

CRITERIA FOR CLASSIFICATION OF MICRO- ORGANISM

Presented by:-

SREEREMYA.S

M.phil

Taxonomy

- Organizing, classifying and naming living things
- Formal system originated by Carl von Linné (1701-1778)
- Identifying and classifying organisms according to **specific** criteria
- Each organism placed into a classification system



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Western blot



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Taxonomy

- Domain
- Kingdom
- Phylum
- Class
- Order
- Family
- Genus
- species

3 Domains

- Eubacteria
 - true bacteria, peptidoglycan
- Archaea
 - odd bacteria that live in extreme environments, high salt, heat, etc. (usually called extremophiles)
- Eukarya
 - have a nucleus & organelles (humans, animals, plants)

Taxonomy

- 4 main kingdoms:
 - Protista
 - Fungi
 - Plantae
 - Animalia
 - *Algae*

Naming Microorganisms

- Binomial (scientific) nomenclature
- Gives each microbe 2 names:
 - **Genus** - noun, always capitalized
 - **species** - adjective, lowercase
- Both italicized or underlined
 - *Staphylococcus aureus* (*S. aureus*)
 - *Bacillus subtilis* (*B. subtilis*)
 - *Escherichia coli* (*E. coli*)

CRITERIA FOR IDENTIFICATION AND CLASSIFICATION

- Morphological Characteristics.
- Differential Staining.
- Biochemical Tests.
- Serology: Slide Agglutination Test, ELISA, Western Blot.
- Phage Typing.
- Amino Acid Sequencing



a. **Morphological characteristics**

- Cell shape and size, arrangement of cells, arrangement of flagella, capsule, endospores, mechanism of motility

b. **Differential staining**- staining properties
– e.g., **Gram stain reaction** and acid-fast stain reaction

Gram stain is an example of **differential staining**—procedures that are used to distinguish organisms based on their staining properties

Use of the Gram stain divides *Bacteria* into two classes—
gram negative and gram positive.

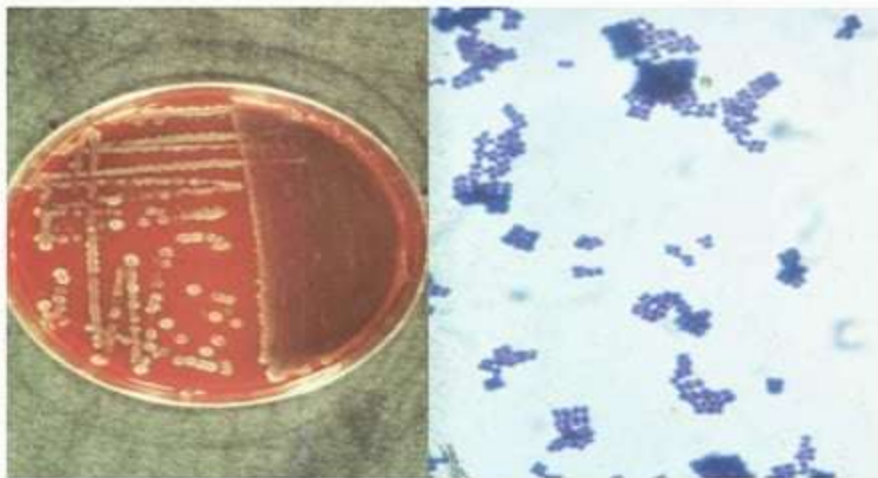
Gram Stain

- Based on cell wall composition and peptidoglycan thickness
- Gram positive cell wall
- Gram negative cell wall

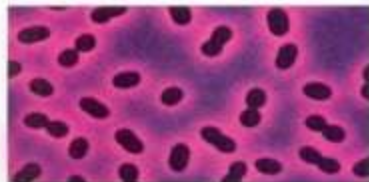
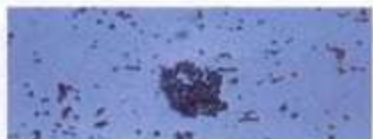


Morphological Characteristics

Colony Isolation & Gram Stain



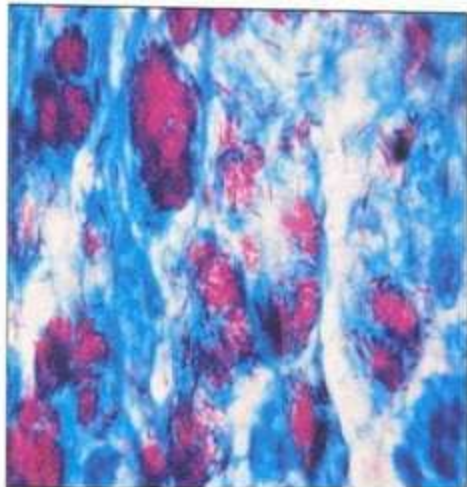
Gram-Stained Rods and Cocci




100x
200x
400x
600x
800x
1000x

Acid Fast Stain

- Red dye basic carbolfuchsin is the principal stain
- Background is counterstained with methylene blue
- Stain based on the mycolic (glycolipid) acid content of the cell wall
- Mycobacterium species is stained red, while background is stained blue

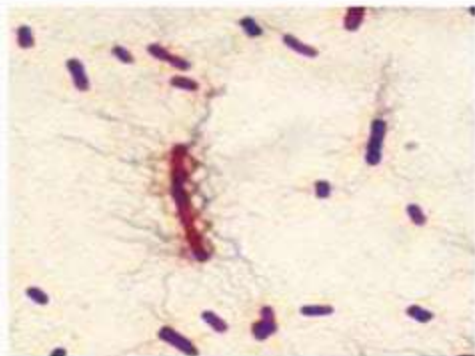




Acid-fast staining is another important differential staining procedure. It is most commonly used to identify *Mycobacterium tuberculosis* and *M. leprae* the pathogens responsible for tuberculosis and leprosy, respectively.

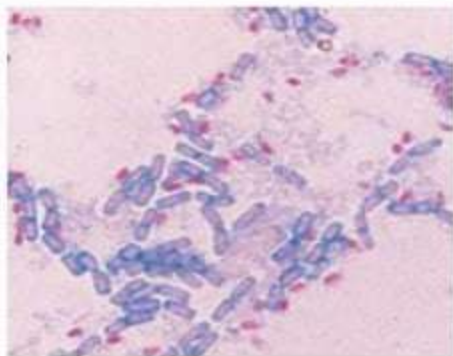
Other types of Stain

Flagella stain with
carbolfuchsin dye




Flagellar stain of *Proteus vulgaris*.
A basic stain was used to build up the
flagella

Endospore stain with
Schaeffer-Fulton stain



Endospore stain, showing endospores
(red) and vegetative cells (blue)



Endospore staining, like acid-fast staining, also requires harsh treatment to drive dye into a target, in this case an endospore.

An endospore is an exceptionally resistant structure produced by some bacterial genera (e.g., *Bacillus* and *Clostridium*). It is capable of surviving for long periods in an unfavorable environment and is called an endospore because it develops within the parent bacterial cell.

Quality assurance- PROTOZOA


*Microscope counts. Care must be taken to ensure that the particles being counted are (oo)cysts, to determine whether or not they contain sporozoites, and to exclude algae and yeast cells from any counts that are made. The criteria used for determining that a particle is in fact a *Cryptosporidium* oocyst or a *Giardia* cyst vary between laboratories. Some workers use only the fact that (oo)cysts fluoresce when labelled with a fluorescein isothiocyanate-tagged anti-*Cryptosporidium* or anti-*Giardia* monoclonal antibody and that it is in the proper size range for a cyst or oocyst. Others will additionally use differential interference contrast microscopy or nucleic acid stains to ascertain that the particles counted are indeed (oo)cysts. This more detailed analysis allows the confirmation of the counted particles as presumptive (oo)cysts.*

Many factors influence the microscope counts: the amount of background debris and background fluorescence, the experience and alertness of the technician who performs the count, the intensity of fluorescence after staining with the monoclonal antibody, and the quality of the microscope. Quality assurance protocols should define how these factors are addressed

NEGATIVE STAINING-

Negative staining is an easy, rapid, qualitative method for examining the structure of isolated organelles, individual macromolecules and **viruses** at the EM level. However, the method does not allow the high resolution examination of samples – for this more technically demanding methods, using rapid freezing and sample vitrification are required. Also, because negative staining involves deposition of heavy atom stains, structural artefacts such as flattening of spherical or cylindrical structures are common. Nevertheless, negative staining is a very useful technique because of its ease and rapidity,

For **bright field microscopy**, negative staining is typically performed using a black ink fluid such as **nigrosin**. The specimen, such as a wet **bacterial** culture spread on a glass slide, is mixed with the negative stain and allowed to dry. When viewed with the **microscope** the bacterial cells, and perhaps their **spores**, appear light against the dark surrounding background. An alternative method has been developed using an ordinary waterproof marking pen to deliver the negative stain.



ALGAL STAIN-The viability of algae belonging to divisions *Cyanophyta*, *Chlorophyta* and *Xantophyta* has been assessed by chlorophyll autofluorescence and after staining with fluorescent dyes rodamin B, neutral rot, calcofluor white and fluorescein diacetate. For this purpose the fluorescence of algal cells from fresh and old cultures was scored. It was established that chlorophyll autofluorescence could be recommended as the most convenient method for differentiating living and dead algal cells.

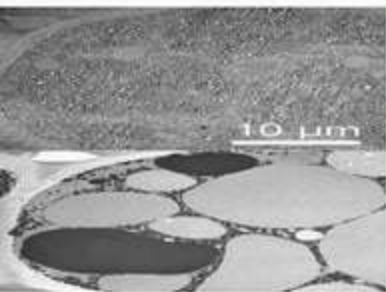
The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls.

Go to:

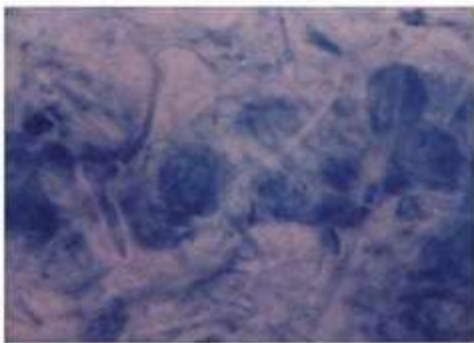
Procedure for corneal scrape material:


Place a drop of 70% alcohol on a microscope slide. Immerse the specimen/material in the drop of alcohol. Add one, or at most two drops of the lactophenol/cotton blue mountant/stain before the alcohol dries out. Holding the coverslip between forefinger and thumb, touch one edge of the drop of mountant with the coverslip edge, and lower gently, avoiding air bubbles. The preparation is now ready for examination.

NEGATIVE STAIN




ALGAL STAIN





Serotyping – Identifying a microorganism based on its reaction to particular antibodies. The antibodies are used to identify microorganisms carrying particular antigens. Techniques like the Western blot or Enzyme Linked Immunosorbent Assay (ELISA)

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Phage typing – determines the susceptibility of a bacterium to a particular phage type.

Highly specialized and usually restricted to the species level and lower

Viruses that attack members of a particular bacterial species are called bacteriophages .

Phage typing is based on specificity of phage surface receptors to cell surface receptors.

Only those bacteriophages that can attach to these surface receptors can bind or infect bacteria and cause lysis.

Flow cytometry

Easy, reliable and fast way to detect single or multiple organisms in clinical sample

Micro-organisms are identified on the basis of their unique cytometric parameters or by dyes called flurochromes

Flurochromes bind to independently with specific antibodies or oligonucleotides

Flow cytometer forms a suspension of cells through a laser beam and measures the light they scatter or the fluorescence they emit as they pass through the beam

Species and Subspecies

- **Species**

- collection of bacterial cells which share an overall similar pattern of traits in contrast to other bacteria whose pattern differs significantly

- **Strain or variety**

- culture derived from a single parent that differs in structure or metabolism from other cultures of that species (biovars, morphovars)

- **Type**

- subspecies that can show differences in antigenic makeup (serotype or serovar), susceptibility to bacterial viruses (phage type) and in pathogenicity (pathotype)

DNA:DNA hybridization - genomic DNA from one organism is labeled and hybridized with the genomic DNA from another organism. This technique measures the similarity between the two DNAs. Does not work well for comparing distantly related microorganisms.

Nucleic acid base composition

- $G + C$ content = the percent of $G + C$ in the DNA Can be determined by hydrolysis of DNA and HPLC analysis of the resulting bases or by melting temperature determination (T_m)
- Organisms with that differ in their $G + C$ content by more than 10% are likely to have quite different base sequences

DNA chip reader

DNA chip technology has made it possible to "print" many different species specific probes (> 10,000) onto a glass slide (i.e., the "chip"). Genomic DNA is extracted from an unknown organism and labeled with a fluorochrome. The labeled genomic DNA is hybridized with the probes on the chip. Hybridization reactions fluoresce and can be identified by reading the DNA chip with an instrument known as a DNA chip reader.

Conclusion

- Thus from initiating the simple technique of staining to identify the microorganisms to the complex molecular level analysis is being carried out to confirm the microbial species

A grayscale micrograph showing a cross-section of plant tissue. The upper portion shows a single cell with a granular cytoplasm. The lower portion shows a cluster of cells with large, clear central vacuoles. A red oval with the text 'THANK YOU' is centered in the upper half. A white scale bar labeled '10 μm' is located in the lower right quadrant.

THANK YOU

10 μm