1

#	DATE	EXPERIMENT.
1	31-8-16	PREVELENCE OF E.Coli IN DRINKING & WASTE WATER.
2.	21-9-16.	ISOLATION, IDENTIFICATION & OPTIMIZATION OF BACTERIA.
3.	28-9-16.	HOW TO AVOID CONTAMINATION.
4		Isolation and pure culturing of anaerobic bacteria to be used in different biotechnological processes.

OBSERVATIONS:-

In drinking water Ecoli was nil while countable colonies of Ecoli were seen in sewage water sample.

Drinking water = Nil.
Sewage water = 259 colonies per 0.5 ml.

1-8-16.

PRACTICAL # 1.

PREVELENCE OF E-COLI IN DRINKING AND WASTE WATER.

MATERIAL:-

Water sample, autoclave, hot air oven, EMB agar, distilled water, Petri plates, Micropipette, Aluminium foil, Conical flask, weighing balance, flame, safety cabinet, 70% alcohol / Ethanol, cotton, tips, incubator.

PROCEDURE:-

- Took 250 ml distilled water in a conical flask and added weighed media (EMB agar - Eosine methylene Blue) in it and mixed well.
- Covered the conical flask with cotton and wraped aluminium foil on it.
- Placed this flask in autoclave for Sterilization
- In autoclave, first release pseudo-Pressure, when needle came on 5
- Wait until needle gone to 115 psi and Then remove air after 15 minutes.

- Placed petri plates in hot air oven for 45 minutes at 180°C.
- Took out media from autoclave and petri plates from hot air oven when time completes.
- Pour 10-12 ml media (estimated) in petri plate under safety cabinet. Covered Allowed it to solidify.
- Poured drops of water on petri plate with the help of micropipette and cover
- Both waste water and drinking water samples poured seperately on petri plates.
- Placed these plates in incubator for 24 hours at 37°C
- Observed these plates after 24 hours and counted if colonies were visible.

-9-16.

PRACTICAL # 2.

ISOLATION, IDENTIFICATION AND OPTIMIZATION OF BACTERIA.

IN This practical, we studied These headings mentioned below:-

- SOURCE OF ISOLATION.
- ENRICHMENT CULTURE DEVELOPEMENT.
- PURE CULTURING.
- IDENTIFICATION.
- OPTIMIZATION.

1 SOURCE OF ISOLATION:-

Isolation means separation of desired bacteria. For Example, Bacteria for bion (clean water Through biological agents), waste water treatment. Source should be envisonmente that have Elements which is going to be clean.

2 ENRICHMEN CULTURE DEVELOPMENT:-

For enrichment culture development liquid medium should be used called Broth. Noted the temperature of Environment from where sample / bacteria taken. Placed sample with broth at That- temperature for one day. Growth in medium will be checked

from turbidity of medium. Note broth OD on Spectrophotometer. (OD - Optical density).

3 ⇒ **PURE CULTURING:-**

When OD of sample high then move it to solid media from liquid media (agar). Selective or differential medium should be used for specified bacterial growth. Selective medium (autoclaved) placed in petriplate (sterilized). After 10-15 min. medium solidify. Took 0.1-1ml from broth and poured on plate (spread through rod). Incubated it for 24 hours.

There are different species of different shapes of colonies. Lawn of colonies were not considered. Considered these plates for further identification that have 30-300 colonies.

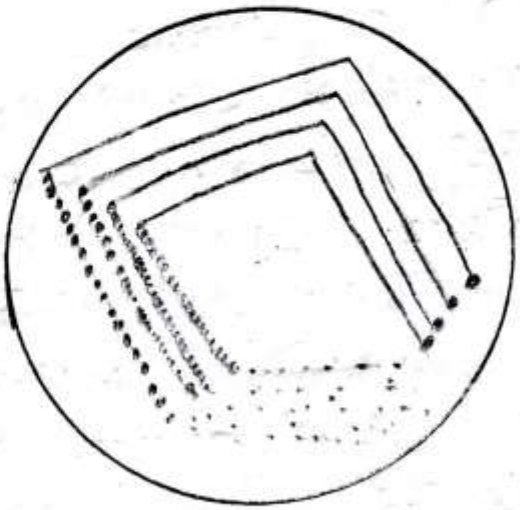
⇒ Preventions from Lawn:-

* Make dilutions.
* Used less quantity of sample (e.g: 50 µl instead of 100 µl)
* Identify their shapes and grow them separately.

Picked colony with the help of innoculating loop and streaked them.

4 ⇒ **IDENTIFICATION:-**

Identification also called characterization.



⇒ Streaking

Shapes:-

Rod shape

circular

Spiral

Vibrio

Characterization of bacteria is of two types:-

1 ⇒ Phenotypic
2 ⇒ Genotypic.

1 ⇒ PHENOTYPIC :-

Bacteria characterized according to their physical appearance that may includes-

- Shape
- Motility
- Staining.
- Endospore staining.

If tie between two / more groups during characterization then further biochemical tests will be performed.

2 ⇒ GENOTYPIC :-

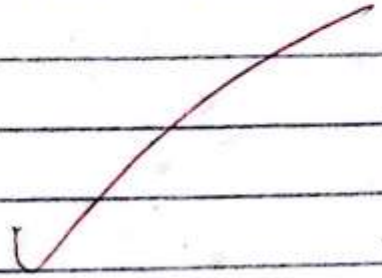
Bacteria characterized according to their genetical basis. Checked their DNA, amplify it and checked the sequence. Submit it in Data basis and checked with which they are matched.

3 ⇒ OPTIMIZATION :-

For these parameters of bacterial bacterial growth optimize

- pH
- Temperature
- Innoculum size.

Checked the OD of sample for pH and then temperature etc. with respect to pH.



3-9-16.

PRACTICAL # 3.

HOW TO AVOID CONTAMINATION.

There are several ways to avoid contamination during bacterial culturing.

1⇒ Wash hands before doing work.
2⇒ Wear gloves and a lab coat.
3⇒ Keep long hair tied back.
4⇒ Take weighing balance, before weighing media.
5⇒ Close bottles of reagents and media after taken them.
6⇒ Do not poured back to media bottle Took enough that is used.
7⇒ Wipe down working surface with 70% Ethanol
8⇒ Used sterilized Equipments.
9⇒ Used distilled water for media preparation.
10⇒ Do not ~~Covered~~ open sterilized petriplate in air
11⇒ Work in a laminar flow hood when passaging cells.
12⇒ Stay as organized as possible - label every thing

13⇒ Inspect all Equipments and media for contamination before use.

14⇒ If you must completely remove a lid from a tube, plate or bottle, set it down with the open surface facing up.

15⇒ Do not pass your hands / arms over any open bottle, plate or Tube.

16⇒ Placed bottles, plates / Tubes on one side before used and on another side after use. (left & right).

17⇒ Use proper antibiotics in your culture media.

18⇒ Turn on the UV lamp within laminar flow for 10 minutes to Sterilized the area.

19⇒ Invert petri plate after 10-15 minutes of pouring (when solidify)

20⇒ Placed petri plates in oven in such a manner that lid cover the base.

21⇒ Do not open mouth during pouring.

22⇒ Clean ~~autoclave~~ incubator with Ethanol before placing petri plates.

23⇒ When finished, dispose of materials properly, wipe down working surfaces with Ethanol.

04/10/16

Practical # 4.

ISOLATION AND PURE CULTURING OF ANAEROBIC BACTERIA TO BE USED IN DIFFERENT BIOTECHNOLOGICAL PROCESSES.

For isolation of pure culturing of anaerobic bacteria some controlled conditions required, Such as,

→ Temperature
→ Absence of O_2
→ PH
→ Inoculum Size etc

• For this purpose simple culturing methods not used, because in these methods, O_2 may enter and purpose of pure culturing of anaerobes not completed. First of all got sample from that area which was completely O_2 deficient like, stagnant water body (bottoms) or from soil depth.

• Different methods should be used for culturing of anaerobes such as given below:-

i ⇒ Anaerobic Chamber
ii ⇒ Sealed vial
iii ⇒ Sealed vial with Oil layer
iv ⇒ Pour plate method

v ⇒ Anaerobic jar.
vi ⇒ Agar dilutions

⇒ **ANAEROBIC CHAMBER:-**

Specific chamber designed for the cultivation of anaerobes which is expensive and need expertise to handle. Evacuated and two cylinders, one with nitrogen and other with carbon dioxide (both have fixed flow) (like a safety cabinet), Spark with Electricity or Ultra violet radiation that sterilized the equipments. Work as safety cabinet as well as incubator. Only anaerobes grow in this chamber.

⇒ **SEALED VIAL:-**

Used autoclaved vial with broth (liquid medium). Sealed the vial with stopel and sealing. Add sample in it and remove O_2 with the help of syringe. Only anerobes grow in this vial. Incubate at specific temperature.

⇒ **SEALED VIAL WITH OIL LAYER:-**

Used sterilized media (broth) and vial, added sample and than added 3-5mm oil layer on it. Oil prevents penetration of Oxygen inside, no need of Excavation.

⇒ POUR PLATE METHOD:-

Added sample in a sterilized plate (petri plate) and than pour media on it (solid media-agar). Oxygen automatically removed by pressing of media. Aerobes grew only but colonial study is limited

⇒ ANAEROBIC JAR:-

Sample placed on solid media and spread. All petri plates with sample collected and placed in a jar and closed with a lid that prevents O_2 passage. Anaerogin kits used to absorb already present O_2 (packets).

⇒ AGAR DILUTIONS:-

Used basically for isolation of specific colonies and their characteristics study. Sample mixed with molten agar in a tube and made dilutions. Incubated and checked colony characteristics in tubes (dilutions). Pure culture will be obtained from this by breaking tube at the point of specific colony. Carefully pick the specific area and grow in selective media for further studies.

15/11/16

Practical # 5

PURE CULTURING OF SALMONELAI

MATERIAL:-

Petri plates, distilled water, pipettes, Bismith sulfide, weighing balance, conical flask, stopper, ~~autoclave~~, hot air oven, safety cabinet, hot plate.

PROCEDURE:-

- Sterilized petri plates in hot air oven at 180°C
- Weighed bismith sulfide media (agar) and mixed with distilled water in conical flask.
- Placed conical flask on hot plate till boiling.
- Poured agar in petri plates under safety cabinet, and allowed them to solidify
- Put 0.5 ml sample for dilutions and poured 100 µl from each dilution in petri plate ~~set~~ & labelled respectively.
- Incubated petriplates for 24 hours and than for 48 hours
- Observed the colonies of Salmonela.

16/11/16