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.

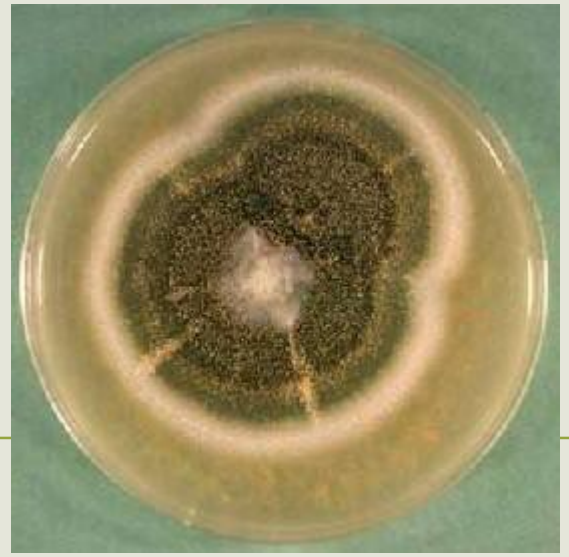
- **Inoculum:**

A few number of cells transferred to other media for isolation.

Types of Culture

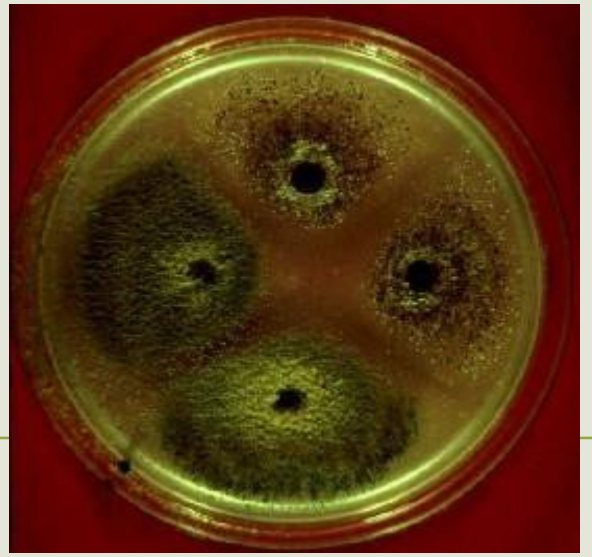
1.Pure Culture:

Only one type of microorganism growing on the media plate



2.Contaminated (mixed) culture

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

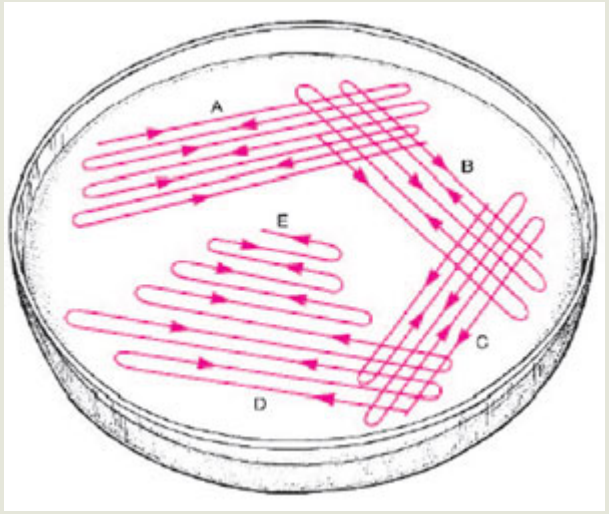


How Does the Microorganisms Live in Nature?

- 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 Microorgansims ?

- **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.**

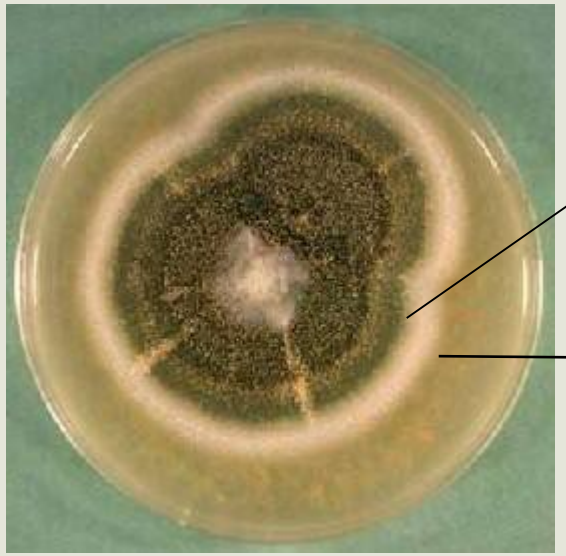


The Experiment

Purification of Microorganisms to get Pure Cultures.

Purification of microorganism
A.Fungi by Disc Transfer :

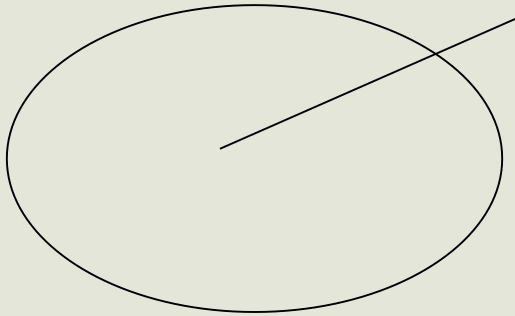
- Use a cork borer or pasture pipet.
- Flame cork borer using alcohol and allow to cool.
- Cut few discs from the edge of an actively growing fungal colony.
- Inoculate it (surface facing down) on the center another media plate with the help of flamed forceps
- Incubate it for 2-3 days
- Pure culture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



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

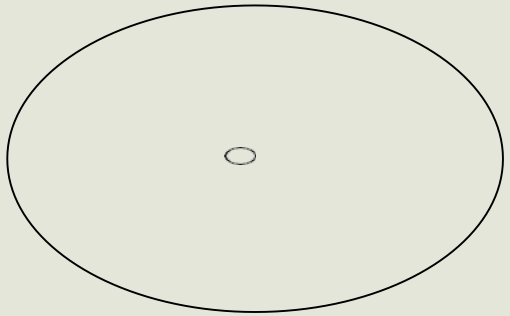
Edge of actively growing fungal colony

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



A sterile media plate (PDA) being inoculated

3- Incubation

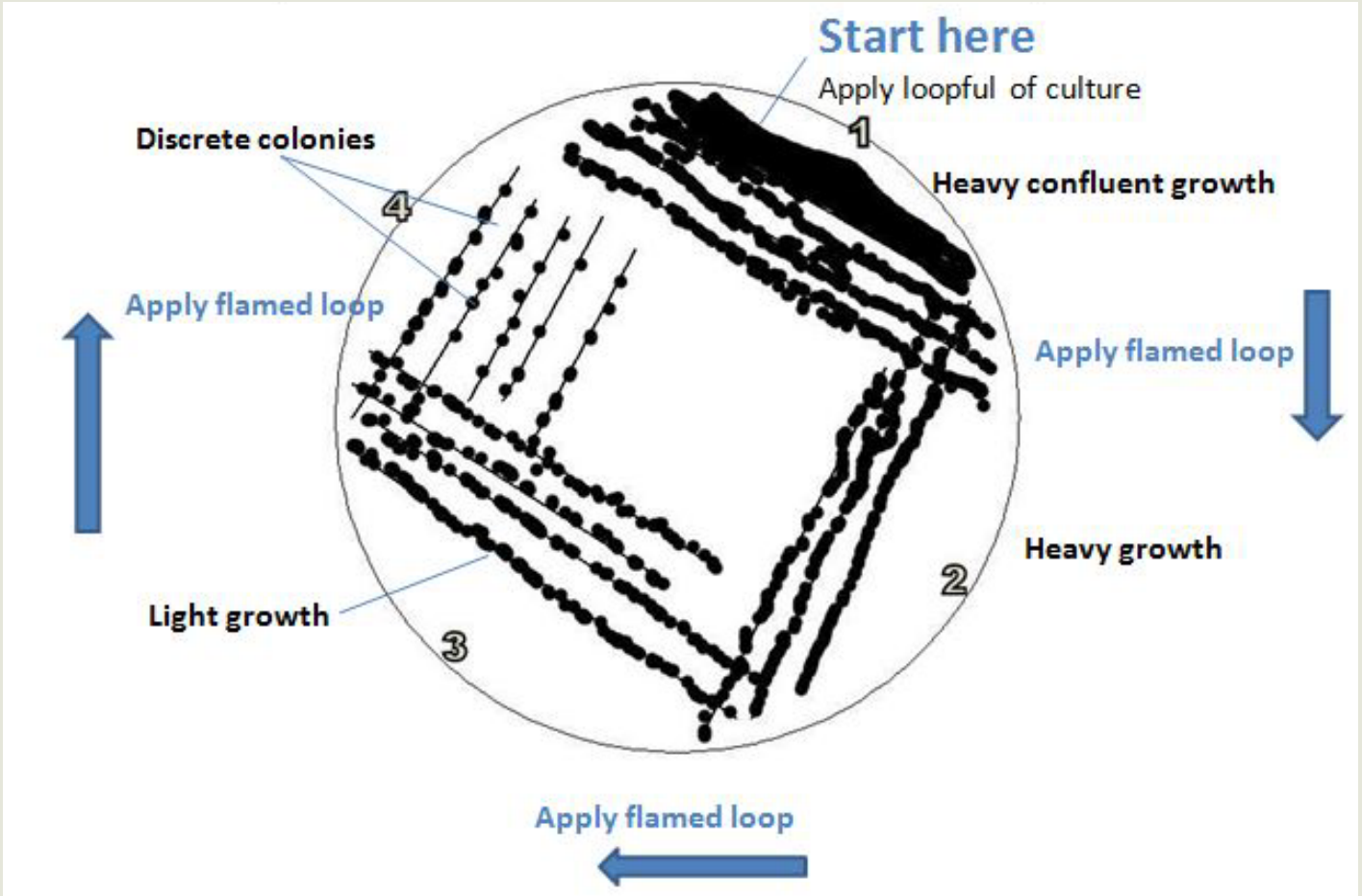


Single colony of organism

Purification of microorganism
B. Bacteria- by Streak plate Method:

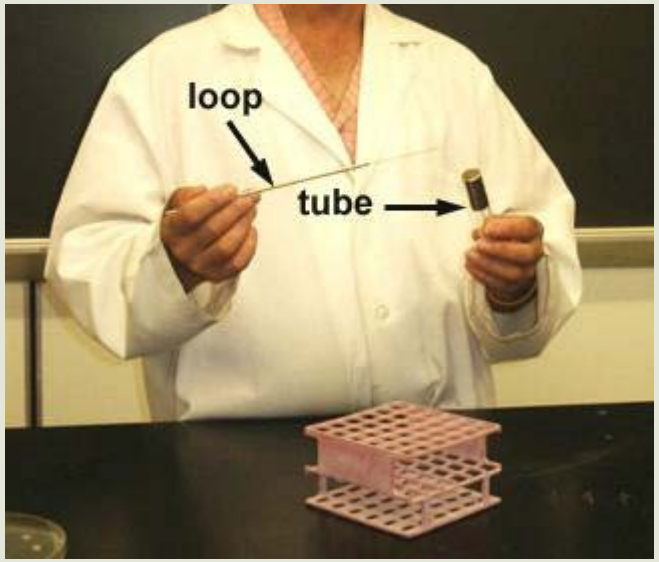
- As the loop streaks across the agar surface
- More and more bacteria are rubbed off
- Until individual separated organism are deposited on the agar
- After incubation, the area at the beginning of the streak pattern will show mix growth,
- At the end of the pattern, a single colony will be observed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

Quadrant Streaking for Isolation into Pure Cultures



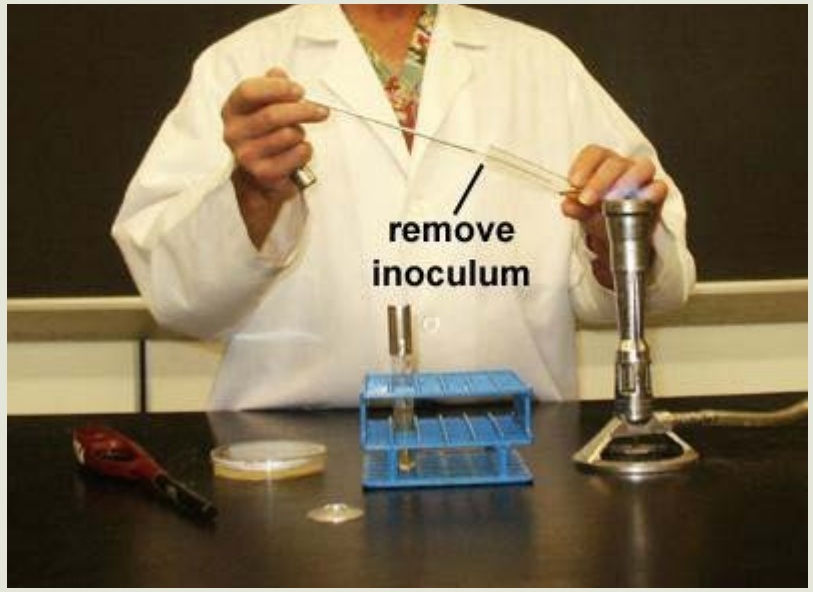
1- Inoculating from Broth culture

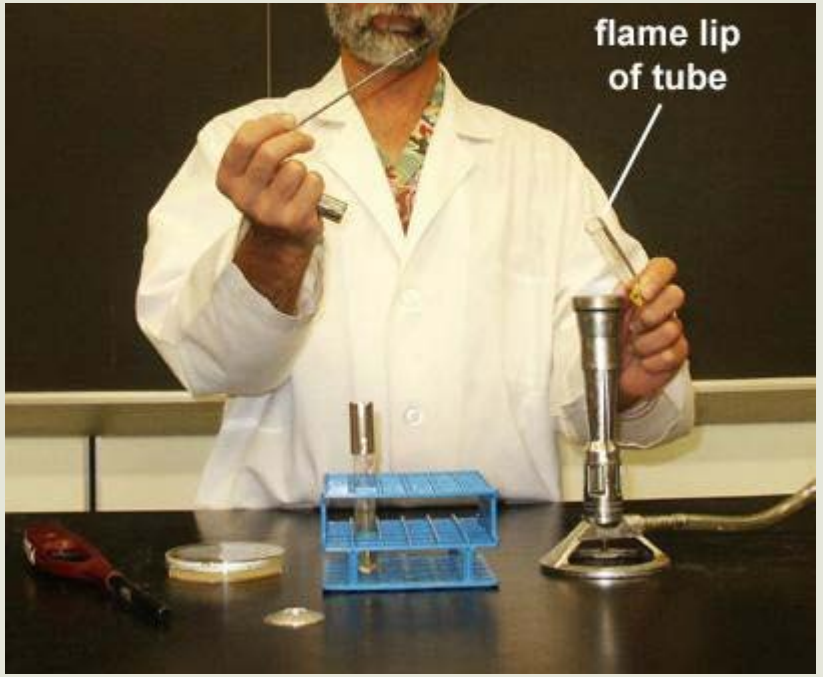
1. Hold the culture tube in one hand and in your other hand hold the sterilized inoculating loop then



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

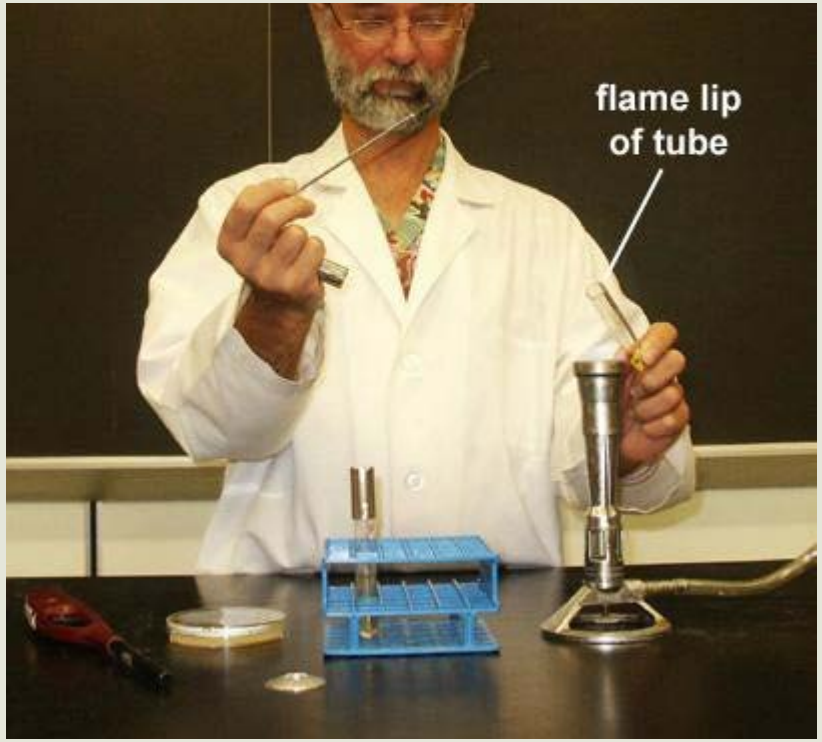


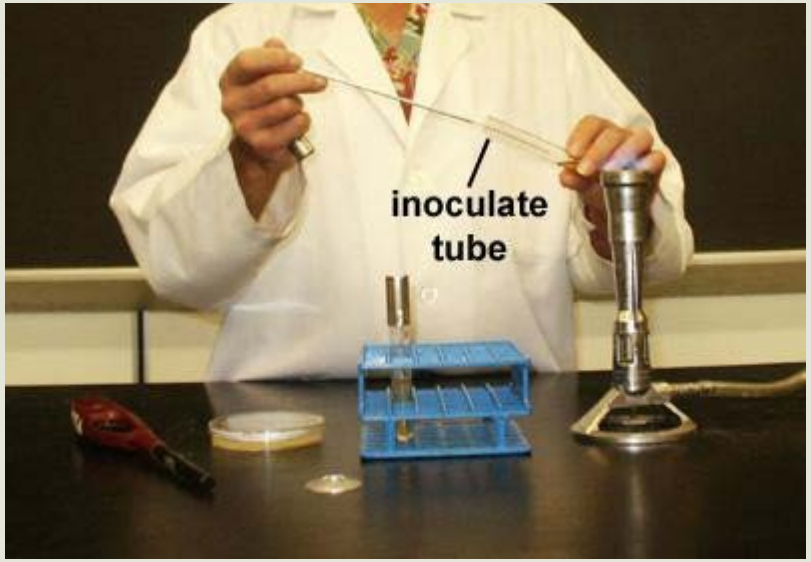


Remove a loopfull of bacteria from your pure culture

1- Inoculating from Broth culture

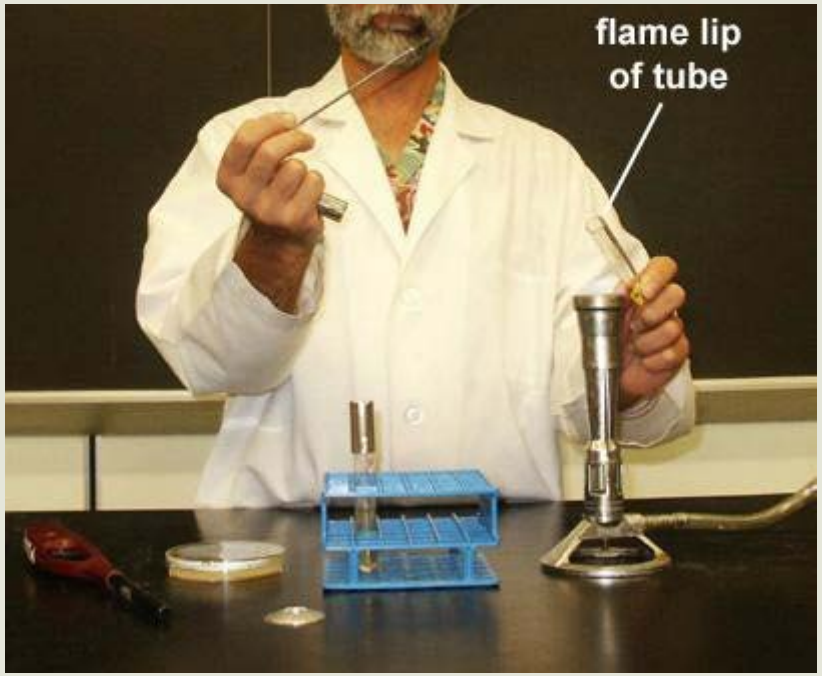
3.Transferring the inoculum into a broth tube Pick up the sterile broth tube and remove the cap with the little finger then flame the lip of the broth tube. After that, 8.Place the loopful of inoculum into the broth and withdraw the loop.





1- Inoculating from Broth culture

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



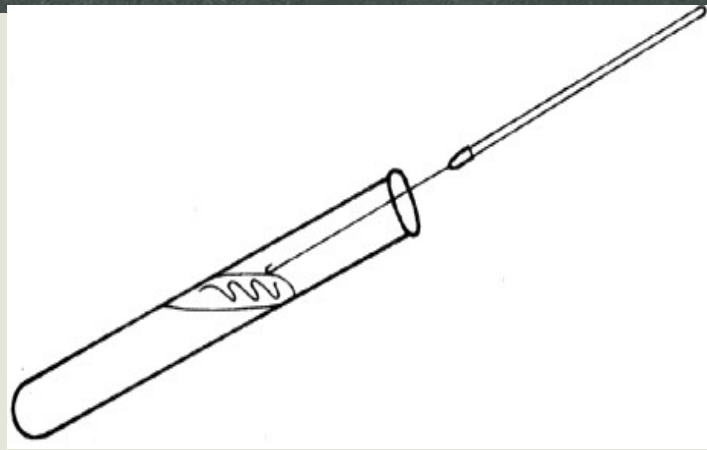
2- Removing inoculum from a plate:

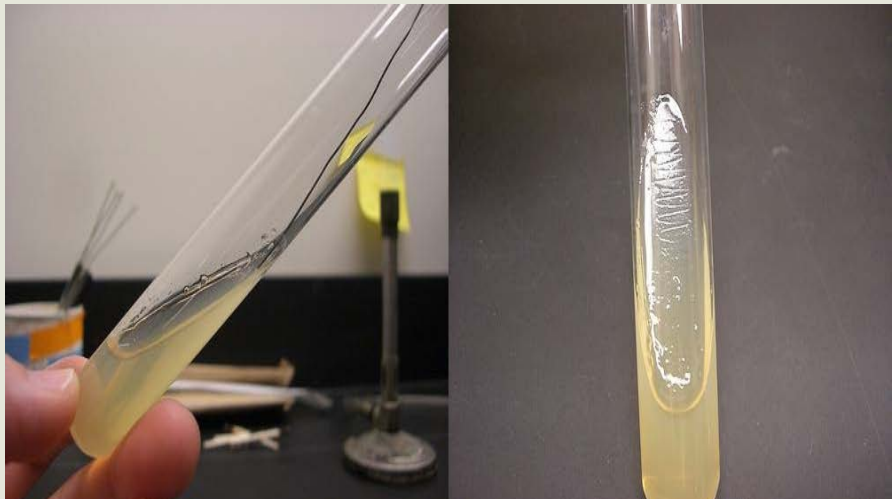
- If the microorganisms growing on an agar surface in a petri plate. Follow the steps under aseptic conditions:

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.

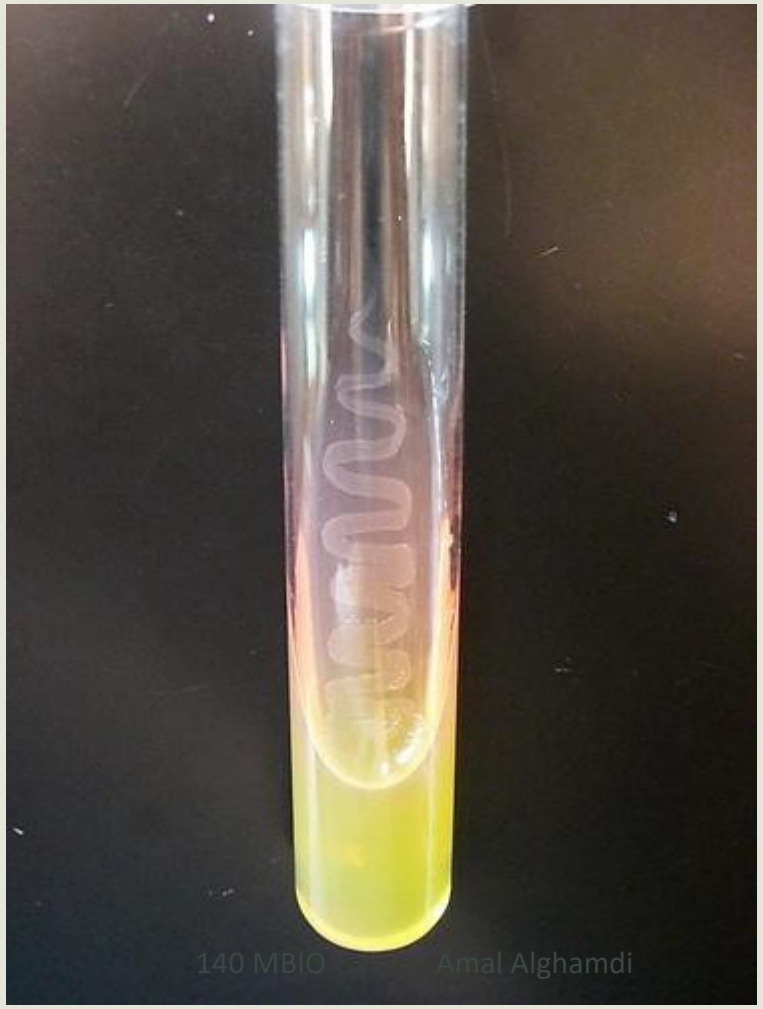
3- Inoculating an Agar Slant:

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 $37^{o}C$ for 24- 48 hours.
6. Observe the slant for growth.





Inoculated Agar Slant, after incubation = Slant Culture



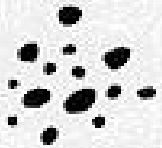
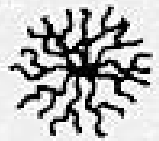
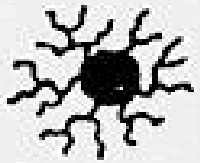
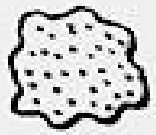
COLONY MORPHOLOGY ON AGAR PLATE CULTURES

Bacillus subtilis

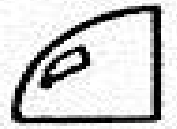
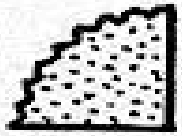
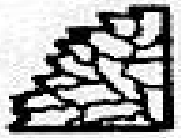
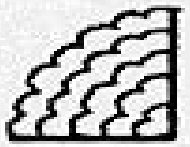
Round yeast colonies

Colony Morphology Characterization

Form of Colony

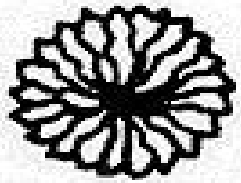
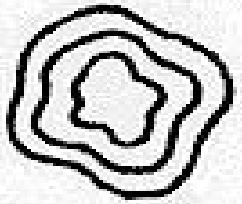
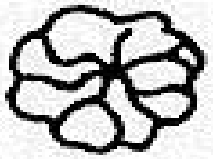
punctiform		under 1mm in diameter
circular		
filamentous		long, irregular, interwoven threads
rhizoid		irregular, branched
irregular		

Margin of Colony

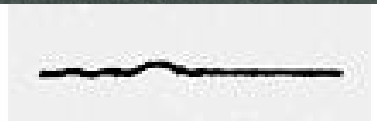
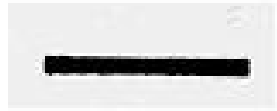
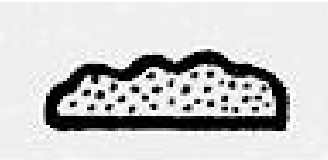
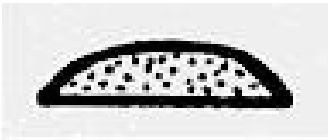
entire	
undulate	
erose	
filamentous	
curled	

Colony Morphology Characterization

Surface of Colony

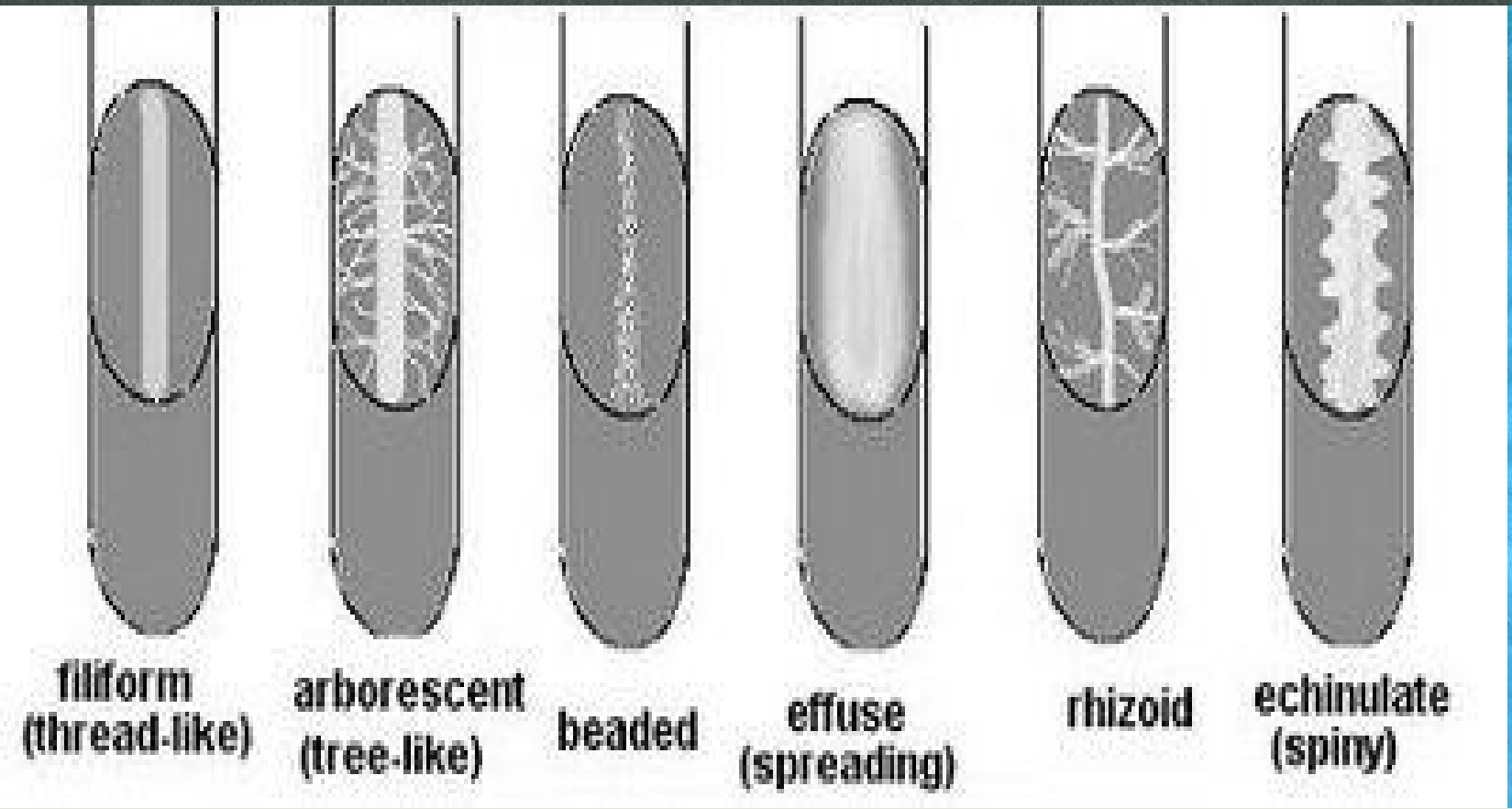
smooth		
contoured		undulating
radiate		radiating rings
concentric		concentric rings
rugose		wrinkled

Elevation of Colony

effuse		very thin, spreading
flat		
raised		
convex		
umbonate		

Colony Morphology Characterization

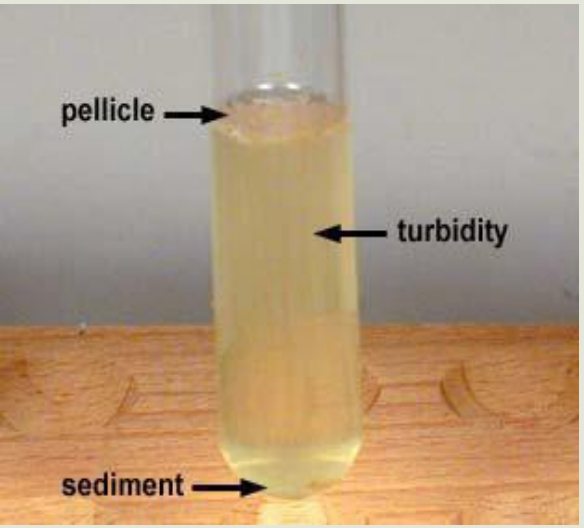
MORPHOLOGY ON SLANT MEDIOM

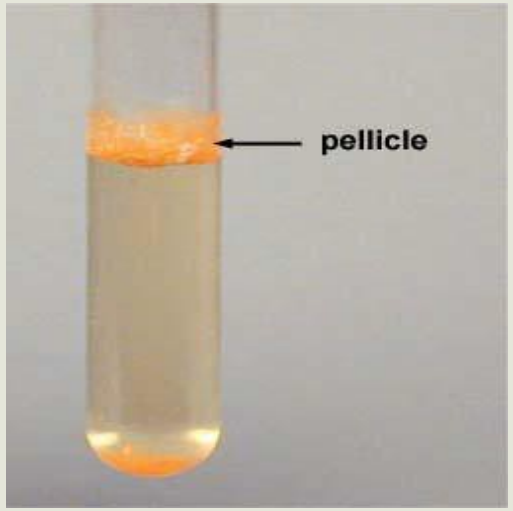


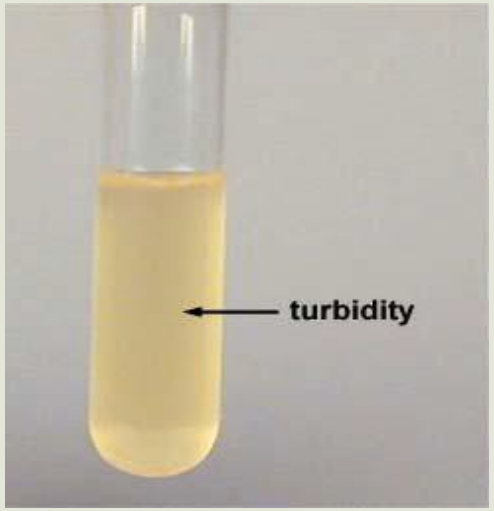
In a liquid medium, the region in which the organism grows depends on the oxygen requirement of that particular species.

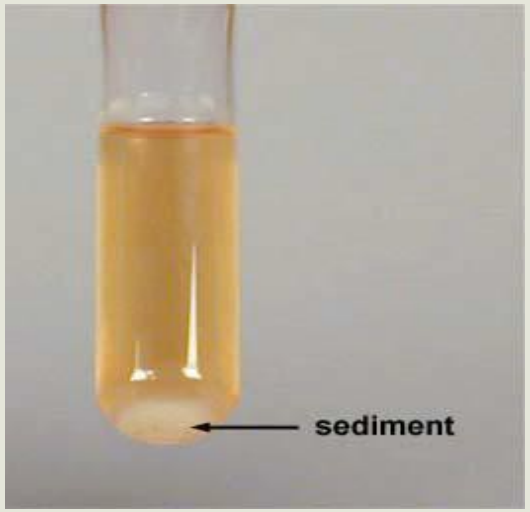
Types of growth on Liquid medium:

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









Thanks for Listening!

For any question, please contact:
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