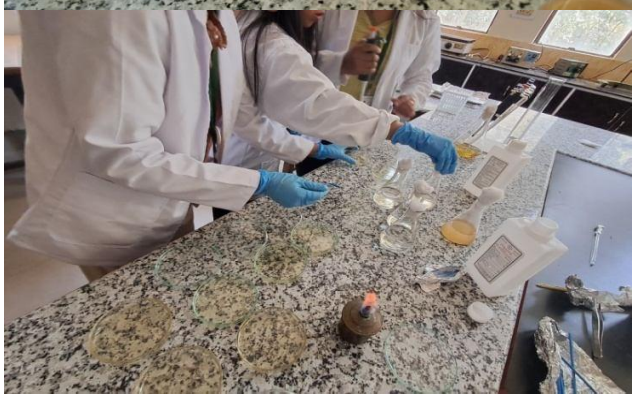


Report on isolation of bacteria from soil obtain from fieldtrip Location: Rangkylliaw (Mawrah), Khatarshnong by the serial dilution plating method

Date: 11th March 2025

Department Of Bio-Chemistry
Lady Keane College, Shillong

Batch2022-2025

DEPARTMENT OF BIO-CHEMISTRY

LADY KEANE COLLEGE

FIELD TRIP TO RANGKYLLIAW

11TH MARCH 2025

B.Sc6th SEMESTER

On the 11th of March 2025, we the students of B.Sc. Bio-chemistry department (including all the even semester i.e. the 2nd, 4th and 6thsemester students) along with our teachers went on a field trip to ***Rangkylliaw (Mawrah), Khartarshnong*** located approximately 38 km from Shillong, with an aim to look for a sample for the examination of microbes present on the soil and water from that surrounding.

B.Sc4TH SEMESTER

We arrived the location around 11:20 am. We had our breakfast and then we started strolling around the place. The place is known for its picturesque landscapes, vibrant culture, and rich biodiversity. It is also known for its bridge, a local community – maintained structure which provides a stunning view of the valleys and surrounding landscape.

After walking around the places we search for a soil that suits for our practical purposes. After collecting the soil we wrapped it properly in a bag and went to take some pictures and rest for a while, at the meantime our juniors and our teachers were helping in preparing food for us all. After the trip, we reach our college around quarter to7pm.We did really enjoyed our trip, it wa such a beautiful experience for us students to learn something outside the classroom and the most important thing is we were abl to build a good bond with other classmates and collect a beautiful memories together.

B.Sc2ND SEMESTER

With the help of our lab attendant, we were able to keep th soil in an oven so that it dries up overnight for our further experiment.

The aim of the experiment that we performed is:

Isolation of bacteria from soil by the serial dilution plating method.

PRINCIPLE: The serial dilution – agar plating method or viable plate count method is oneofthecommonlyusedproceduresforthisisolationandenumerationoffungi,bacteria and

Img: Collecting soil sample

actinomycetes which are the most prevalent microorganisms. This method is based upon the principle that when material containing microorganisms is cultured each viable microorganism will develop into colony, hence the number of colonies appearing on the plates represent the number of living organisms present in the sample.

In this method, a known amount of soil sample is suspended or agitated in a known volume of 0.85% NaCl to make a microbial suspension. Serial dilution10^{-2},10^{-3}……. 10^{-11}, are made by pipetting measured volumes of the microbial suspension into additional 0.85%NaCl dilution blanks.

Different media are employed to support the growth of the three–types of microorganisms. Nutrient agar for the isolation of bacteria, glycerol yeast agar for the isolation of actinomycetes, Sabauraud agar for the isolation of fungi. The dilutions $10^{-2}$to $10^{-5}$are selected for enumeration of fungi, 10^{-3} to 10^{-6} for actinomycetes and 10^{-4} to 10^{-7} for bacterial relative to their proportions in soil. After incubation, the number of colonies appearing on dilution plates are counted and observed.

The reagents required for the experiment are listed below:

1) 0.85% sodium chloride.
2) Nutrient agar – 7g of nutrient agar powder was dissolved in 250ml of distilled water. It is then heated to boiling to dissolve the powder completely. After the powder was completely dissolved we kept it aside for the solution to cool down(approx.45-50^{o}C).
3) 95%ethanol.

Some of the other requirements required for this experiment are:

1) Soil Sample
2) Bunsen Burner
3) Micropipettes
4) Conical flasks
5) Petriplates
6) Spreader
7) Autoclave
8) Incubator
9) Colony counter

Img: Preparing Reagents

The ***procedures*** that we used for the isolation of bacteria from soil by the serial dilution plating method are:

- ✓ The soil sample collected from the field trip was kept overnight in an oven in a sterile container in order for the soil to become dry.
- ✓ After the soil is completely dry, we weighed 1g of finely powdered air-dried soil sample and we transfer it into a conical flask which contains 99ml of 0.85%NaCl,we labeled this flask as 1 with dilutions of 1:100 (10^{-2}).
- ✓ After that we mixed the diluted soil thoroughly and shaking it for 30 times with the elbow resting on the table to obtain a uniform suspension of microorganisms and then incubate at 37ºC.
- ✓ We labeled four conical flask as 2, 3, 4 and 5with each containing 99ml of 0.85%NaCl.
- ✓ After the nutrient agar medium is boiled it is being kept in an autoclave alongside with the petriplates and measuring cylinder in order to get rid of contamination. Before keeping in an autoclave the necessary things are being wrapped in a foil properly.

- ✓ On the flask labeled as 2 we add 1ml of the diluted soil sample and then vigorously shaken, apparently 30 times, with the elbow resting on the table.
- ✓ From the flask 2 we took 1ml of the dilution and transferred it to flask 3 and vigorously shaken as we did before. The final dilution is 1:10,00,000 (10^{-6}).

- ✓ From the flask 3 we took 1ml of the dilution and transferred it to flask 4 and vigorously shaken as we did before. The final dilution is 1:100,000,000 (10^{-8}).

- ✓ From the flask 4 we took1ml of the dilution and transferred it to flask 5 and vigorously shaken as we did before. The final dilution is 1:100,00,000,000 (10^{-10}).
- ✓ We later took 8 petriplates and labeled as10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} and 10^{-11}.
- ✓ After the nutrient agar medium is cooled(45-50ºC), we took approximately 15ml and added to each of the petriplates that we labeled before.
- ✓ Intothepetriplateslabeledas10^{-4},10^{-5},10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} and 10^{-11}, the following dilutions of the inoculums was pipetted and placed at the centre of the plate.

a) 1ml of dilution from flask 2 was transferred to the plate labeled 10^{-4} to effect a10^{-4}dilution.

b) 0.1ml of dilution from flask 2 was transferred to the plate labeled 10^{-5} to effect a10^{-5}dilution.

c) 0.1ml of dilution from flask 3 was transferred to the plate labeled 10^{-6} to effect a10^{-6}dilution.

d) 1mlofdilutionfromflask3wastransferredtotheplatelabeled10^{-7}toeffecta 10^{-7}dilution.
e) 0.1mlof dilution from flask 4was transferred to the plate labeled10^{-8} to effect a 10^{-8}dilution.
f) 1mlofdilutionfromflask4wastransferredtotheplatelabeled10^{-9}toeffecta 10^{-9}dilution.
g) 0.1ml of dilution from flask 5 was transferred to the plate labeled 10^{-10} to effect a10^{-10}dilution.
h) 1mlof dilutionfromflask5wastransferredtotheplatelabeled10^{-11}toeffecta 10^{-11}dilution.

- ✓ After completing the above steps we close the petriplates and sealed them with paraffin and kept them overnight on an incubator and set it at 37ºC for the microorganisms to multiply in number and form colonies.

OBSERVATION: After1 or 2days the following was observed

1) All colonies on the plates were observed.

2) Statistically viable plate counts are only obtained from bacterial cell dilutions that yield between 30 and 3oo colonies. Plates with more than 300 colonies cannot be counted and are designated as too numerous to count (TNTC); plates with fewer than 30 colonies are designated as too few to count (TFTC). Therefore, only plates containing between 30 and 300 colonies were counted. All subsurface as well as surface colonies are to be counted.
3) It was also found that in the petriplates labeled as 10^{-10} and 10^{-11} microorganism were identified.

TABULATION

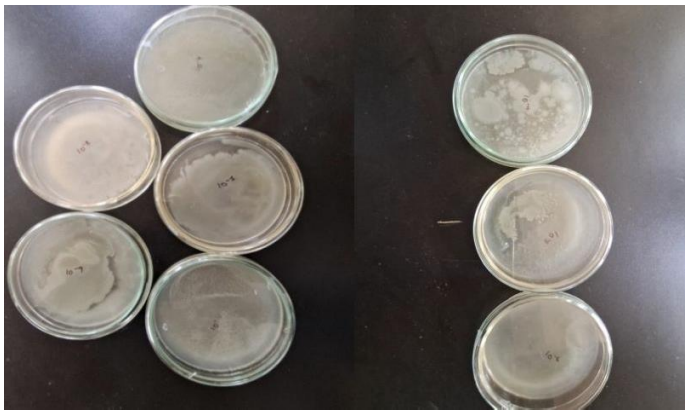
Plate no	Dilution	Vol^{m} of Dilution (ml)	Dilution flask	No. of colonies in plates	Bacterial count per ml of sample (CFµ/ml)
1	10^{-4}	1	10^{4}	TNTC	TNTC
2	10^{-5}	0.1	10^{5}	TNTC	TNTC
3	10^{-6}	1	10^{6}	TFTC	TFTC
4	10^{-7}	0.1	10^{7}	TFTC	TFTC
5	10^{-8}	1	10^{8}	TNTC	TNTC
6	10^{-9}	0.1	10^{9}	TNTC	TNTC
7	10^{-10}	1	10^{10}	TFTC	TFTC
8	10^{-11}	0.1	10^{11}	TNTC	TNTC

TNTC=Too numerous to count
TFTC= Too few to count

RESULTS

The bacterial count per ml of sample (CFU/ml)was found out to be

1. 10^{-4}dilution=TNTC
2. 10^{-5}dilution=TNTC
3. 10^{-6}dilution=TFTC
4. 10^{-7}dilution=TFTC
5. 10^{-8}dilution=TNTC
6. 10^{-9}dilution=TNTC
7. 10^{-10}dilution=TFTC
8. 10^{-11}dilution=TNTC

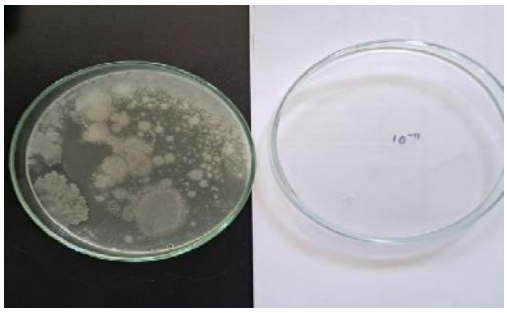


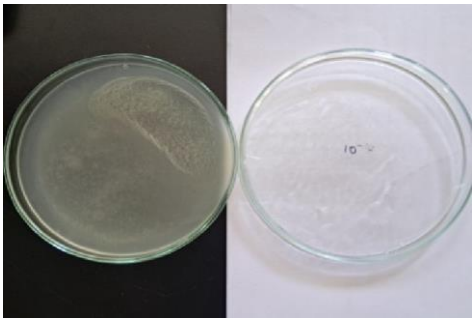
It was also found that in the petriplates labeled as 10^{-10} as well as 10^{-11} microorganism like *Pseudomonas aeruginosa* as well as *Bacillus cereus* bacteria were found out to be present respectively.

- ✓ In the petriplate labeled as 10^{-10} consist of *Pseudomonas aeruginosa* which is a common encapsulated, gram-negative, aerobic – facultatively anaerobic bacterium known for producing the blue-green pigment pyocyanin, especially when grown on certain media like nutrient agar.

- ✓ In the petriplate labeled as 10^{-11}consist of *Bacillus cereus* which is a gram positive bacterium, known for its ability to form spores, which allows it to survive various food-processing methods.

Petriplatelabeledas10^{-11}





Petriplatelabeledas10^{-10}

CONCLUSION

Typically bacteria are found in soil at densities of 10^{-8} to 10^{-9} colony forming units (CFµ) per gram of dry soil. Isolating bacteria from soil is a valuable process for discovering new microbial species with potential applications in various fields. Soil bacteria play a crucial role in nutrient cycling, plant health and decomposition, making them a rich source of novel bioactive compounds and enzymes.