Culture Media

- In this lab we learn about different types of media that are used to grow bacteria. Some types of media will grow just about any type of bacteria and the others are more selective and only grow specific types of bacteria

Types of Media

- There are generalized media, like (Nutrient ager)that will grow many different types of microbes.
- This media is the type most often used to culture bacteria

Selective Media

- culture medium that allows the growth types of organisms, while inhibiting the growth of other organisms

Example:

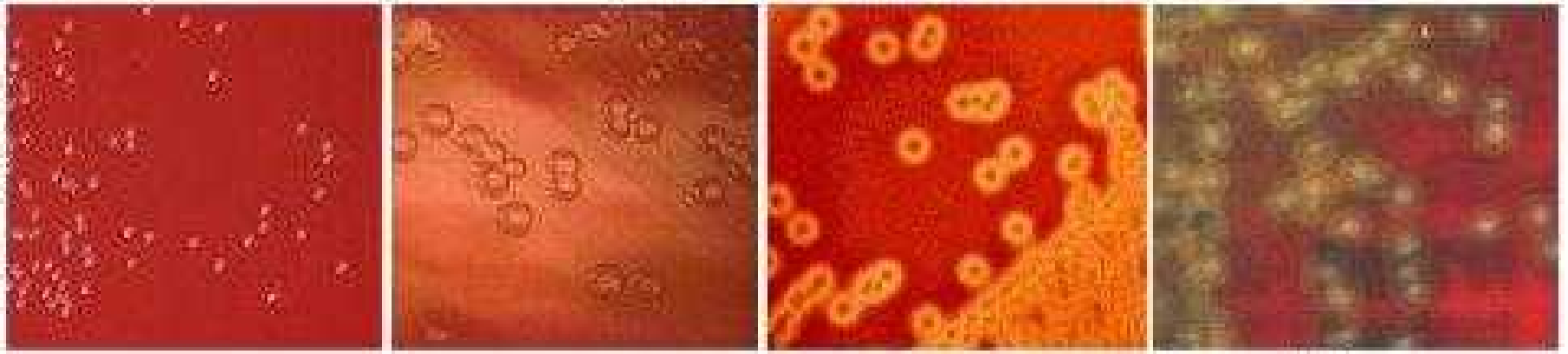
EMB (Eosin Methylene Blue)
dyes inhibit Gram (+) bacteria
selects for Gram (-) bacteria

Differential Media

culture medium that includes ingredients, such as chemical indicators , that produce observable differences between species of bacteria

Differentiates between different organisms growing on the same plate
Example: **Blood agar**

- This media is **differential** because:
- Certain bacteria produce enzymes (hemolysins…) that act on the red cells to produce either:
- **Beta hemolysis**: Enzymes lyse the blood cells completely, producing a clear area around the colony.
- **Alpha hemolysis**: Incomplete hemolysis produces a greenish discoloration around the colony
- **Gamma hemolysis**: No effect on the red cells.



gamma gamma beta alpha

ere are also (selective) and

-

(differential)media

MacConkey's

- MacConkey's is both a **selective** & **differential** media.

1.

- MacConkey's is **selective** media because it inhibits the growth of some organisms [Gram positive bacteria].

2. MacConkey's is **differential** media

- "lactose fermenters" bacteria will grow in red colonies while" non-lactose fermenters" will be colorless and clear.

So if there are colonies of bacteria growing on MacConkey's,
it's understood that they are Gram-
If those colonies are colorless, they are not lactose fermenters.
If the colonies have a pinkish appearance, they are lactose fermenters


Klebsiella pneumoniae
on Macconkey Agar Plate

MacConkey Agar

left: no lactose fermentation

right: lactose fermentation

FORMS OF CULTURE MEDIA

broth : liquid medium

most common growth media for microorganisms are
(liquid nutrient medium) nutrient broths

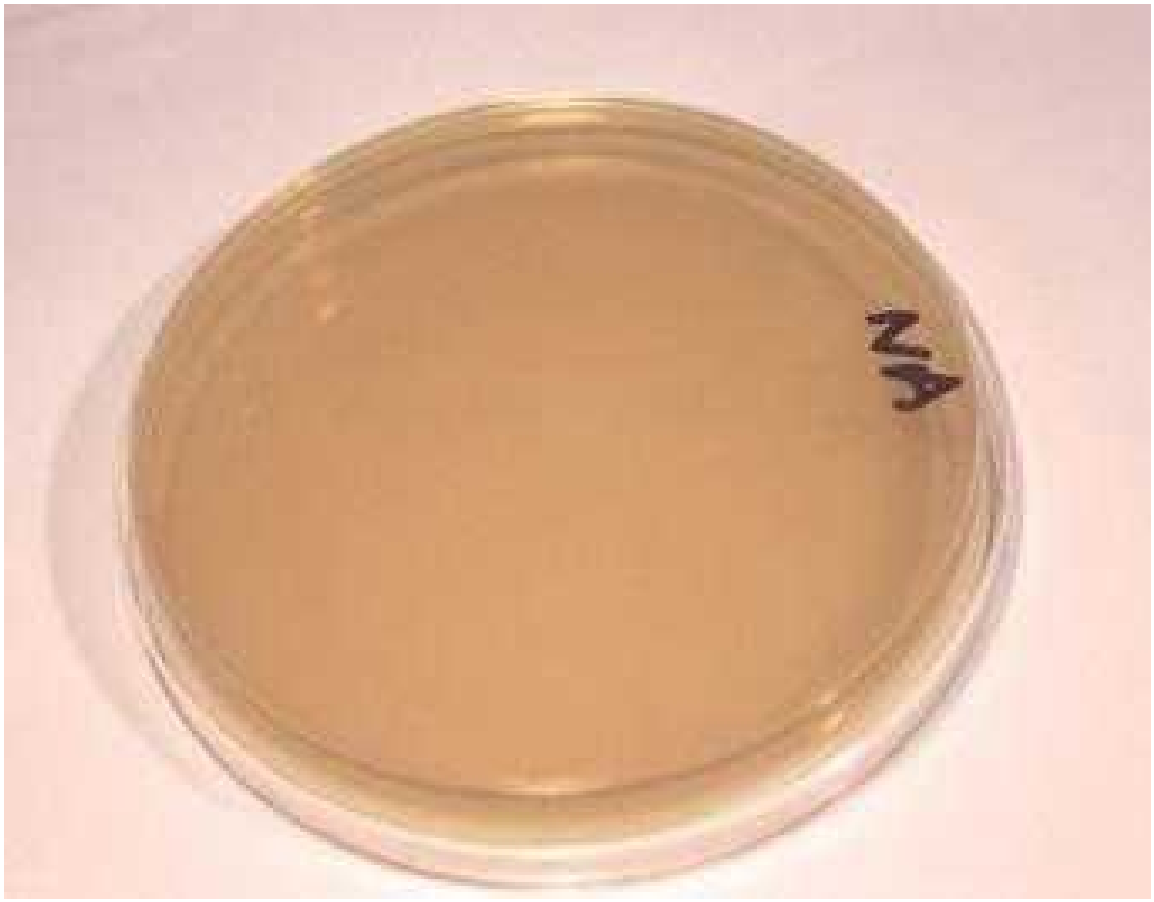
Solid media

- Solid media commonly contain 1.5% agar per weight to solidify the liquid media.
- After sterilization, the media is poured into sterile Petrie plates.

Agar is liquefies at 100 C and solidifies at 40 C

General Media:

Nutrient Broth and Nutrient Agar



- or After autoclaving the media(in tube) for 20 minutes, the tubes are placed in a slanted position to allow the agar to solidify. These tubes are called slants

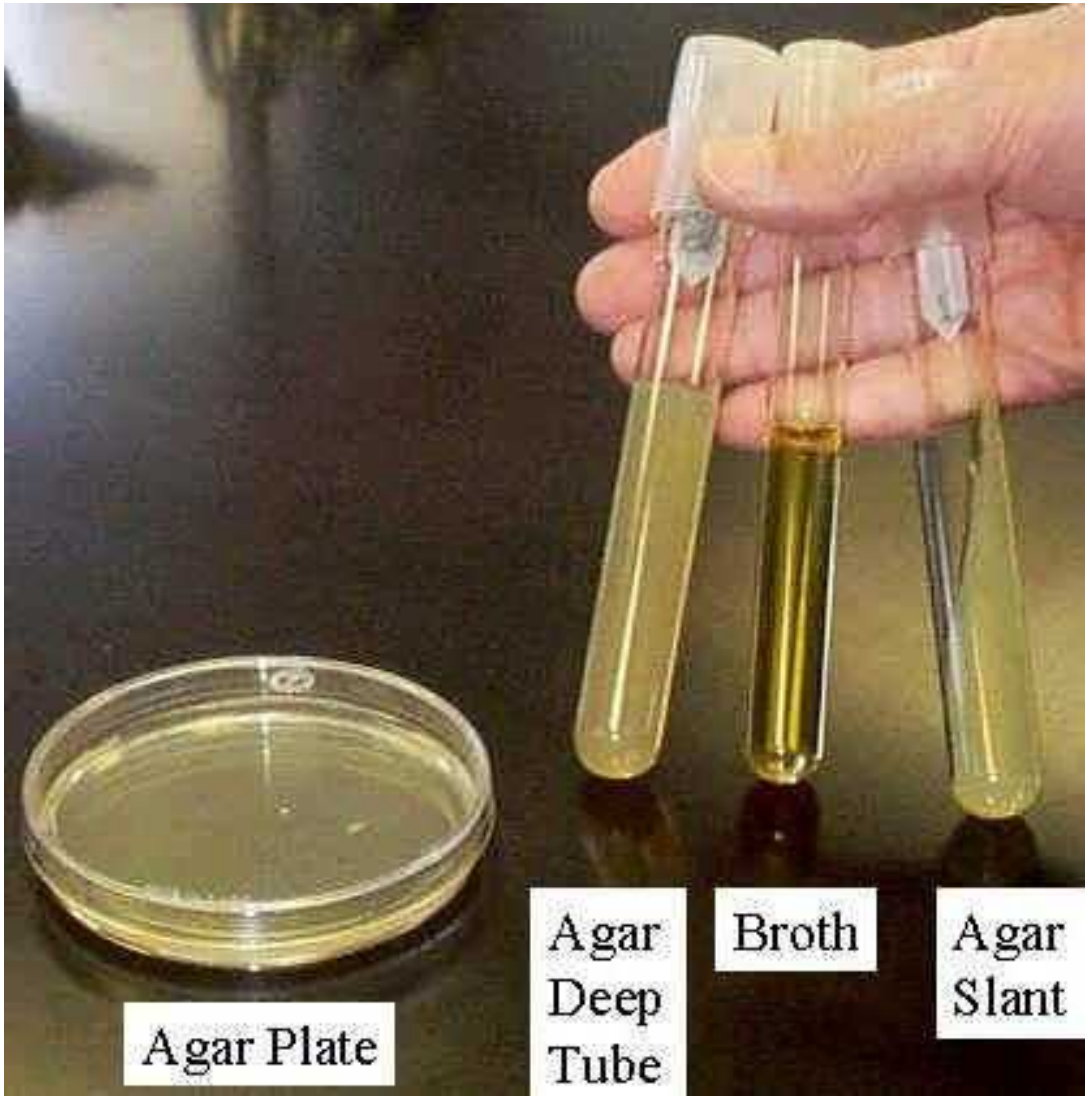
Slant tubes:

are tubes containing a nutrient medium plus a solidifying agent, (agar-agar. The medium has been allowed to solidify at an angle in order to get a flat inoculating surface

Agar Plate:

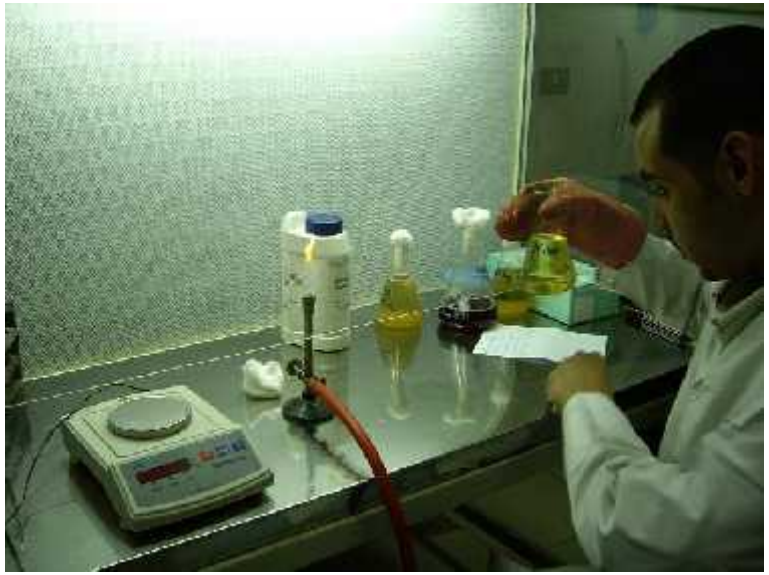
are sterile petri plates that are filled with a melted sterile agar medium

Microorganisms grow on the surface of agar plates and slants


Agar Plate
Agar Deep Tube
Broth
Agar Slant

How is media made?

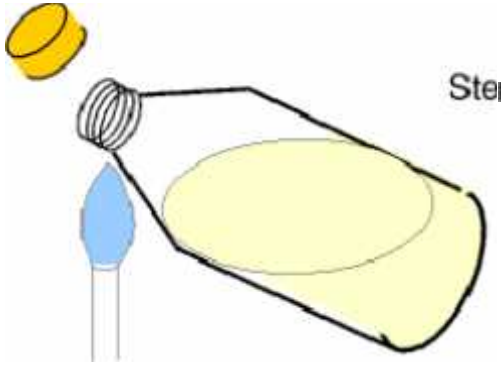
- When lab personnel make media they measure out a quantity of **dry powdered nutrient media**, add **water** and **check the pH(7)**.
- They dispense the media into bottles(flask,tube), cap it and **autoclave**. The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
- Once the media is **autoclaved** it is sterile
- (all microoranism forms killed)




200 mL
±5%
150
100
50

Aseptically pouring agar plates

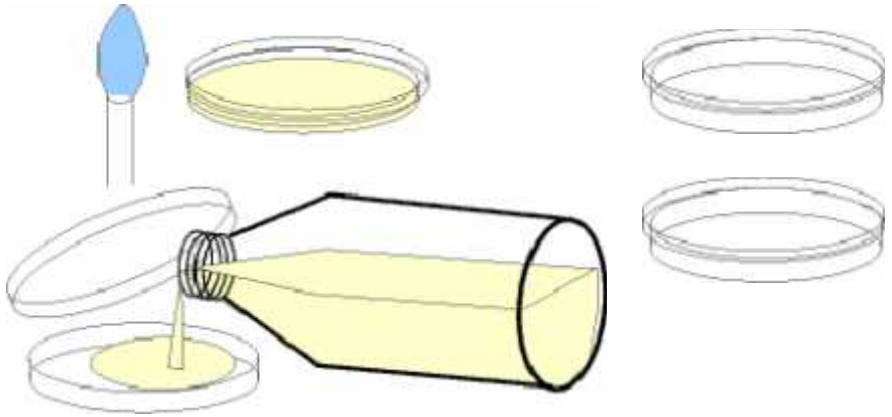
"Pouring a Plate"



Neck of agar bottle is passed through flame

Sterilised molten agar is poured in and left to set.

Petri dish lid is opened as little as possible, angled and kept over the base.



Each Petri dish hold about 20 ml, so 200ml will do for 10.

- All labeling is done on the **bottom** of the agar plate

1. Initials
2. Date (mm/dd/yy)
3. Code # or letter

Isolation of Bacteria

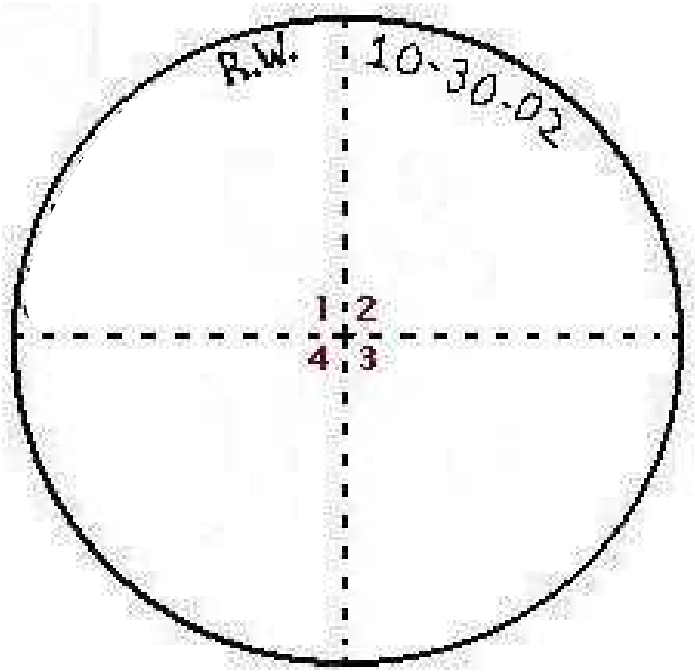
- Environmental Sample

After agar in plate has cooled and set:

Label the Plates! Using a wax pen, divide the bottom (the part of the plate that contains the media)

Surface samples are normally taken using sterile swabs

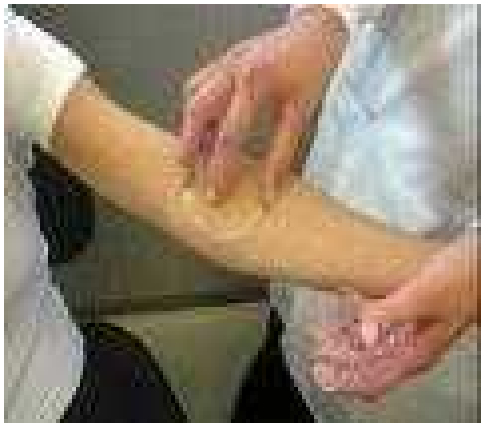
Environmental sampling



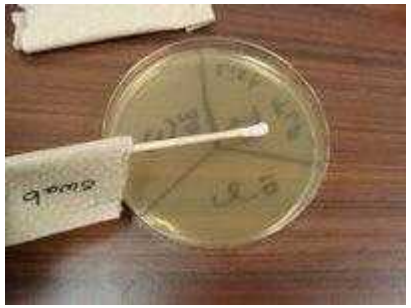
Surface samples are normally taken using sterile swabs

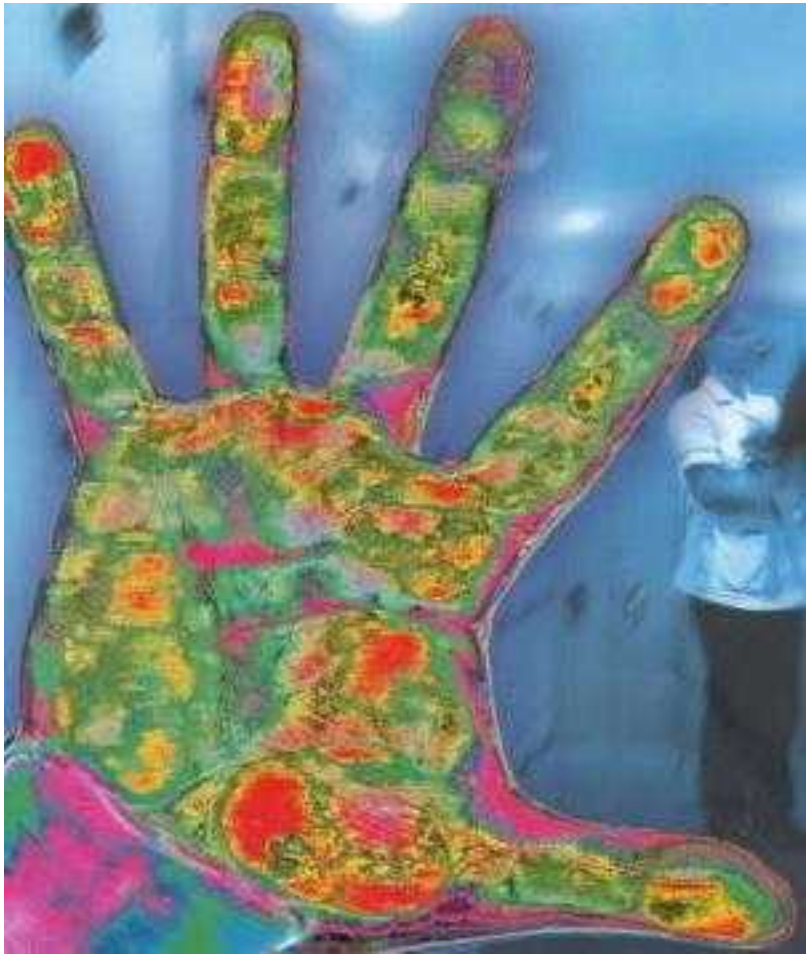
Normal Flora Samples

- Important to remember that microbes are (everywhere)!
- We are inhabited (covered) by many different bacteria. .
- Most of the symbiotic relationships that we have with microbes are beneficial to both the microbe and us!
- In today's lab we will examine **normal flora** (hand.hair.skine)



.Applying oral sample to surface of agar





.Sterilize the inoculating loop •

The inoculating loop is sterilized by passing it at an angle through the flame of a gas burner until the entire length of the wire becomes orange

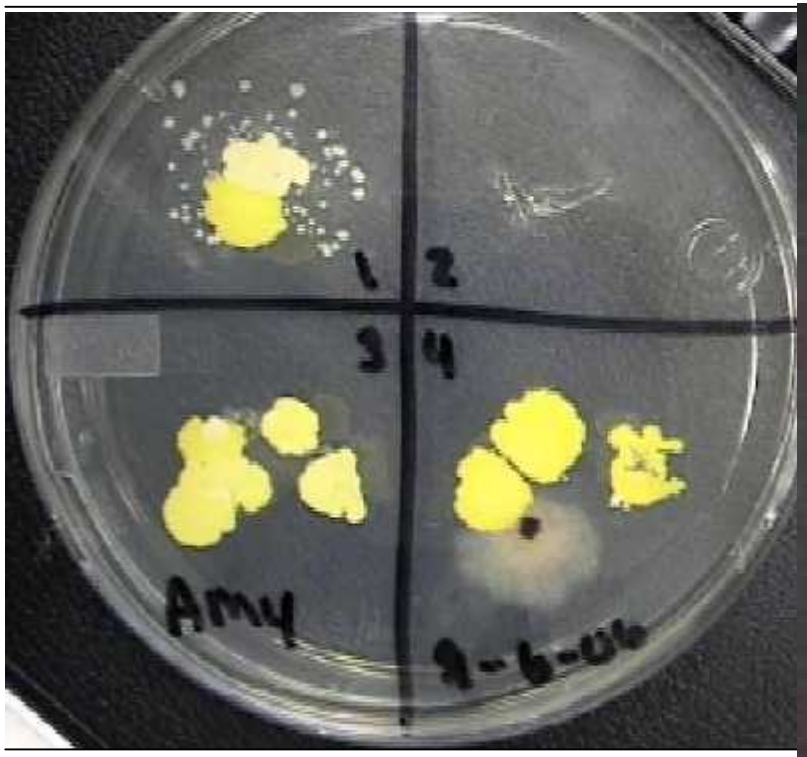
In this way all contaminants on the wire are incinerated
Never lay the loop down once it is sterilized .
or it may again become contaminated. Allow the loop
. to cool a few seconds to avoid killing the inoculum

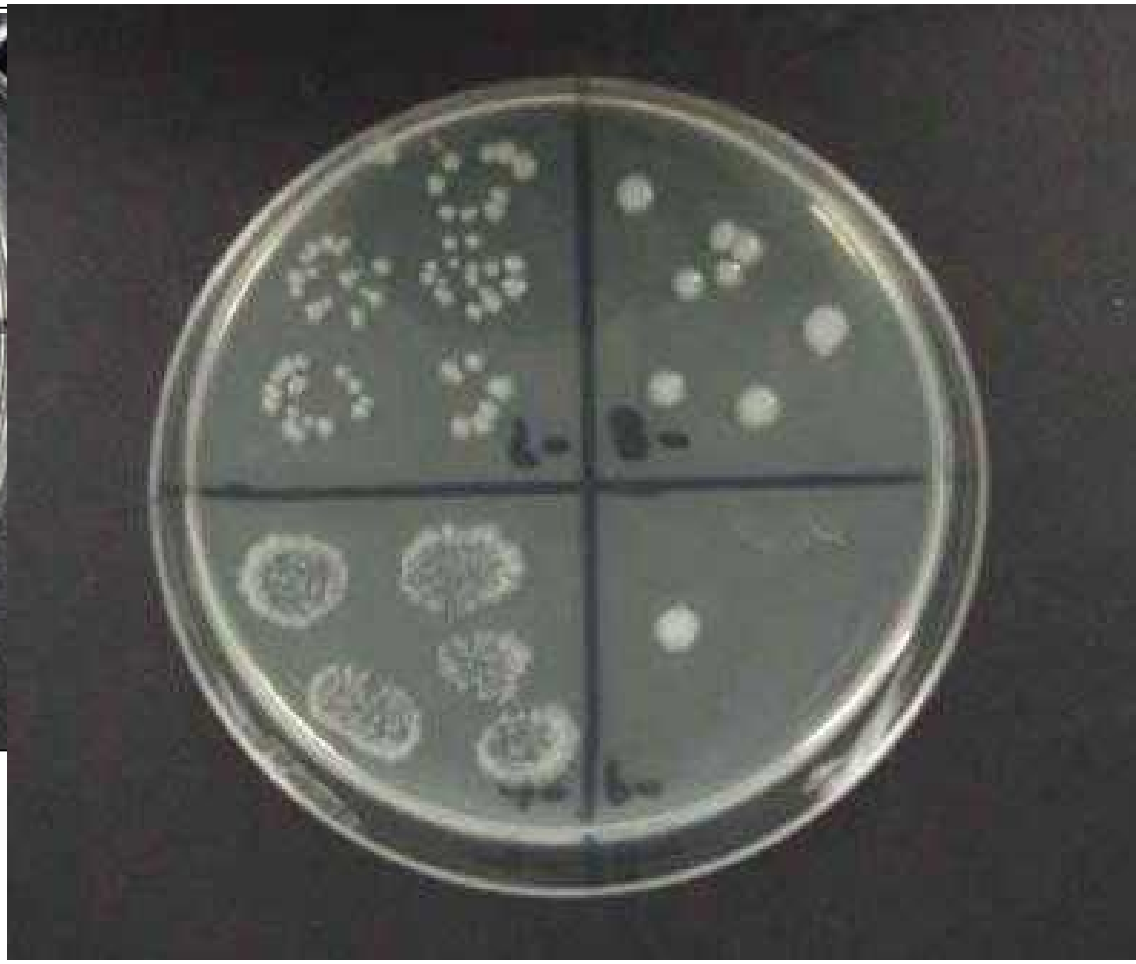
- Place all inoculated material in **incubator** **Culture tubes** should be stored **upright in plastic beakers**, while **Petri plates** should be **incubated upside-down** (lid on the bottom)

Agar plates are stored upside down to prevent condensation.

- These plates will be incubated at
- 37° C for 24 hours and then stored at refrigerater until next week when you will observe for results.

Typical environmental sampling results



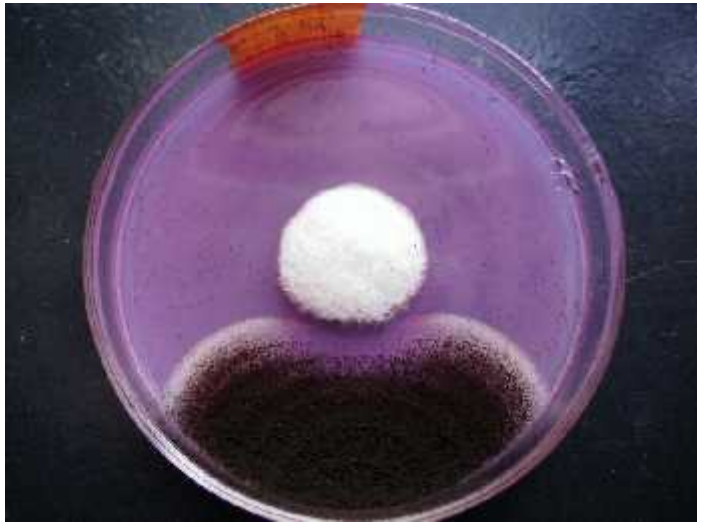


Isolation of fungi

- soil sample
- fungi are commonly found in highly localised concentrations In soil

- Soil Plating
- you can place a small fragment of soil on an agar plate(PDAagar)or(Czapek dox)

- Air sample
- the air we breathe contains spores of many different fungi.
- air spore can be sampled by exposing an agar plate (for30minute)then incubated at in25°c for7days



A fungal colony surrounded by bacterial colonies from dirty hands and house