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Isolation of *Rhizobium* Bacteria from Chickpea (*Cicer Arietinum*) and Slant Culture Preparation

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Introduction:

Chickpea (*Cicer arietinum* L.), a member of the familyLeguminosae, is an important pulse crop valued as a rich source of proteins [Maatallah *et al*.,2002]. It is a cool-season legume crop that isgrown in many countries around the world as a food source [Berger and Turner, 2007]. It has an important role in the maintenance of soil fertility, particularly in dry, rain-fed areas, and can resist drought [Katerji *et al*., 2001]. Chickpea is a staple basic food crop in many tropical and subtropical Afro-Asian countries and is one of the world's major pulse crops which are traditionally cultivated in marginal areas and semiarid regions. chickpea is rich in fiber, minerals (phosphorus, calcium, magnesium, iron and zinc) β-carotene and large amount of unsaturated fatty acids (Gaur,2010). Besides playing an important role in human diet it also improves soil fertility by fixing the atmospheric nitrogen (Siddiqi and Mahmood, 2001; Kantar *et al.*, 2007). Maximum nitrogen requirement (4-85%) ofchickpea as a legume is obtained through symbiotic Nitrogen fixation in association with compatible *Rhizobium* strain (Chemining and Vessey, 2006). The genus Mesorhizobium includes species with high geographical dispersion and able to nodulate awide variety of legumes, including important crop species, like chickpea. It has beenestimated that 1g of soil may contain a community of 10^9 microorganisms with *Rhizobia* representing around 0.1% of soil microbes or $10^6 g^{-1}$ soil.

Rhizobia are one of the most efficient bacterial symbionts of legumes that fix atmospheric nitrogen by the process of biological nitrogen fixation (BNF). *Rhizobia* are able to metabolize atmospheric nitrogen and convert it into plant usable form in specialized structures called nodules where aerobic condition are maintained by leghaemoglobin. In return, *Rhizobia* utilize the carbon substrates derived from the plant photosynthesis. In agriculture,

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perhaps 80%of the biologically fixed nitrogen comes from symbiosis involving leguminous plants and bacteria of family *Rhizobiaceae*.

Materials and Methods

Collection and extraction of root nodules from the chickpea plants:

The experimental material for the present study was collected from crop cafeteria of Jaipur national university, Jaipur. Plants possessing healthy nodules with pink colouration were selected and Brought to the lab.

Carefully remove the soil around root nodules, and remove root nodules with the help of forcep and placed carefully in a sterile Petri-plate. Root nodules of chickpea plant (*Cicer arietinum*) were used as study material for isolation and further morphological and physiological characterization of *Rhizobium*strain. The roots were first washed thoroughlywith sterile distilled water.

Fig 1: - Collection of root nodules from *Cicer arietinum*

Preparation of Congo Red-Yeast Extract Mannitol Agar:

- **Cryema :-** Congo Red- Yeast Extract Mannitol Agar is a specialized growth medium use for the cultivation and isolation of *Rhizobium* bacteria. It contains specific nutrients and indicators to facilitate the growth & identification of these microorganisms.
- **Ingredients required for preparing Cryema:-**

S.NO.	Media composition	Amount(gm/250ml)
1	Yeast extract	0.25

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2	Mannitol	2.5
3	Dipotassium phosphate	0.125
4	Magnesium sulphate	0.05
5	Sodium chloride	0.025
6	Congo red	0.0625
7	Agar	5.0
8	pH	7

Step – by- step procedure for preparing 'Congo Red Yeast Extract Mannitol Agar':-

1. **Mixing ingredients: -** Measure and combine the base ingredients in 250ml distilled water
2. **Adding Congo Red: -** Dissolve & incorporate the Congo Red & other Components into the mixture.
3. **Gelling process: -** Boil the mixture to dissolve the agar & pour it into petri dishes to solidify & also in test tube.

- **Sterilization and storage of 'Congo Red Yeast Extract Mannitol Agar': -**
  1. **Sterilization: -** Place the prepared media in an autoclave at 121.6^{o}C for 15 min & subject them to high- pressure steam for sterilization.
  2. **Storage: -** After the sterilization, store the agar plates in a cool, dry place.

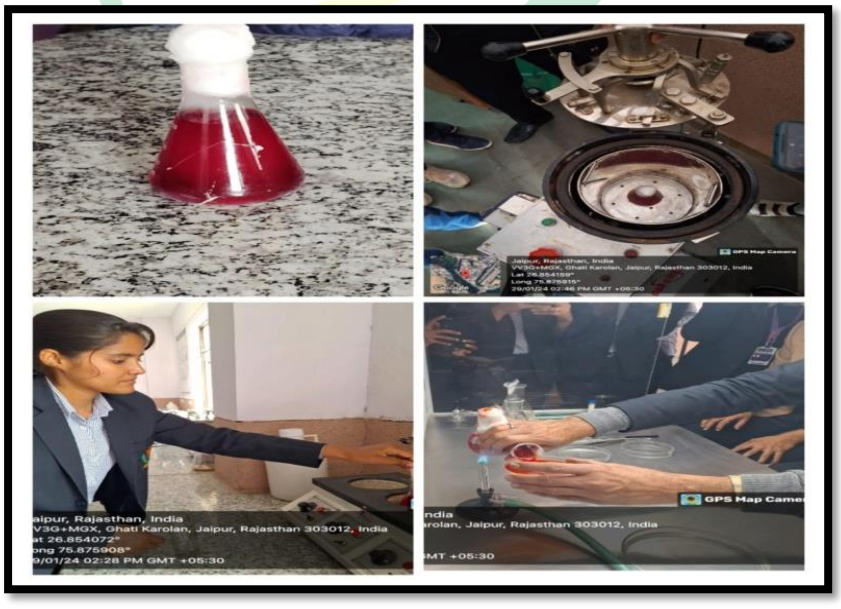

Fig 2: - Preparation of growth (CRYEMA) media

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Isolation and Culturing of *Rhizobium* bacteria: -

Rhizobium was isolated from root nodules of two healthy & actively growing plants by carefully uprooting whole plants at 45 days after emergence, which is characterized by maximum nodulation.

- **Requirement: -** Legume plant roots, Sterile distilled water, petriplates, test tubes, Cryema plates, 70% ethanol, 0.1% Mercuric Chloride solution.
- **Procedure: -**
  1. **Sterilizing root nodules for culturing: -** Detached nodules were firstly dipped in 0.1% mercuric chloride solution for 30 sec, followed by 70 % ethanol for another 30 sec and thoroughly rinsing 3 times with sterilized distilled water.
  2. **Culturing *Rhizobium* in Cryema:-** Surface disinfected nodules were then transferred to test tubes containing 5 ml of sterilized distilled water where they were crushed with a sterilized glass rod to obtain a milky suspension. A 0.1 ml aliquot of this suspension was placed on CRYEMA medium plates. All of the work was carried out in laminar air flow chamber.

Fig 3: Step by step procedure of Rhizobium isolation and culturing

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Preparation of Bacterial slant Culture:

The slant culture is done basically under the test tube and it is done to increase the surface area that helps in the inoculation of the bacteria.

Procedure: -

1. **Selecting a suitable medium: -** We have the pre-made Cryema media that was melted on the heating plate.
2. **Sterilizing equipment's: -** Sterilize all equipment, including test tubes, and inoculating loop or needle, typically by autoclaving.
3. **Preparing agar slant: -** The Cryema media were filled in the test tube. Cover the surface of test tube with the cotton plug and paraffin tape. Then test tube were kept in the autoclave for one day for proper sterilization.

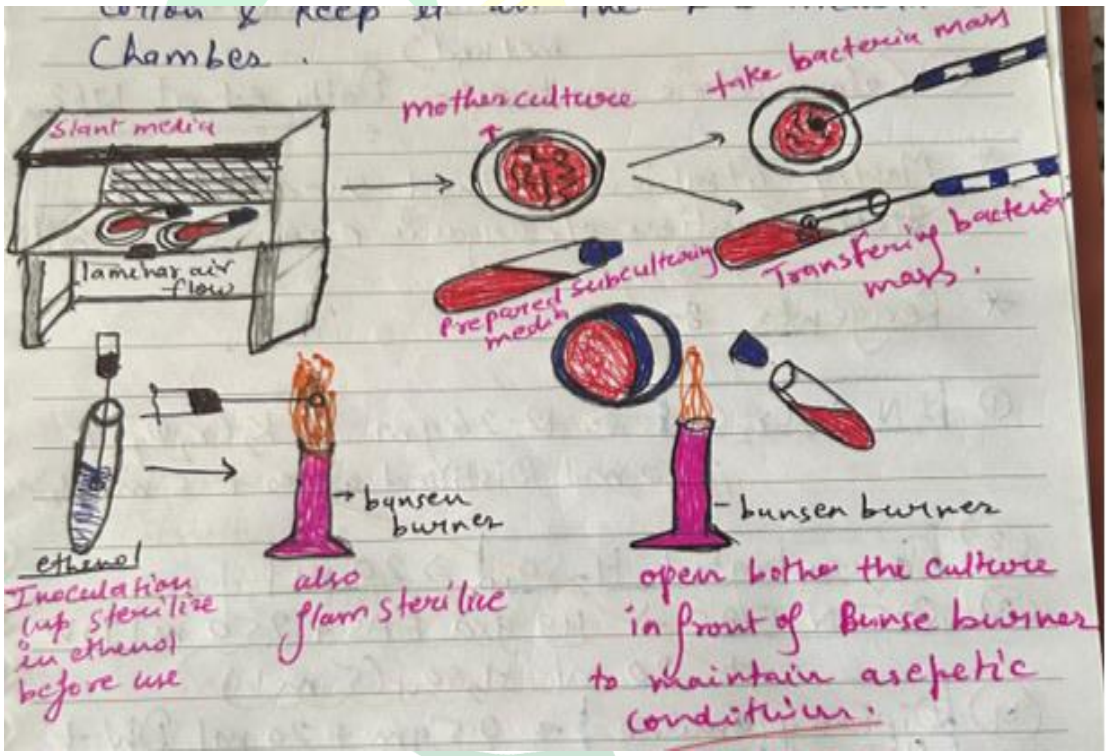

Fig 4: Bacterial Slant Culture

4. **Inoculation: -** After the sterilization is done, the next day test tube containing CRYEMA media were placed in the laminar air flow chamber in the slanted position and maintaining 45^{o} angle. Then we put the nodules of *Rhizobium* in 0.1% $HgCl_2$ Solution then in 70% ethanol solution followed by washing with distilled water. The Petri-plate containing nodules suspension was smeared on the inoculation loop

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followed by streaking carefully on the test tube. It was carried out near the flame so there is no contamination.

5. **Incubation: -** Place the inoculated slant tubes in an incubator set to the appropriate temperature for the growth of the bacteria Culture. Allow the bacteria to grow for the specific incubation period.
6. **Observation: -** Within 2-3 days the grown culture can be observed. Rhizobia appeared as white coloured colonies.
7. **Storage: -** After incubation, store the slant culture in a refrigerator or at the appropriate temperature for future use.

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