

قسم الميكروبيولوجيا

Lab 5: Purification of microorganism from mixed cultures

140 MIC
Practical

Glossary

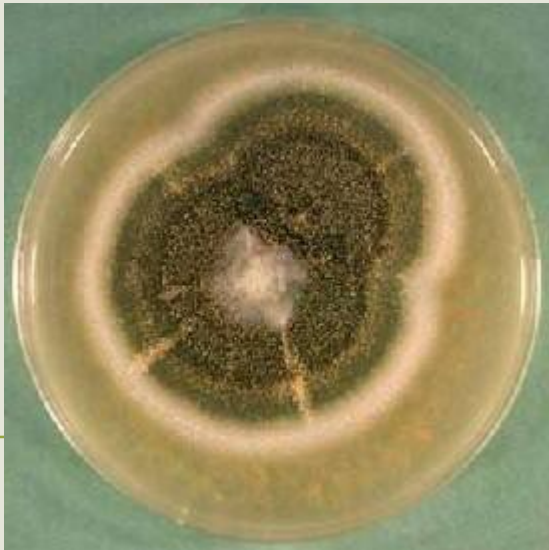


- **Culture**
The growing organism onto the media plate.
- **Colony**
The number of cells of any organism living together.
- **Broth culture:**
Microorganisms growing in a liquid medium.
- **Inoculum:**
A few number of cells transferred to other media for isolation.

Types of Culture

1. Pure Culture:

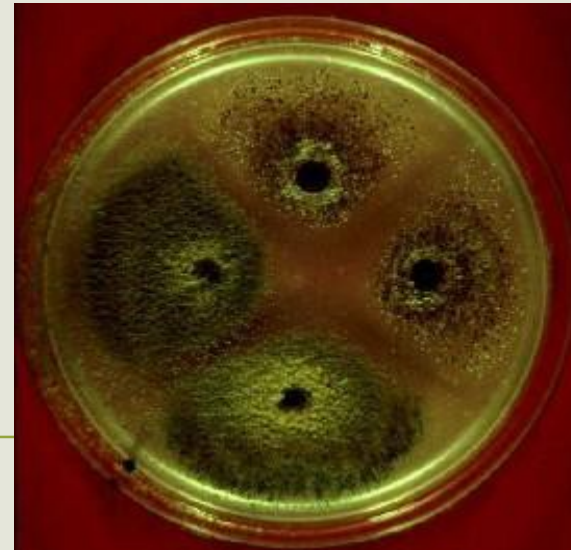
Only one type of microorganism growing on the media plate



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2. Contaminated (mixed) culture

More than one type of microorganism growing on the media plate.



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Amal Alghamdi

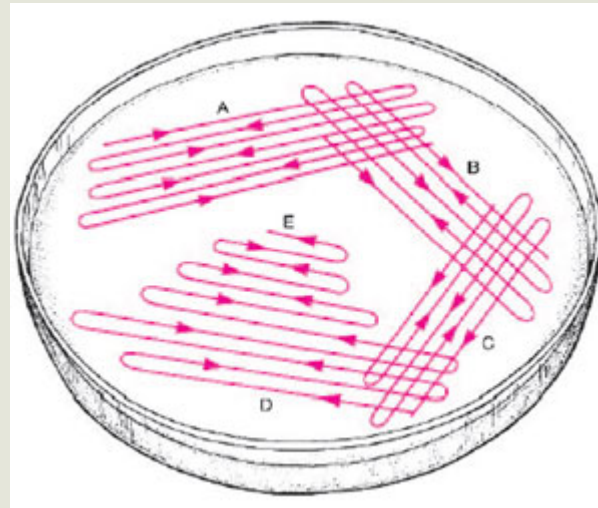
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How Does the Microorganisms Live in Nature?

- microorganisms exist in nature as mixed populations.
- For example, a **mixed culture** contains two or more bacterial species.
- However, to study microorganisms in the lab we must have them in the form of a **pure culture**.

How to separate Microorganisms ?

- **Streak plates** allow for the growth of **isolated** colonies on the surface of the agar.
- An **isolated colony** is a colony that is not touching any other colonies and is assumed to be a **pure culture**.



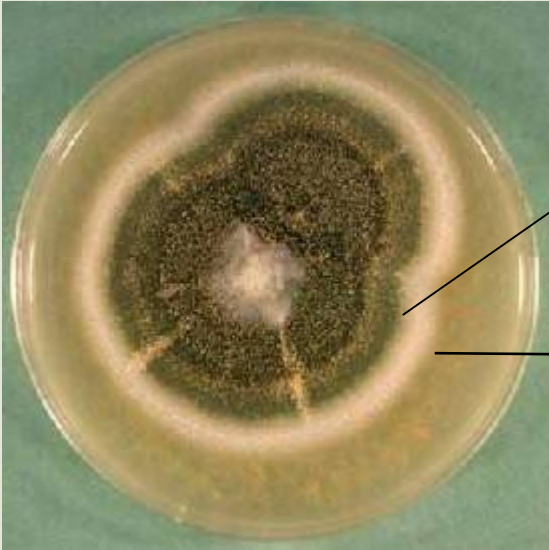
The Experiment

**Purification of Microorganisms
to get Pure Cultures.**

Purification of microorganism

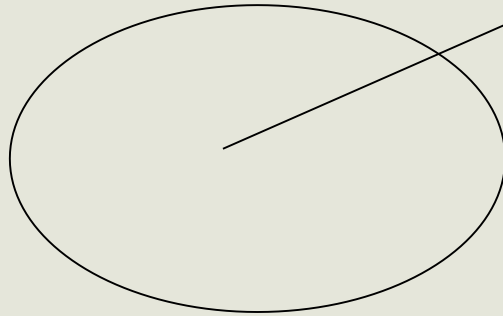
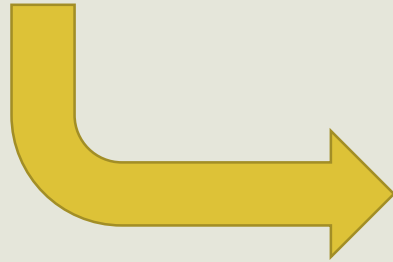
A. Fungi by Disc Transfer :

- Use a cork borer or pasture pipet.
- Flame cork borer using alcohol and allow to cool.
- Cut few discs from the **edge** of an actively growing fungal colony.
- Inoculate it (surface facing down) on the center another media plate with the help of flamed forceps
- Incubate it for 2-3 days
- Pure culture of the organism will grow.



1- Use a cork borer to pick up some material from the colony

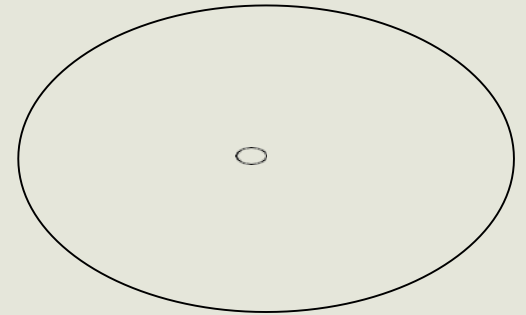
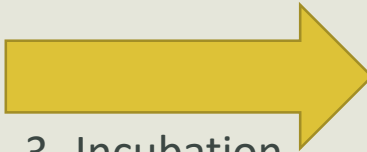
Edge of actively growing fungal colony



A sterile media plate (PDA) being inoculated

2- A fungal colony Disc transferred Aseptically to the centre by loop

3- Incubation



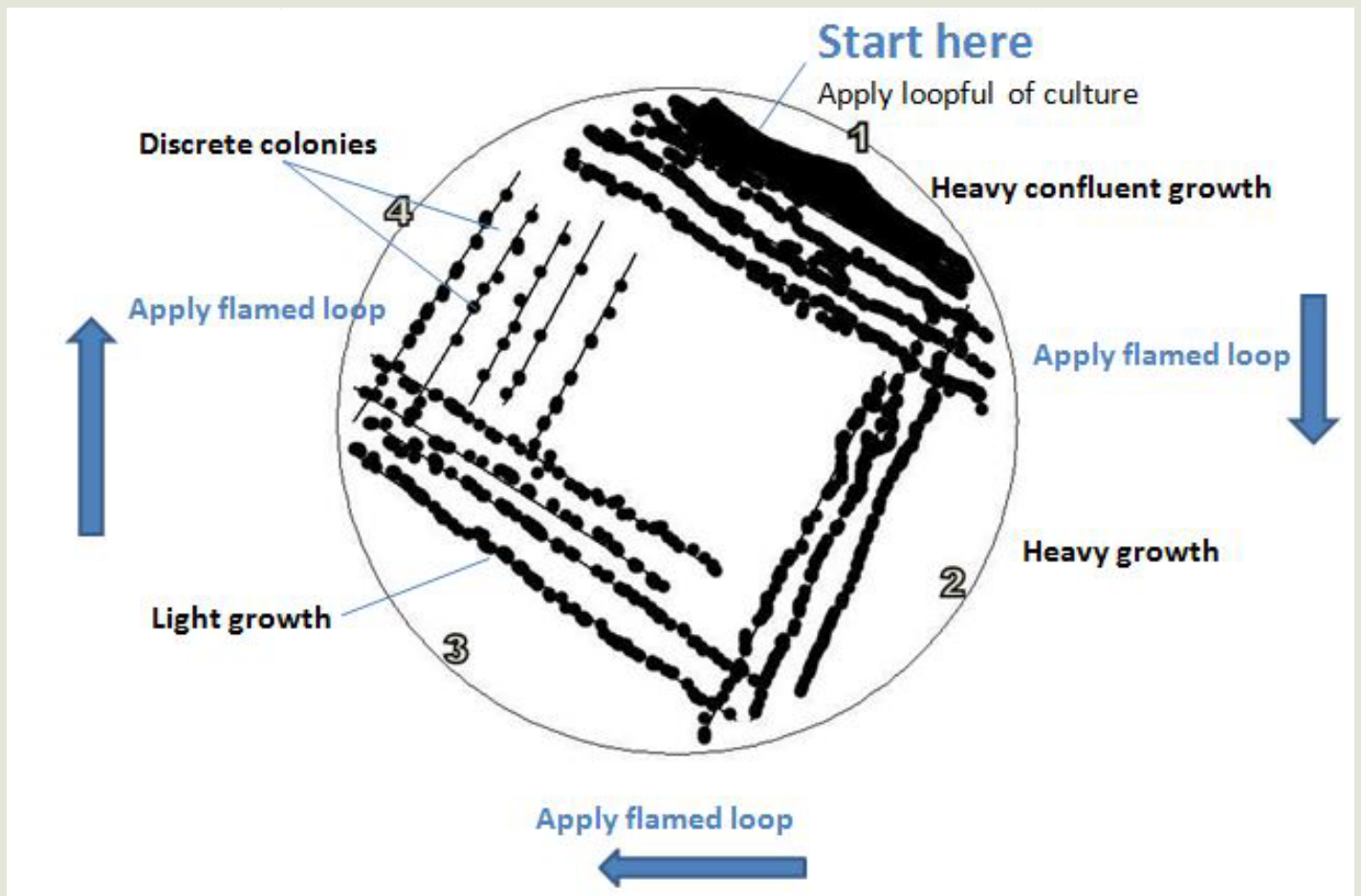
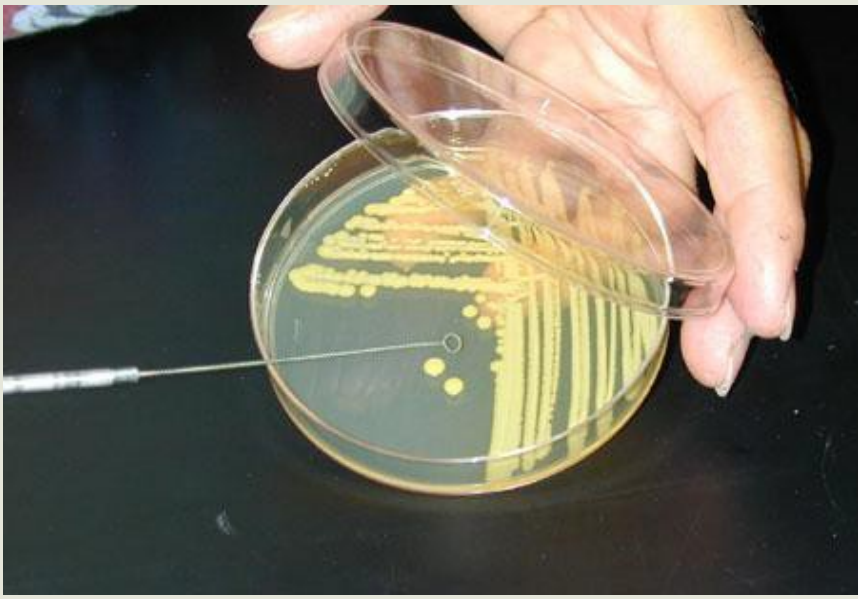
Single colony of organism

Purification of microorganism

B. Bacteria- by Streak plate Method:

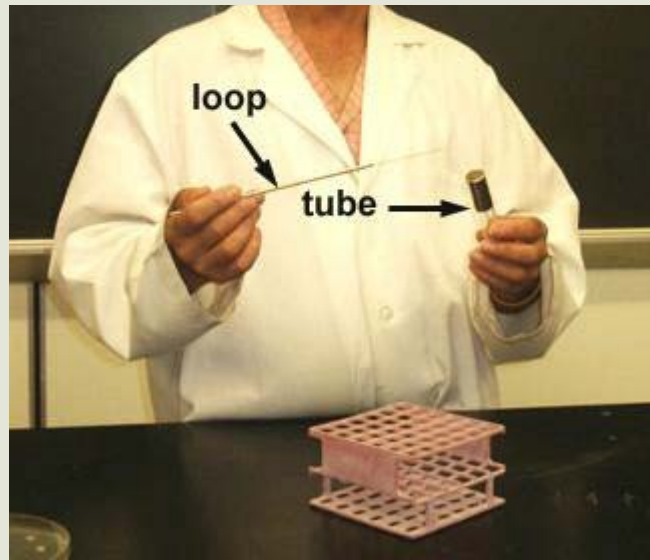
- As the loop streaks across the agar surface
- More and more bacteria are rubbed off
- Until individual separated organism are deposited on the agar
- After incubation, the area at the beginning of the streak pattern will show mix growth,
- At the end of the pattern, a single colony will be observed after incubation period.

Quadrant Streaking for Isolation into Pure Cultures



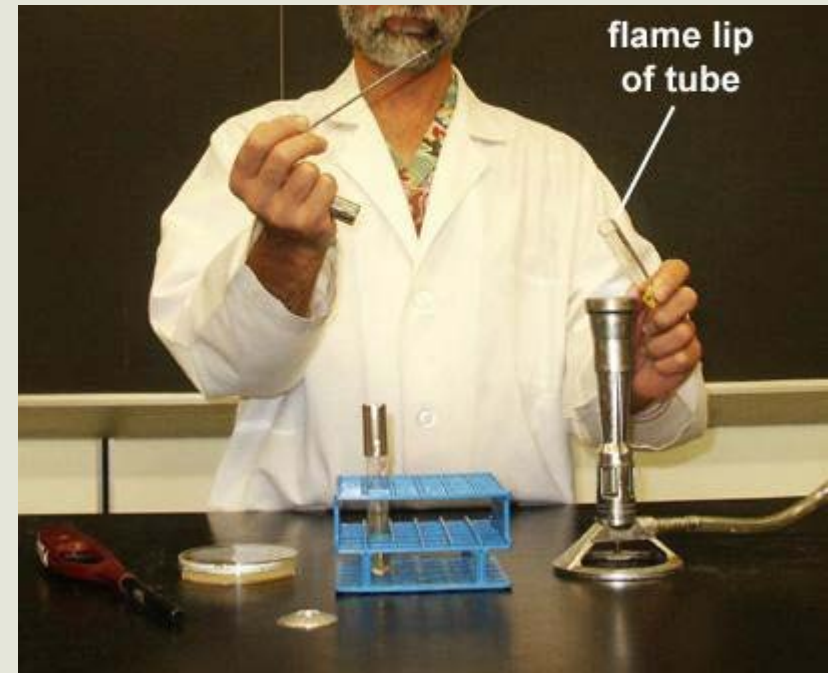
1- Inoculating from Broth culture

1. Hold the culture tube in one hand and in your other hand hold the sterilized inoculating loop then



1- Inoculating from Broth culture

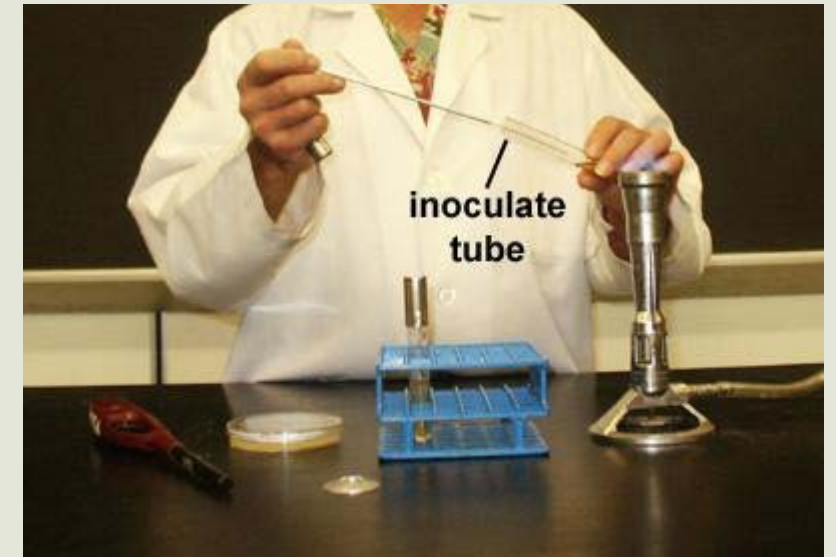
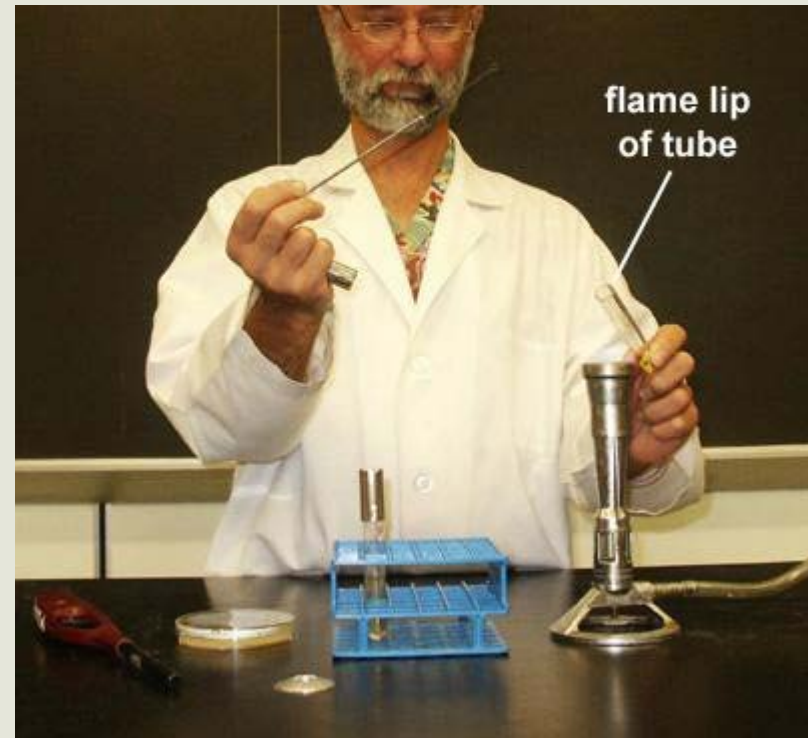
2. Keeping the culture tube at an angle, insert the inoculating loop and remove a loopful of inoculum **Again flame the lip of the culture tube and Replace the cap** flame the lip of the culture tube



Remove a loopfull of bacteria from your pure culture

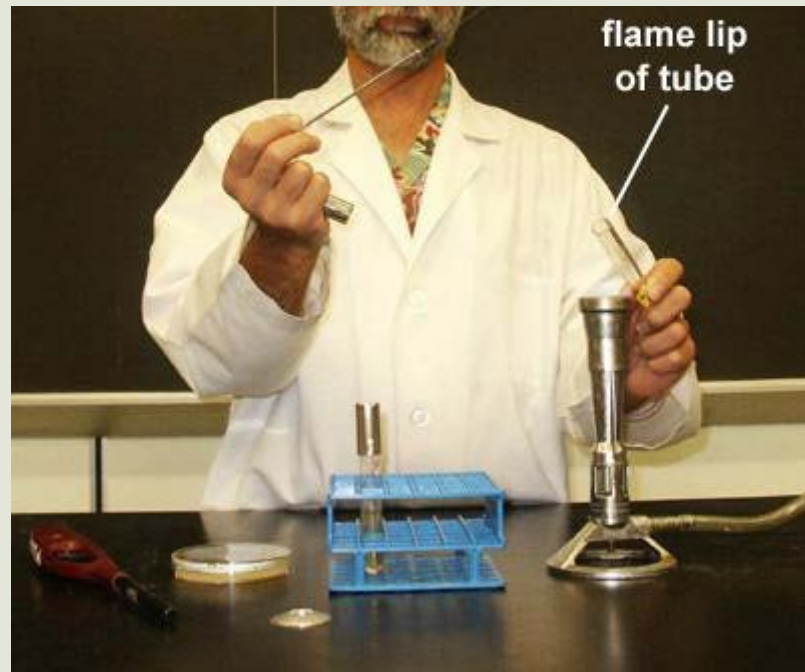
1- Inoculating from Broth culture

3. Transferring the inoculum into a broth tube Pick up the sterile broth tube and remove the cap with the little finger then flame the lip of the broth tube. After that, 8. Place the loopful of inoculum into the broth and withdraw the loop.



1- Inoculating from Broth culture

4. Again flame the lip of the tube and Replace the cap



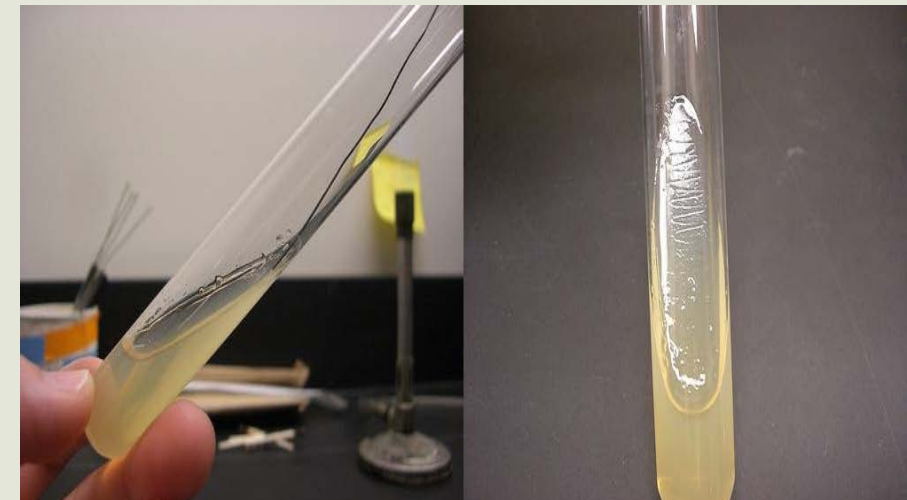
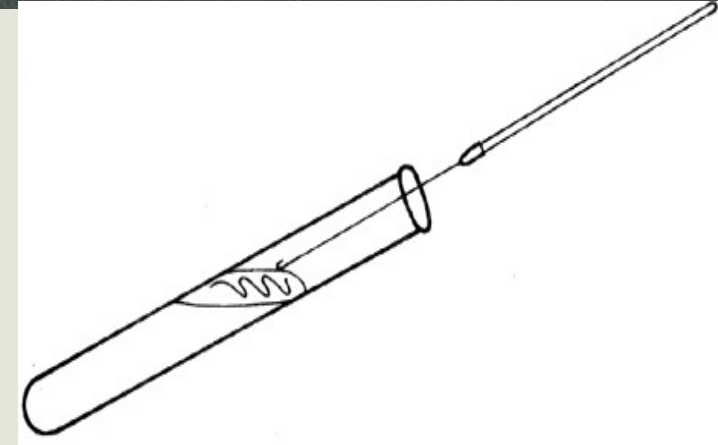
2- Removing inoculum from a plate:

- If the microorganisms growing on an agar surface in a petri plate. Follow the steps under aseptic conditions:
 1. Sterilize the inoculating loop in the flame
 2. Lift the lid of the culture plate and stab the loop into the agar away from any growth to cool the loop
 3. Scrape off a small amount of the organisms and close the lid.

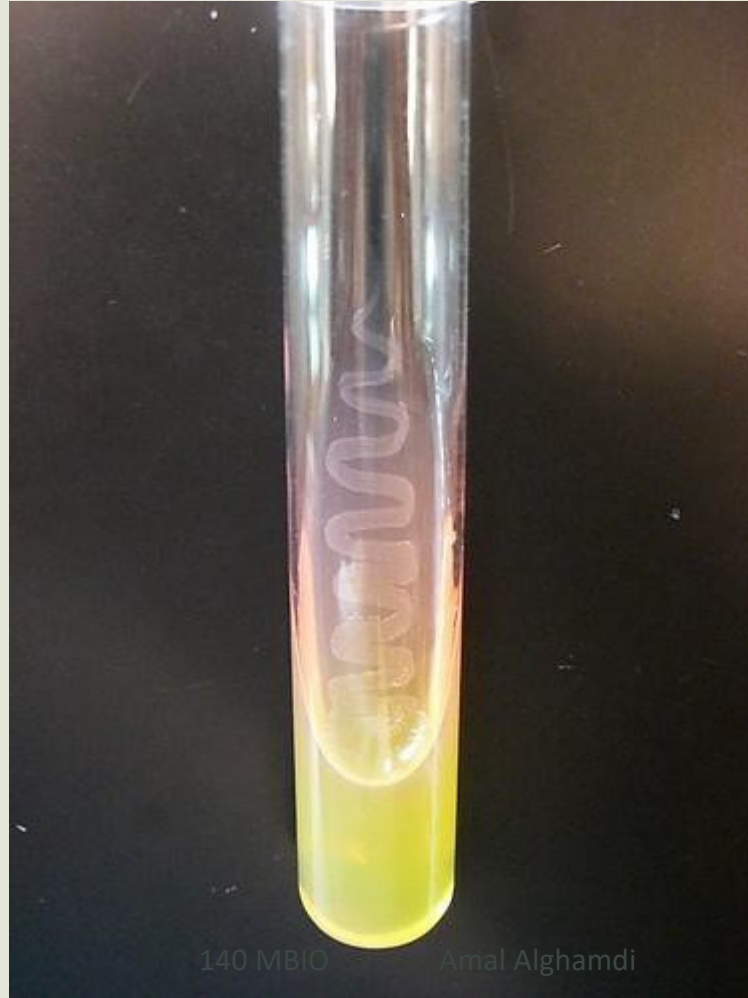


3- Inoculating an Agar Slant:

1. Label the sterile nutrient agar slant with the source of the culture and your Initials.
2. Sterilize the loop.
3. Using appropriate aseptic technique, remove a loopful of broth from the culture tube.
4. Insert the loop into the sterile agar slant tube and starting at the base of the slant, draw the loop up the slant. Do not penetrate the agar. Then, Sterilize the loop.
5. Incubate the slant at 37°C for 24- 48 hours.
6. Observe the slant for growth.



Inoculated Agar Slant, after incubation = Slant Culture



COLONY MORPHOLOGY ON AGAR PLATE CULTURES



Bacillus subtilis

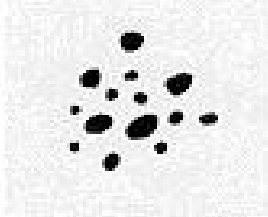


Round yeast colonies

Colony Morphology Characterization

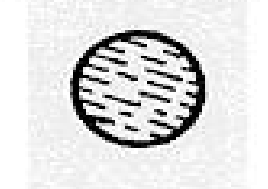
Form of Colony

punctiform

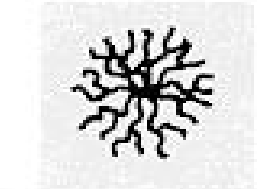


under 1mm in diameter

circular

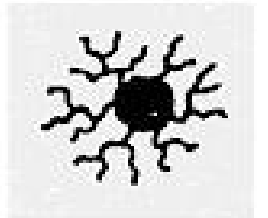


filamentous



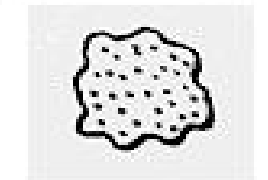
long, irregular, interwoven threads

rhizoid



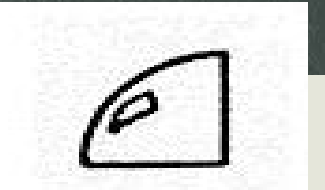
irregular, branched

irregular

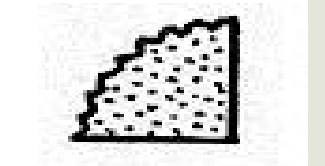


Margin of Colony

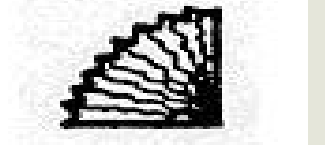
entire



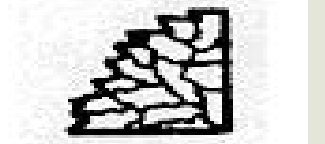
undulate



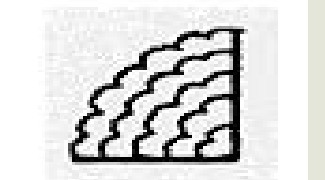
erose



filamentous



curled



Colony Morphology Characterization

Surface of Colony

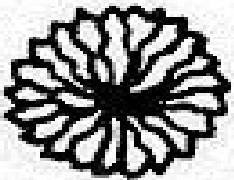
smooth



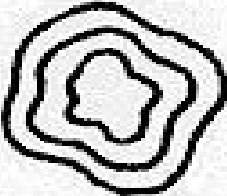
contoured



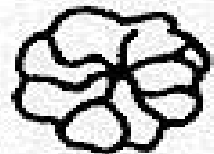
radiate



concentric



rugose



undulating

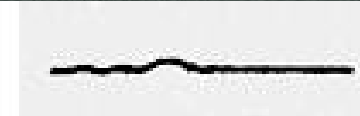
radiating rings

concentric rings

wrinkled

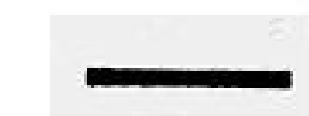
Elevation of Colony

effuse

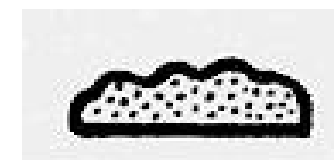


very thin, spreading

flat



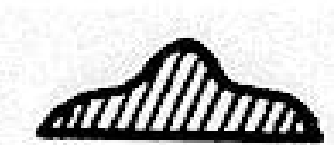
raised



convex

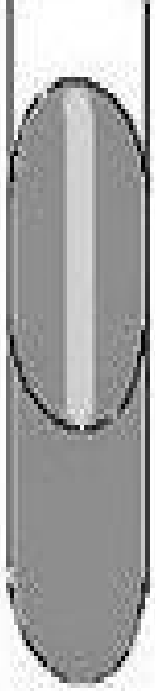


umbonate

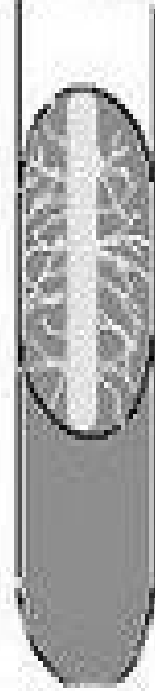


Colony Morphology Characterization

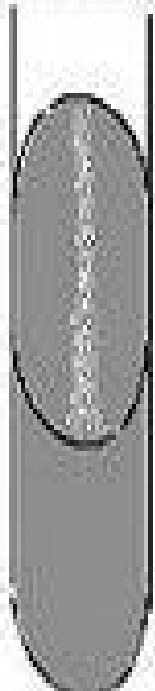
MORPHOLOGY ON SLANT MEDIUM



**filiform
(thread-like)**



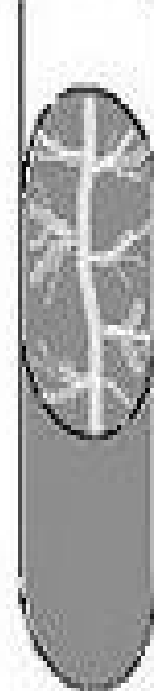
**arborescent
(tree-like)**



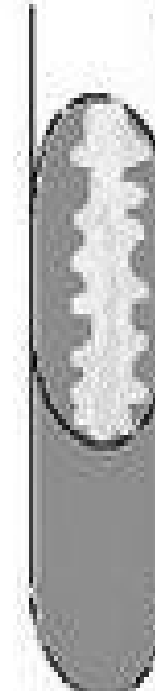
beaded



**effuse
(spreading)**



rhizoid



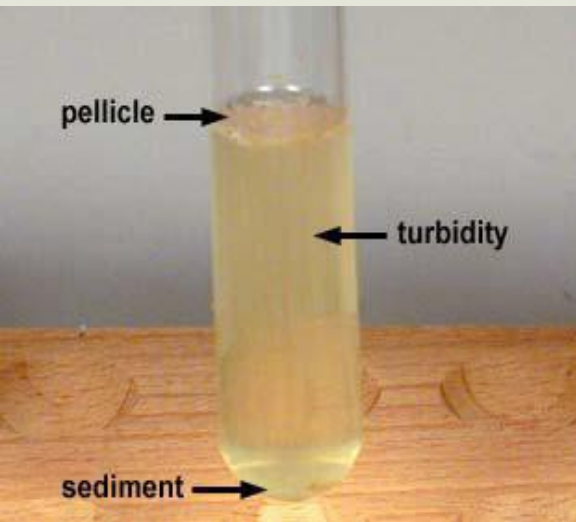
**echinulate
(spiny)**



In a liquid medium, the region in which the organism grows depends on the oxygen requirement of that particular species.

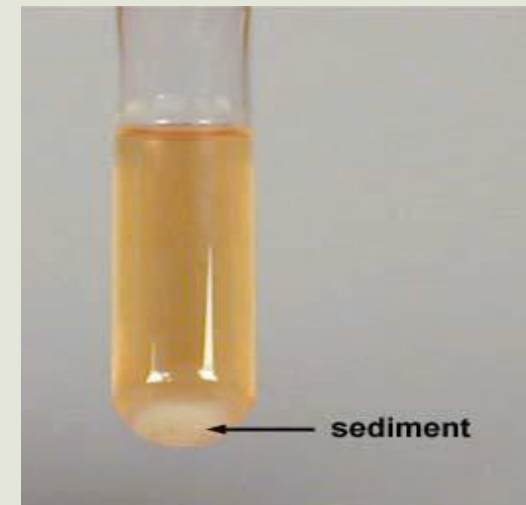
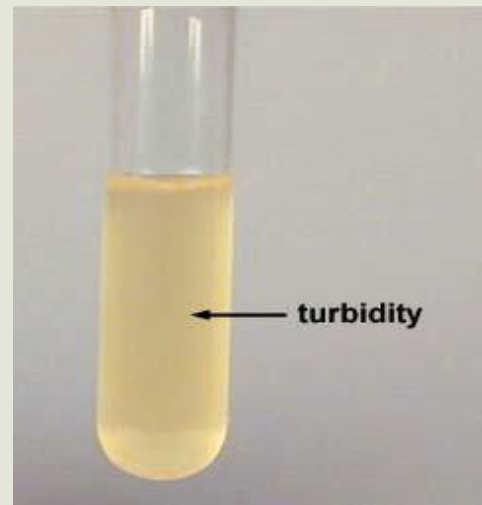
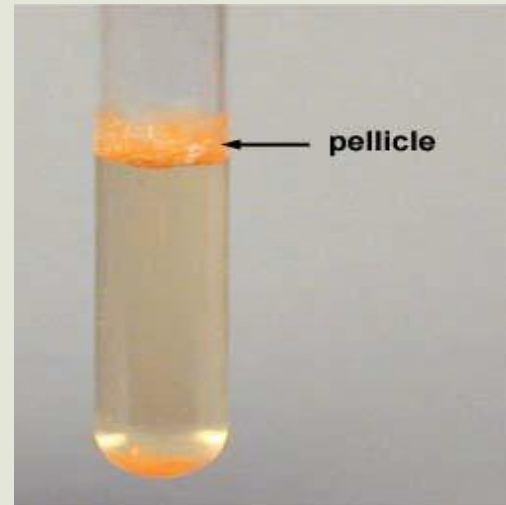
Types of growth on Liquid medium:

1. Turbid
2. Pellicle (thick growth at the top of the tube)
3. Sediment



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Amal Alghamdi
Amal Alghamdi

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Thanks for Listening!

**For any question, please contact:
ahamdan1@ksu.edu.sa**