بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

140 Micro

Lab 5: Purification of microorganism from culture

Purification of microorganism

- **Culture**

  The organism growing on the media plate is called as culture

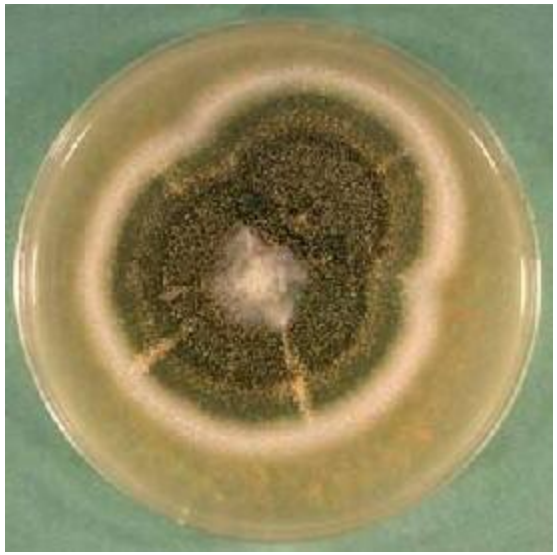
- **Colony**

  The number of cells of any organism living together

Types of Culture

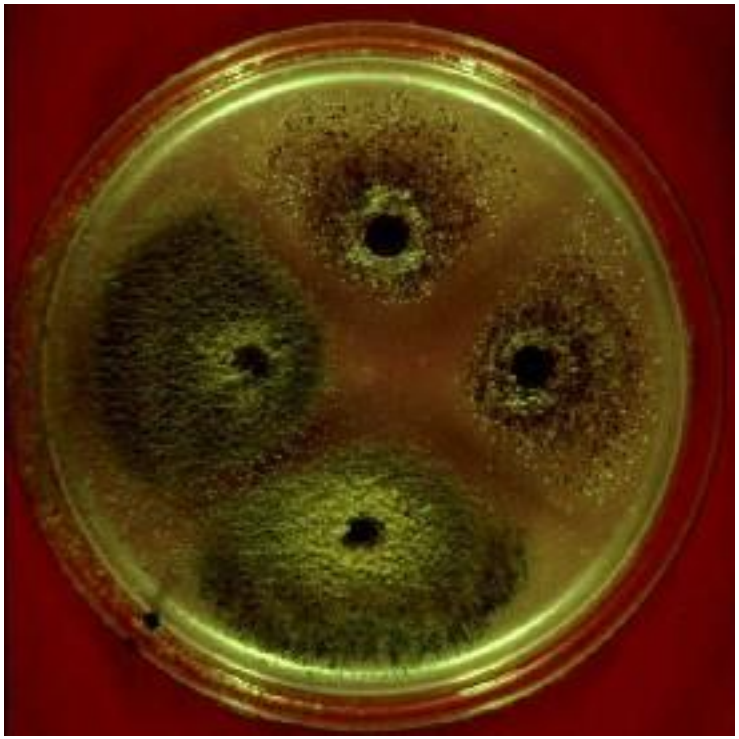
1. Pure Culture:

Only one type of microorganism growing on the media plate



2.Contaminated culture

More than one type of organism growing on the media plate



- **microorganisms exist in nature as mixed populations(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.**

- **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**

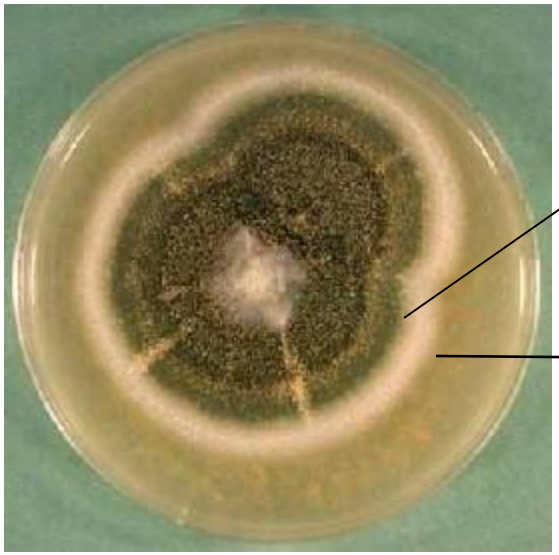
COLONY MORPHOLOGY ON AGAR PLATE CULTURES

Bacillus subtilis

Round yeast colonies

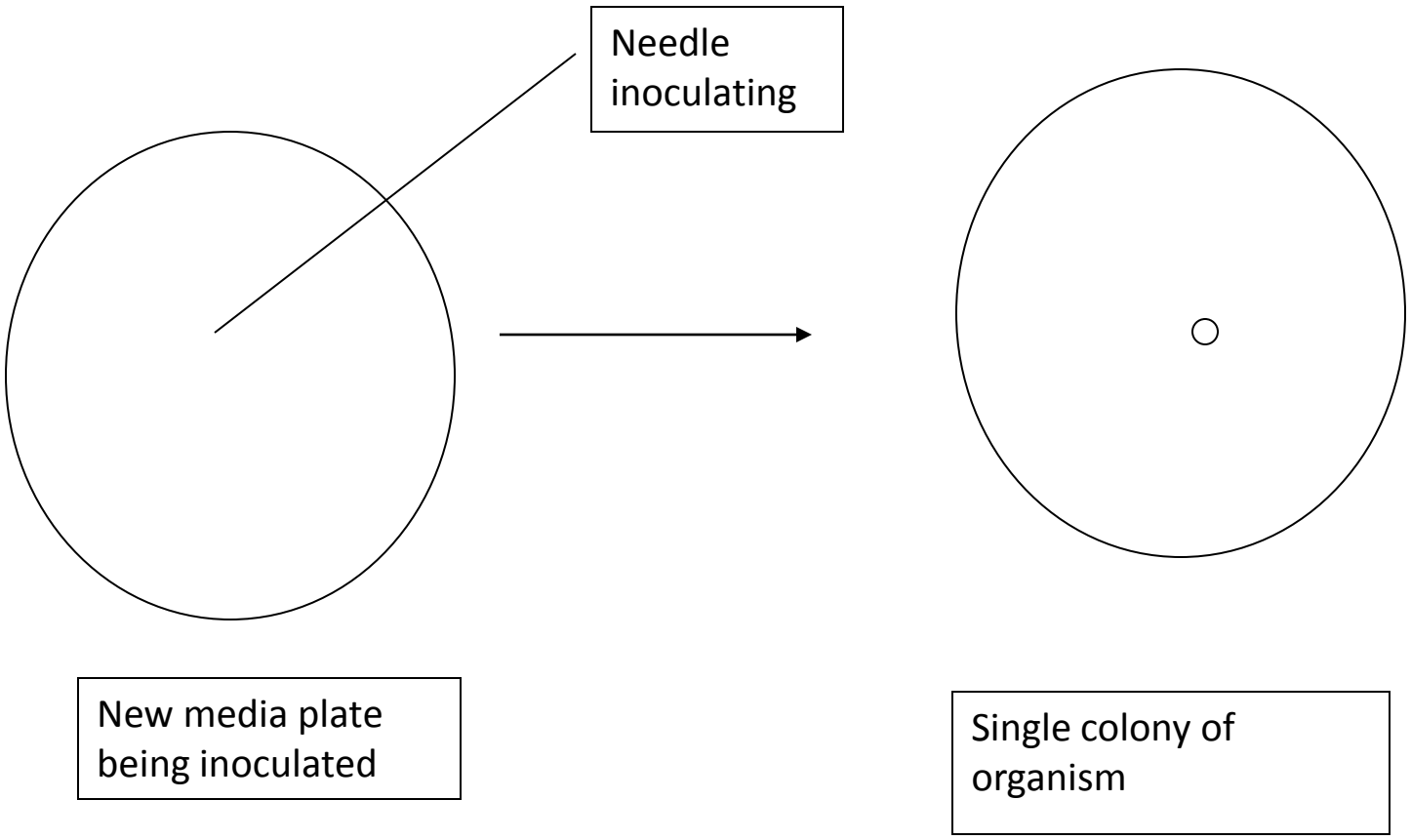
A.Purification of microorganism Fungi :

- 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.



Needle picking up some material from the colony

Actively growing fungal colony


Needle inoculating
New media plate being inoculated
Single colony of organism

B.Bacteria-Streak plate Method:

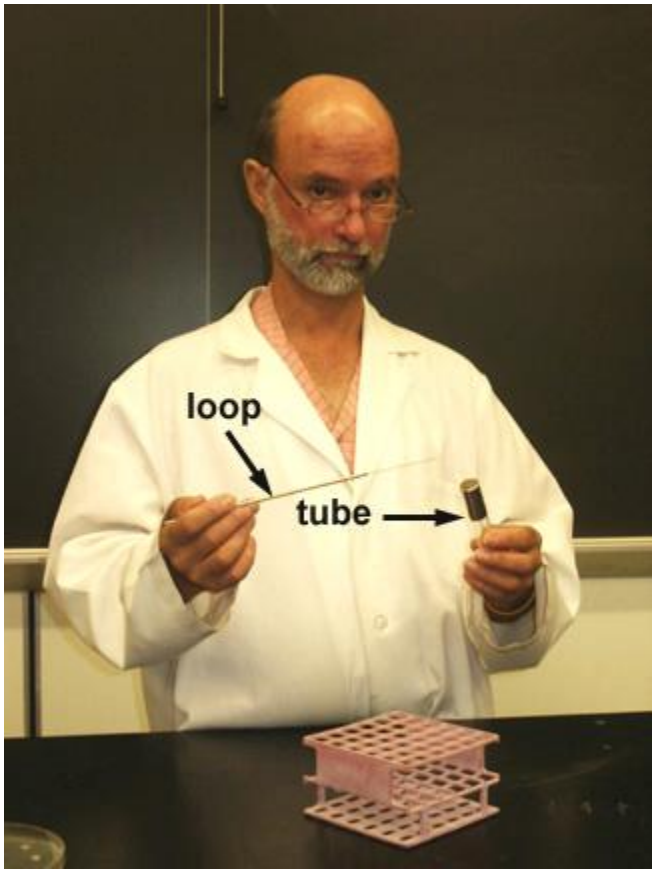
- As the loop streaks across the agar surface
- More and more bacteria are rubbed off
- Until individual separated organism are deposited on the agar
- After incubation, the area at the beginning of the streak pattern will show mix growth,
- At the end of the pattern , a single colony will be observed

ASEPTIC TECHNIQUE

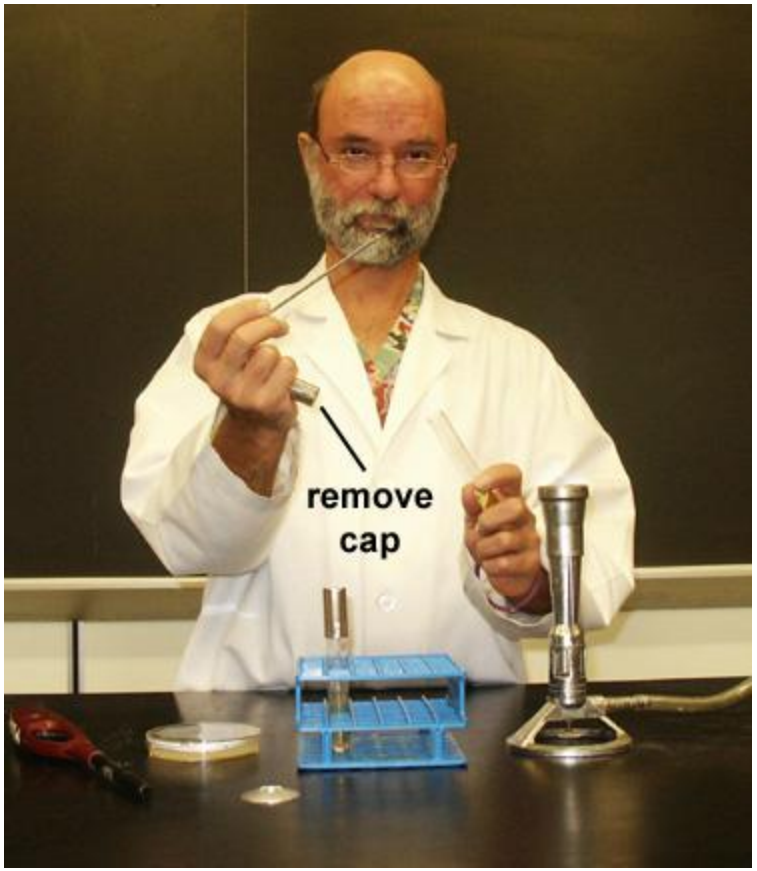
Removing inoculum from a broth culture

organisms growing in a liquid medium

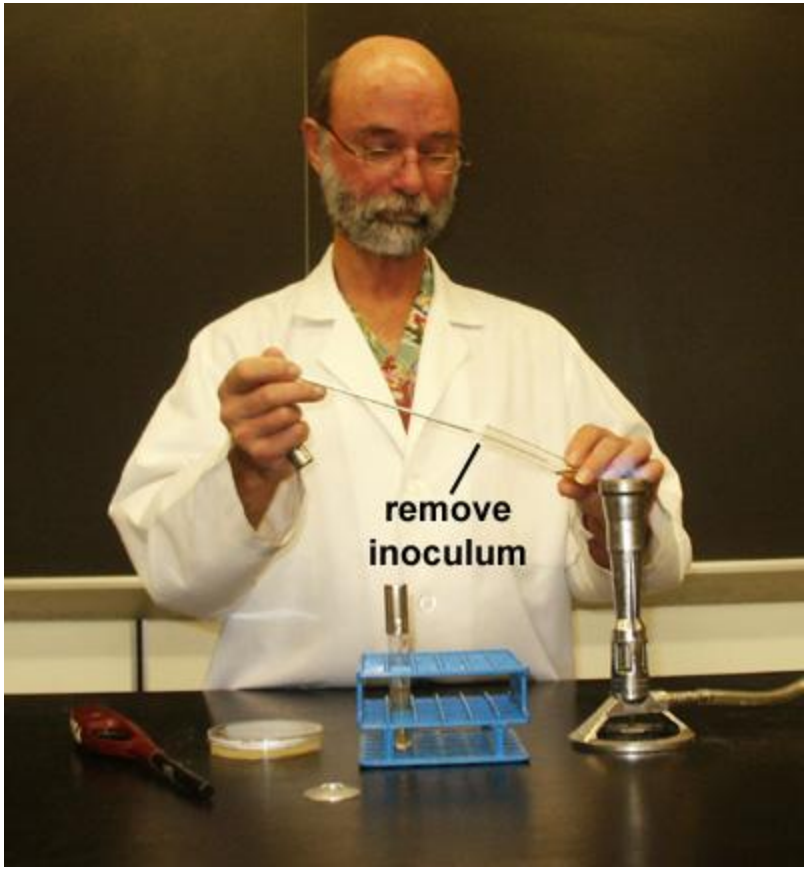
1. Hold the culture tube in one hand and in your other hand hold the sterilized inoculating loop



2. Remove the cap of the pure culture tube with the little finger of your loop hand



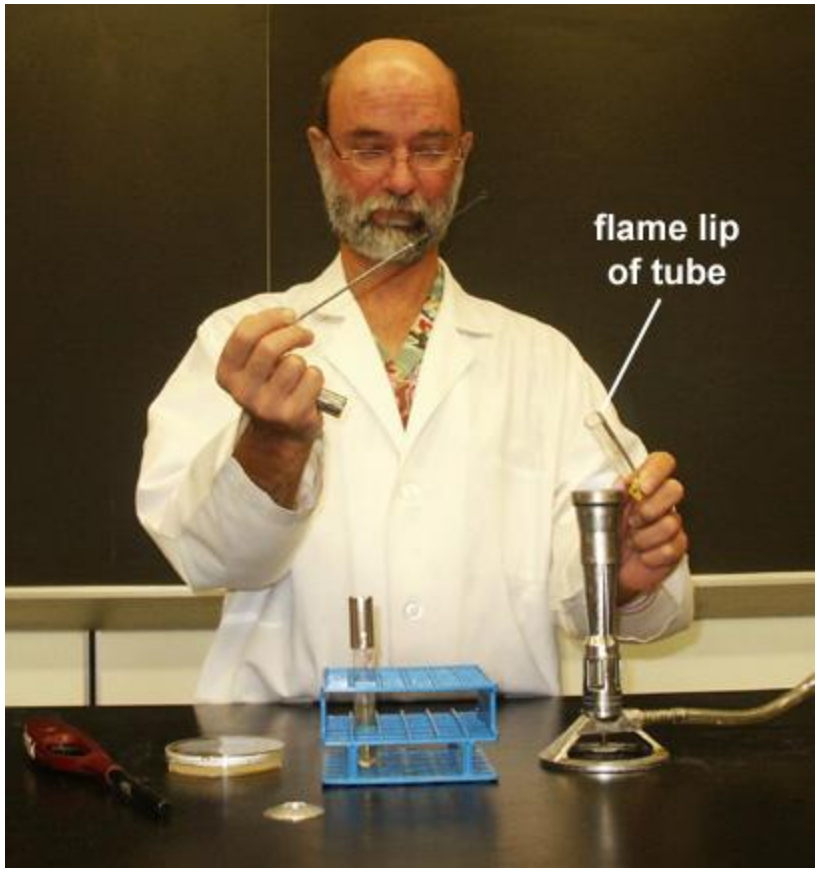
3. Keeping the culture tube at an angle, insert the inoculating loop and remove a loopful of inoculum



Remove a loopfull of bacteria from your pure culture

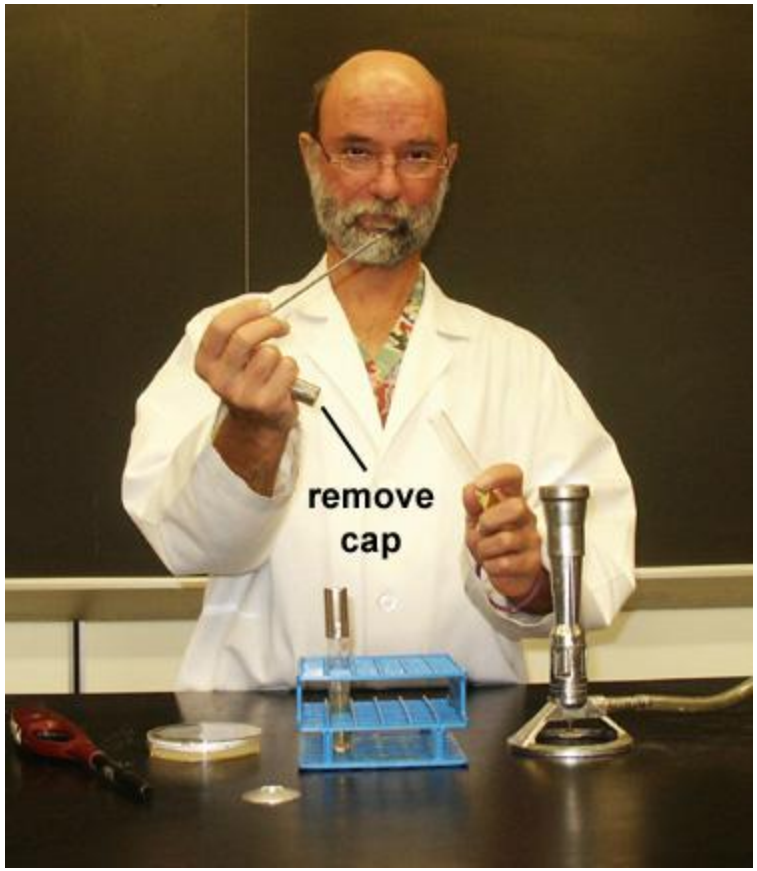
4. **Again flame the lip of the culture tube and Replace the cap**

5. flame the lip of the culture tube

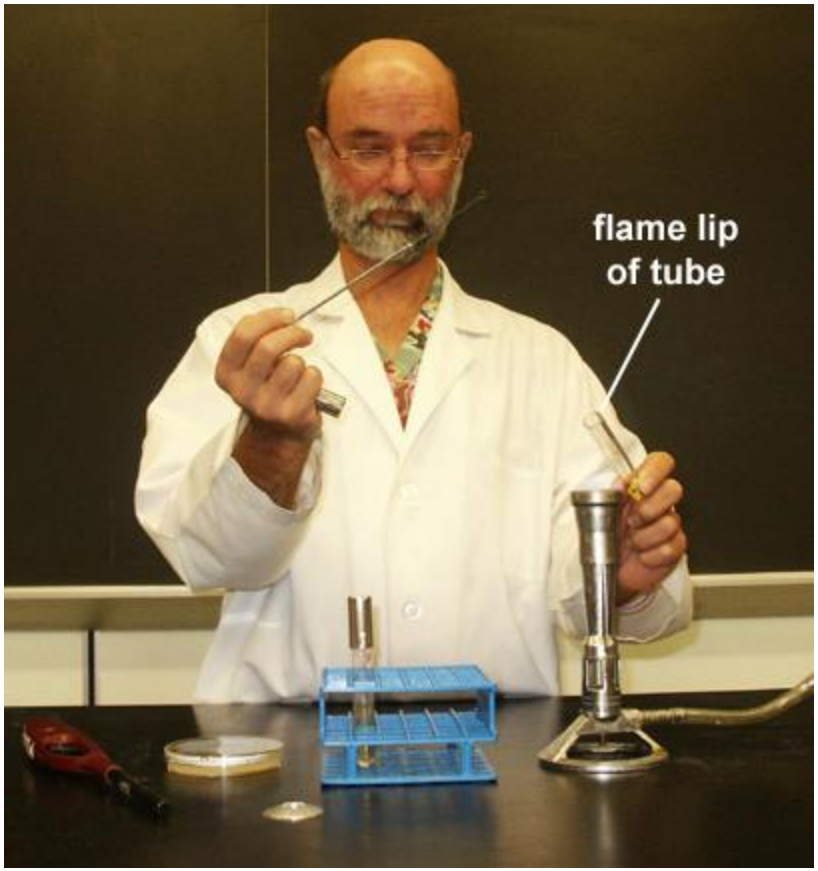


6.Transferring the inoculum into a broth tube

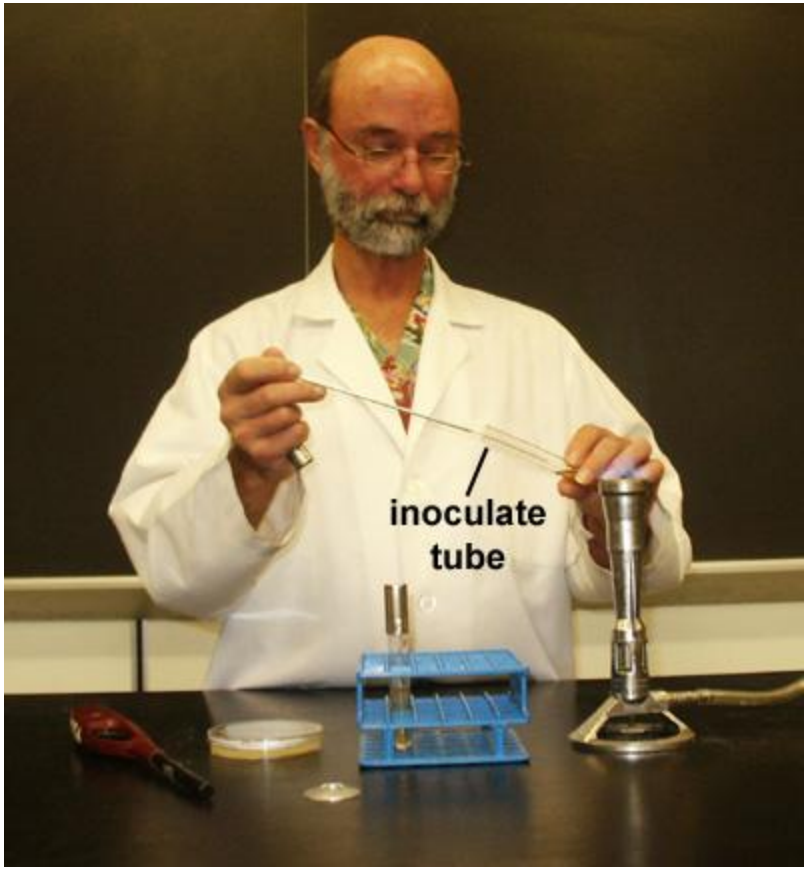
Pick up the sterile broth tube and remove the cap with the little finger



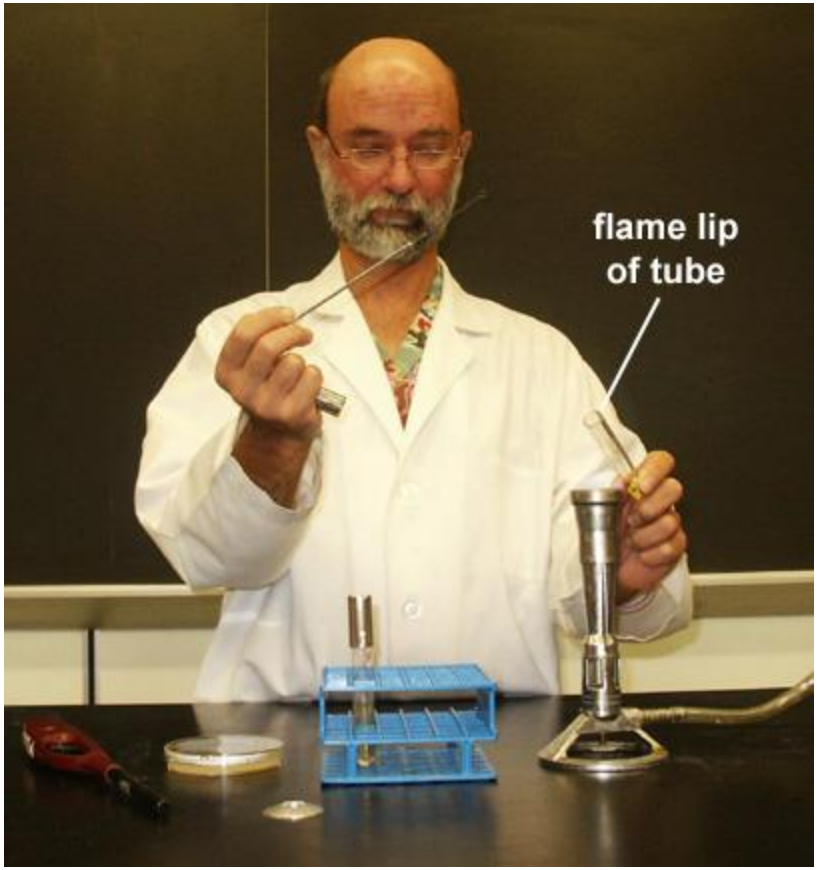
7. flame the lip of the broth tube



8.Place the loopful of inoculum into the broth and withdraw the loop



9. Again flame the lip of the tube



Replace the cap

Removing inoculum from a plate

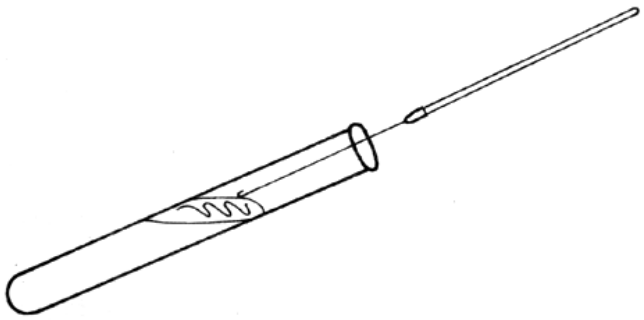
- **organisms growing on an agar surface in a petri plate**

1. Sterilize the inoculating loop in the flame

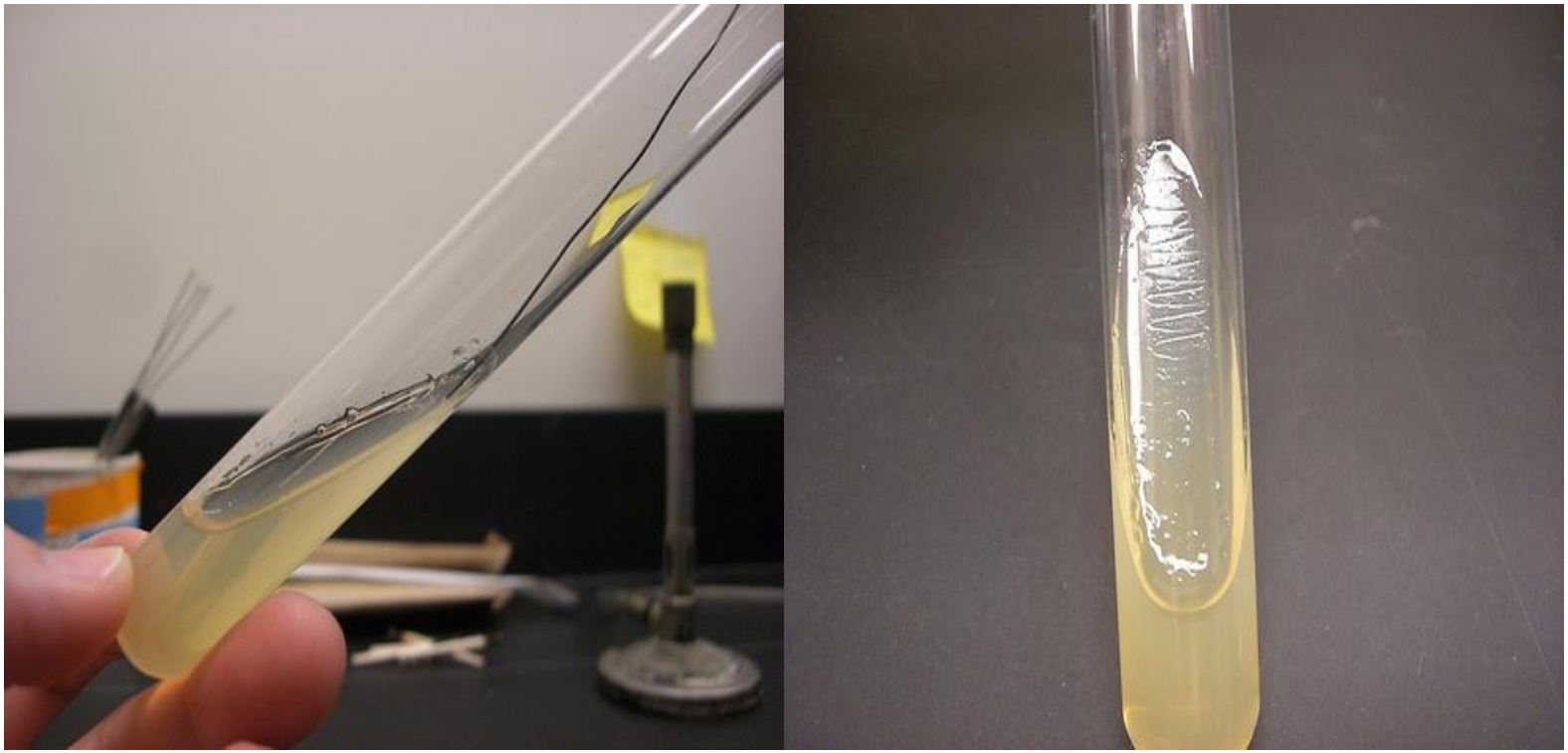
2. Lift the lid of the culture plate and stab the loop into the agar away from any growth to cool the loop

3.Scrape off a small amount of the organisms and close the lid

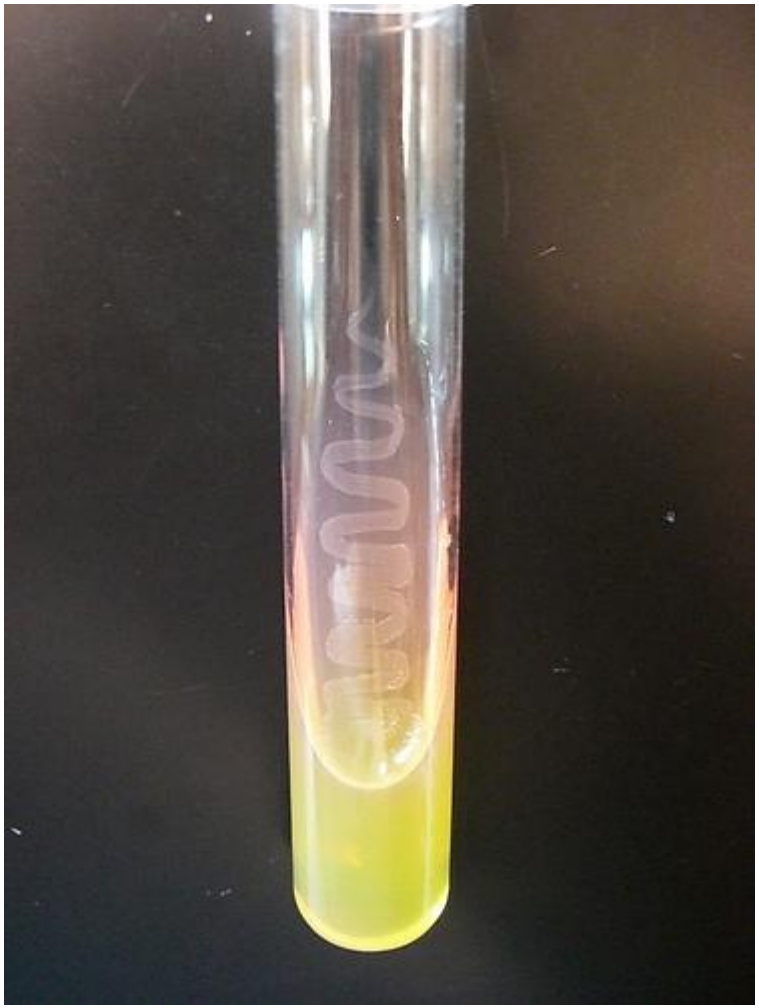
Inoculating an Agar Slant



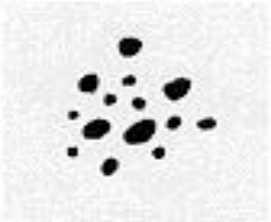
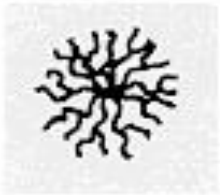
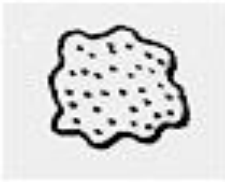
1.Label the sterile nutrient agar slant with the source of the culture and your
Initials.
2. Sterilize the loop.
3. Using appropriate aseptic technique, remove a loopful of broth from the culture tube.
4. Insert the loop into the sterile agar slant tube and starting at the base of
the slant, draw the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



Inoculated Agar Slant, after incubation

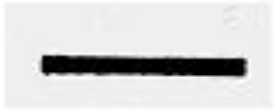


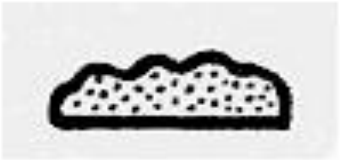
Form of Colony

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

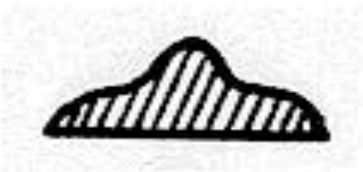
Elevation of Colony

effuse  very thin, spreading

flat 

raised 

convex 

umbonate 

Margin of Colony

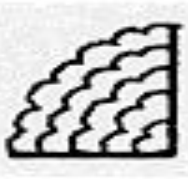
entire

undulate

erose

filamentous

curled



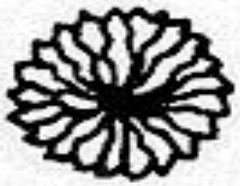
Surface of Colony

smooth

contoured

undulating

radiate

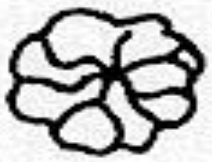


radiating rings

concentric

concentric rings

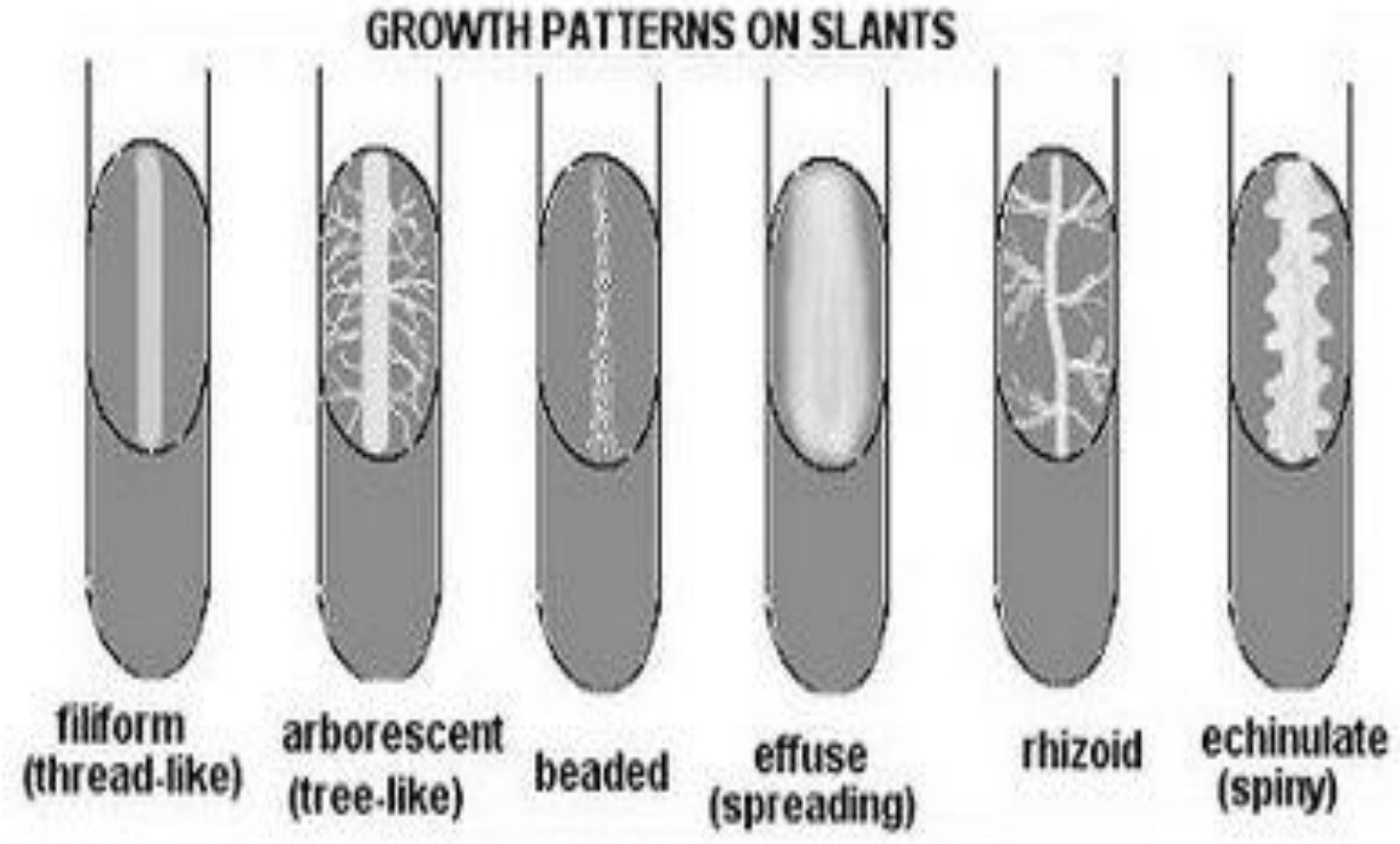
rugose



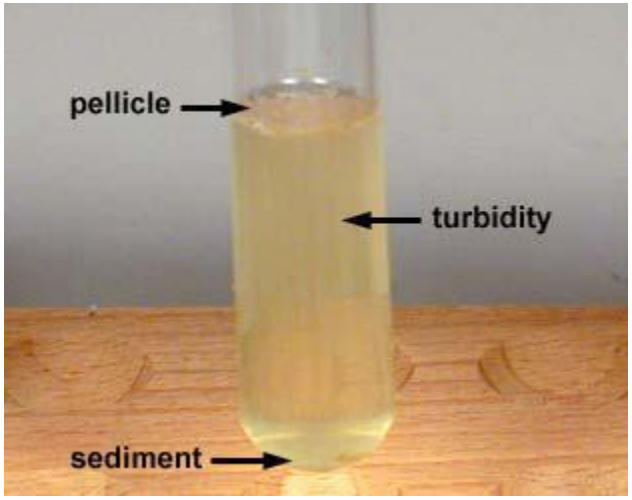
wrinkled

MORPHOLOGY ON SLANT MEDIOM

√√


GROWTH PATTERNS ON SLANTS
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.



Liquid medium √√

*Turbid

*Pellicle

) (thick growth at the top of the tu

*Sediment