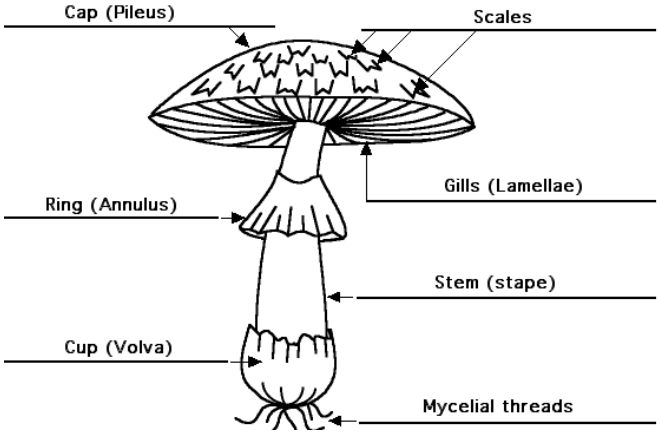
The branch of biology which deals with the study of microorganism are called as “Microbiology”. the biosphere i.e the biotic and abiotic component has a variety of microorganisms that exhibit beneficial activities. They include small algae, fungi, bacteria, protozoans, mycoplasma and related organisms. A large number of microbes help human civilization through their useful activities. These activities are either of domestic, industrial or commercial importance. It has several applied branches such as medical microbiology, food microbiology, industrial microbiology, etc.

Microbial identification is **a clear characterization of a certain microorganism through an appropriate test method able to return the name of the analyzed species**. What are the three methods of microbial identification? The three methods used for microbial identification are genotypic, proteotypic, and phenotypic.

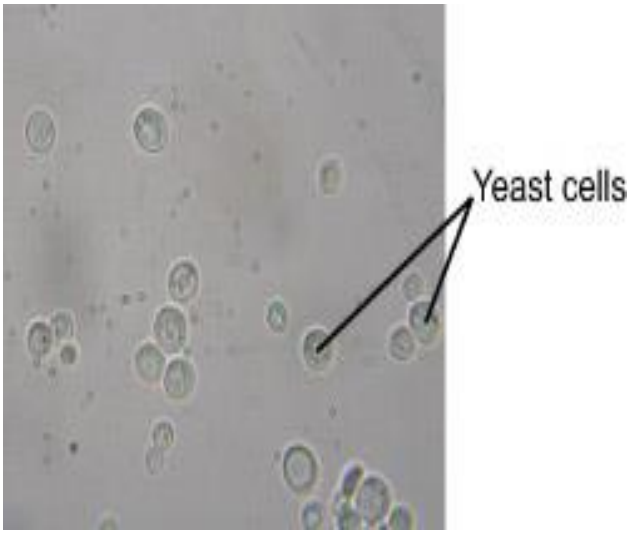
1. Algae are classified into four major groups (palmer 1962): **Blue-greens, greens, diatoms and Flagellates**. By knowing the major group of your algae, verification and identification to the genus and species level with the use of a microscope and classification key becomes easier.
2. The conventional method of ECM fungal identification involves noting the morphological characteristics of mushrooms such as their size, color, presence or absence of volva, stipe, ring, scales, reticulum, zonation, striation, warts, cap, areolae, and gills. Diagnosis of fungal infection has relied primarily on methods such as **direct microscopic examination of clinical samples, histopathology, and culture**. Such approaches are dependent on personnel with relatively high levels of specific mycology training.



3. Yeast identification can be achieved by **using biochemical criteria i.e. ability of yeasts to assimilate certain carbon and nitrogen compounds (Assimilation reactions) and to ferment sugars (fermentation tests). To observe the yeast under the microscope:**

a. Place a drop of the yeast mixture on the microscope slide (it might be necessary to dilute it a bit more with water).

b. Place a coverslip on top and observe under different magnifications. High magnifications will be needed to see the yeast well.



4. Bacteria are identified routinely by **morphological and biochemical tests, supplemented as needed by specialized tests such as serotyping and antibiotic inhibition patterns**. Newer molecular techniques permit species to be identified by their genetic sequences, sometimes directly from the clinical specimen. Isolation:

Identification of microbes- Microbes due to their size their identification is difficult process because odf their use in human civilization the identification of microbes is th is the important process through which only identical strain of microbes can collect for their uses in industry and human welfare. The many techniques like staining, identification of their products and several instrumentation process are applying for the identification of microbes. Methods for microorganism identification:

A. Chromogenic media (Chromogenic media **utilize synthetic chromogenic enzyme substrates in order to specifically target pathogenic species (or groups of species) based on their enzyme activity**. Such enzyme activity is never completely species specific, necessitating the use of complementary enzyme substrates and/or selective agents)**.**

B. Microscopy

C. Biochemical

D. Molecular techniques.

E. Traditional Methods for Identifying Microbes

a. Examining Agar Cultures- Agar, or agar-agar, is **widely used as a culture medium for growing micro-organisms**. The stuff itself is also the product of micro-organisms. Examples are following

I. XLD agar -Salmonella

II. S_1 medium- (Sucrose, SLS, casamino acids, Trimethoprim)-Fluorescesnt Pseudomonads

III. Agar medium containing K+ buffers, Mg_2+ salts, Trace elements, $NaNO_2$, Bromothymol blue Nitrosomonas and Nitrobacter

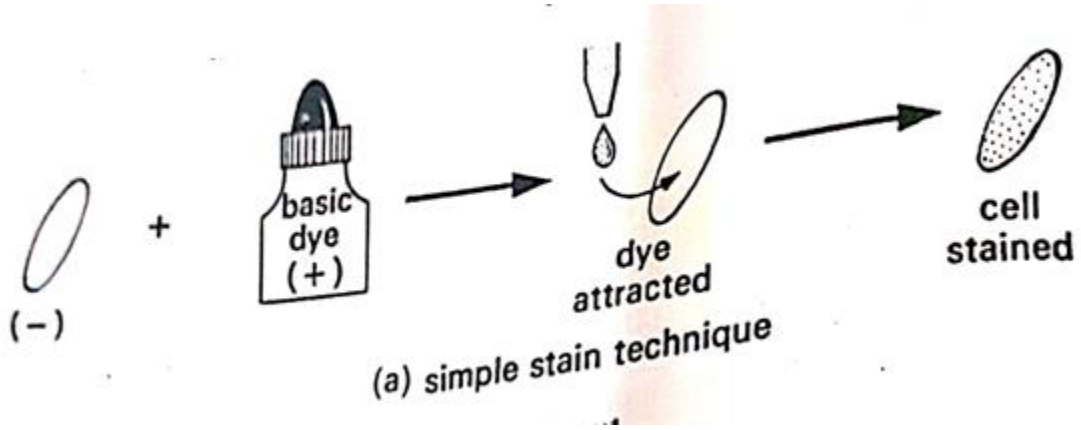
IV. BG_{11} medium BGA Bold's Basa medium (+2000-3000 lux light)

V. Green and yellow-green- Algae

VI. PDA, MEA (+Rose Bengal) -Fungi

b. Simple stain technique- A small amount of bacteria is placed in a droplet of water on glass slide. It is air dried slide passed through a flame for heat fixation on slide, kill organism and prepare them for staining.

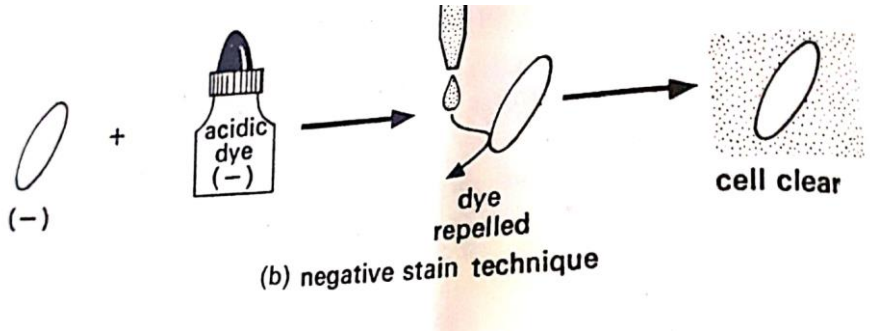
Now the slide flooded with basic dye such as crystal violet or methylene blue for a second or minute. Possitive charged dye which attracted to the negative charged cytoplasm of bacteria will take the stain> it is effective for vegetative cells, stain does not penetrate spores**.**



(a) simple stain technique

c. Negative stain technique- Bacteria mixed with slide with an acidic dye such as congo red, nigrosin . A small amount of bacteria is placed in a droplet of water on glass slide.It is air dried.slide passed through a flame for heat fixation on slide, kill organism and prepare them for

staining Negative charged stain is repelled by bacteria which has negatively charged cytoplasm but the stain will gathered around the bacteria.



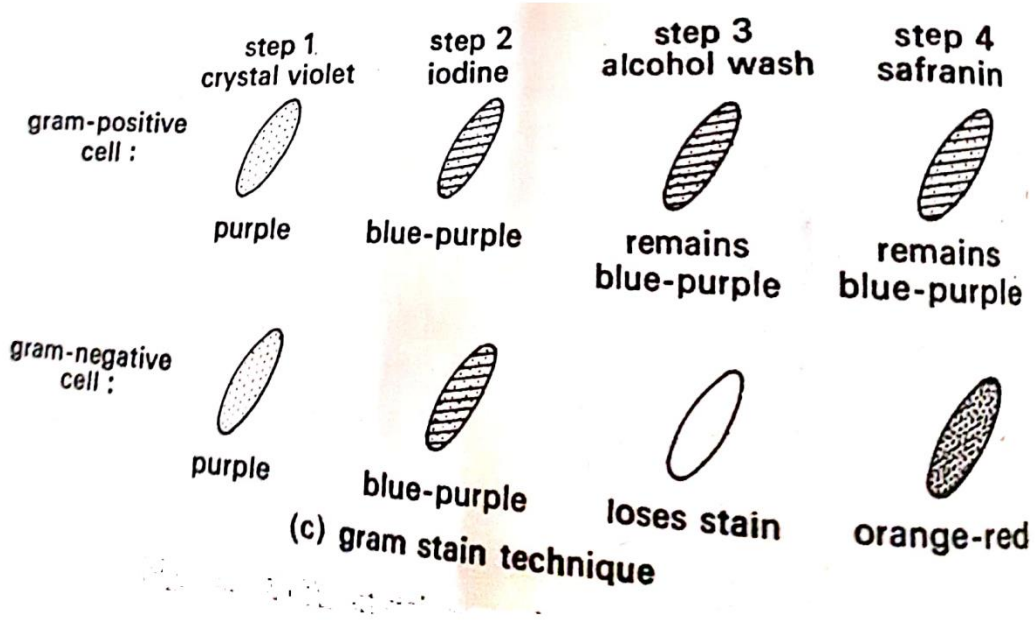
(b) negative stain technique

d. Gram Staining- **Gram staining technique-** A Danish sholar Christian Gram in 1884 devised differential stain g procedure which differentiate gram positive and gram negative bacteria.

In this process

a) a thin smear of bacterium is prepared on glass slide.
b) Crystal violet stain is applied on smear for 30 seconds.
c) Clean the slide rinsed with distilled water
d) Apply the iodine solution for 30 seconds
e) Blue purple color will appear on smear
f) Rinsed the slide with 95% ethyl alcohol

Microscopic examination will show violet color represent the appearance of Gram positive bacteria while Gram negative will show their natural color or colorless.



e. **Endospore Staining**- Endospore staining is a technique used in bacteriology to identify the presence of endospores in a bacterial sample. Within bacteria, endospores are protective structures used to survive extreme conditions, including high temperatures making them highly resistant to chemicals.

Application	Reagant	Cell Color	
		Vegetative Cell	Endospore
Primary Stain	Malachite Green	Green	Green
Mordant	Heat(Steam)	Green	Green
Decolorizer	Distilled Water	Colorless	Colorless with Green endospore
Counter Stain	Safranin	Pink	Pink with Green endospore

f. **Ziehl-Neelsen Staining**- Conventional smear microscopy with the Ziehl-Neelsen (ZN) stain is a rapid and practical method for **detecting acid-fast bacilli (AFB)**, especially in low-income countries, due to its rapidity, low cost, and high positive predictive value for tuberculosis

I. Step 2: Smear Preparation (Review) ...
II. Cover the smear with carbolfuchsin dye. ...
III. Dry heat for 2 minutes.
IV. Cool and rinse with water. ...

V. Wash the top and bottom of slide with water and clean the slide bottom well.

VI. Counterstain with Methylene Blue for 30 seconds to 1 minute.

g. **Stains for Fungi and Yeast**- The Grocott's silver (GMS) stain is probably the most widely used fungal stain, however, the somewhat capricious nature of silver staining may challenge even the most experienced of histology practitioners. o determine the viability of your yeast, you must stain it with either methylene violet or methylene blue. For brewer's yeast, the best staining technique requires using methylene violet.

h. **Catalase Testing**- The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. If an organism can produce catalase, it will produce bubbles of oxygen when hydrogen peroxide is added to it. The catalase test facilitates the detection of this enzyme in bacteria. It is essential for differentiating catalase-positive Micrococcaceae from catalase-negative Streptococcaceae. While it is primarily useful in differentiating between genera, it is also valuable in speciation of certain gram positives.

i. **Oxidase Testing-** Oxidase Test. The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. (note: All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration.

j. **Substrate Utilization** Tests- Bacteria have developed specific substrate utilization profiles which allow them to grow only under specific conditions. They assimilate different organic substrates as carbon and energy sources for their growth and biomass production.

k. **Microscopic techniques** Bacteria visualized under compound microscope for motility, size, shape, spores, capsulation and morphological appearance of their colony. Motility is studied by hanging drop , whereas size and shape etc can be stained and identified under microscope,

1. **Hanging drop method**- It require cavity slide, inoculation loop, cover slip, inoculums, Parafin wex, microscope. In this process

I. Take the sterilized inoculation loop

II. Collect the film of inoculums with the help of loop.

III. Drop the culture at center of cover slip

IV. Placed the wax on all four corners of coverslip

V. Now invert the coverslip on cavity slide as inoculums drop ids hanging on the center of slide

VI. Observed under microscope on 10x20 or 10x100 magnification.

VII. Ife bacteria is moving across the microscope field shows that bteria is motile

2. Bacterial motility- On the basis of flagellum bacteria are of following types

I. Atrichous- Flagella absent

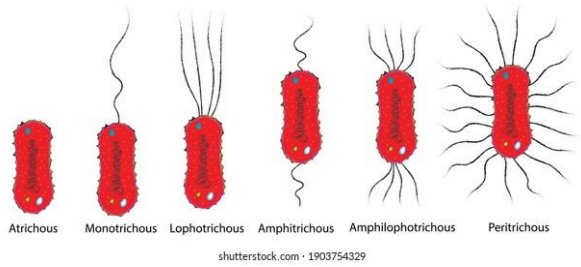
II. **Monotrichus-** Singlr flagrlla

III. Lophotrichous- A group or tuft of flagella is found on one end

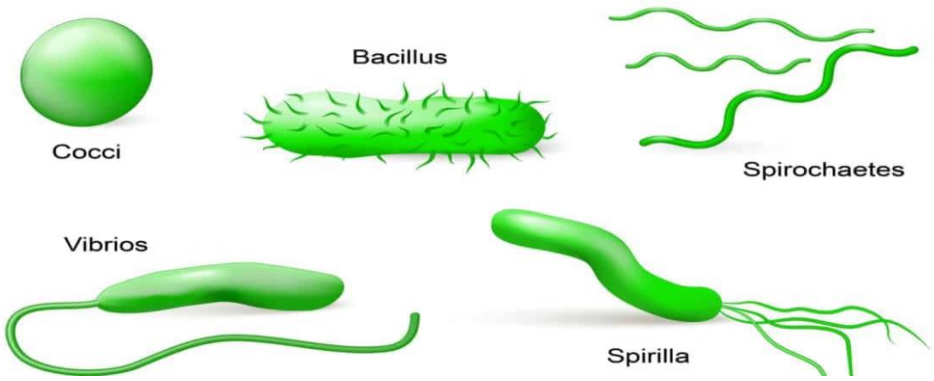
IV. Amphitrichous- flagellum at each side or end

V. Peritrichous -Flagellum found on all surface of bacterium'

VI. Cephalotrichoud- A tuft of flagellum found on both the end



1. **Spherical- Cocci**
2. **Rod-shaped- Bacilli**
3. **Spiral bacteria**
4. **Comma shaped- Vibrio**



F. Modern approaches for the rapid identification of bacteria use molecular techniques, such as **16S ribosomal RNA gene sequencing based on polymerase chain reaction or electromigration, especially capillary zone electrophoresis and capillary isoelectric focusing**

a. Biochemical method- turbidimetry and colorimetry methods

b. **Molecular Method-** Molecular techniques have proven to be particularly useful in the identification of microorganisms that are difficult to detect by phenotypic methods. Molecular biology methods can be used to confirm the presence of a specific microorganism in the tested material, as well as screen research. The most commonly used molecular methods include **single gene sequencing (e.g., by sequencing ribosomal RNA encoding 16S, 23S, 18S, and ITS gene), multiple gene sequencing (housekeeping and pathogenic genes), and whole-genome sequencing**. amplification of species-specific PCR products (15), PCR-based methods, and 16S rRNA/16S ribosomal DNA (rDNA) gene sequencing (currently considered the gold standard in identifying microorganisms; 48–52).

c. **Spectrometric Techniques** MALDI-TOF MS is the latest next generation tool being used for the rapid identification and classification of microorganisms (Figure 1). The principle of this method is based on the gentle ionization of intact microorganism cells with short laser pulses and then accelerating the particles in a vacuum by way of an electric field. As a result of microorganism ionization, a specific molecular fingerprint (spectra profile) of the microorganism can be registered (82). Identification of the microorganism occurs as a result of comparing the spectral profile of the analyzed microorganism against a database using an automated program (82). The most commonly used MS technique for the identification of microorganisms is protein analysis (83)

d. **Electromigration Techniques**- In recent years, electromigration techniques have become a tool of increasing interest for the analysis of microorganisms. These techniques are based on the migration of charged particles under the influence of a homogeneous electric field