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Isolation and Identification of Bacteria from Different Soil Samples of Telangana and Andhra Pradesh States

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ABSTRACT

The word agriculture is more related to natural activity and to nature like plant life cycle, ecosystem, green earth, vegetables, fruits, soil, water & natural environment. Plants have a number of relationships with fungi, bacteria, and algae. These are known to deliver a number of benefits including plant nutrition, disease resistance, tolerance to adverse soil and climatic conditions. These techniques have proved to be successful biofertilizers that form a healthy relationship with the roots. Biofertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers.

In the present studies various soil samples were collected from actively cultivated lands after a thorough survey of the areas. The places from where these soil samples were collected include Gandipet, Shamirpet areas in Hyderabad, Chevella on the outskirts of Hyderabad and Adilabad district of Telangana State and Kurnool, Kadapa, Gudipadu areas of Andhra Pradesh State. Most of the soils thus obtained were seem to be supporting agriculture actively. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. Characterization and microscopic observation of the culture was done and the bacteria identified were *Streptomyces sp., Staphylococcus sp., Azotobacter sp., Leptothrix sp., Derxia sp., Bacillus sp., and Sphaerotilus sp.* Of the above isolated organisms *Azotobacter sp. Streptomyces sp. and Bacillus sp*. are familiar to be used as biofertilizers in various crop fields. *Derxia sp.* is a Gram negative, nitrogen fixing bacteria but not much known as a biofertilizer, which seems to be very interesting to scientist to work on it. Hence it is important to study its role in agriculture.

Keywords: Soil samples, Biofertilizers, Bacteria, Crop fields, Farmers.

I. INTRODUCTION

One of the major concerns in today's world is the pollution and contamination of soil. [1] The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. While manure, cinder and iron making slag have been used to improve crops for centuries, the use of biofertilizers is arguably one of the great innovations. [2] [3] Minerals, organic components and microorganisms are three major solid components of the soil. They profoundly affect the physical, chemical, and biological properties and processes of terrestrial systems. Biofertilizer are the products containing cell of different types of beneficial microorganisms. Biofertilizers are classified as Nitrogen fixers which includes Bacteria like *Rhizobium, Azotobacter, Mycobacterium and Blue Green Algae* like *Anabaena , Nostoc etc.* Further depending upon the fixing capacity they are divided as Phosphate solubilizing fertilizer, Plant growth promoting Rhizobacterim (PGPR), Sulphur solubilizing microbes etc.

Biofertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting

rhizobacteria "(PGPR).[4] These are some of bacteria that can grow in the root environment and be effective on plant growth. Mechanisms that can promote plant growth include production of phytohormones, biological nitrogen fixation and increased solubility of insoluble elements in soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, biofertilizers do not contain any chemicals which are harmful to the living soil.

Several soil bacteria help the crops to fix the atmospheric nitrogen and the organic phosphate. Hence these potential bacteria would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers.[5] It also means that nature grows only when there is balance of activities between natural things only like animals, plants, microbes and environment! These potential bacteria as biological fertilizers can be important components of integrated nutrient management.

II. METHODS AND MATERIAL

A. Collection of soil samples

Various soil samples were collected from actively cultivated lands after a thorough survey of the areas. The places from where these soil samples were collected include Gandipet, Shamirpet areas in Hyderabad, Chevella on the outskirts of Hyderabad and Adilabad district of Telangana State and Kadapa, Kurnool, Gudipadu areas of Andhra Pradesh State. Most of the soil thus obtained was seem to be supporting agriculture actively. The land was dug as deep as 10 – 15 cm for the collection of soil samples. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. The soil samples were wetted from time to time to help in the sustenance of the microorganisms as moisture is also a very important factor that plays an important role in the survival of microorganisms.

B. Characterization of the soil samples

The soil samples were then studied for the soil type, colour and the crops that they support for cultivation. The soils were then typed as red soil and black soil. Crops like Green leafy vegetables, Onion, Turmeric, Ground nuts, Rice, Pea plants and Tomato were being cultivated on these soils. The soils were then labelled and segregated according to the crops and place from where they were obtained.

C. Preparation of culture media

Nutrient agar media was used for primary isolation and Jensen agar media ,William and Kusters media for their sub culturing.

D. Isolation and characterization of bacteria

Serial dilution agar plating was done for the isolation of soil microbes. Suspension was diluted up to 10^{-10}. The aliquots were cultured for bacteria. For primary isolation of bacteria Nutrient broth, Nutrient agar media and specific medias like Jensons media and William and Kusters media for the growth of *Azotobacter sp. and Actinomycetes sp.* were used. The inoculum was spread with the help of a spreader evenly on the media. The inoculated plates were then incubated at 37°C for 24 h. Growth of the microorganisms is observed in the form of turbidity and colony growth respectively. Characterization and microscopic observation of the culture was done.

III. RESULT AND DISCUSSION

One way to increase crop yield is using the beneficial microorganisms. Biofertilizers help the crops to fix the atmospheric nitrogen and the organic phosphate. They maintain a healthy symbiotic relationship with the crops thus helping to increase the yield of the crops. This symbiotic relationship proves beneficial both to the organism contributing for their sustenance and also in turn increases the soil fertility thereby helping to obtain an increased, disease free, resistant crop type [6]. Biofertilizers such *as* Rhizobium, Azotobacter, Azospirilium and blue green algae(BGA) have been in use for a long time. And also there must be some organism having direct or indirect association with

plants which need to be investigated thoroughly. For this one has to study the organism present in the different soil samples of various plants.

In the current studies various soil samples were collected from actively cultivated lands after a thorough survey of the areas. The first step of this project is to isolate and identify bacteria from different soil samples like Gandipet, Shamirpet areas in Hyderabad, Chevella on the outskirts of Hyderabad and Adilabad district of Telangana State and Kurnool, kadapa, Gudipadu areas of Andhra Pradesh State. Most of the soils thus obtained were seem to be supporting agriculture actively. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. Characterization and microscopic observation like Gram stainig, Spore staining, Capsular staining and biochemical test were performed. It is evident from the Table 1. the organism isolated and identified from Telangana State of Gandipet black soil rice field is *Streptomyces sp.,* to confirm this organism it was inoculated in to Williams and kusters media which had shown the orange pigmented smooth, entire mucoid and translucent colonies, characteristic feature of *Streptomyces.* According to literature *Streptomyces sp.* isolated (Tinatin Doolotkeldieva et al., 2015)[7] from the rhizosphere has improved the composition of rhizosphere microflora, attracting saprophytic microorganisms: ammonificators and oligotrophs. The presence of the biocontrol microorganism *Streptomyce sp.* in the rhizosphere plays an important role in enhancing the growth and development of useful groups, such as nitrogen-fixing bacteria [7]. Organism identified from Tomato plants of Shamirpet black soil is *Staphylococcus sp.* Chevella black soil, showed the growth of *Azotobacter sp.* in pea plant and the organism isolated from leafy green vegetables of Adilabad red soil are *Leptothrix sp.* and *Azotobacter sp.*

Leptothrix sp. is a sheathed filamentous bacterium that can generally be found in different types of aquatic environments with sufficient organic matter. It is known to be capable of oxidizing both iron (II) and manganese (II), unlike other sheathed bacteria. Crops inoculated with *Azotobacter* and *Azospirilla* reviewed by Wani (1990) indicated that Pearl millet and Sorghum, which are grown as dry land crops showed 11-12% increased yields due to inoculations. [8] Table 2. shows that an organism isolated from Andhra Pradesh State of Kurnool black soil Rice is *Azotobacter sp.* and from Ground nut is *Derxia sp.* The organism *Azotobacter sp.* are Gram-negative bacteria[9] found in neutral and alkaline soils, in water and in association with some plants. It plays a vital role in every ecosystem, it is found worldwide, in climates ranging from extremely northern Siberia to Egypt and India. [10]. It is used as biofertilizers for the development of various vegetable plants such as mustard, maize, wheat, cotton etc. The most dominant *Azotobacter sp.* which was isolated from Chevella, Adilabad and Kurnool districts is confirmed by inoculating in to specific Jensen agar media. *Derxia* is a genus of Gram-negative, nitrogen-fixing bacteria from the family of Alcaligenaceae.[11] [12] [13] Rennie (1980) examined sugarcane rhizosphere soil samples from Brazil and found them to contain equal populations of *Derxia*gummosa, Enterobacter cloacae, Bacillus polymyxa and Azotobacter vinelandi.[14]And organism isolated from Ground nuts of Kadapa red soil are *Bacillus sp.* and *Staphylococcus sp.* Literature shows that *Bacillus* and *Staphylococcus sp.* bacteria have potential to fix atmospheric nitrogen, able to produce IAA with the range of 15.13± 0.2 to 33.1± 0.2 µg/ml when supplemented with 100 µg/ml of tryptophan and showed some P-solubilizing activity which have been claimed by manufacturers. [15] Sample collected from Gudipadu black soil of Onion and Turmeric crop field identified as *Bacillus sp.* and from Ground nuts are *Sphaerotilus sp.* and *Bacillus sp*.

Sphaerotilus sp.[16] is a filamentous bacterium that is covered in a tubular sheath and can be found in flowing water and in sewage and waste water treatment plants. A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant growth promoting rhizobacteria or PGPR (Schroth and Hacock, 1981). PGPR[17] belong to several genera e.g. *Agrobacterium, Alcaligenes,Arthrobacter, Actinoplanes, Azotobacter, Bacillus, Pseudomonas sp., Rhizobium, Bradyrhizobium, Erwinia, Enterobacter, Cellulomonas, Flavobacterium, Streptomyces and Xanthomonas sp.* (Weller, 1988). Several soil bacteria and fungi notably species of *Pseudomonas, Bacillus, Penicillium* and *Aspergillus* etc., secrete organic acids and lower the pH in their vicinity to bring about solubilization of bound phosphates in soil (Sundara Rao and Sinha, 1963)[18]. Habibi *et al.* (2011) strongly suggested that using

biofertilizers (combined strains) plus half a dose of organic and chemical fertilizers have resulted in the greatest grain yield and oil yield in medicinal pumpkin[19]. Studies showed that the inclusion of wheat plant with PGPR increased the growth characteristics of wheat [20].

The second step of the project is to check potential use of these bacteria as biofertilizers on various crop fields. Furthermore, the phosphate solubilization capacity and nitrogen fixing ability of *Azotobacter* and other organisms isolated from various soils are to be tested and employed as biofertilizers during field trials on various crop plants which will be taken up as next phase of project work. Biofertilizer containing those microorganisms would play a great role in crop improvement. Crop yield can be increased by 20-30% if they are used properly. Hence these potential bacteria would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers.[21]

Table 1: Organism isolated from Telangana State.

Area / Type of soil		Type of crop	Media used	Colony morphology	Microscopic Observatios	Organism identified
Gandipet	**Black Soil**	Rice	Nutrient agar	Orange pigmented smooth, entire mucoid and translucent colonies.	Gram-positive, filamentous bacteria with well-developed vegetative hyphae with branches. Spore surfaces were hairy, smooth, and spiny .	*Streptomyces sp.*
		Rice	William and kusters media	Orange pigmented smooth, entire mucoid and translucent colonies.	Gram-positive, filamentous bacteria with well-developed vegetative hyphae with branches. Spore surfaces were hairy, smooth, and spiny .	*Streptomyces sp.* is confirmed
Shamipet Black Soil		Tomato Plant	Nutrient agar	Golden yellow colonies	Gram positive cocci in clusters	*Staphylococcus sp.*
Chevella	**Black Soil**	Pea plant	Nutrient agar	Non pigmented, smooth, mucoid and translucent colonies.	Gram negative thick sporulating bacilli arranged singli.	*Azotobacter sp.*
		Pea plant	Jensen agar media	Non pigmented, smooth, mucoid and translucent colonies.	Gram negative, thick sporulating bacilli arranged singli.	*Azotobacter sp.* is Confirmed
Adilabad	**Red Soil**	Green leafy vegetables	Nutrient agar	Non pigmented medium sized, round, entire margin colonies.	Gram negative filamentous network.	*Leptothrix sp.*
		Green leafy vegetables	Nutrient agar	Non pigmented, smooth, mucoid and translucent colonies.	Gram negative, thick sporulating bacilli arranged singli.	*Azotobacter sp.*

Table 2: Organism isolated from Andhra Pradesh State

Area / Type of soil		Type of crop	Media used	Colony morphology	Microscopic Observatios	Organism identified
Kurnool	**Black Soil**	Ground nut	Nutrient agar	Small,mucoid, pin pointed, opaque , smooth.	Gram negative slightly curved rods with rounded ends. Cells appear as dividing pairs.	*Derxia sp.*
		Rice	Nutrient agar	Non pigmented smooth, mucoid and translucent colonies.	Gram negative thick sporulating bacilli arranged singli.	*Azotobacter Sp.*
Kadapa	**Red Soil**	Ground nut	Nutrient agar	Non pigmented pale, smooth mucoid and opaque.	Gram positive thick sporulating bacilli.	*Bacillus sp.*
		Ground nut	Nutrient agar	Round, flat,serrated margins, yellow pigmented colonies.	Gram positive Cocci arranged in cluster.	*Staphylococcus sp.*
Gudipadu	**Black Soil**	Onion, Turmeric and Ground nut	Nutrient agar	Non pigmented pale, smooth, mucoid and opaque colonies.	Gram positive thick sporulating bacilli.	*Bacillus sp.*
		Ground nut	Nutrient agar	Large irregular , mucoid, rough and opaque colonies.	Gram negative sheated bacilli	*Sphaerotilus sp.*

IV. CONCLUSION

The purpose of this study was to investigate the bacteria associated with different soil samples of various crop fields of Telangana and Andhra Pradesh States. The bacteria isolated and identified were *Streptomyces sp., Staphylococcus sp., Bacillus sp., Azotobacter sp., Leptothrix sp., Derxia sp., Bacillus sp.,* and *Sphaerotilus sp*. Of the above isolated organism there is no evidence of using *Leptothrix sp.*and *Sphaerotilus sp.* as biofertilizers. *Azotobacter sp.*is used as biofertilizers for the development of various vegetable plants. *Derxia sp.* is a Gram negative, nitrogen fixing bacteria but not much known as a biofertilizer, which seems to be very interesting to scientist to work on it. Hence it is important to study its role in agriculture. Furthermore, the phosphate solubilization capacity and nitrogen fixing ability of *Azotobacter*, *Derxia* and other organisms isolated from various soils are to be tested and employed as biofertilizers during field trials on various crop plants which will be taken up as next phase of project work.

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