A

PROJECT REPORT

ON

ISOLATION OF NATIVE MICROORGANISMS FROM GARDEN PLANTS RIZOSPHERE

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CERTIFICATE

This is to certify that the project report title “ISOLATION OF NATIVE MICROORGANISMS FROM THE GARDEN PLANT RHIZISPHERE” was completed by Miss. A.Navya, Miss L.Swathi, Miss B.Devalaxmi, B.Sangeetha, B.Suma under the Guidance of KRajani and U.Swathi. This has not been submitted to any other institution or university for the award of any Degree.



SIGN OF THE GUIDE **HOD** **PRINCIPAL**

Abstract:

As a part of isolating the rhizosphere microorganisms in the understanding the microbes present in the rhizosphere soil of garden plant, sample was collected from the garden plant roots and transferred in to beaker. Later the sample was taken for serial dilution and then inoculated using spread plate technique. After incubation period the isolated colonies are taken for smearing and stained with differential staining and after identifying the microbes weather gram positive or negative.

Introduction:

The rhizosphere is the region surrounding the plant root where the nutrients secreted by the plant roots and other chemicals are accumulated and became a good substrate for the microbial propagation. The term Rhizosphere was first used by the scientist Lorenz Hiltner. In Greek “Rhiza” means root.

The root region is directly influenced by the root exudates. Due to high amount of nutrients such as proteins, amino acids, carbohydrates and other chemicals many microorganisms accumulates near the Rhizosphere. Due to high accumulation of microbiota the zone also contains their metabolic products which are helpful to the plant specially gives immunity towards diseases and also acts as antibiotics.

Collection of Soil Sample:

The soil was collected from the roots of a garden plant in the college garden. The plant was uprooted with the whole root system along with the adherent soil. Three soil samples were made as Rhizosphere soil, Rhizoplane soil and endorhizosphere soil. And these soil samples are collected in sterilized glass beakers.

Culturing and Isolation:

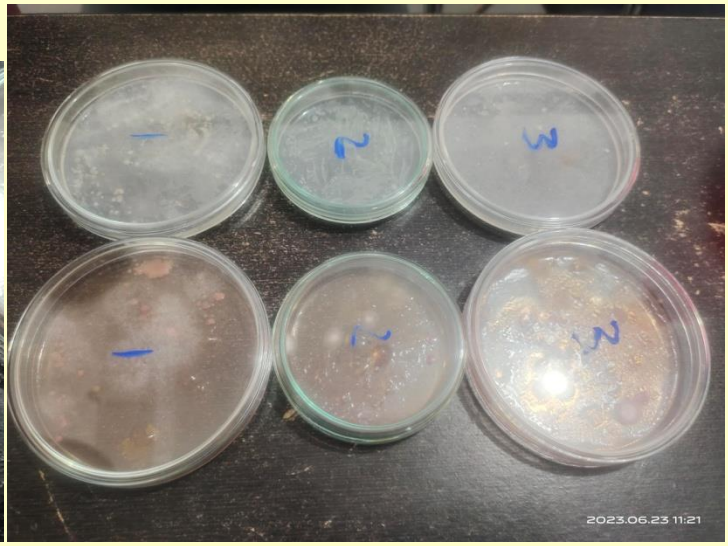
- The soil sample that is loosely attached to the root was collected and added distilled water to the sample
- The sample for Rhizoplane was taken by collecting the soil that is closely attached to the roots after removing the loosely attached soil.
- The collected sample was added to distilled water
- For collecting the endorhizosphere soil the roots are sterilized with the ethanol and there after the roots are crushed using pestle and jar and then added 9ml of water.
- Three sets of test tubes taken in each of the tube 9 ml distilled water taken.
- In the 1st tube of 2 sets, 1gm of soil sample was taken and in the 3rd set 1ml of the sample taken and then the bottle was mixed vigorously.
- Later 1 ml sample from the 1st tube was transferred in to the 2nd tube and so on diluted up to 9th tube.

- Nutrient agar medium was prepared and poured in to the petri plates and given numbering.
- 0.1ml of sample from each test tube was poured to the each petri plate and spreaded through the spreader. Then each petriplate was named with the type of sample taken and dilution taken.
- The plates are incubated in the incubator for 48Hrs.

S.NO	Name of the plate for Rhizosphere soil	Name of the plate for Rhizoplane soil	Name of the plate for Endorhizosphere soil
1	RS1	RP1	ER1
2	RS2	RP2	ER2
3	RS3	RP3	ER3
4	RS4	RP4	ER4
5	RS5	RP5	ER5
6	RS6	RP6	ER6
7	RS7	RP7	ER7
8	RS8	RP8	ER8
9	RS9	RP9	ER9
10	RS10	RP10	ER10

COLONY COUNTING:

- After incubation period the developed colonies are counted using colony counter and noted according to the dilution.



Purification of colonies by Streak plate Method:

- The single and distinct colonies developed on the media were picked with the sterilized loop and inoculated on the another petri plate.
- The plate was also named with the dilution rate.
- The plates were incubated for 48 Hrs.

Gram's Staining:

- The single colonies developed on the media were picked with the help of inoculation loop and made smear on the slide.
- The slide was the heat fixed and stained with crystal violet followed by grams iodine and safranin and finally let the slides dry.

Result:

- The slides from all 3 kinds of samples were checked and the results are as mentioned below.

S.NO	PLATE-RS		PLATE-RP		PLATE-ER	
1	RS1	Pink	RP1	Pink	ER1	Pink
2	RS2	Violet	RP2	Pink	ER2	Pink
3	RS3	Pink	RP3	Pink	ER3	Pink
4	RS4	Pink	RP4	Pink	ER4	Pink
5	RS5	Pink	RP5	Violet	ER5	Pink
6	RS6	Violet	RP6	Pink	ER6	Violet
7	RS7	Pink	RP7	Pink	ER7	Pink
8	RS8	Pink	RP8	Pink	ER8	Pink
9	RS9	Violet	RP9	Pink	ER9	Pink
10	RS10	Pink	RP10	Pink	ER10	Pink

Conclusion:

In conclusion this research indicates the presence of more gram negative bacteria compared to Gram positive bacteria.

THANK YOU