Topic: Expt - 4

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Aim: To prepare a bacterial culture (of Bacillus subtilis) by ξ - Streaking and observing it's growth in the presence of Antibiotic.

Principle:

In this experiment we use ξ - streaking method, which is a common method in Microbiology. ~~It is used for isolation of single colonies of microorganisms, which can be from same~~. Streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. ξ - streaking method is a useful method to distribute the food (LB Agar Media) evenly to all the bacterial cells. Our LB Agar Media consists of -

- Yeast Extract
- NaCl solution (Osmolyte)
- Agar
- Tryptone.
- Distilled water.

Materials Required!

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(i) Spirit Lamp.

(ii) LB Agar Media Composition in a petri dish, which has been autoclaved

(iii) Bacterial solution (Bacillus Subtilis)

(iv) Antibiotic

(v) A metal loop used for streaking

(vi) Tweezers; (vii) Micropipette.

Method:

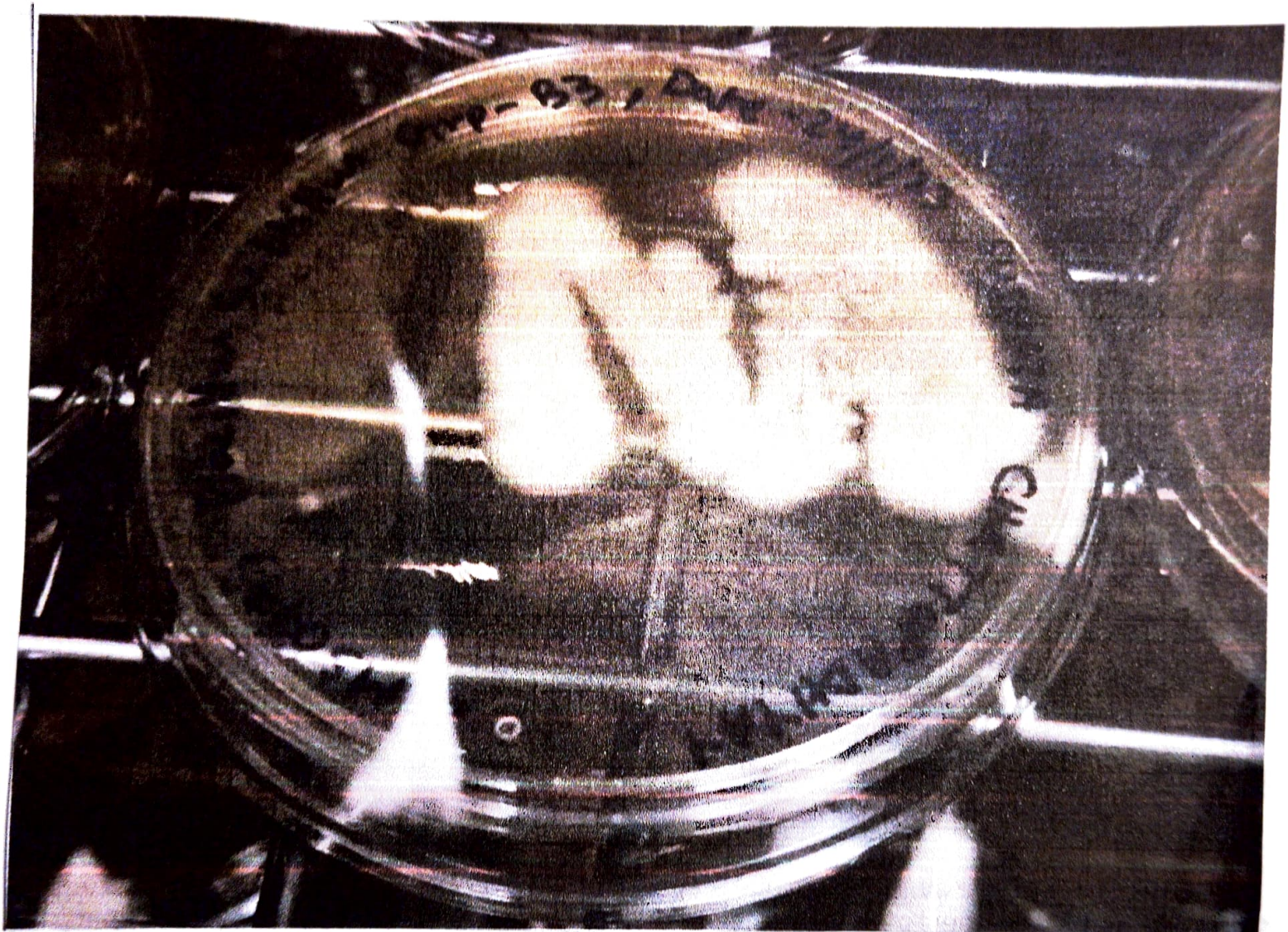
(i) We sterilise our hand under the fume hood, using Ethanol Spray, ~~and the metal loop using in the~~

(ii) We disinfect the metal loop and the tweezers ~~but~~ by putting them on the spirit lamp flame until they are red hot.

(iii) Label the petri dish given on both sides with name, Roll No., Date, Group No., Bacterial species.

(iv) Micropipette out some bacterial solution and put it on the ~~petri dish~~ LB Agar Media.

(v) Use the metal loop and carefully streak the drop, in the shape of Z. Beware not to disturb the Media.



Growth of Bacterial Culture.

(vi) Using tweezers, dip a small piece of blotting paper in antibiotic solution and put it on the Z - streak inside petridish.

(vii) Close the lid, and put ~~it inside~~ it upside down inside the ~~a~~ incubator, which ~~is~~ maintains a constant optimum temperature and humidity.

(viii) Click a picture on the day of streaking and another picture on the next day.

Observation

After about 24 hours, we observed dense white growth of bacterial culture on the media, in the shape of Z. But the bacterial growth ~~is inhibit~~ appears somewhat diminished near the antibacterial paper.

Conclusion.

The antibacterial solution contains Ampicillin which ~~inhibit~~ inhibit bacterial cellwall ~~bi~~ biosynthesis, thus resulting in diminished growth of their culture.

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Aim:

Perform Gram staining on our prepared bacterial culture, in order to differentiate between Gram positive and negative bacteria.

Principle:

In microbiology Gram's stain, Laboratory staining technique distinguishes between two groups of bacteria by the identification of differences in the structure of their cell wall. Gram positive bacteria remain colored after the staining procedure, while Gram negative bacteria do not retain dye. This is because the gram (+) bacterial cells have a thick peptidoglycan layer in the cell wall that retains primary stain, crystal violet. Gram (−) cells have a thinner peptidoglycan layer that allows Crystal violet to wash out on addition of decolourisers. They are stained pink or red by the counterstain, commonly safranin. Iodine soln is added after addition of crystal violet to strengthen the stain with the cell membrane.

Counterstained gram-negative cells appear red, and gram-positive cells remain blue.

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Method:

1. Take a little bit of ~~culture~~ LB Agar Media from our petri dish and put

Materials Required :-

(i) Spirit Lamp
(ii) Gram slide
(iii) Distilled water
(iv) Deoimised water.
(v) Metal Loop
(vi) Reagents - Gram crystal violet solution, gram iodine Sol^{n}, gram decolouriser solution (50% Ethanol + Acetone Mixture), safranin dye solution.

Procedure:

1. Take a little bit of LB Agar Media using metal loop from our petri dish, and carefully and evenly spread it over a drop of deionised water on Gram slide.

2. Then take a small piece of bacterial culture from a single colony and apply it evenly. Dry it using the spirit Lamp.

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3) Once dried, add 2-3 drops of crystal violet stain over the fixed culture, and let it stand for 60 sec.

4) Pour off the stain and gently rinse the excess stain with a stream of water.

5) Add about 3 drops of Iodine solution on the smear, enough to cover the fixed culture. Let it stand for 30 sec.

a) Pour off the iodine solution, rinse the slide with running water and shake off excess water.

(b) Put few drops of decolorizer, and rinse it off with water after 5 seconds. The exact time to stop is when the solvent is no longer colored as it flows over the slide.

(c) counter stain with 3 drops of safranin solution for 40s.

(d) Air dry the slide after washing off the red Safranin solution.

6) Examine the finished slide under microscope.

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Observations –

Bacterial cells were observed taking violet - blue stain.

Conclusion –

Our bacterial sample, contained gram (+)ve cells.