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Research Article

STUDY OF MICROBIAL ISOLATION FROM SOIL OF LEGUMINOUS PLANTS COLLECTED FROM PATTOKI

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A R T I C L E I N F O

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A B S T R A C T

Background: Soil isthe region where most of the physical, biological and biochemical reactions related to decomposition of organic matter, degradation, cycling of nutrients, mineral and metal transformation occur.The soil environment with diverse microbial population responsible for nitrogen fixation, decomposition and mineral transformation which aid in maintaining a sustainable soil environment.All nutrients that plants absorb have to pass a region of intense interactions betweenroots, microorganisms and animals, termed the rhizosphere.Leguminous plants belong to family *Fabaceae*and includes peas, lentils, beans, chickpeas and soybeans etcSome groups of bacteria grow in roots of legumes which aid in crop rotation. The study aims are to isolation, identification and characterization of nitrogen fixing bacteria. Determine prevalence of high number of *Rhizobium* in soil samples of leguminous plants.

Methods: The observational and prospective study was conducted on soil samples collected from Pattoki pea, soybean, chickpeas, lentil and beans field in the period of two days and examined from 1st March to 30thMay 2019. For prevention of contamination, glass wares (such as conical flask, test tube, pipette, petri dish etc) was washed and sterilized before taking bacterial sample**.**Media are the synthetic mixtures of bacto-peptone 10g, NaCl 5g, Yeast extract Powder 0.5g in a liter of distilled water, Agar powder of 105g was added in 100 ml for solidification of liquid medium. These were used for culturing the bacteria in glass- tubes and in petri dishes.

Results:*E.coli, Rhizobium, Pseudomonas* and *Proteus* specie of bacteria were identified in soil samples. *E.coli* obtained in highest quantity (35%) then *Rhizobium* (33%), *Pseudomonas* (28%)and *Proteus species* (4%) as shown in graph.Their presence confirm by Catalase, Oxidase, Voges-Proskauer and Indole tests. The biochemical tests performed on the isolates (*Rhizobium, E.coli, Proteus and Pseudomonas)*showed that most were positive for Catalase, Oxidase, Voges – Proskauer and Indole tests. Sugar tests positive during isolation and characterization of *Rhizobium meliloti* on most of leguminous plant roots. All identified species are motile and gram negative rods in nature. *Pseudomonas and Rhizobium* are aerobic while *Proteus* and *E.coli* are facultativeanaerobe and aerobe respectively. Colony morphology of *E. coli* on blood agar was slightly convex, grey and moist with hemolytic property of some strains. *Proteus*showed swarming over entire surface and the ripples on water on blood agar. The colonies of *Pseudomonas*were observed to slightly opaque colony while showed large, flat, spreading colonies with clear zones of hemolysis. *Rhizobium* with mucoid colony characteristics has morphology onblood agar was non hemolytic and on MecConkey agar was non lactose fermenting. Complex microbial and faunal interactions with plant roots accompanied and shaped the evolution of land plants. In future we recommend to use these beneficial bacteria instead of organic or synthetic fertilizers.

Conclusion:*Rhizobium* is an important microorganism for the environment because of its nitrogen-fixing ability when in symbiotic relationship with plants (mainly legumes). This study confirmed that the root nodules of leguminous plant harbour the nitrogen-fixing bacterium- *Rhizobium.* It also showed that leguminous plants when inoculated with *Rhizobium* isolates perform better. This organism will greatly enhance agricultural production, if they are often used to inoculate legume plants, thereby reducing the environmental threat of synthetic nitrogen fertilizers.

INTRODUCTION

Leguminous plants belong to family *Fabaceae* and seed of such plant called pulse. *Fabaceae* includes peanut, soybean, fieldbeans, fieldpeas, limabeans, cowpeas, mungbeans, alfalfa, clover, peas, lupin beans, chickpeas, broadbeans, pigeonpeas, tamarind and lentil(Donahue *et al.,* 1983).

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Leguminous plants are a source of organic material with significant benefits for soil and crops, due to the high nitrogen fixation capacity, nutrient cycling and contribution to soil cover (Cobo *et al*., 2002). These are major source of protein, carbohydrates, dietary fiber and dietary mineral in nutrition(USDA Nutrient Database, 2015). Some groups of nitrogen fixing bacteria are attached to the roots of legumes. Leguminous plants also play key role in crop rotation and referred as green manure. These are natural fertilizer source to provide high quality forage and play role in soil stabilization

and erosion prevention. Leguminous plants possess a unique ability to establish symbiotic association with nitrogen-fixing bacteria of the family *Rhizobiaceae.Rhizobium* inoculants significantly improves yield in many leguminous crops and can minimize the use of synthetic fertilizer which is rather expensive and deteriorates soil properties(Mahdhi *et al.,* 2008).

The ball of Earth contain roots of plants and microorganisms that accompany them. The rhizosphere contains abundant bacteria, fungi, protozoa and nematodes. Some nematodes are feeding on bacteria and fungi(Thom and Smith, 1939). Microbes mostly reside in radius of 50µm(Pinton *et al.,* 2001). About 200 million tons biological nitrogen fixation had come from symbiotic relation of *Rhizobium* and leguminous plants per year. Rhizobial species *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* lead to symbiotic interactions with legumes and result in root nodule formation (Mishra *et al*., 2009).

Once leguminous plant dies, the nodule breaks down and release the *rhizobia* back into the soil(Herridge and David, 2013). Grain legumes are cultivated on about 1.5 million km^2 of land per year.The amount of nitrogen fixed annually is about 44–66 million tons worldwide, providing almost half of all nitrogen used in agriculture(Alberton *et al.,* 2006).After doing studies on nodulating bacteria of leguminous plants we are able to increase the fertility of soil and also enhance the growth of plant. These bacteria isolate from soil to multiply and increase the amount of this beneficial bacteria. The goal of this research is to increase the plants' productivity without using fertilizers.

MATERIAL AND METHOD

The observational and prospective study was conducted on twenty (20) soil samples of pea, soybean, chickpea, lentil and beans fieldcollected from Pattoki.Dissolved 28 gm of prepared Nutrient agar in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilized it by autoclaving at 121^0C and 15 Ibs pressure for 15 minutes and mixed it well and poured it into sterilized Petri plates.

Media were the synthetic mixtures of bacto-peptone 10g, NaCl 5g, Yeast extract Powder 0.5g in a liter of distilled water, Agar powder of 105g was added in 100 ml for solidification of liquid medium. These were used for culturing the bacteria in glass- tubes and in petri dishes. The media were sterilized in an autoclave at 1200ºC under 15lbs pressure for 15 min. Both solidified and liquid culture media were used for study of rhizosphere bacteria. Pots were filled with these soils and filter papers were put appressed on the soil and sterile liquid bacterial culture medium was pipette on the filter paper to allow growth of bacteria in colonies on the filter paper from the soil.

The pots were put on the laboratory bench at room temperature (30-350c). Well-grown bacterial colonies were picked up with a sterile wire loop and cultured separately in liquid culture tubes. These were numbered numerically. Streaking method was used for making bacterial cultures in plates. Soil were sterilized at 1200ºC under 15lbs pressure for one hour, then cooled. Thepots were filled with sterile soil as the purpose of the experiments.

Figure 1 Media, Plates and Flame

Figure 2 Soil In Pots

Figure 3 Colonies on Mannitol agar

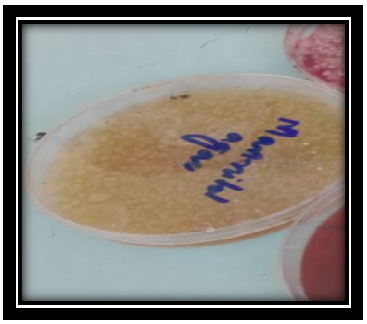


Figure 4 Picking up Colony

Figure 5 Inoculate Colonies From Different Media

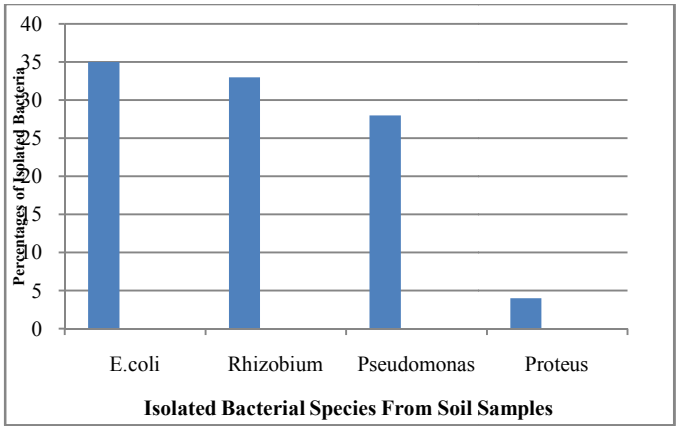
Figure 6 Overnight Incubation of Culture Plates

RESULTS AND DISCUSSION

The nitrogen fixing bacteria in leguminous plants proved more helpful as compared to synthetic fertilizers. The present study was conducted to isolate and identify different bacteria. Most of the nitrogen fixers obtained from root's soil of leguminous plants. *E.coli, Rhizobium, Pseudomonas* and *Proteus* specie of bacteria were identified in soil samples.

Table 1 Site of Soil Sample Collected

Leguminous Plants	Location	No. of Samples
Peas	4cm away from roots	7
Chickpeas	8 cm away from roots	3
Lentil	12cm away from roots	*3*
Soybean	6cm down from roots	2
Beans	12cm down from roots	5



Graph 1 Prevalence of Isolated Soil Bacteria

Hardly there is any soil without microorganisms, mainly the bacteria. The microflora in the soil benefits to the plants by protecting the roots from infection of pathogenic bacteria, fungi and other parasites. Plant roots excrete organic substances, although in smaller amounts, in the soil around the roots.These includes sugars, organic acids, amino acids and vitamins which stimulate the development of microflora of non-nitrogen fixing microorganisms.

We are also convincedthat further work in this line with large-scale experimental work in fields, also with other crops will bring about improvements in our agriculture by minimizing application of organic manure and chemical fertilizers.

Rhizosphere bacteria may, therefore, be used as a substitute for fertilizer, because along with the roots they produce CO_2 around the roots by respiration and this in turn is converted to carbonic acid with water ($CO_2+H_2O=H_2CO_3$). Carbonic acid converts the nutrients of the soil to the soluble form for absorption by roots (Berkeley, 1971).Organic manuring does not harm the rhizosphere flora. Moreover, application of rhizosphere bacteria in the soil increases the crop yield. The shortage of available N due to high leaching, a decrease in organic matter mineralization and an increase of denitrification favors the legume–bacteria symbiosis (James, 2001).

The biochemical tests performed on the isolates (*Rhizobium, E.coli, Proteus and Pseudomonas)* showed that most were positive for Catalase, Oxidase, Voges – Proskauer and Indole tests. These findings are in close agreement with Javed and Asghari, who have previously characterized the *Rhizobium* from soil and root nodules of leguminous plants with same positive biochemical tests(Javed and Asghari, 2008).Similarly, the nodulation pattern in legume plants by screening through the same tests and reported similar results.

The characterization of *Rhizobium* strain from the roots of leguminous plant bacterial species. These findings corroborate with the results of Singh(Singh *et al*, 2008). Sugar tests positive during isolation and characterization of *Rhizobium meliloti* on most of leguminous plant roots(Erum and Bano, 2008).

Recommendation

In addition to previous studies in this area, it was demonstrated that more education is needed on the current recommendations and consensuses the management of nitrogen fixers in leguminous plants. Furthermore, as our evidence-based knowledge is limited, more well-designed studies are urgently needed. The usage of organic or synthetic fertilizers in our local crops of the world needs to be replaced with beneficial bacteria *Rhizobium*. Nitrogen from atmosphere is fixed and at the end become part of atmosphere again.

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