Pure culture technique:

- Streak plate method
- Pour plate method

EXPERIMENT NO-12 DATE-23.9.15

AIM OF THE EXPERIMENT

To study the streaking method of isolation.

THEORY

This is the simplest and most applicable method for the isolation of single spore from the main culture of bacteria or fungi.

REQUISITES

1) Potato-Dextrose agar media
2) Sterile Petridish - 2 pairs
3) Sterile inoculating needle
4) Spirit and spirit lamp
5) Main culture

PROCEDURE

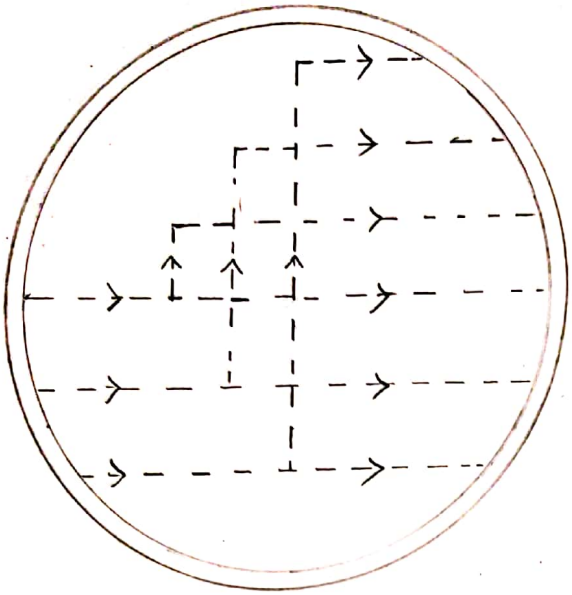
Sterile melted media is poured in two sterile petridishes. Petridishes are moved in a rotating motion in order to uniform distribution of the media. The media is now allow to cool and allowed to solidified.

Single spores can be isolated by two means—

(i) Simple streak and
(ii) Cross streak

SIMPLE STREAK

Inoculating media is sterilized in the flame dipping the same in spirit. The needle is dipped in the main culture and streaked over the medium in a zig-zag manner as shown in the figure.



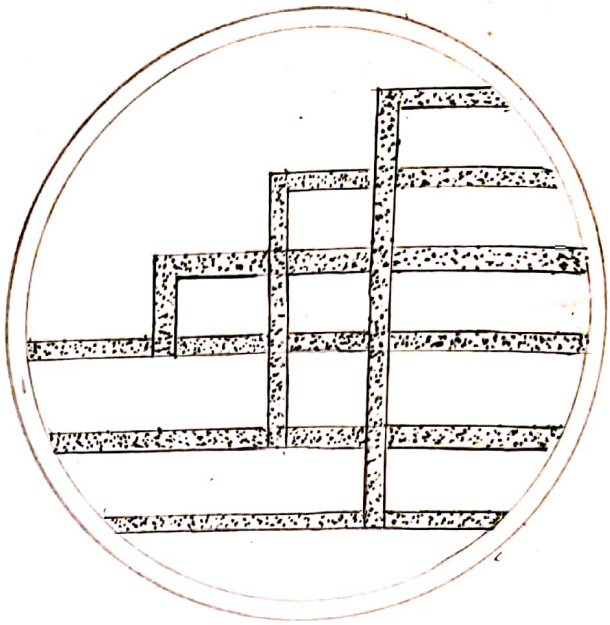
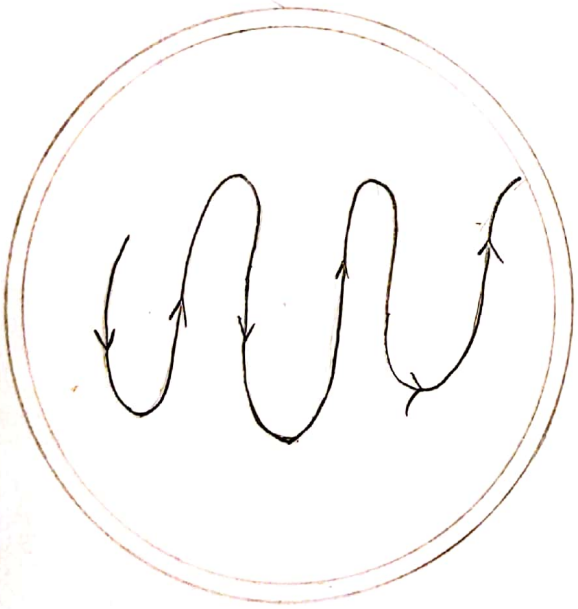


FIG- CROSS STREAK



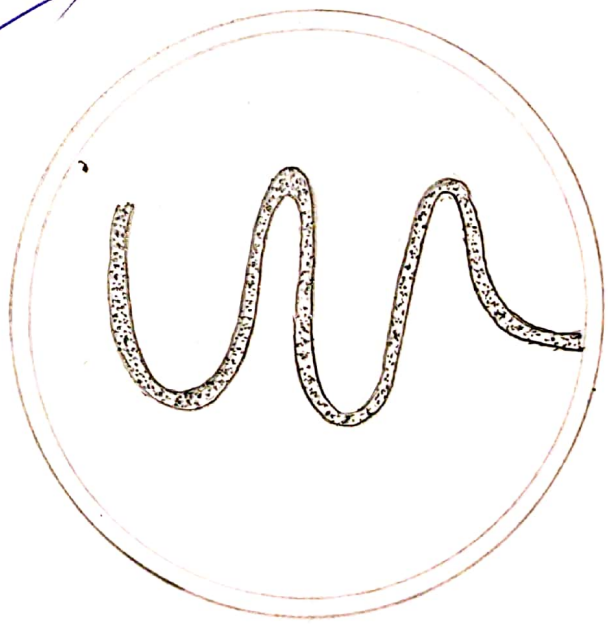


FIG- SIMPLE STREAK

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CROSS STREAK

With flame sterilized bent needle the inoculum is taken from the main culture. Three straight streak are made with the loop on the medium of the petridishes.

The needle is flame again and allow to cool, again deeped in the inoculum and other three streaks are made with the loop starting from three successively but vertical to them of they three downward streaks (Petridishes are inoculated at 30° for 24-48 hours).

OBSERVATIONS

The petridishes are examined at first naked eye and then under the low power microscope without removing the cover.

In simple streaking region of streaked line is crowd with colonies and number of things off as the line near the end. Towards the end region colonies are well apart and single spore is found to grow at the end.

In cross streaking, colonies were found to be crowded in the streaking region but gradually thined out as the loop travelled more distance on the medium. Cross streaks also show more crowded colonies at the starting region.

PRECAUTION

1) Everything must be done aseptically.

2) At the time of streaking, care should be taken so that the agar medium is not cut deep into the thickness. Only superficial line of streaking is sufficient.

3) At the time of streaking the needle should be remove intermittently.

3/10/23

EXPERIMENT NO-13 DATE - 7.10.15

AIM OF THE EXPERIMENT

To prepare pour plate method of isolation.

THEORY

When a suspension of bacteria or other materials like fungal spores of different types are diluted successively individuals are seperate as the dilution proceeds. This is the basis of experiment.

REQUISITES

1. Sterile petridishes
2. Nutrient agar-tubes (sterile)
3. Inoculating loop
4. Spirit
5. Fungal suspension.

PROCEDURE

The nutrient agar in the tubes are melted and cooled down to a temperature 45°-50°C and maintained at this temperature. So that the medium is in liquid state and not enough to kill the fungus. A suspension of the material to be studied as made in a tube. The prepared fungal suspension is transferred to a tube of sterile nutrient agar. The second tube is also shaken for uniform distribution and then one loopful of this is transferred to a third tube. In this way several dilution are made in the tubes. In this way several dilution are made in the tubes, which are marked "NO-1", "NO-2" etc respectively and allowed to solidify. The sterile nutrient agar of a tube is poured into a petridishes which is labelled as "control". These dishes are then incubated for 3 days at about 27°C.

RESULT

It is observed that the minimum growth of fungal colonies takes place in NO-1 petridishes; gradually number of fungal colonies decreasing from NO-1 to last number of petridishes.

PRECAUTION

1) Cotton plug must be heated in the flame.

2) The media must be cooled before inoculation.

3) The cover of the petridishes is lifted just enough to permit the tube neck.

4) The petridishes should not be distributed until the agar solidified.

9/10/15