INTRODUCTION TO BASIC MICROBIOLOGICAL LAB TECHNIQUES

By,

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STERILIZATION AND DISINFECTION

- A **disinfectant** is a chemical or physical agent that is applied to inanimate objects to kill microbes
- Typically an **antiseptic** is a chemical agent that is applied to living tissue to kill microbes
- **Sterilization** is the killing of all microorganisms in a material or on the surface of an object
- **Disinfection** means reducing the number of viable microorganisms present in a sample
- **Sanitization** is the cleaning of pathogenic microorganisms from public eating utensils and objects such as that done by the kitchen of a restaurant

ANTIMICROBIALS

- Antimicrobials are used to simply kill or inhabit the growth of microorganisms

- All antimicrobial agents act by harming microorganisms in some manner

  - Protein denaturation
  - Membrane disruption
  - Nucleic acid damage
  - Inhibition of metabolism

TYPES OF ANTIMICROBIALS

- PHYSICAL ANTIMICROBIALS
- CHEMICAL ANTIMICROBIALS

CHEMICAL ANTIMICROBIALS

- Surfactants
- Various organic acids and bases
- Heavy metals
- Halogen-containing compounds
- Alcohols
- Phenol and phenol derivatives
- Oxidizing agents
- Alkylating agents
- Certain dyes
- Other agents

PHYSICAL ANTIMICROBIALS

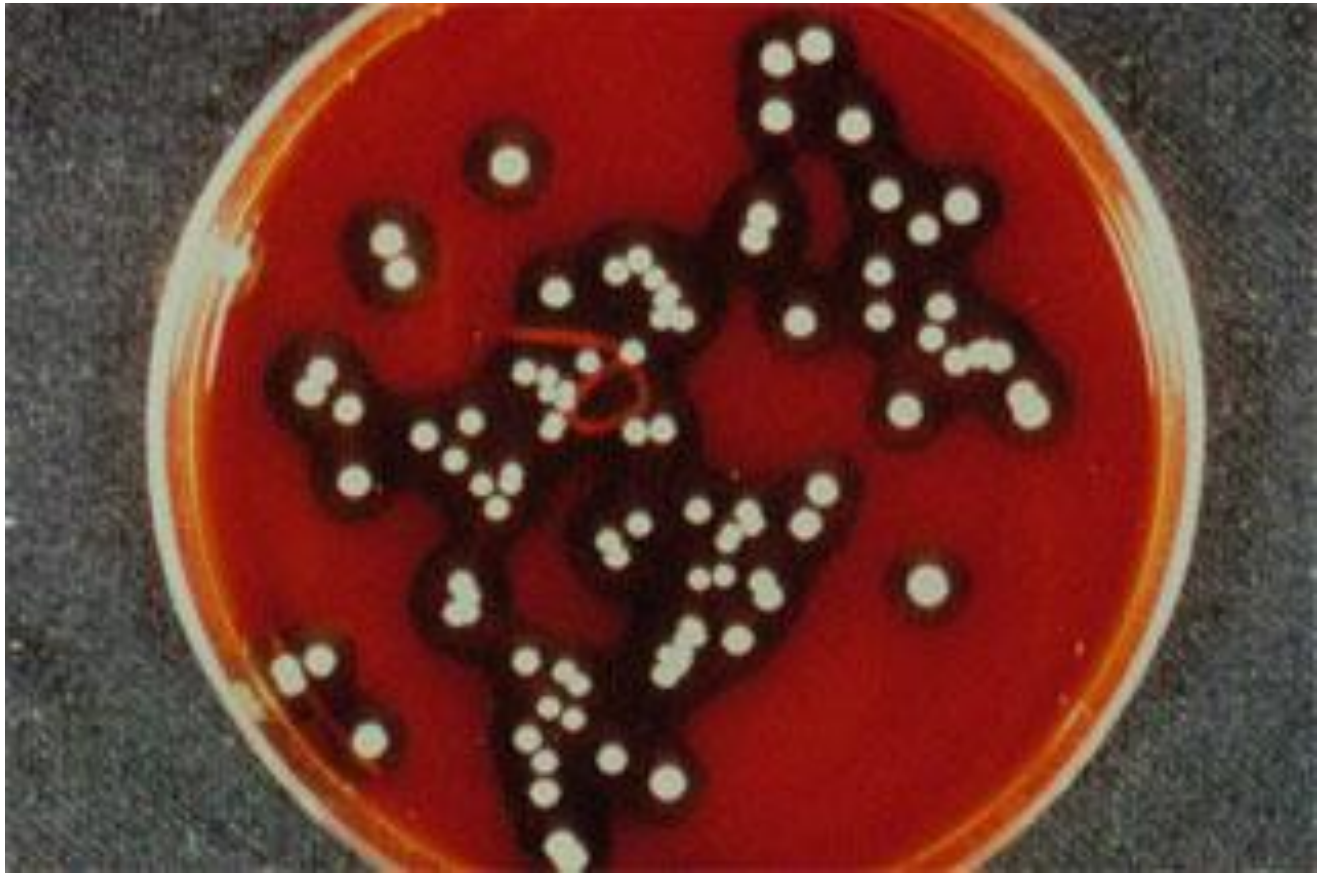
- Sunlight
- Drying
- Heat
- Filtration
- Freezing and deep freezing
- Radiation

BACTERIOLOGICAL MEDIA

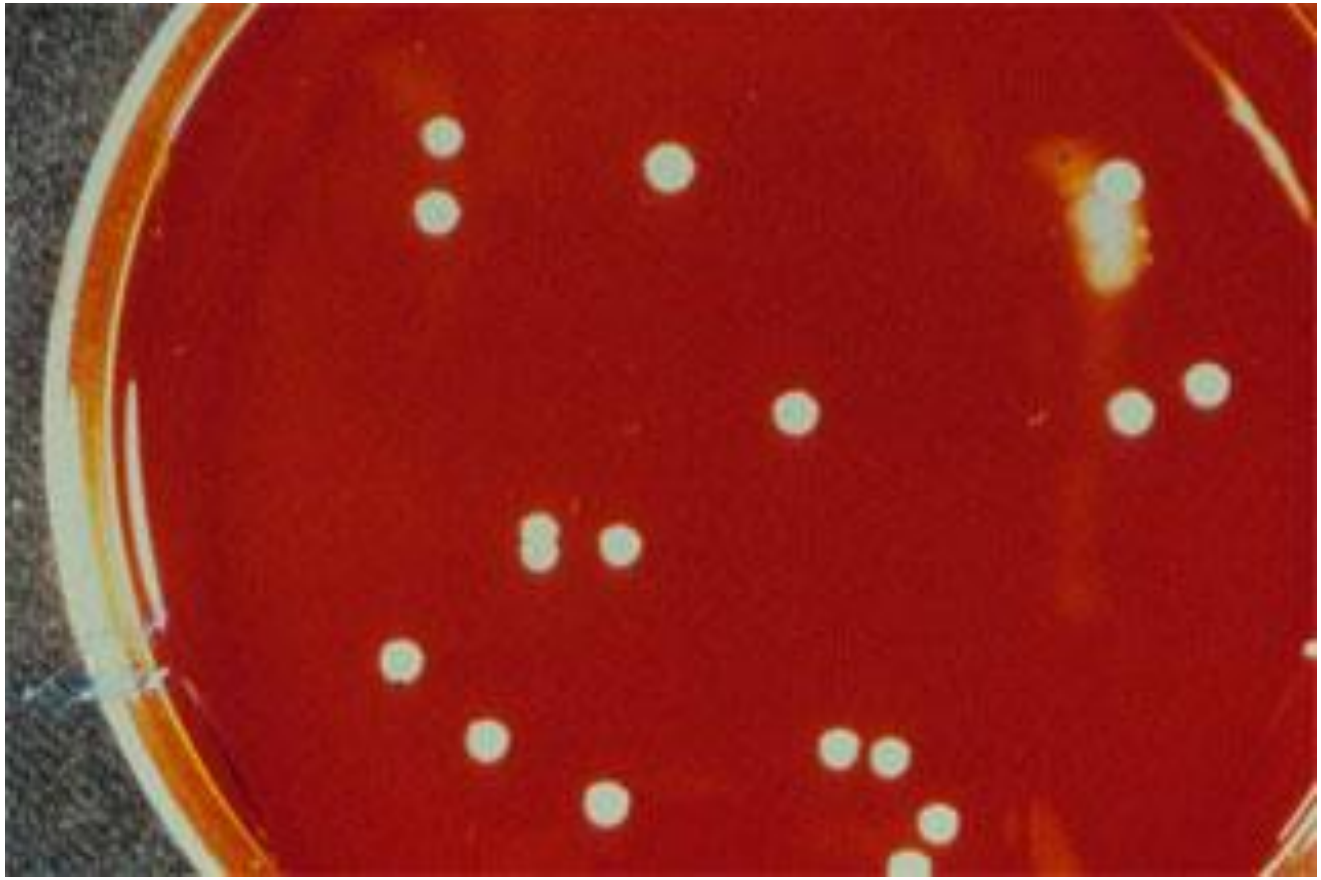
- Nutrient Media
- Enriched Media
- Selective/Differential Media

STREAK ISOLATION ON NUTRIENT AGAR - TRYPTICASE SOY AGAR (TSA)

HEMLOYSIS ON ENRICHED AGAR - BLOOD AGAR



NON-HEMLOYTIC GROWTH ON ENRICHED AGAR - BLOOD AGAR



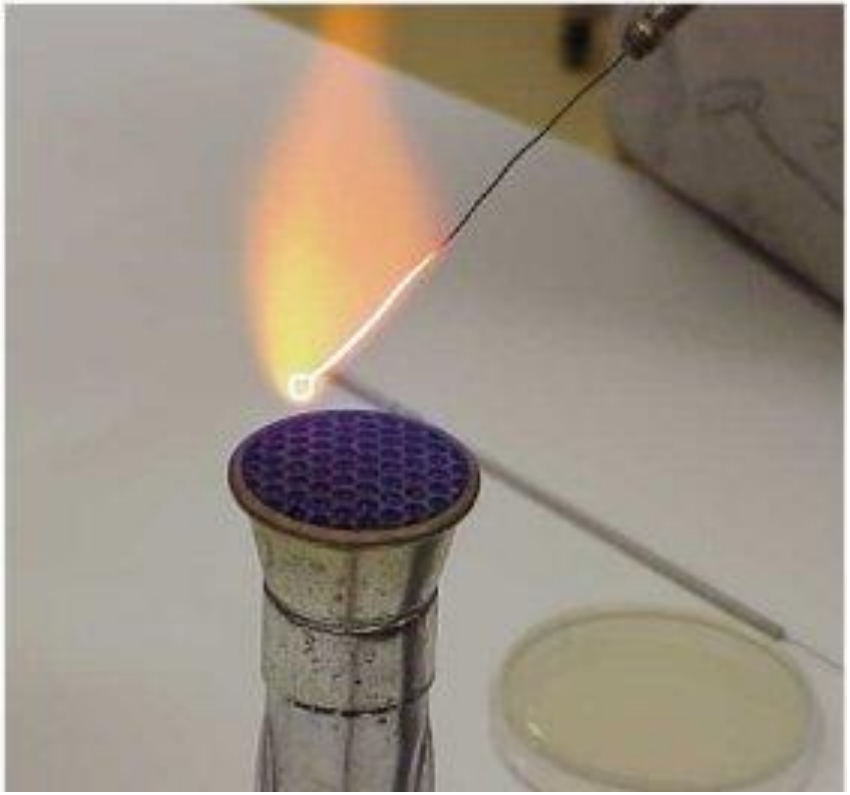
E. COLI AND PROTEUS ON SELECTIVE/DIFFERENTIAL MEDIA - MACCONKEYS

S. EPIDERMIDIS AND S. AUREUS ON SELECTIVE/DIFFERENTIAL - MANNITOL SALT AGAR (MSA)

GRAM POSITIVE ORGANISMS ON SELECTIVE AGAR - COLISTAN NALDIXIC ACID AGAR (CNA)

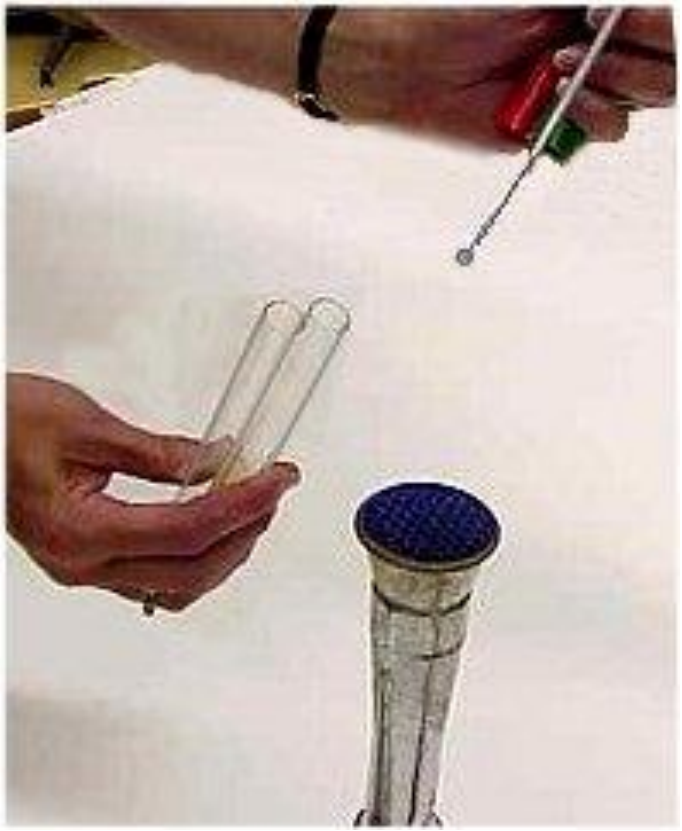
CULTURING MICROORGANISMS

ASEPTIC TRANSFER OF BACTERIA



- Heat the inoculating wire of the loop or needle until red-hot

ASEPTIC TRANSFER OF BACTERIA



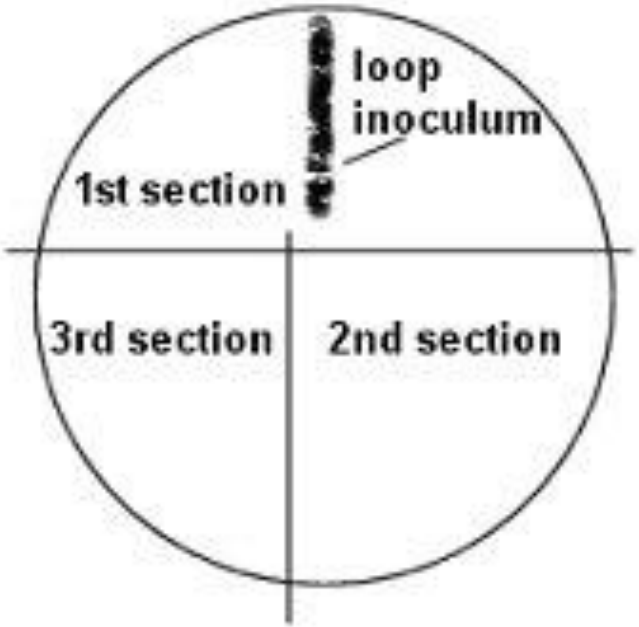
- Keeping the sterile inoculation instrument in your hand, remove both tube caps with your little finger

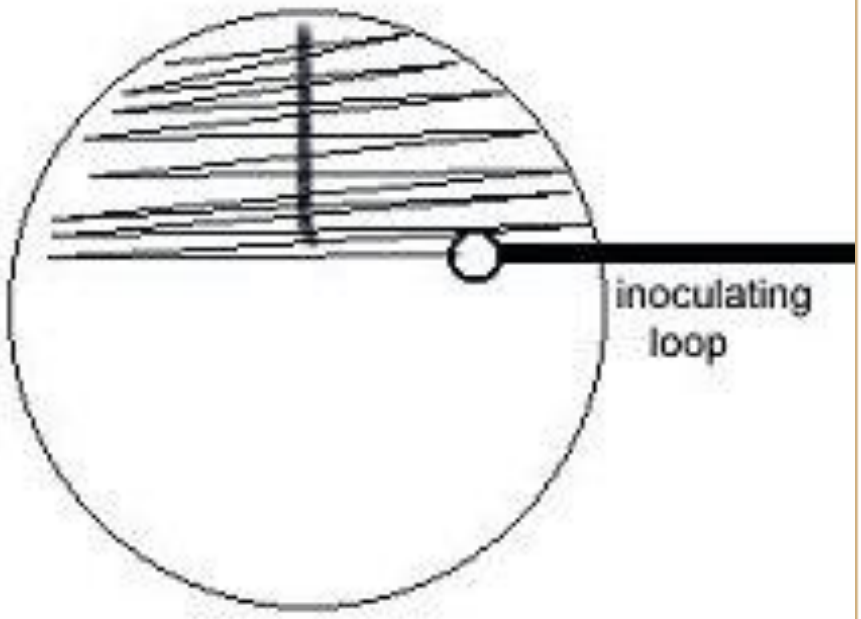
ASEPTIC TRANSFER OF BACTERIA

- Take the inoculum and quickly place it into the new medium tube

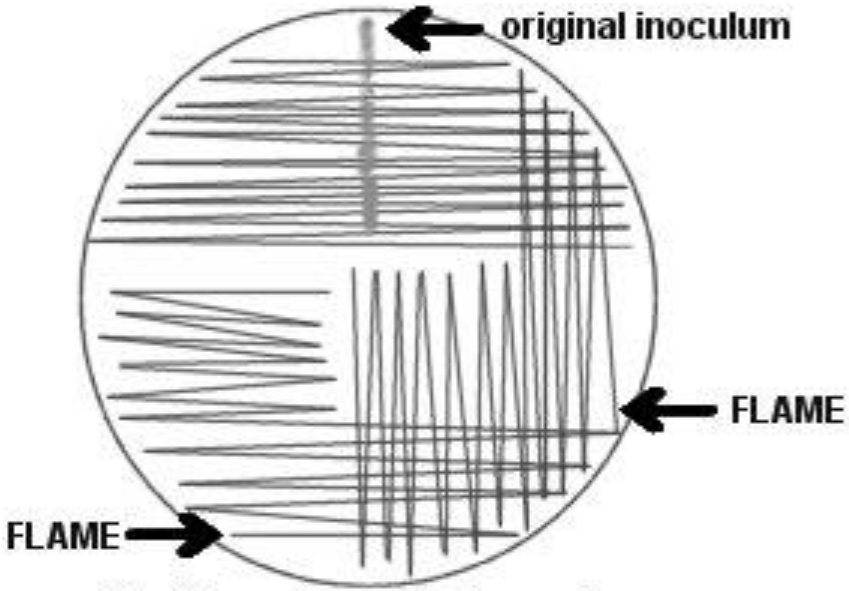
CULTURING BY STREAKING

- Form imaginary sections on the agar plate
- Move the loop in a zig-zag pattern across the agar until 1/3 of the plate is covered

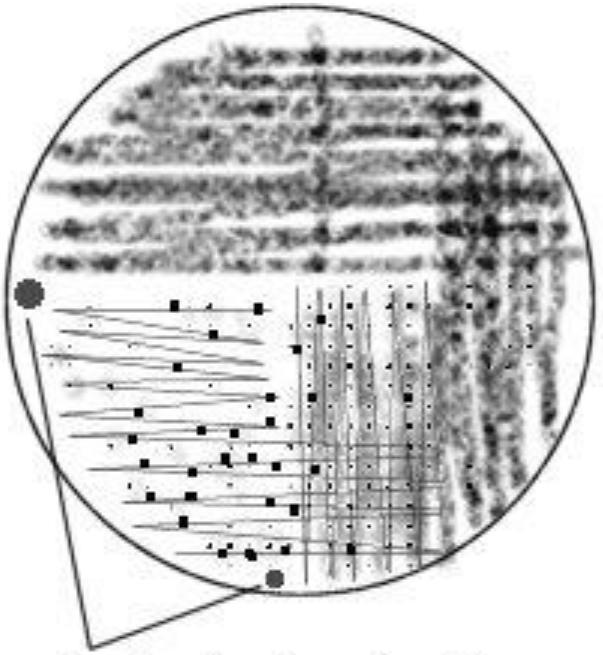




CULTURING BY STREAKING



original inoculum picked up only once
loop glides over top of agar medium
loop flamed at beginning of new section
3-5 crossovers from new section into previous
then streak only within that section

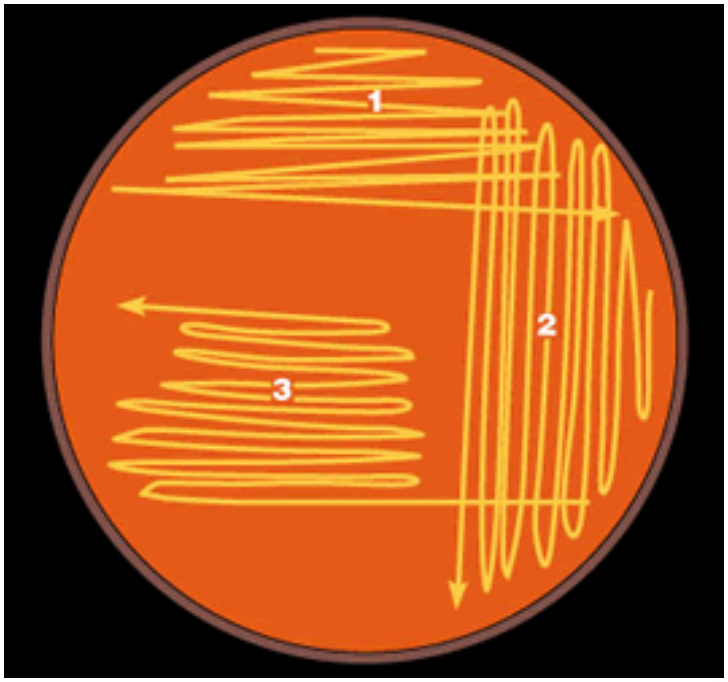


contaminants not on streak lines

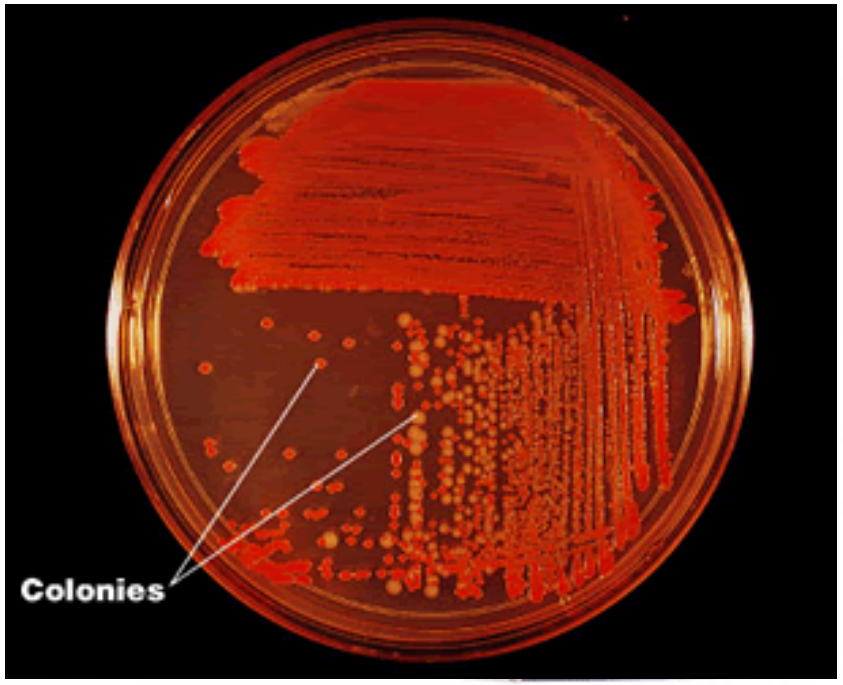
well-isolated colonies in 3rd section

CULTURING BY STREAKING

- Streak Dilution Technique



- Isolated colonies of two different types of bacteria

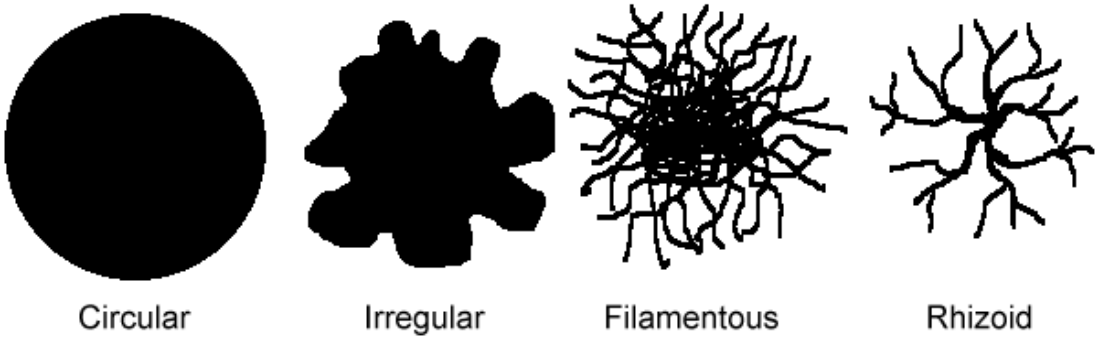


INTERPRETATION OF CULTURES BY GROWTH PATTERNS

- **Form** - The basic shape of the colony (circular, filamentous, etc)
- **Elevation** - The cross sectional shape of the colony. Turn the Petri dish on end.
- **Margin** - The magnified shape of the edge of the colony.
- **Surface** – The appearance of the surface of the colony (smooth, glistening, rough, dull, rugose/wrinkled)
- **Opacity** - The transparency (clear, opaque, translucent, iridescent, etc)
- **Chromogenesis** – The pigmentation (white, buff, red, purple, etc)

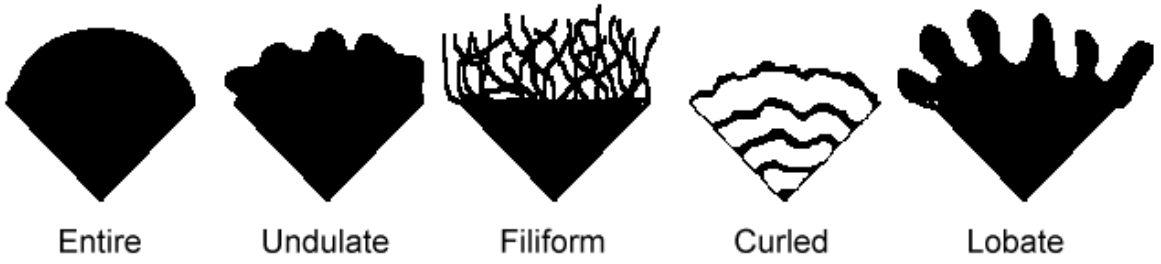
INTERPRETATION OF CULTURES

Form



Elevation

Margin



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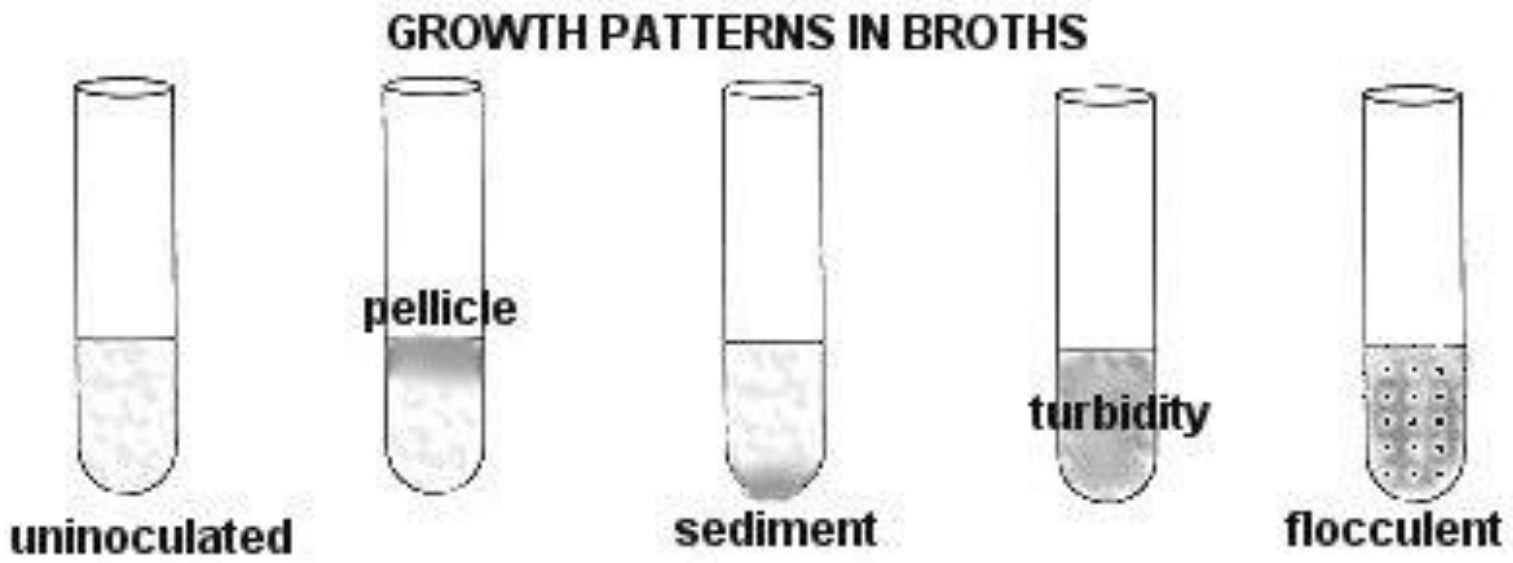

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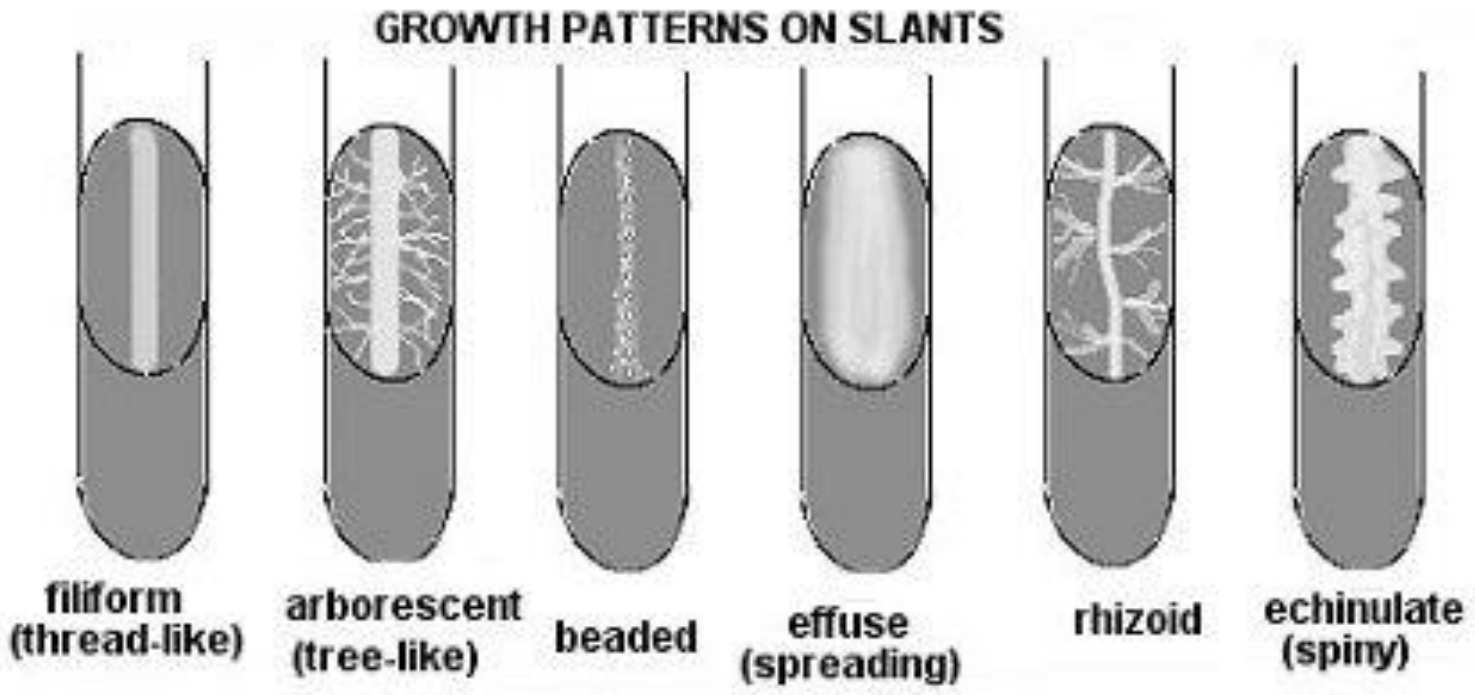
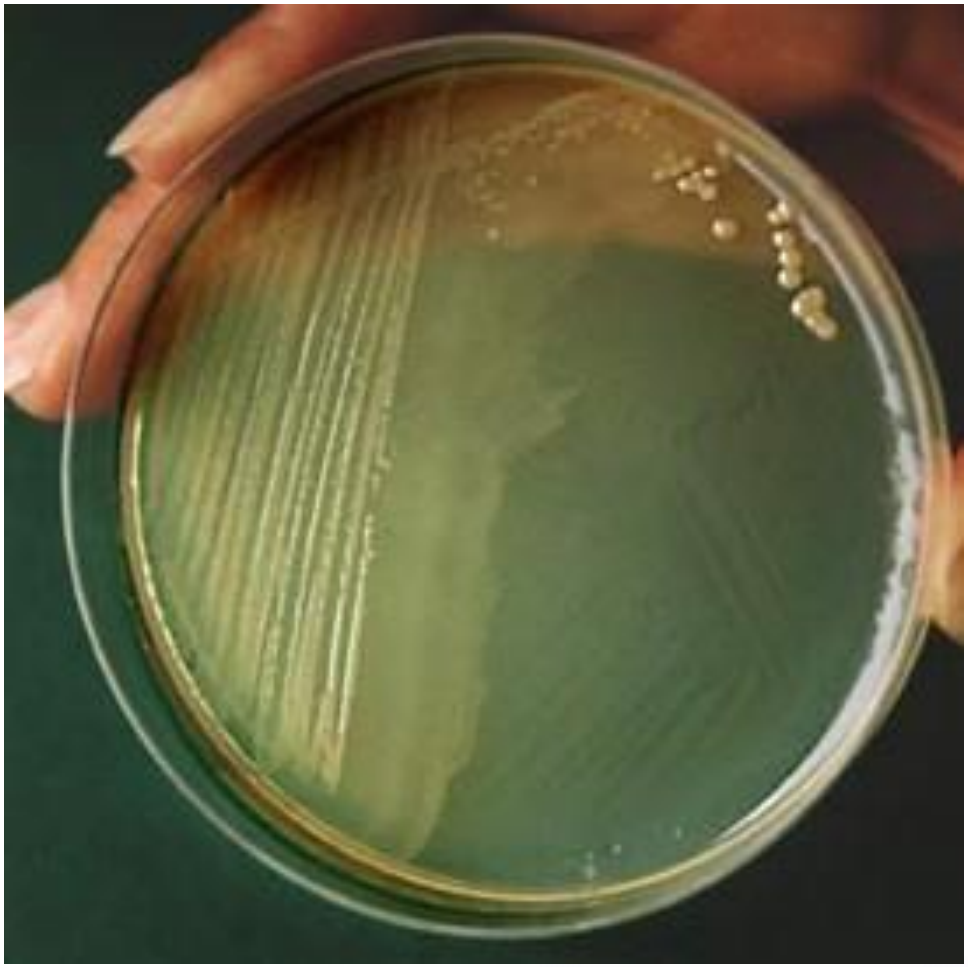


BACTERIA - *Bacillus subtilis*

BACTERIA - *Proteus vulgaris*



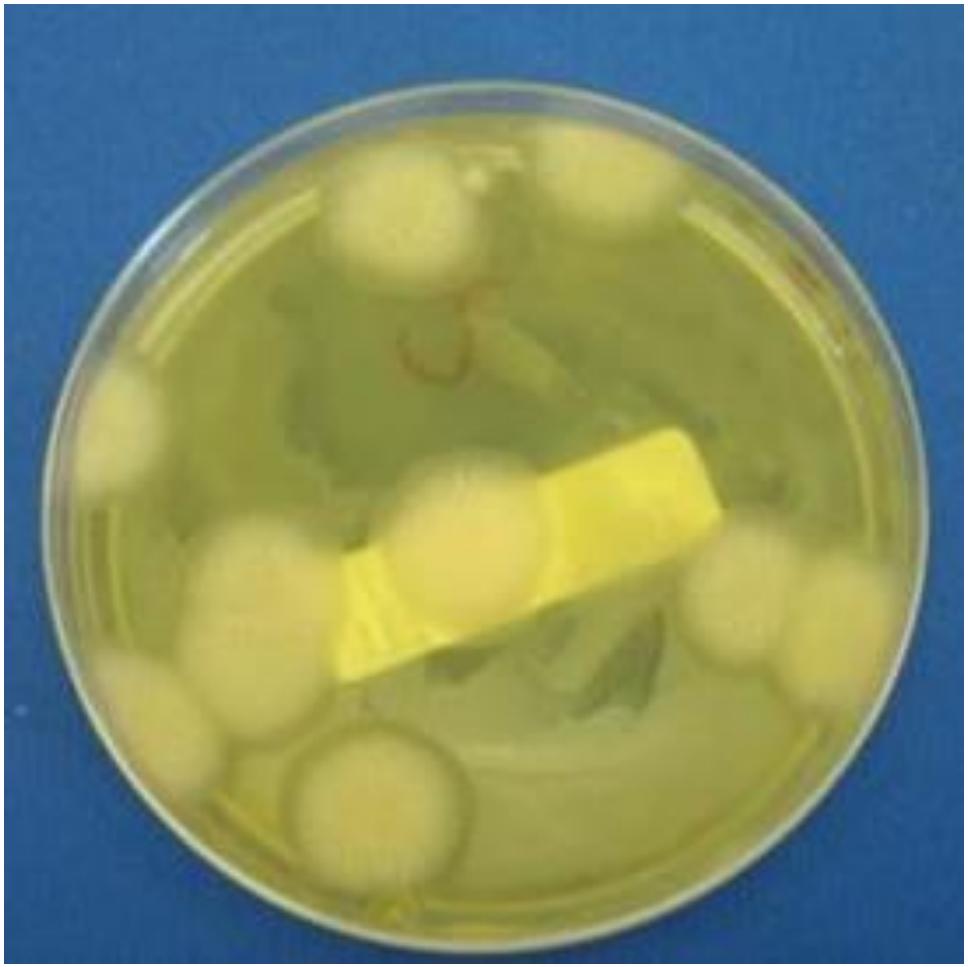
BACTERIA - *Staphylococcus aures*

BACTERIA - *Streptococcus pyogenes*

YEAST - *Candida Albicans*

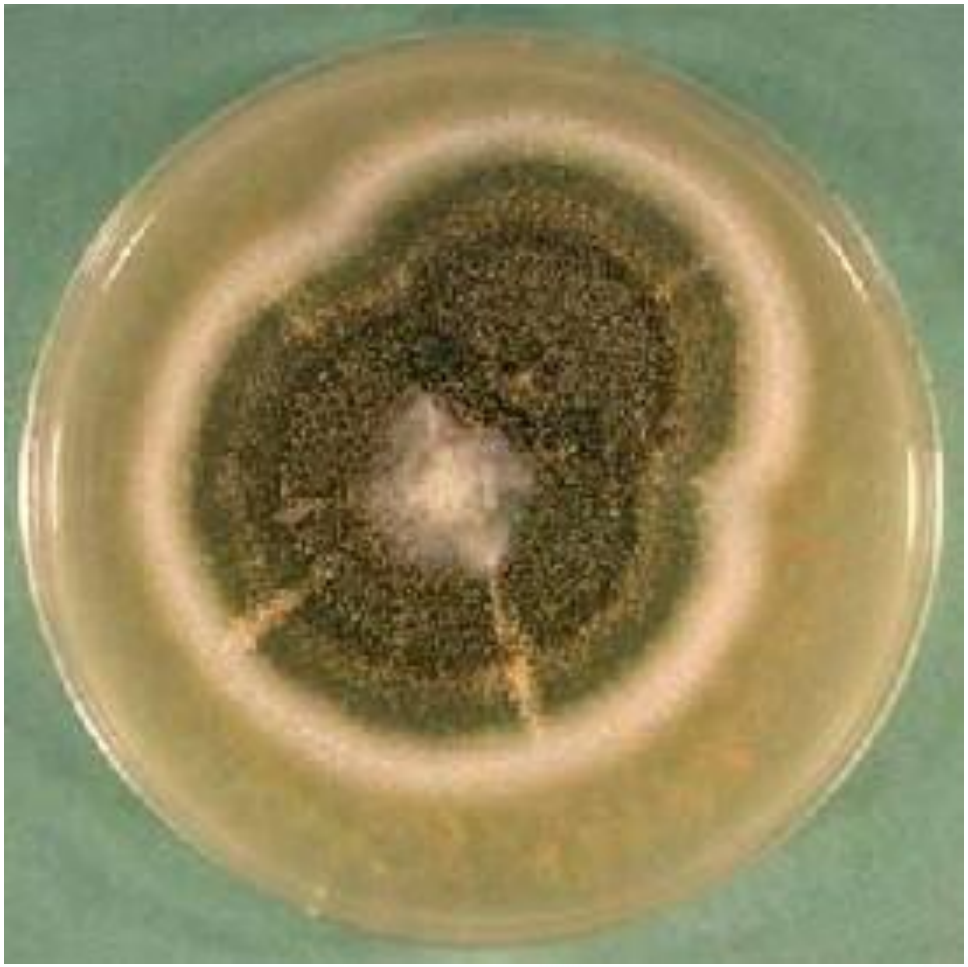
YEAST - ROUND YEAST

YEAST - PINK YEAST

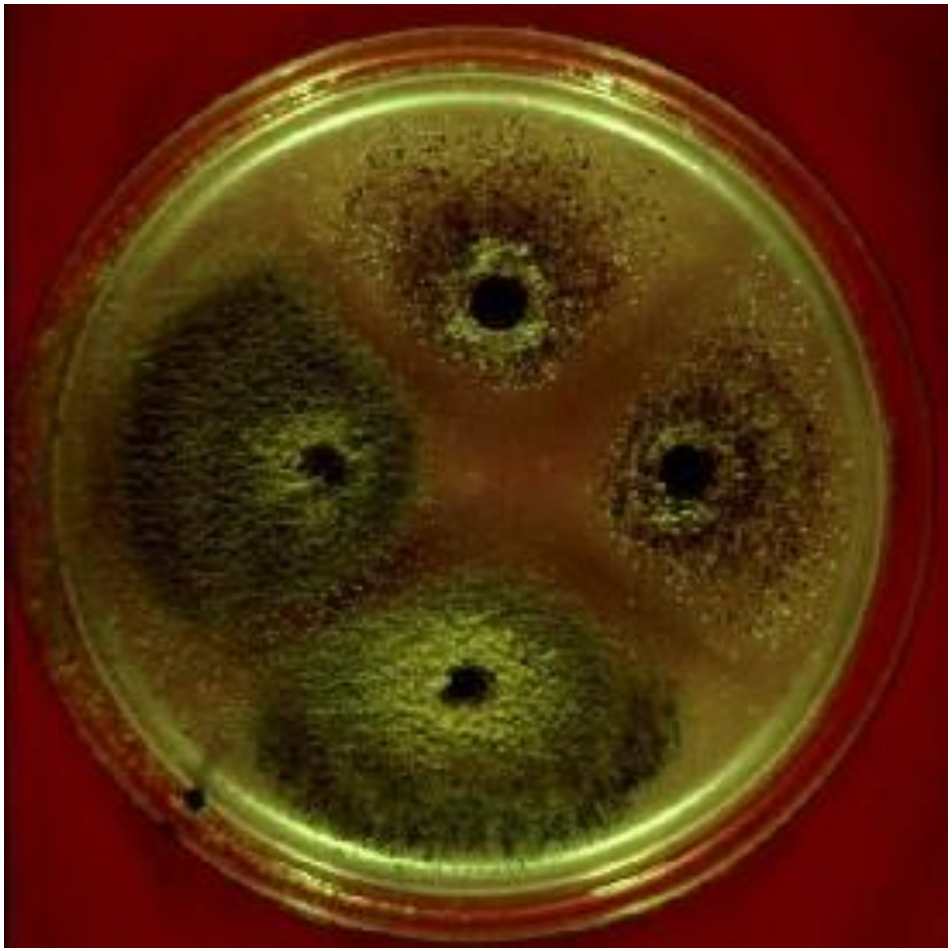


MOLD – GREEN MOLD

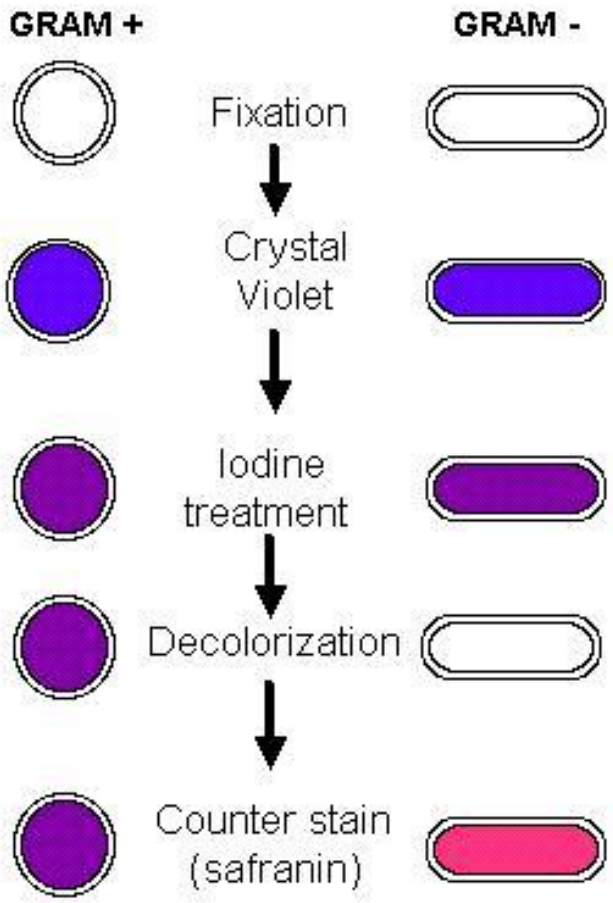
MOLD – BLACK MOLD



OTHER FUNGI



GRAM BACTERIAL STAINING



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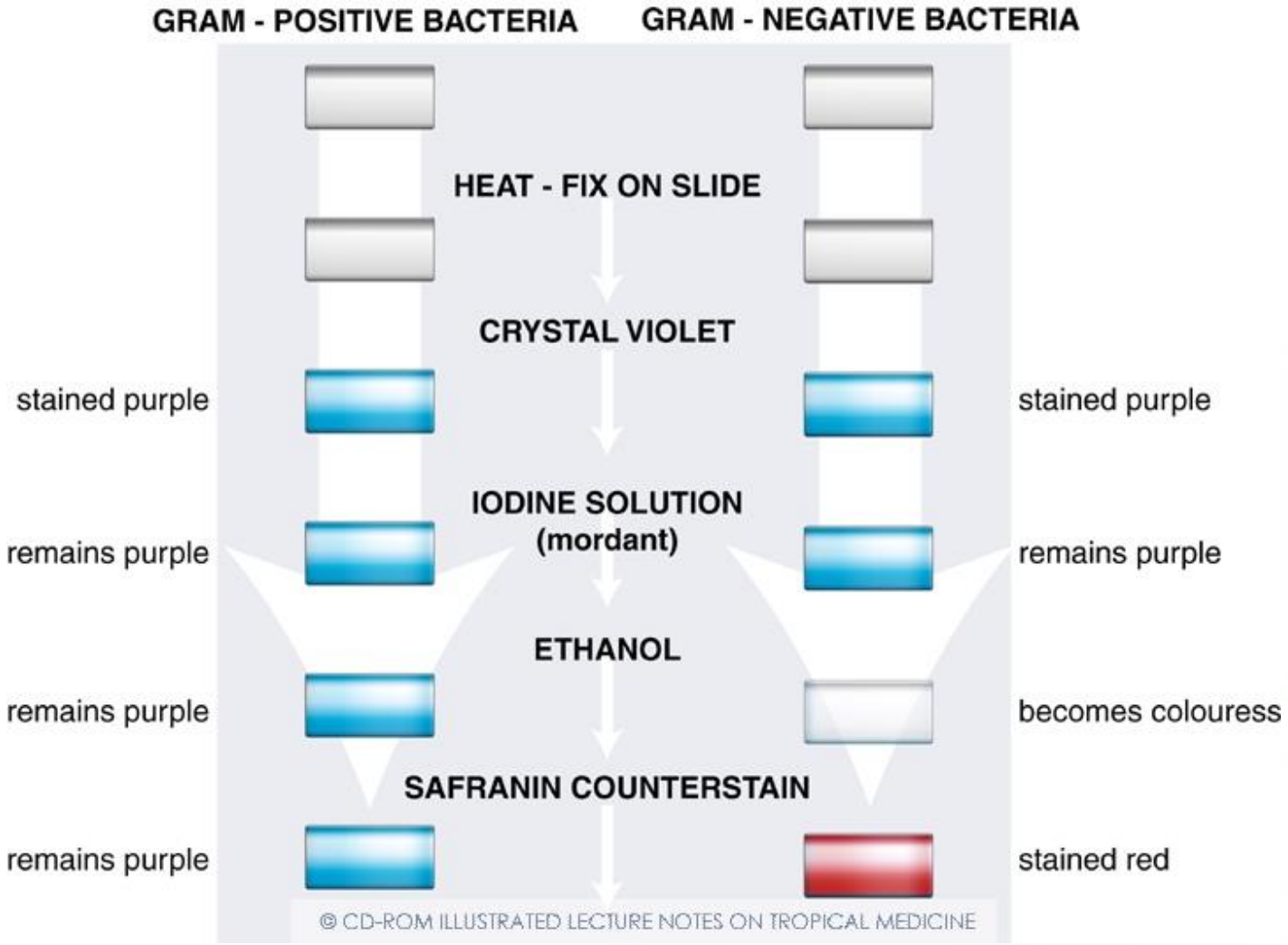

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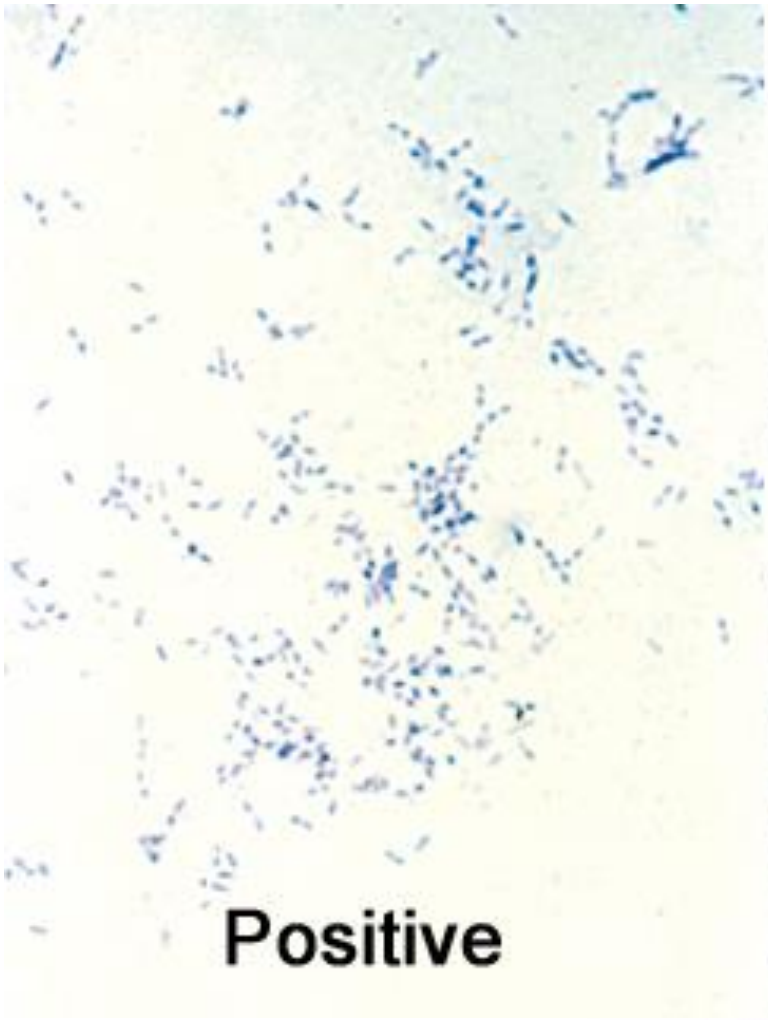
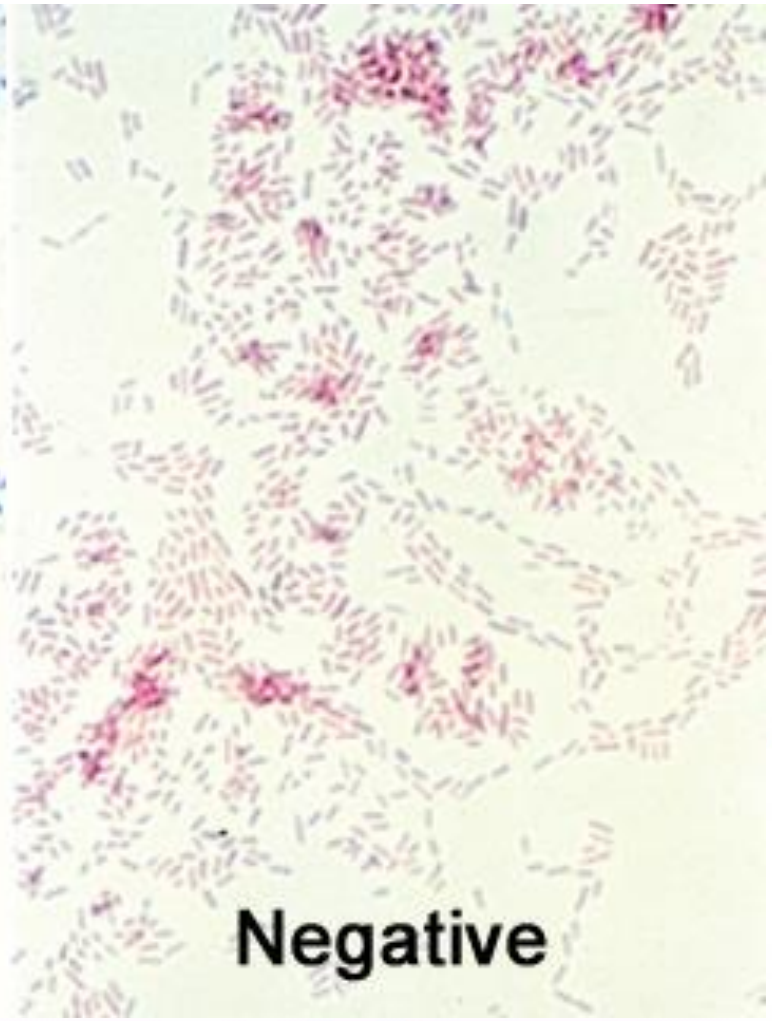




ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

- Gather the apparatus and prepare the agar plates

ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

- Take one drop of the water sample which contains the algae to be isolated and place it on the agar plate

ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

- Heat the inoculation loop in an alcohol flame until the wire loop is red

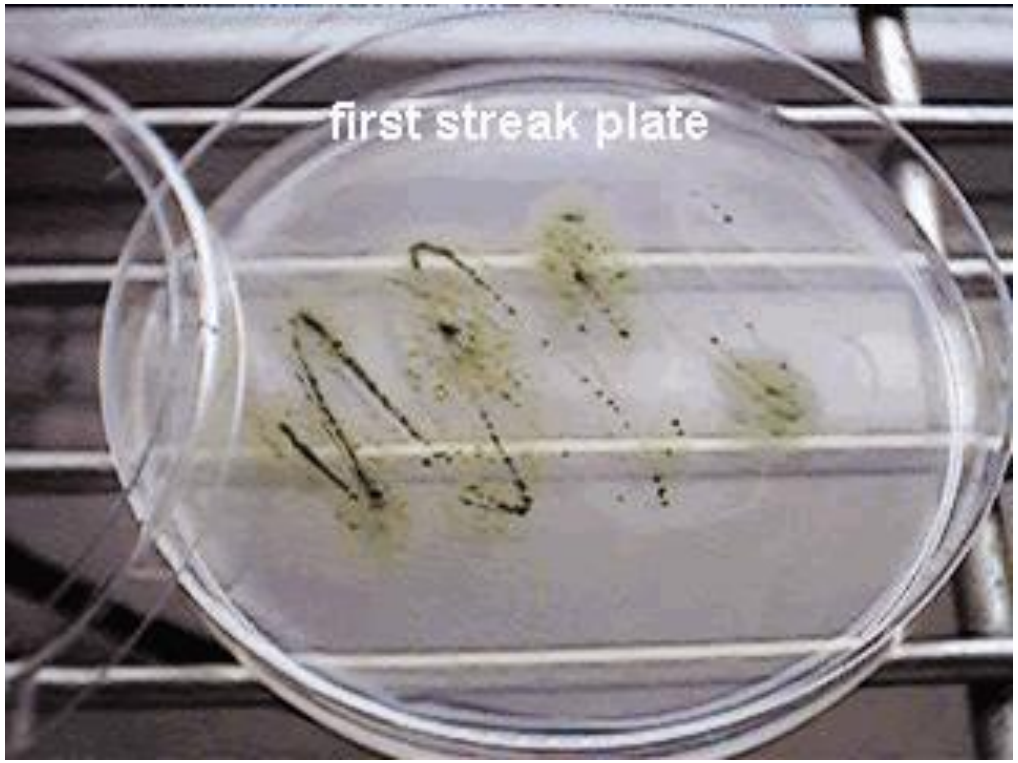
ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

- Spread out the algal cells floating in the water drop

ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

- Place the lid back on the Petri plate and place masking tape along the edge of the plate to stop it from drying

ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

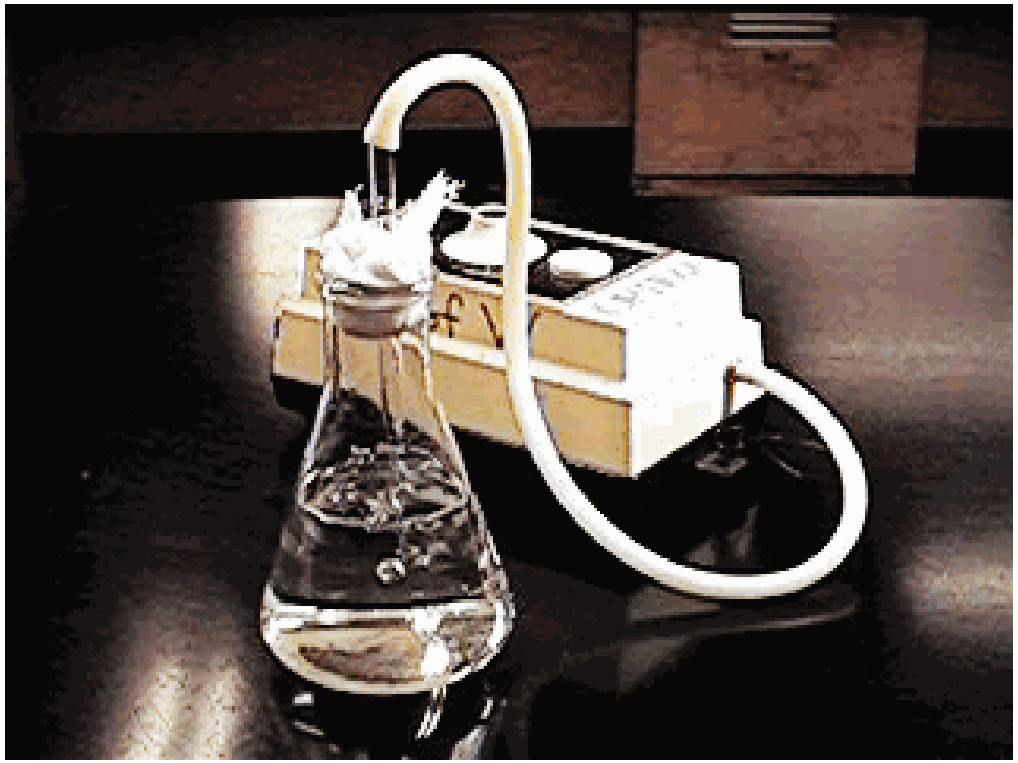


- The first streak plate often tend to grow together

ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE

- Scrape some colonies off the first plate and re-streak them on a second plate

ISOLATION OF A SINGLE ALGAL SPECIES FROM A WATER SAMPLE



- Transfer one pure colony into a flask of liquid medium

THANK YOU

THE END