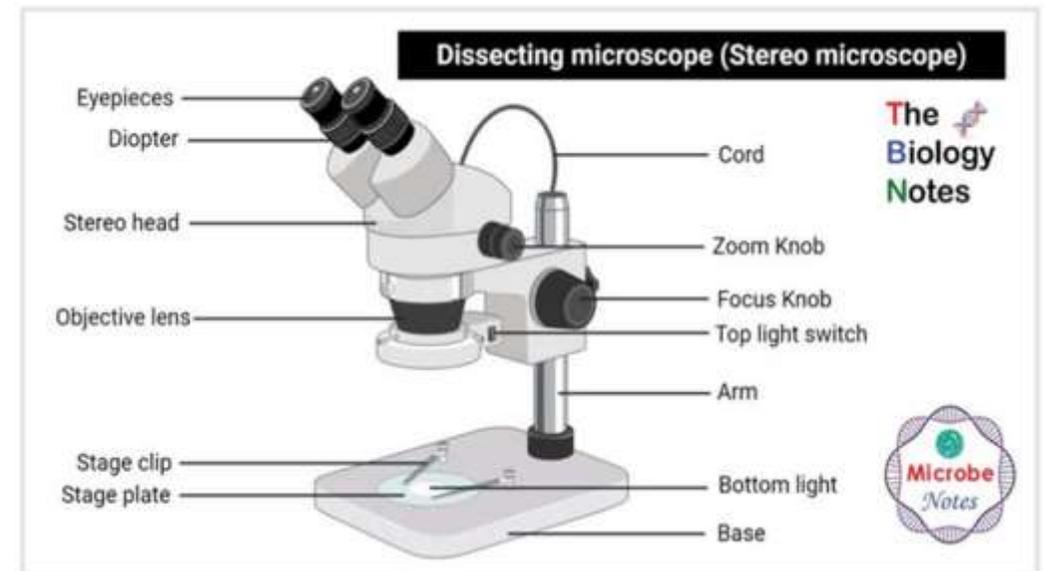




## 2. Stereoscopic (Dissecting) microscope:

It is two microscopes in one, and uses **two separate optical** Bathways shafts, which focus on the same point from different angles to produce a **three-dimensional visualization of the examined sample**. It provides slightly different viewing angles to the left and right eyes because using two separate optical paths with two objectives and two eyepieces.

Stereo microscope is relatively **low power compared with compound microscopes (below 100 X)**. It is often used to study the **surfaces of solid specimens** or to carry out close work such as sorting, dissection, microsurgery, small circuit board manufacture or inspection, and the like.





### 3. Inverted Microscope:

This kind of microscope views objects from an inverted position than that of regular microscopes.

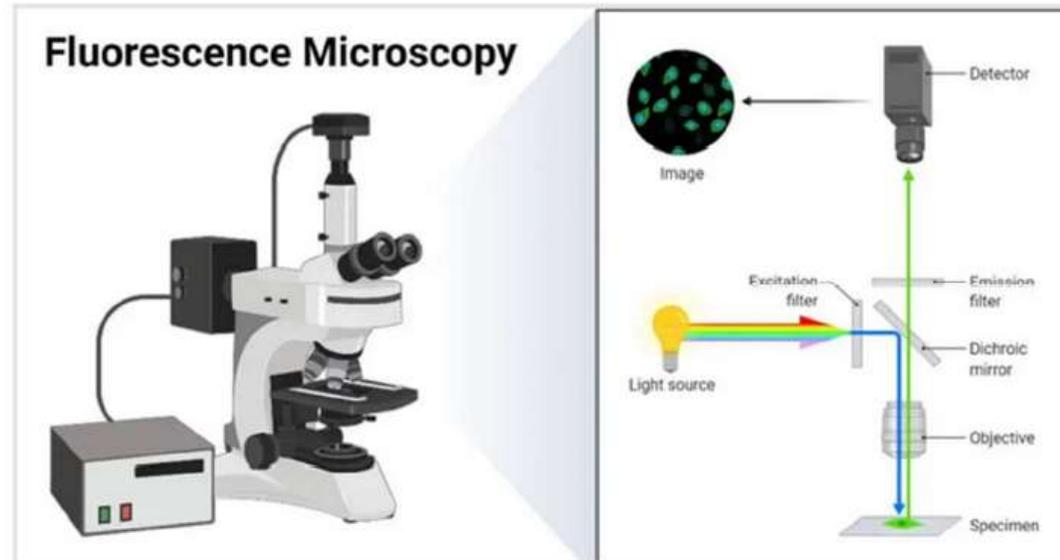
- It used to the study cell cultures in liquid media





## 4. Fluorescence microscope

- Fluorescence microscope is widely used device in the [life sciences and biology](#).
- It is a light microscope used to study properties of organic or inorganic substances using the phenomena of [fluorescence and phosphorescence](#).
- Both of an [excitation and emission filters](#) used in the fluorescent microscope.





## 5. Digital microscopes

- Traditional optical, stereoscopic and inverted microscopes have been recently modified into digital microscopes.
- These modified microscopes constructed from computer units attached to camera devices.
- Computer software converts the images to be displayed on a high resolution LCD monitor instead of direct viewing.



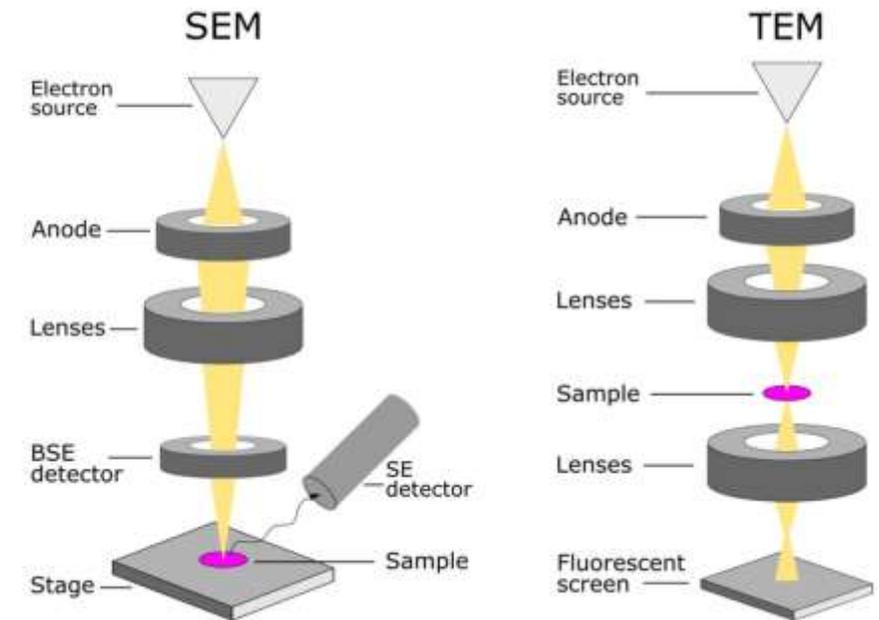


## 6. Electron microscopes

- Electron microscopy employs electron waves running parallel to a magnetic field providing higher resolution.
- Electron microscopy allows one to visualize objects that are as small as 1 nm.
- Electron microscopy is a high- cost technology use very expensive materials such as osmium gold-palladium or carbon or platinum.

1-Scanning electron microscope (SEM):

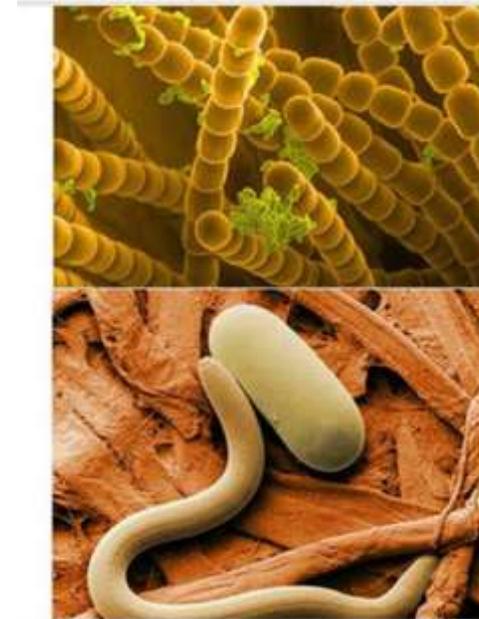
2- Transmission electron microscope (TEM):





## 1-Scanning electron microscope (SEM):

Scanning electron microscope used to visualize the **surface of tissues, macromolecular aggregates.**





## 2. Transmission electron microscope

- Used to study the inner structure of objects (tissues, cells, viruses).



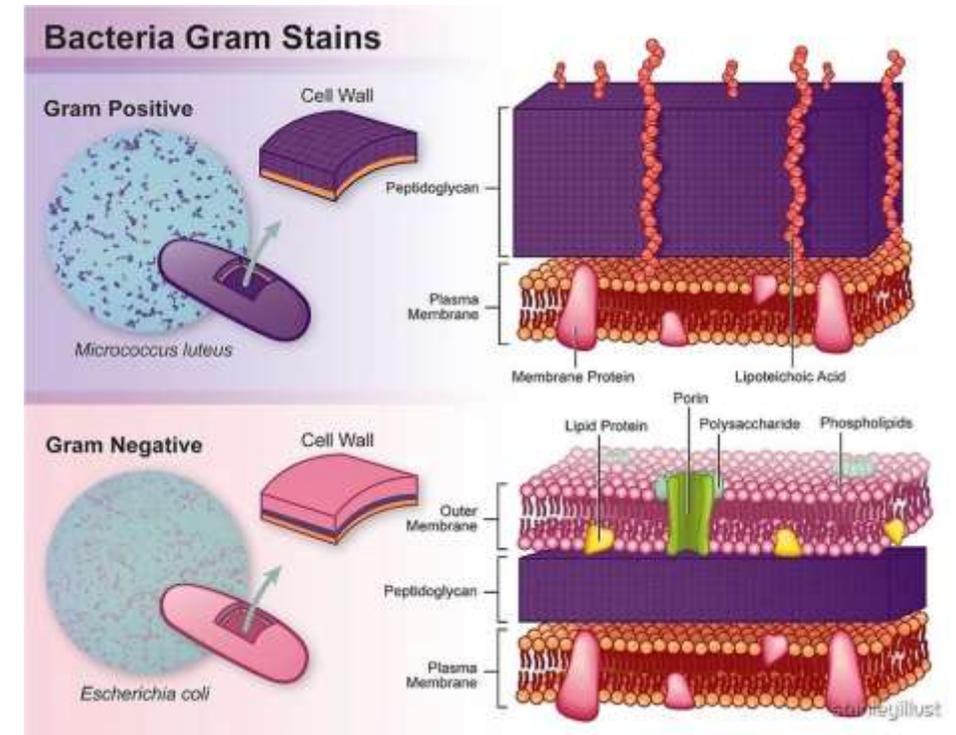


# Gram Staining

- It separates bacteria into two major groups:

- Gram +ve Bacteria.
- Gram -ve Bacteria.

- This technique was invented by Danish **Dr Hans Christian Gram in 1884.**





# Principle

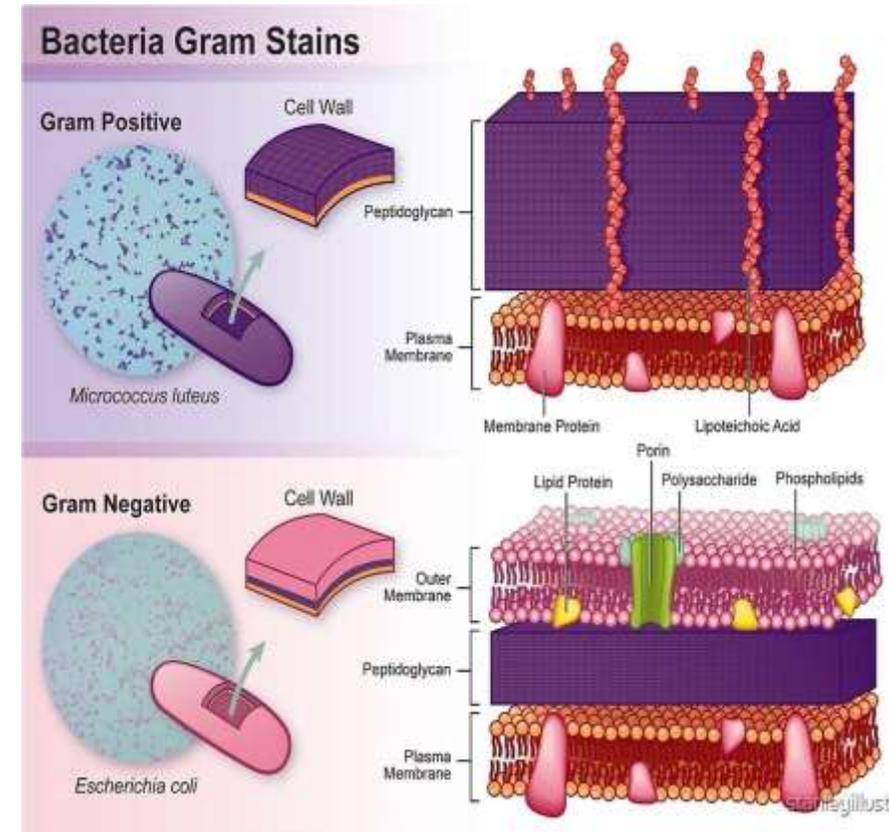
• The Gram stain technique is based on the differential structure of the cellular membranes and cell walls of Gram +ve and Gram -ve bacteria.

• Gram +ve Bacteria:

• Gram-positive organisms contain a thick and highly cross-linked layer of peptidoglycan that retains/hold the primary dye, Crystal Violet (CV), following the application of the mordant, iodine (I).

• The iodine and crystal violet form a complex (CV-I) within the peptidoglycan.

• When decolorizer is applied to the cells, the CV-I complex remains within the cell, making it appear dark purple to blue.



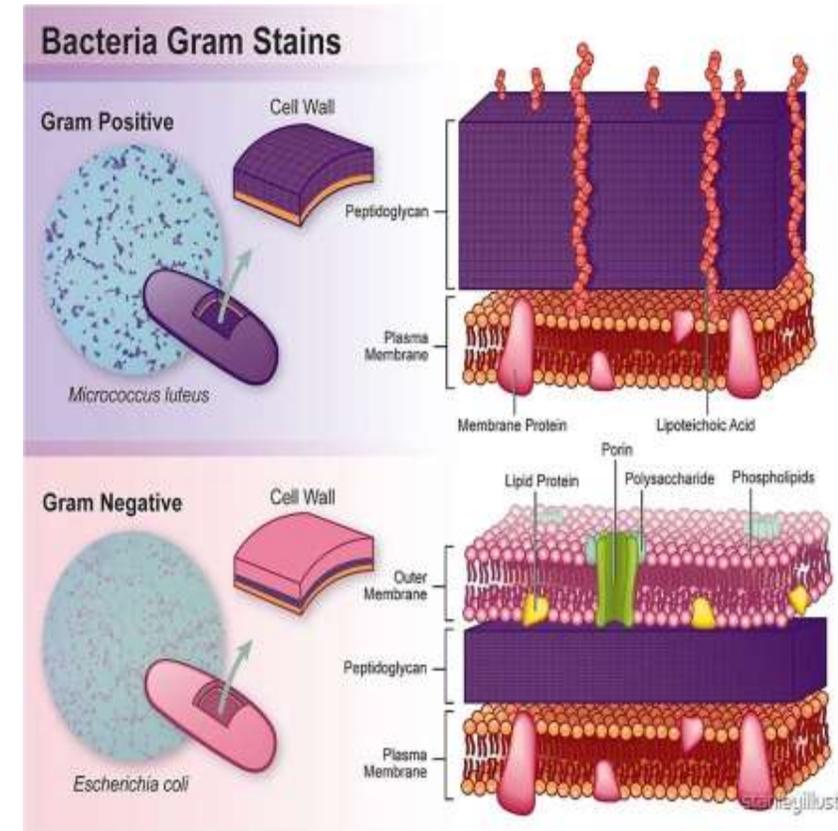


# Principle

## • Gram -ve Bacteria:

- The gram-negative organisms **do not** contain a thick cross-linked layer of peptidoglycan, which is loosely distributed.
- Following the application of the crystal violet and iodine, the CV- I complexes are not trapped within the peptidoglycan.
- Application of the acid-alcohol decolorizer dehydrates the outer cellular membrane, leaving holes in the membrane and effectively washing or removing the CV-I complex from the cells.

Then, a secondary/counter stain, safranin, is applied, making the Gram-negative cells pink/red.





## **Materials:**

Glass slides and cover slips.

Bacterial loop.

Sterilized distilled water.

Staining rack

**Crystal violet (Primary Stain)**

**Gram's iodine (Mordant)**

**Alcohol (decolorizer)**

**Safranin**

**(Secondary/counter stain)**

Bibulous paper

Compound microscope

## **Procedure of Gram Staining**

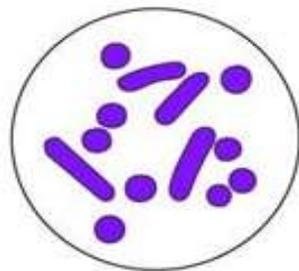
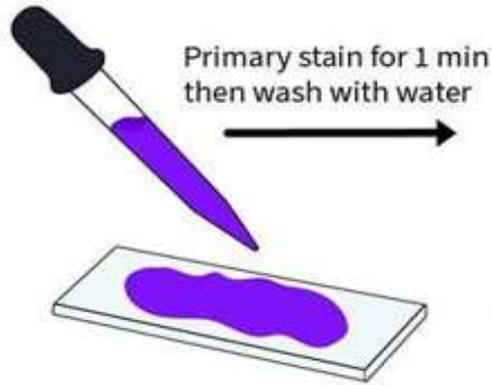
1. Take a clean, grease free slide.
2. Prepare the **smear** of suspension on the clean slide with a loopful of sample.
3. **Air dry** and **heat fix**
4. **Crystal Violet** was poured and kept for about 30 seconds to 1 minutes and rinse with water.
5. Flood the **gram's iodine** for 1 minute and wash with water.
6. Then, wash with **alcohol** for about 10-20 seconds and rinse with water.
7. Add **safranin** for about 1 minute and wash with water.
8. Air dry, Blot dry and Observe under Microscope.



## Gram Staining procedure

1. **Primary Stain:**  
Crystal violet

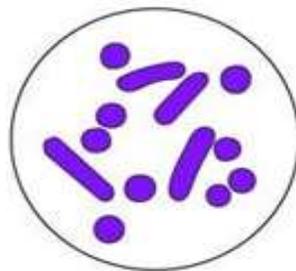
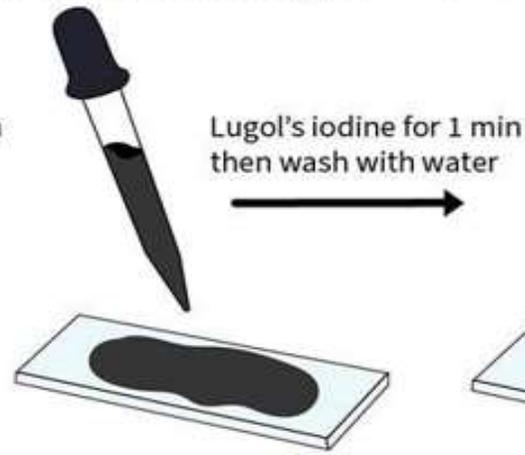
All bacteria will be stained bluish or bluish-purple



Gram +ve: Purple  
Gram - ve: Purple

2. **Mordant:**  
Gram's Iodine

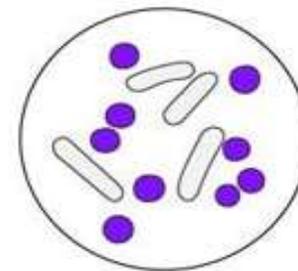
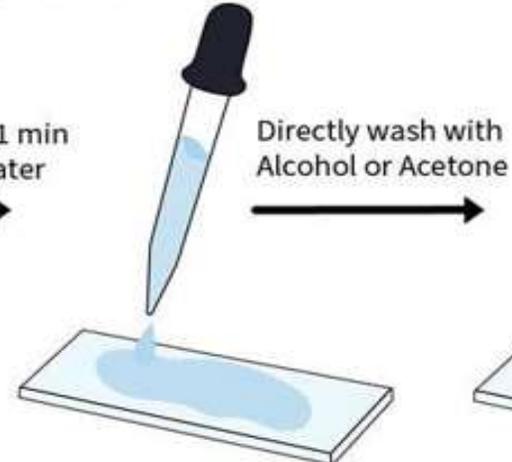
Enhance the Crystal violet staining by forming Crystal violet - Iodine complex



Gram +ve: Purple  
Gram - ve: Purple

3. **Decolorizer:**  
Alcohol/Acetone

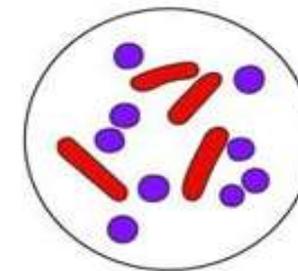
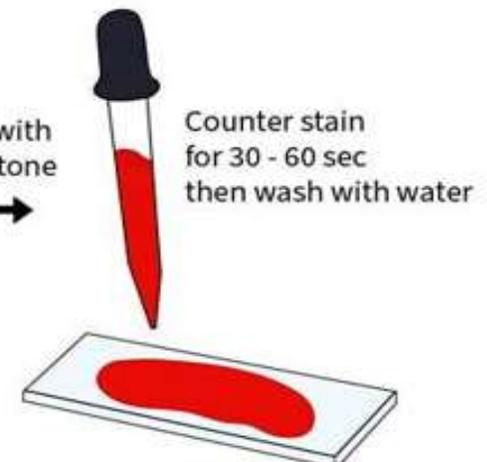
Decolorizer washes away Primary stain from gram -ve bacteria



Gram +ve: Purple  
Gram - ve: Colorless

4. **Counter Stain:**  
Safranin/Carbol fuchsin

Counterstain stains the colorless gram -ve bacteria



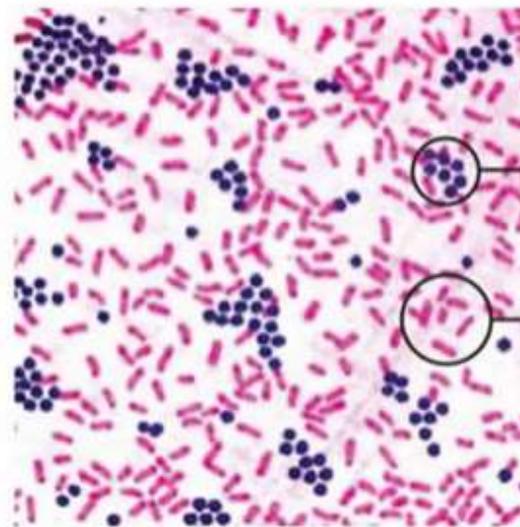
Gram +ve: Purple  
Gram - ve: Red



## Interpretation

Gram Positive: **Blue/Purple Color**

Gram Negative: **Red Color**



Gram-positive  
Cocci (spherical)

Gram-negative  
Bacilli (rod-shaped)

## Examples

**Gram Positive Bacteria:** *Actinomyces*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Gardnerella*, *Lactobacillus*, *Listeria*, *Mycoplasma*, *Nocardia*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, etc.

**Gram Negative Bacteria:** *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, and other Enterobacteriaceae, *Pseudomonas*, *Moraxella*, *Helicobacter*, *Stenotrophomonas*, *Bdellovibrio*, acetic acid bacteria, *Legionella*



# Fungal staining

## Lactophenol Cotton Blue (LPCB) Staining

### **Principle:**

Lactophenol Cotton Blue (LPCB) staining method works on the principle of aiding the **identification of the fungal cell walls.**

- Fungi are eukaryotic organisms with both macroscopic and microscopic characteristics.
- The fungal cell wall is made up of chitin of which the components of the Lactophenol Cotton Blue solution stains for identification.
- The lactophenol cotton blue solution **acts as a mounting solution as well as a staining agent.**
- The solution is clear and blue in color and it is made up of a combination of three main reagents:
  - Phenol: It acts as a disinfectant by killing any living organisms
  - Lactic acid: To preserve the fungal structures
  - Cotton blue: To stain or give color to the chitin on the fungal cell wall and other fungal structures
- The stain will give the fungi a blue-colored appearance of the fungal spores and structures, such as hyphae.



## Fungal staining

### **Objective:**

To learn the simplest preparation technique of stained fungal slides and its value in fungal identification.

### **Materials**

Fungal cultures on PDA agar (Aspergillus, Penicillium, Rhizopus & Saccharomyces).

Clear tape.

Glass slides.

Lactophenol cotton blue (for staining molds).

Light microscope.

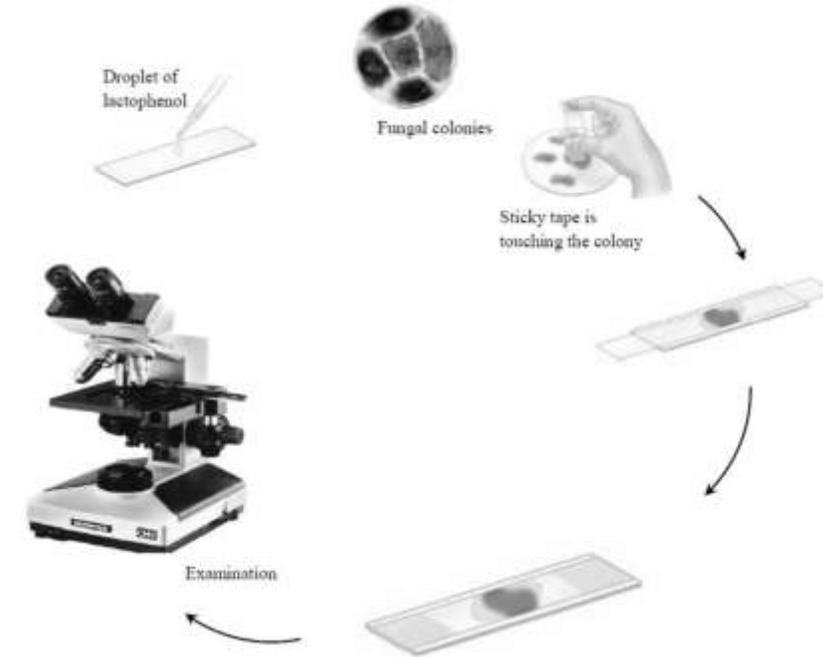
Miscellaneous supplies.

Lens paper.



## Procedure:

1. Examine the plated cultures of the fungi, recording all colony characters that may help recognizing fungal genes.
2. After examining the colonies, make a pressure tape preparation of the fungal cultures as following:
  - a. Place a drop of lactophenol cotton blue on the center of the slide using a fine dropper.
  - b. Hold a piece of clear sticky tape in a U-shape, sticky side down as illustrated in the fig.
  - c. Touch the surface of a fungal colony with the sticky tape.
  - d. Place the tape sticky side down in a lactophenol cotton blue drop.
  - f. Examine this preparation using the light microscope then record your observations.



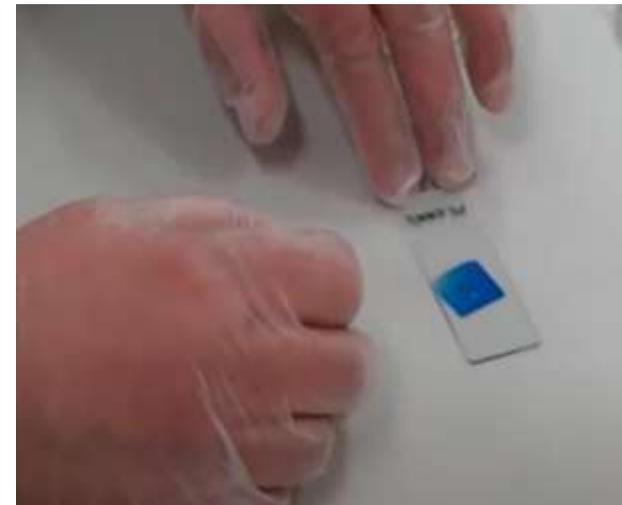
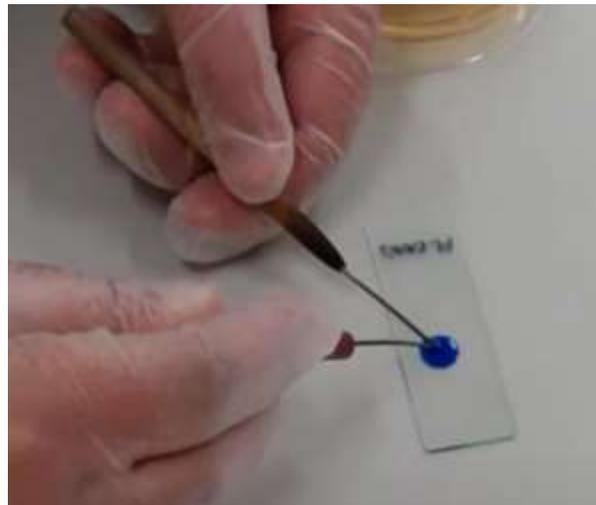


# Microscopic Observation

Using Stereo Microscope &  
Compound Light Microscope

## Lacto phenol Cotton Blue Staining (LPCB)

A portion of mycelial colony is picked with needle mounted on a slide , stained with LPCH and observed under a microscope after placing a cover slip carefully



Slide preparation

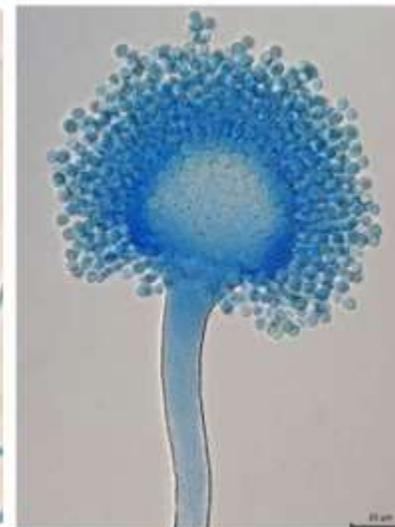
Microscopic observation



*Penicillium sp*



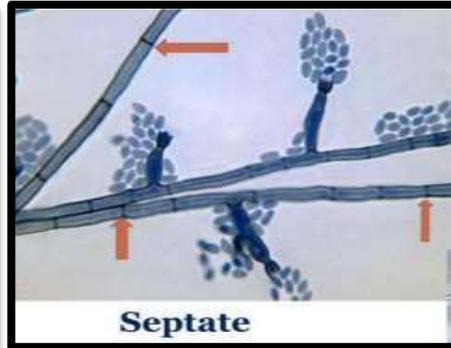
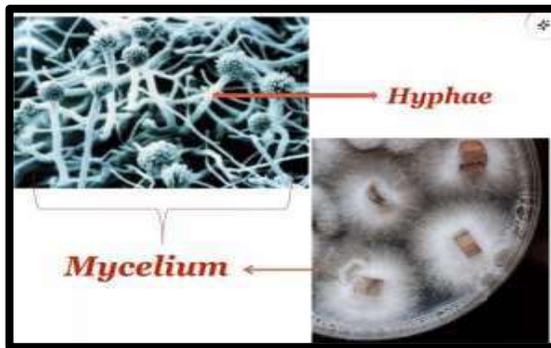
*Fusarium sp*



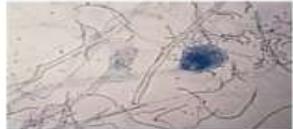
*Aspergillus sp*

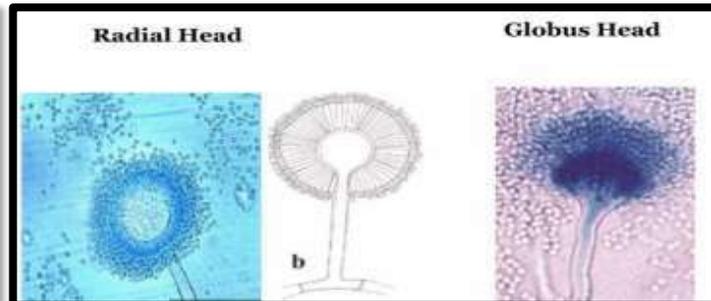
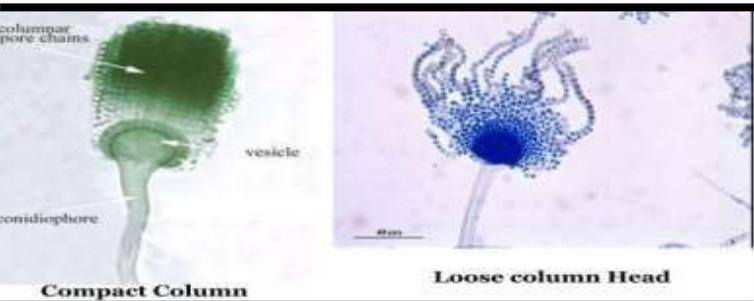


- The following structures are observed under a microscope:
- a) Unicellular or multicellular- (yeast/ molds)
- b) Hyphae-septate/ aseptate/pseudohyphae and branching
- c) Conidia/spores-- shape, size, colour and conidiophores
- d) Presence of macrospores, microspores, arthroconidia,
- E) Fruiting bodies

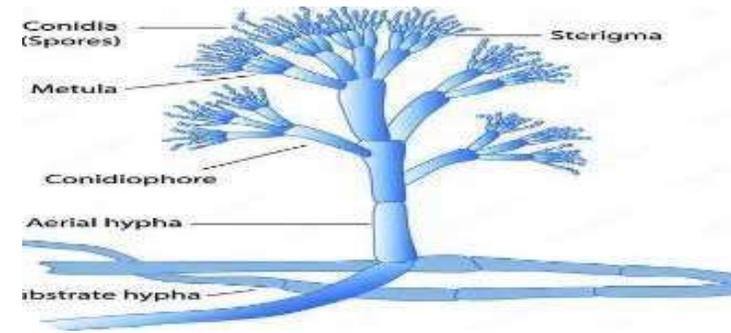


### Hyphae

- Hyaline 
- Dematiaceous 



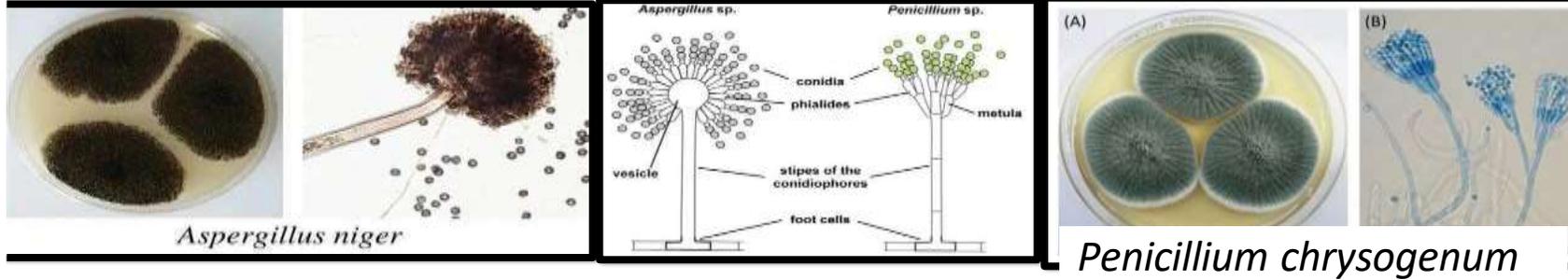
Conidia heads



brush-like conidial arrangement in penicillium.

# Conidiospores-Aspergillus and Penicillium sp.

- **Fungi reproduce asexually by conidiospores and sporangiospores.**



*Aspergillus* and *Penicillium* are common contaminant molds, and they **reproduce by conidiospores**

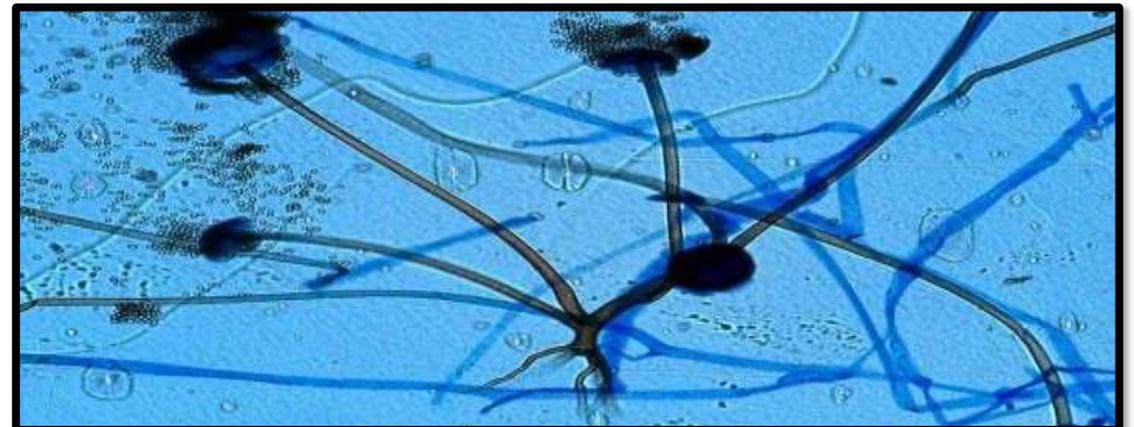
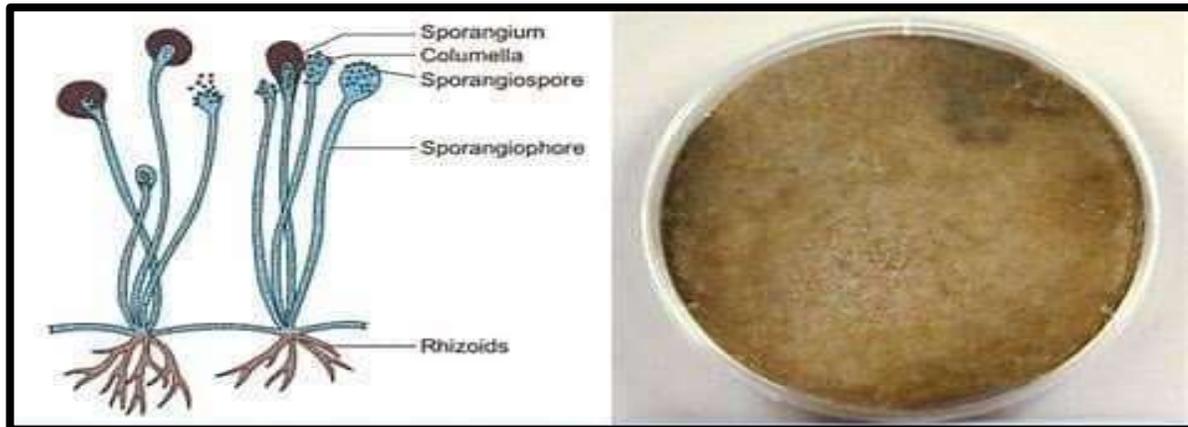
- *Aspergillus*, although nonpathogenic, may become opportunistic and cause severe respiratory tract infection in a compromised host.
- They also produce mycotoxins, which cause serious health issues and cancer.
- The conidiophore terminates in a ball-like structure called a vesicle.
- Its **conidiospores**, which typically appear brown to black, are **produced in chains on phialides coming off the vesicle**
- *Penicillium* is one of the most common household molds and is a frequent food contaminant.

The conidiospores of *Penicillium* usually appear grey, green, or blue and are produced in chains on finger-like projections called phialides coming off the conidiophore.



# Sporangiospores- *Rhizopus stolonifer*

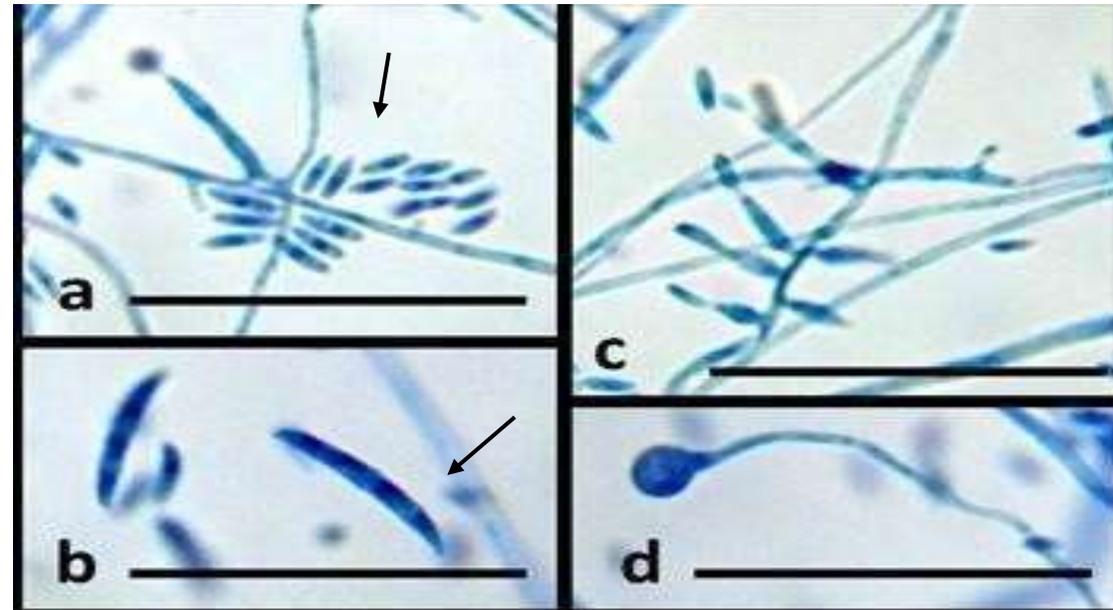
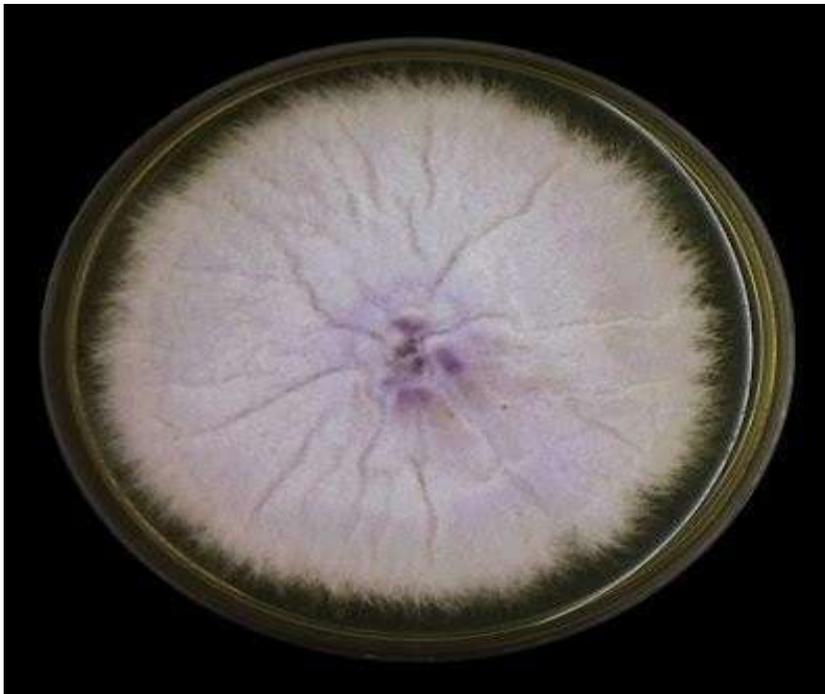
- Sporangiospores are **produced within a sac or sporangium on an aerial hypha called a sporangiophore**
- *Rhizopus* is an **example of a mold that produces sporangiospores**.
- Although usually nonpathogenic, it sometimes causes opportunistic wound and respiratory infections in the compromised host.
  
- At the end of its sporangiophore is **dome-shaped end called a columella** that **extends into a sac-like structure called a sporangium**. Its sporangiospores, typically brown or black, are produced within the sporangium .
- Anchoring structures **called rhizoids** are also produced on the vegetative hyphae.



# Microspores and Macrospores



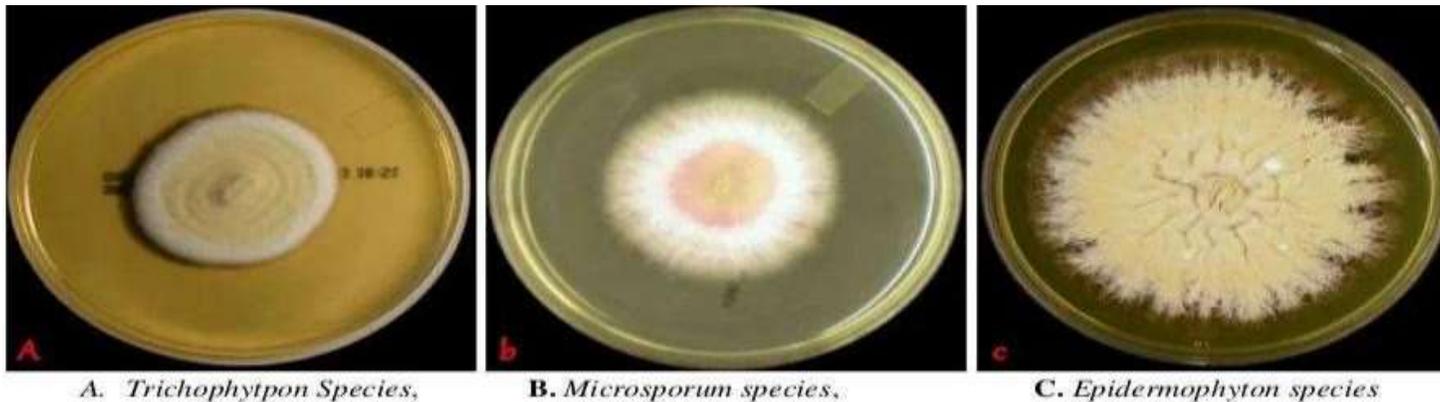
- Fusarium sp. grows really fast with varying colony colour depending on isolates.
- **Woolly to cottony, flat, spreading colonies. Within few days cover the entire agar plate.**
- Conidia are the spores produced by Fusarium.
- They are two types- **macro and micro-conidia** both of which can be seen under microscope.
- Macroconidia are multicelled while micronidia are single celled



Macroconidia and Micro-conidia

# Dermatophytes

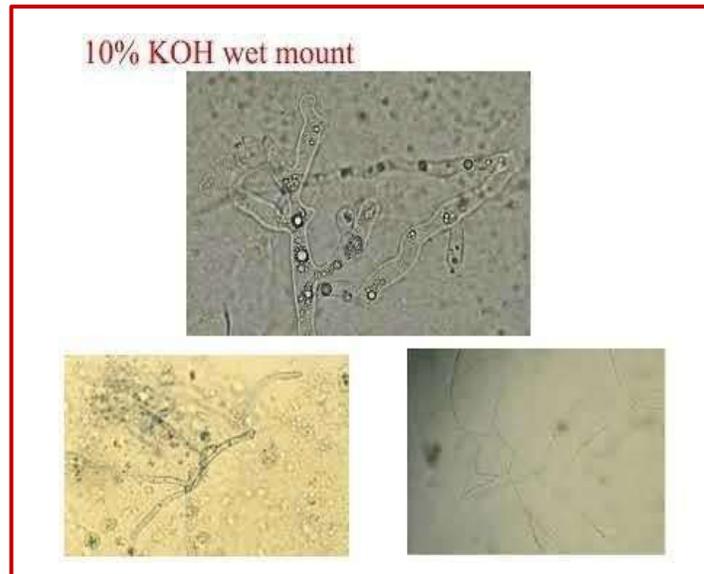
- The dermatophytes are a group of molds that cause superficial mycoses an infection of the hair, skin, and nails.
- They feed on the protein keratin, present in hair, skin, and nails.
- Infections are commonly referred to as ringworm or tinea infections.
- The three common dermatophytes are *Microsporum*, *Trichophyton*, and *Epidermophyton*. These organisms grow well at 25°C.



Macroscopic (colony) appearance of various dermatophytes on SDA.

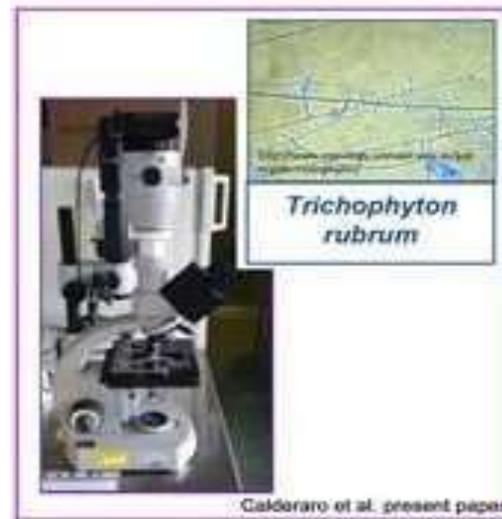


# Identification of dermatophytes



KOH mounts

Microscopy



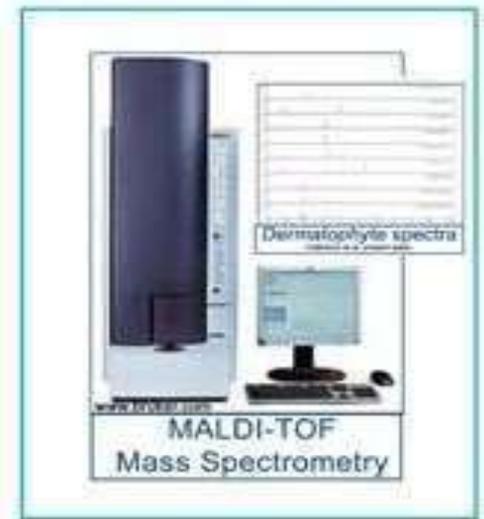
## DERMATOPHYTES IDENTIFICATION



Culture



MALDI-TOF MS



NEW