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Characterization of plant growth promoting rhizobacteria from legume rhizosphere

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ABSTRACT

Legumes have been used in agricultural production since the earliest of civilizations. They have served as the primary source of nitrogen for many cropping systems, as well as providing food for humans and domestic animals. In many developing agricultural regions of the world, legumes are still used extensively for these purposes. However, in the last several decades, the widespread availability of synthetic nitrogen fertilizer has resulted in a major decrease in the cultivation of legumes. Thus it is important both in terms of crop productivity and soil health that biofetilizers should be used. The present study aimed at characterization of growth promotion potential of rhizobacteria of legume crop. The rhizobacterial population (log_{10}cfu) varied significantly from 5.44 to 7.36. The rhizobacterial population was found to vary in species richness from 0d to 90d of cropping. Bacillus was documented to be dominant population of zero day (40%) while Pseudomonas was the dominant population (33%) on 30th day of cropping. On 60th day of cropping Pseudomonas became a predominant population (50%) while on 90th day of cropping Pseudomonas and Corynebacterium were the dominant population (30%). These isolates exhibit a significant plant growth promotion attributes viz., siderophore production, phosphorus solubilization, protease and rhamnolipid production.

Key words: Legumes, Rhizobacteria, Biofertilizers, *Pseudomonas*, *Corynebacterium*, Siderophore, Rhamnolipid, Phosphorus solubilzation.

INTRODUCTION

The intensive use of fertilizers to increase crop production had adverse effects on the soil health. Greater productivity and competitiveness are anticipated to come from increased efficiency through the acquisition and management of new of biotechnologies and crop production strategies [1]. Improvement in agriculture sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological processes and soil biodiversity. In this context, the long-lasting challenges in soil microbiology are development of effective methods to know the types of microorganisms present in soils, and to determine functions which the microbes perform *in situ*.

It is imperative to understand the relationship of soil and plant with the diversity of associated bacteria, defining the roles of plant growth promotory bacteria (PGPR) to evolve strategies for their better exploitation. PGPR live in mutualistic interactions with the plant. They benefit from rhizodeposition-derived nutrients and in some cases from other root derived factors like micro-oxic conditions, growth factor, attractants or even inducers of enzyme activity. In return, these populations may exhibit properties favouring plant growth and productivity. Beneficial rhizobacteria can increase plant vigor and soil fertility [2]. The application of plant growth promoting rhizobacteria (PGPR) as biofertilizers, phytostimulators and biocontrol agents would be an attractive alternative to decrease use of chemical fertilizers which lead to environmental pollution [3].

MATERIALS AND METHODS

2.1 Collection of samples
Samples were collected from the field at different time periods viz., 0d, 30d, 60d, 90d and 120d.

2.2 Physical characteristics of soil
The temperature and pH of soil sample was recorded.

2.3 Recovery of rhizospheric microflora
Rhizospheic soil was separated from roots of legume with the help of brush in a petridish. 10g soil was placed in 100ml sterile phosphate buffered saline (PBS) and was placed in shaker for 1h. 0.1 ml of appropriately diluted sample was spreaded on nutrient agar. All fractions were plated in triplicates. Plates were incubated in a BOD incubator at 28 ± 1^0C for 24h.

2.4 Characterization of isolates
2.4.1 Morphological characterization
Morphological characteristics viz., colony morphology (colour, chromogenesis, shape, margin, elevation and surface) and cell morphology (shape, gram reaction and arrangement) of recovered isolates were studied.

2.4.2 Biochemical characterization
The various biochemical characteristics viz., Oxidase test, IMViC test, TSI test, Uresae test, Catalase test and nitrate reduction test were carried out according to [4].

2.4.3 Functional characterization
The functional diversity amongst recovered isolates was studied by qualitative screening of their ability to solubilize phosphorus, protease, rhamnolipid and siderophore production.
(a) Phosphorus solubilization
The ability of isolates to solubilize phosphorus was estimated according to [5]. Isolates exhibiting clearing zone on Pikovaskya's agar after 96-120h of incubation were considered as positive.
(b) Rhamnolipid production- It was estimated according to [6]. All isolates were inoculated on rhamnolipid production medium. Isolates exhibiting blue colour were considered as positive.
(c) Siderophore production- It was assayed according to [7]. Isolates were spot inoculated on Chromeazurol 'S' agar. Isolates exhibiting an orange halo zone after 48-72h of incubation were considered positive. Their zone diameter was measured.
(d)Protease production- It was assayed on skim milk agar. Isolates exhibiting a clear halo zone after 24h of incubation were considered as positive. Their zone diameter was measured.

RESULTS

Temperature of soil sample of maize varied significantly from 38 ± 0.45^0C (0d) to 24 ± 0.32^0C (90d). pH varied slightly from 7.1 (0d) to 6.7(90d).

3.1 Diversity of rhizobacteria
3.1.1 Structural diversity
The rhizobacterial population (log_{10}cfu) varied significantly from 5.44 to 7.36 (Fig. 1). The rhizobacterial population was found to vary in species richness from 0d to 90d of cropping (Fig. 2). *Bacillus* was documented to be dominant population of zero day (40%) followed by *Pseudomonas* (20%), *Alcaligenes* (20%) and *Corynebacterium* (20%). *Pseudomonas* was the dominant population (33%) on 30^{th} day of cropping followed by *Alcaligenes* (25%), *Serratia* (25%) and *Bacillus* (17%). On 60^{th} day of cropping *Pseudomonas* became a predominant population (50%) followed by *Bacillus* (25%) and *Alacligenes* (25%). On 90^{th} day of cropping *Pseudomonas* and *Corynebacterium* were the dominant population (30%) followed by *Bacillus* and *Serratia* (20%).

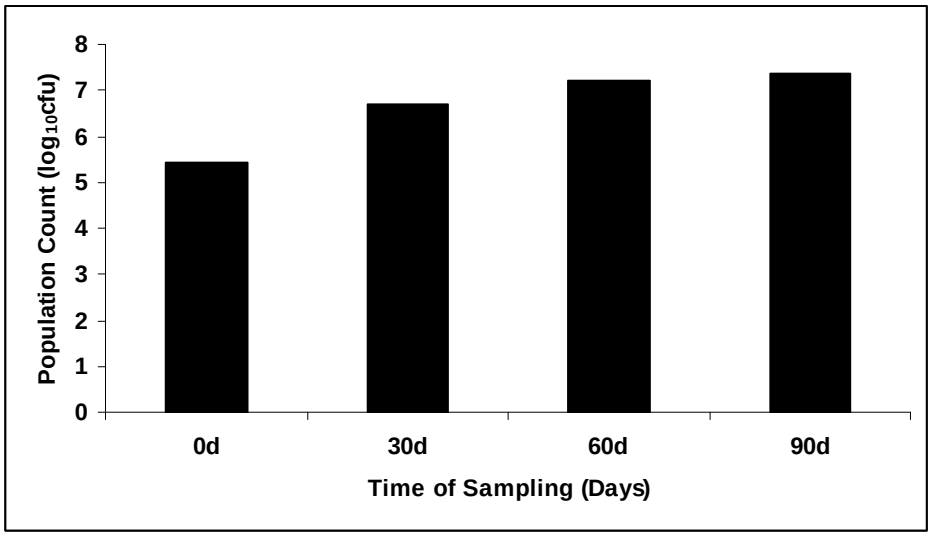
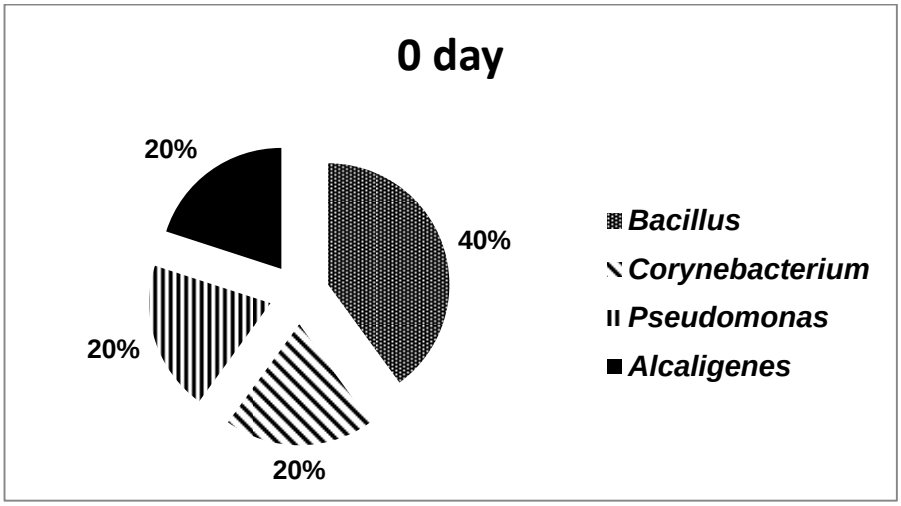
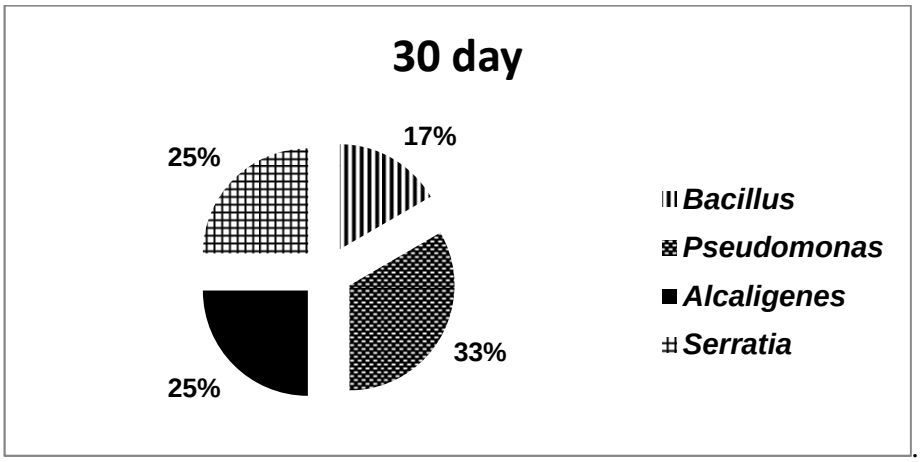


Fig. 1: Population structure of legume rhizobacteria





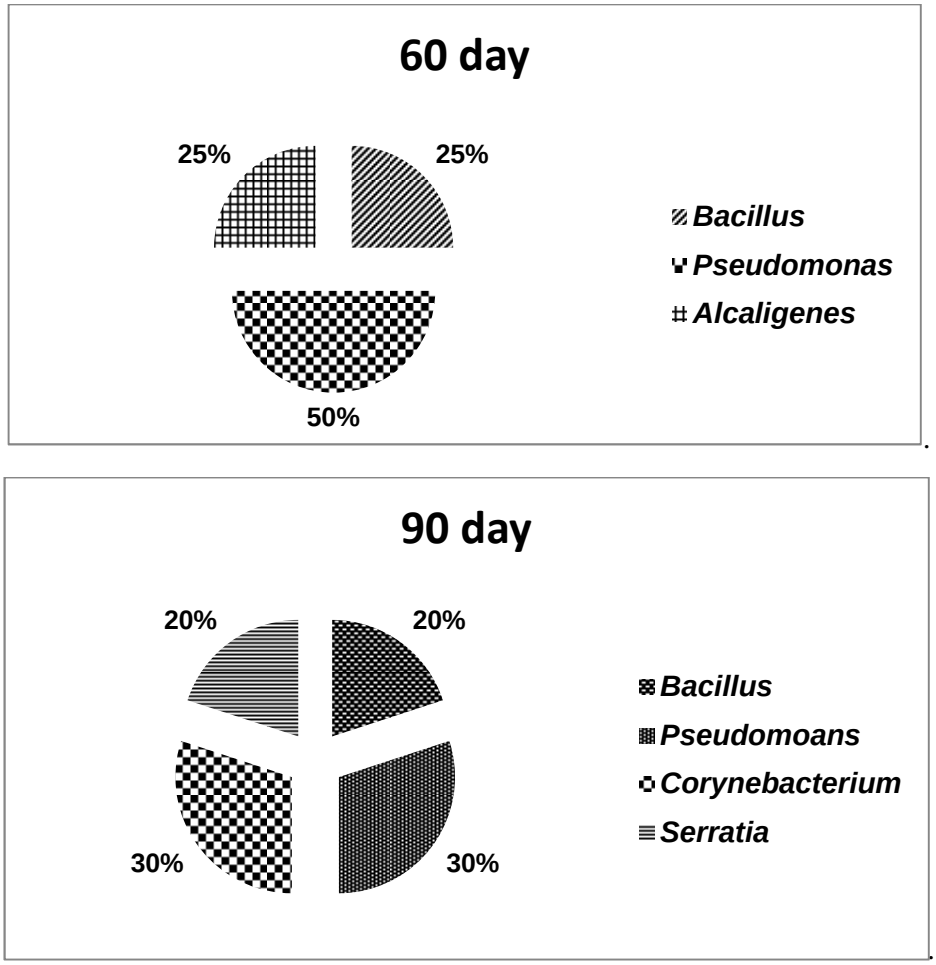


Fig.2: Distribution of legume rhizobacteria at different time intervals in field

3.1.2 Functional characterization of recovered rhizobacteria

The distributional of functional diversity amongst the recovered rhizobacteria is depicted in Fig. 3. Maximum siderophore producers were recovered from 30d, 60d and 90d samples. Maximum P solubilizers were recovered from 60d sample while maximum protease producers were recovered from 60d sample. Maximum rhamnolipid producers were recovered from 60d and 90d samples.

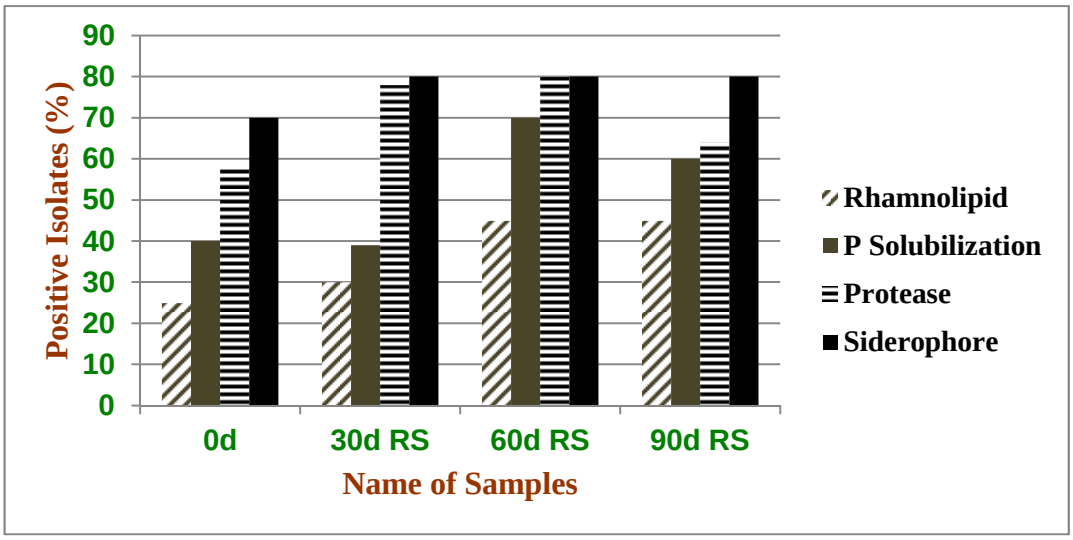


Fig. 3: Functional diversity of recovered rhizobacteria from legume

DISCUSSION

The race for producing more crop yield by adopting intensive agronomic practices and applying more fertilizers is thought to have had adverse effects on the diversity of bacteria in the agriculture fields. The beneficial microorganisms in bulk soil and rhizosphere in natural agroecosystems contributing to soil health and plant productivity can be exploited as bioinoculants to increase more crop productivity. This requires a deeper understanding of relationship between rhizosphere and bacteria. The present work was aimed at characterizing the rhozobacteria of legume crop so that the promising isolates can be exploited as biofertilizers. The population profile of rhizobacteria was observed to change with the age of crops. Rhizobacterial population (log_{10} cfu) varied from 5.44 to 7.36. The distribution of rhizobacteria varied with the age of crops. The species richness varied with the age of cropping. *Bacillus* was documented to be dominant population of zero day while *Pseudomonas* was the dominant population on 30th day. On 60th day of cropping *Pseudomonas* became a predominant population while on 90th day of cropping *Pseudomonas* and *Corynebacterium* were the dominant population. The distribution and dominance pattern of rhizobacteria is usually influenced by the crop. The aging of crop probably change the root exudates and this influence the richness as well as dominance pattern of rhizobacteria. The functional diversity was also observed to be influenced with the age of crop as siderophore producers were maximum on 30d, 60d and 90d of cropping. Sideophore are low molecular weight iron chelating compounds which plays an important role in plant growth promotion [8, 9]. Maximum P solubilizers and protease producers were from 60d of crop. The amount of phosphorus available to plants is very low because of its extreme insolubility. Thus rhizobacteria plays an important role in plant growth promotion by solubilizing phosphate by secreting some acids or by some other means [10, 11, 12, 13]. Maximum rhamnolipid producers were from 60d and 90d crops. Rhamnolipids are a class of glycolipid produced by microorganism. They have a glycosyl head group, i.e. rhamnose moiety, and a 3-(hydroxyalkanoyloxy)alkanoic acid (HAA) fatty acid tail. Rhamnolipid helps in uptake of hydrophobic substrates, exhibit antimicrobial properties, helps in biofilm formation and swarming motility [6]. Thus this study yielded some of the promising isolates which needs to be tested for their *in vitro* plant growth promotion potential.

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