Microbiology – Chapter 3

Culturing Microbes

The Five “I’s

Inoculation: Producing a pure culture

Isolation: Colony on media, one kind of microbe, pure culture

Incubation: growing microbes under proper conditions

Inspection: Observation of characteristics (data)

Identification: use of data, correaltion, to ID organism to exact species

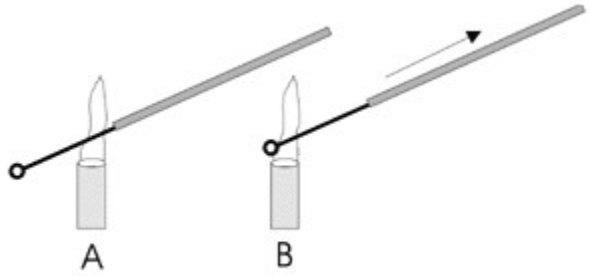
Microbiology – Chapter 3

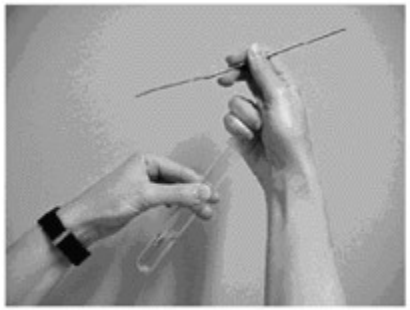
Culturing Microbes

The Five "I's

Innoculation: Producing a pure culture

Introduce bacteria into a growth medium using "aseptic technique" to prevent contamination. Tools: Bunsen burner, loop. Needle, etc.

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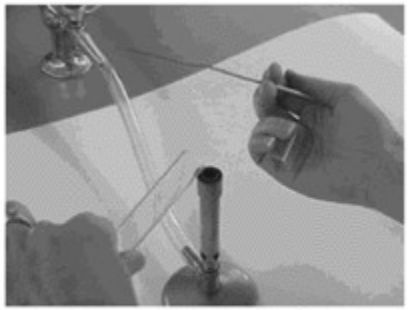
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Growth medium

- A **growth medium** or **culture medium** is a solid, liquid or semi-solid designed to support the growth of a population of microorganisms or cells via the process of cell proliferation, or small plants like the moss *Physcomitrella patens*.
- Different types of media are used for growing different types of cells.

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Innoculation: Producing a pure culture

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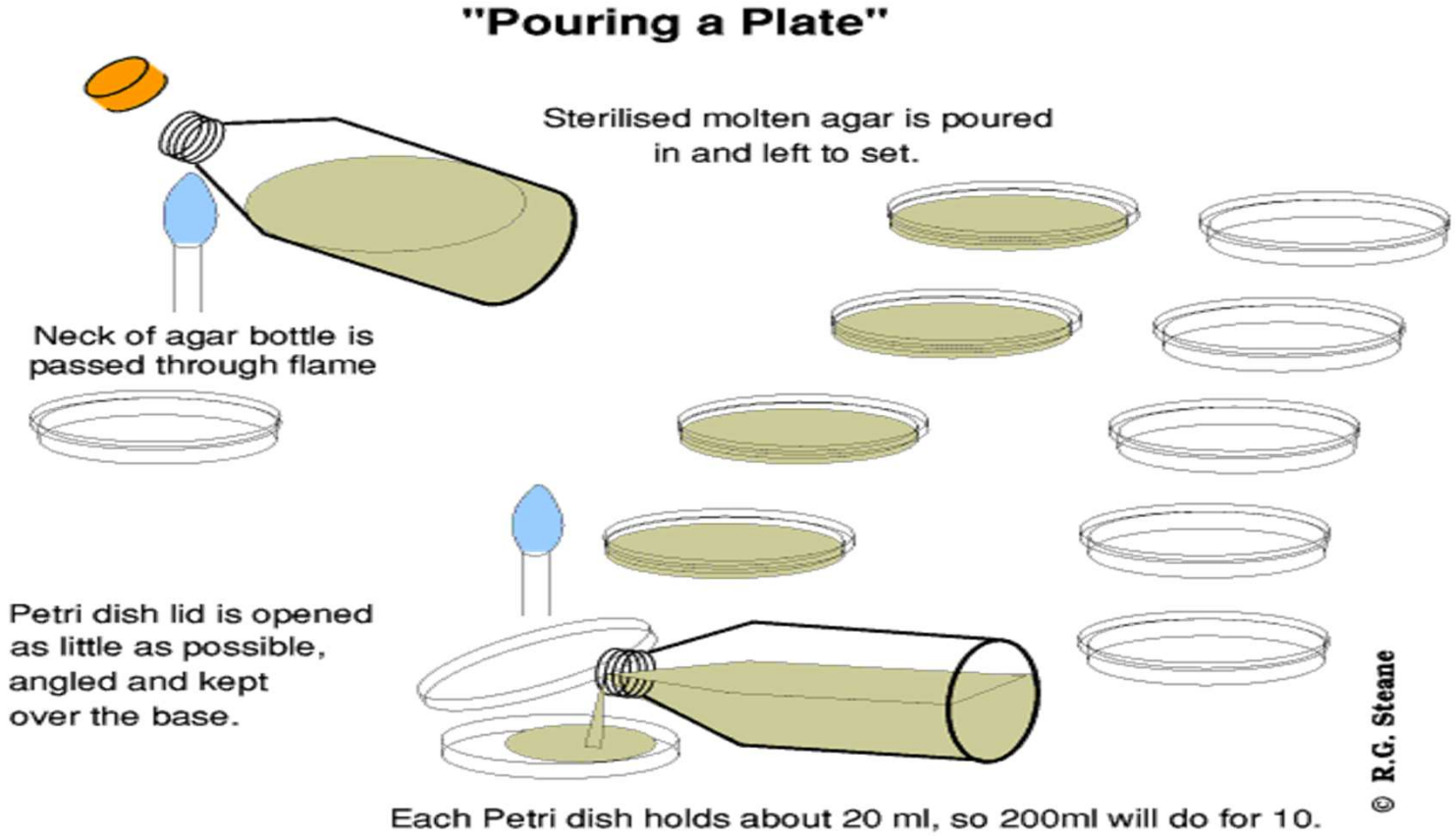


Types of Media

Isolation: Colony on media, one kind of microbe, pure culture: isolation on general and special "differential media"

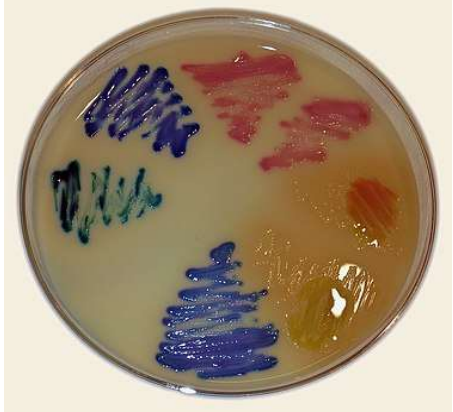
General growth media: Nutrient agar, Trypticase soy agar(TSA)

Minimal media: A defined medium that has just enough ingredients to support growth

Minimal media can also be used to select for or against recombinants or exconjugants. Minimal medium typically contains:

- a carbon source, which may be a sugar such as glucose, or a less energy-rich source such as succinate
- various salts, which may vary among bacteria species and growing conditions; these generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the bacteria to synthesize protein and nucleic acids
- water

Differential: Blood agar (used in strep tests) MacConkey agar is differential for lactose fermentation(Mac), Eosin methylene blue is differential for lactose fermentation (EMB).



These have dyes, salts, inhibiting agents : see differences on plates

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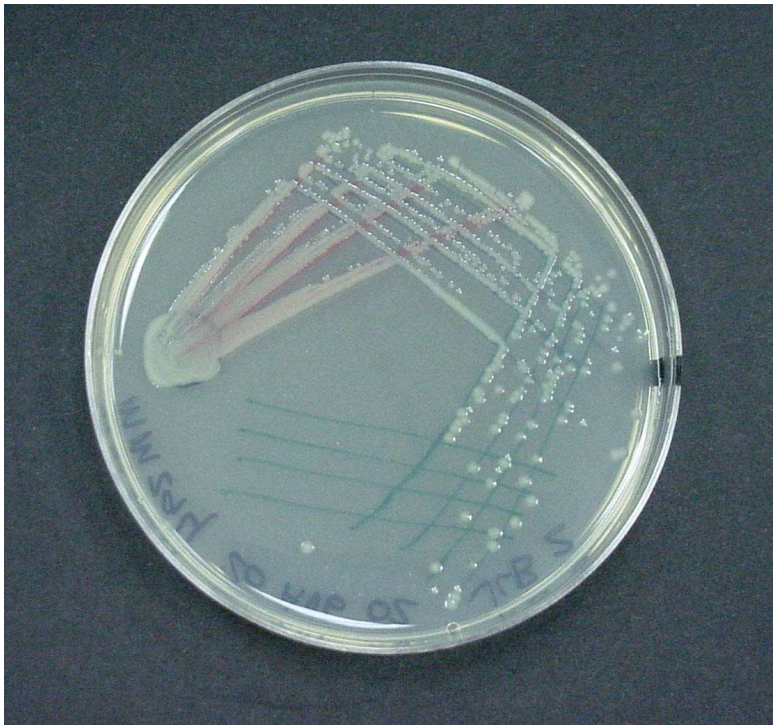
Isolation: Colony on media, one kind of microbe, pure culture



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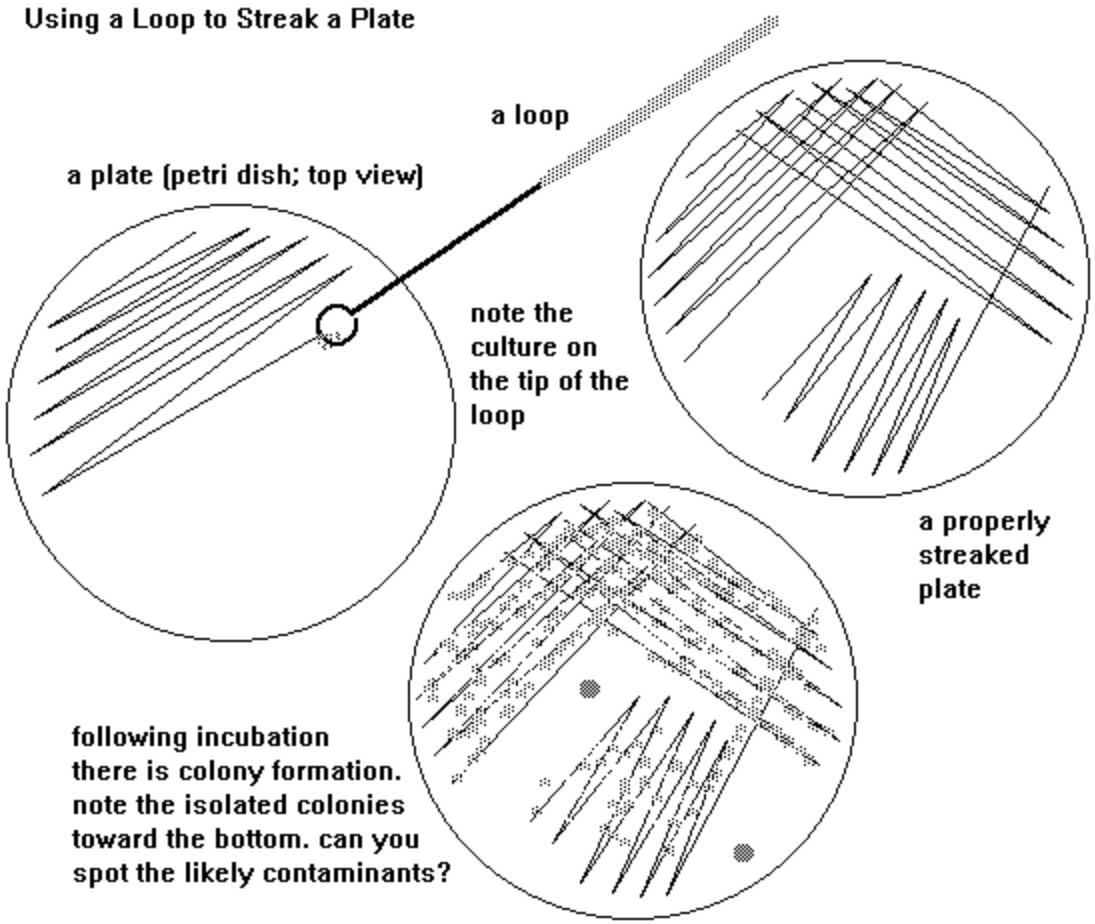
Isolation: Colony on media, one kind of microbe, pure culture – Streak Plates



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Isolation: Colony on media, one kind of microbe, pure culture. Many colonies? Use a needle, pick one, and redo streak plate

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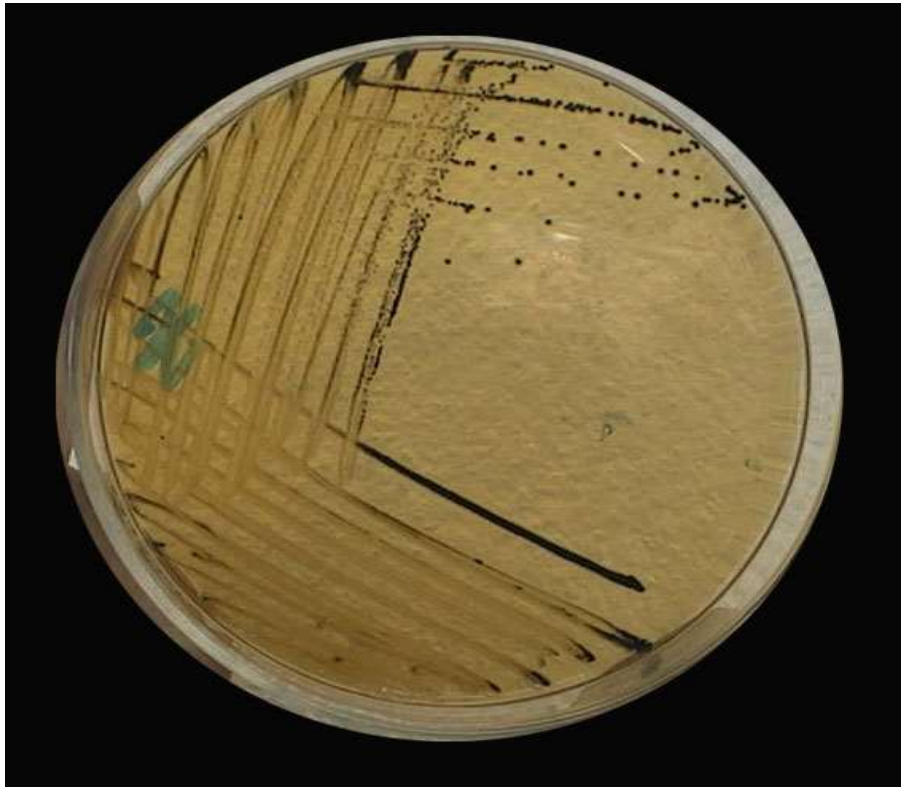
Differential: Mac, EMB

These have dyes, salts, inhibiting agents : see differences on plates



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- Blood agar : rich with nutrients, can see a difference, thus differential; much more later

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- Incubation: Allow organisms to grow under the optimal conditions
- Temperature, with or without oxygen etc

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- Incubation: Allow organisms to grow under the optimal conditions
- Temperature, with or without oxygen etc
- Candle jar reduces oxygen

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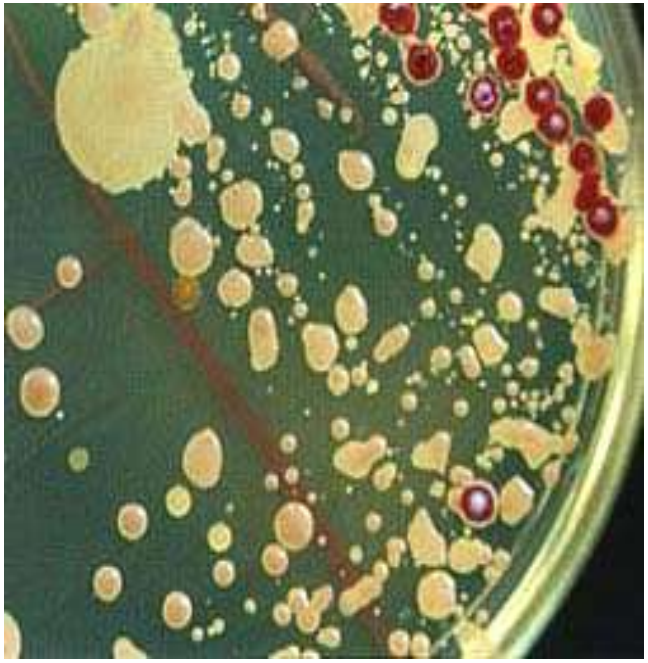
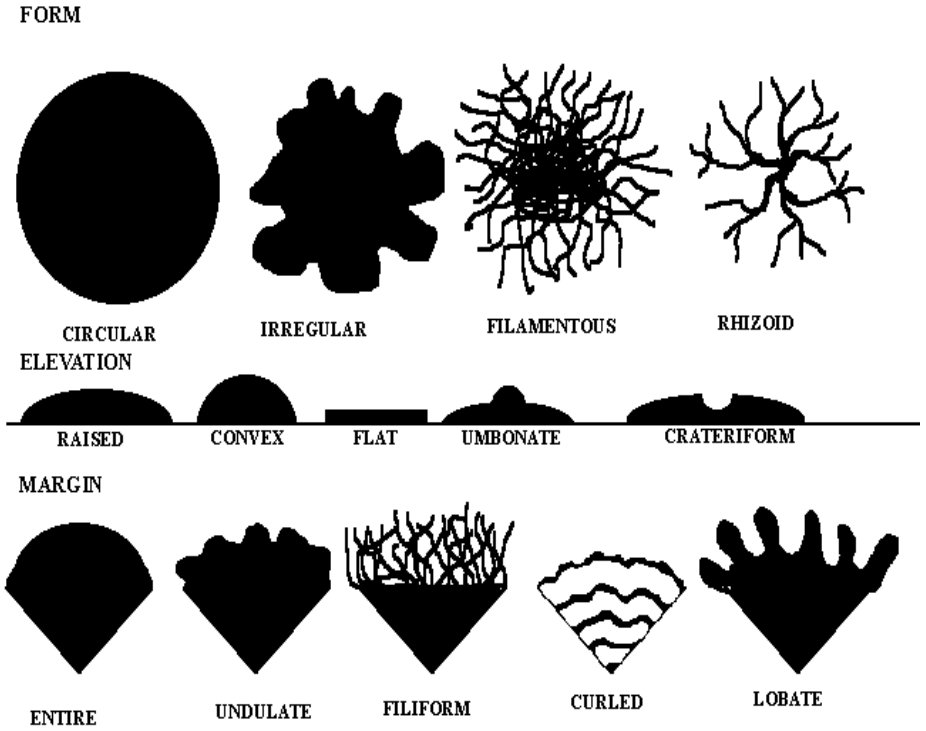
- Inspection: Observation, description
- Colony Morphology, Microscopic examination (grams stain)
- Systematic recording of “DATA”





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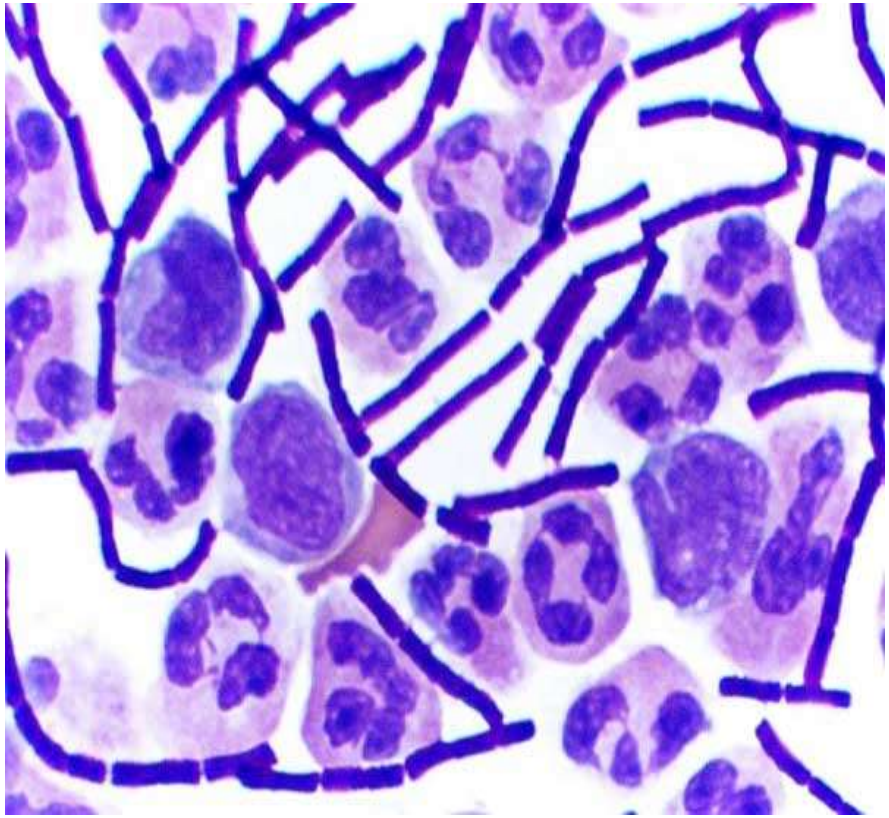
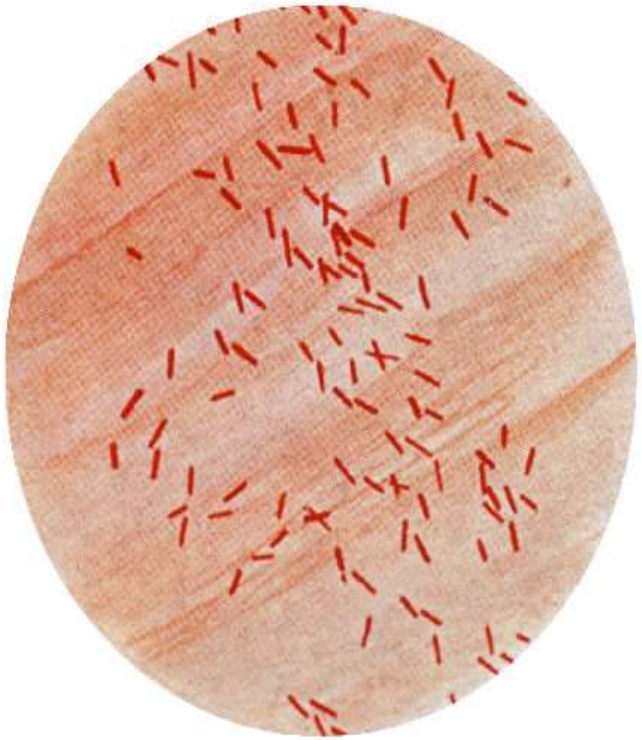
- Microscopic study: Gram + bacilli, Gram - bacilli





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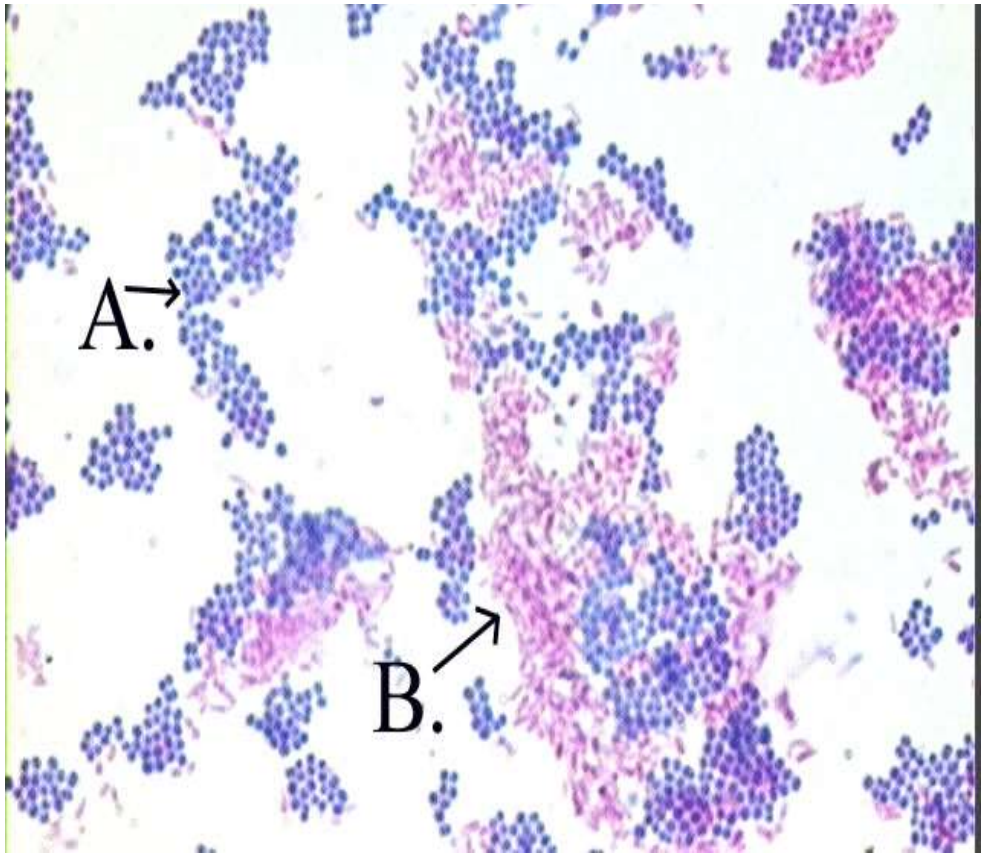
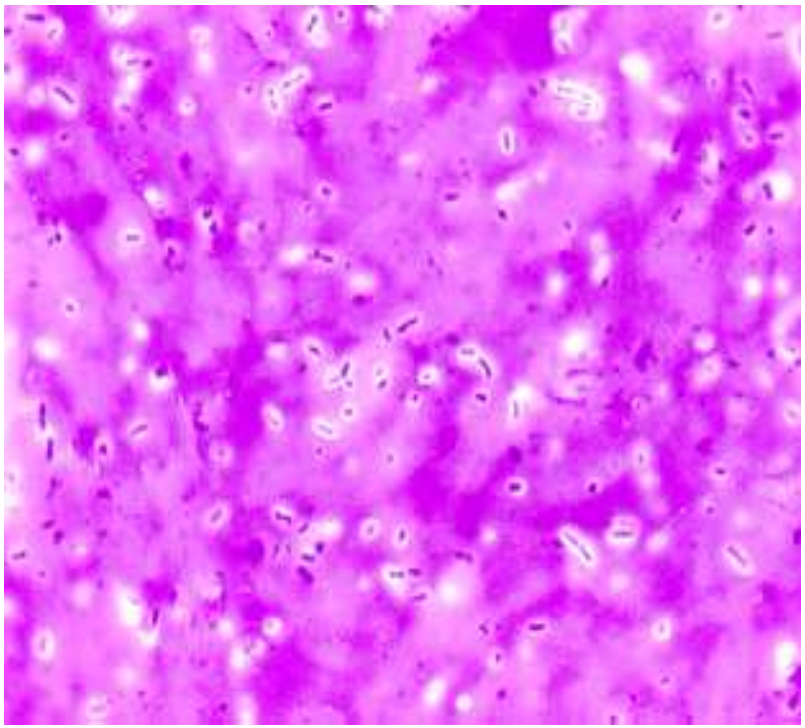
- Microscopic study: Acid fast, and capsule





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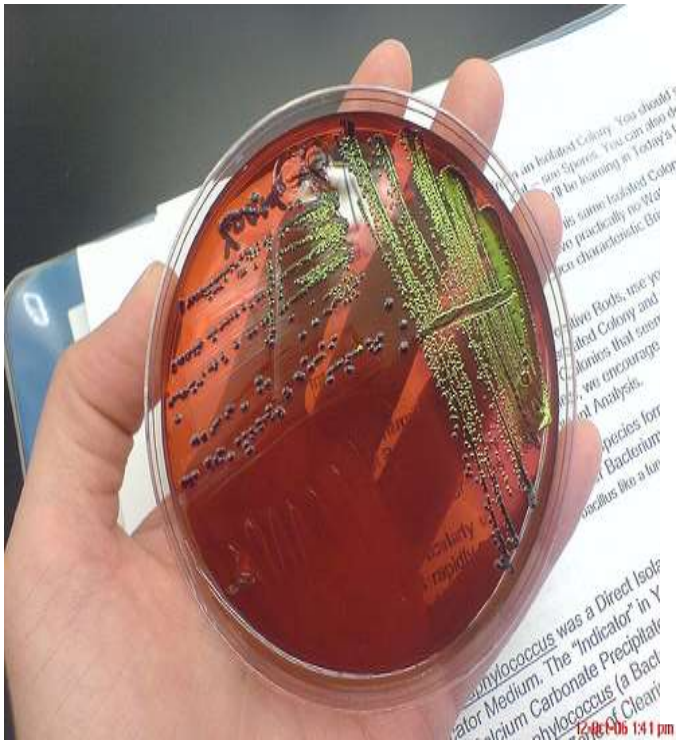
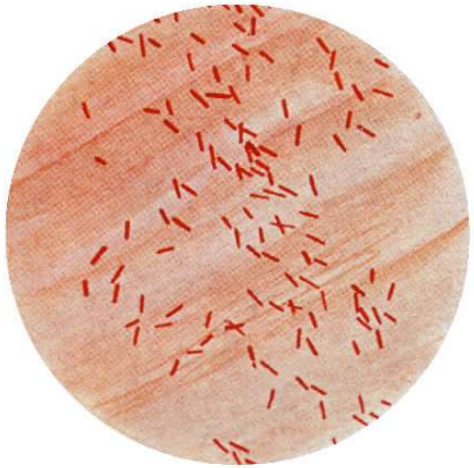
- Identification: Correlating data from all observations to ID organism to species
- Resources: flow charts, Bergey's manual etc.
- Ex. Gram – bacilli, ferments lactose, green sheen on EMB: E.coli





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- Identification: Correlating data from all observations to ID organism to species
- Gram + cocci, grape like clusters, golden yellow colonies, catalase +, coagulase +, resistant to Methicillin (MRSA)
- <u>Staphylococcus aureus</u>