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Bacterial diversity of wheat-maize-legume cropping system

KEYWORDS: Rhizosphere, Plant growth promotoryrhizobacteria, Pseudomonas, Klebsiella, Corynebacterium, Siderophore, Rhamnolipid, Phosphorus solubilzation

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ABSTRACT *Thecropping systems are of central interest to explore for sustainable agriculture.This study focused on the characterization of rhizobacteria of wheat-maize-legume cropping system. In the field chosen for the present study, maize-legume intercropping is done.The wheat rhizobacterial population (log10cfu) varied from 6.48 to 9.57.Bacillus was documented to be dominant population of zero day (40%) whilePseudomonas 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%).The maize rhizobacterial population (log10cfu) varied from 5.4 to 9.5.Alcaligeneswas documented to be dominant population of zero day (40%). Klebsiella was the dominnatrhizobacteria on 30th day (40%) while Pseudomonas and Alcaligenes were equally dominant on 60th day (30%). Pseudomonas was dominant on 90th day (60%) as well as 120th day (47%). The rhizobacterial population (log10cfu) of legume 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.*

1. Introduction

Wheat-Maize-Legume cropping system is of central interest to explore for sustainable agriculture. The intensive use of fertilizers to increase crop production had adverse effects on the soil health. The greater productivity as well as good soil health in long terms necessitates the use of biofertlizers in the field instead of chemical fertilizers (Sinha*et al.*, 2001). In order to develop efficient biofertilizers it is very important to characterize the microflora present in the soil and to determine the role played by them in the niche. PGPR live in mutualistic interactions with the plant. They are benefitted from the rhizodeposition-derived nutrients and in return they may exhibit properties favouring plant growth and productivity.Beneficial rhizobacteria can increase plant vigor and soil fertility. 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.Generally, PGPR function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and preventing the plants from diseases. Some common examples of genera exhibiting plant growth promoting activity are *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Corynebacterium*, *Pseudomonas*, *Rhizobium*, *Serratia* etc. (Glick, 1995; Podile&Koshore, 2007). The present study aimed at characterization of rhizobacteria from wheat-maize-legume cropping system.

2. Materials and Methods

2.1 Collection of samples

Samples were collected from the fieldat different time periods viz., 0d, 30d, 60d, 90dand 120d.

2.2 Physical characteristics of soil

The temperature and pH of soil sample was recorded.

2.3 Recovery of rhizosphericmicroflora

Rhizospheic soil was separated from roots of crop 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. 0.1ml of appropriately diluted sample was spreaded on nutrient agar. All fractions were plated intriplicates. Plates were incubated in a BOD incubator at 28±1^{0}C 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 recoveredisolates 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 (Cappucino and Sherman, 1992).

2.4.3 Functional characterization

The functional diversity amongst recovered isolates was studied by qualitative screening of theirability to solubilize phosphorus, protease,rhamnolipidand siderophore production.

(a) Phosphorus solubilization

The ability of isolates to solubilize phosphorus was estimated according to (Pikovskaya, 1948). Isolates exhibiting clearing zone on Pikovaskya's agar after 96-120h of incubation wereconsidered as positive.

(b)Rhamnolipid production- It was estimated according to (Sharma and Johri, 2002). All isolates were inoculated on

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rhamnolipid production medium. Isolates exhibiting blue colour were considered as positive.

(c) Siderophore production- It was assayed according to (Schwyne and Neilands, 1987). 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.

3. Results

Temperature of soil sample of wheat field varied from 16^{0}C to 38^{0}C and pH varied from 7.9 to 6.3.Temperature of soil sample of maize-legume field varied significantly from 38±0.45^{0}C (0d) to 24±0.32^{0}C (120d) and pH varied slightly from 7.1 (0d) to 6.7(120d).

3.1 Diversity of rhizobacteria

3.1.1 Structural diversity

The wheat rhizobacterial population (log_{10}cfu) of wheat varied from 6.48 to 9.57 (Fig.1).The maize rhizobacterial population (log_{10}cfu) varied from 5.4 to 9.5 (Fig. 2). The rhizobacterial population (log_{10}cfu) of legume varied significantly from 5.44 to 7.36 (Fig. 3).

The distribution pattern of rhizobacteria varied with the cropping system and with the age of crop (Fig. 4-6). In wheat *Bacillus* was the dominanatmicroflora of zero day (30%) followed by *Pseudomonas* (20%), *Staphylococcus*(20%) and *Staphylococcus* (20%) and *Corynebacterium*(10%). On 30th day of cropping *Corynebacterium* and *Pseudomonas* were equally dominant (33%) followed by *Baciilus* (20%) and *Alcaligenes* (14%). On 60th day of cropping *Pseudomonas* was dominant (30%) followed by *Bacillus* (25%) and *Pseudomonas* (25%). On 90th day of cropping *Pseudomonas* was the predominant population (43%) followed by *Serratia*(23%), Klebsiella (17%) and *Corynebacterium* (17%). On 120th day of cropping *Pseudomonas* was dominant (35%) followed by *Corynebacterium* (33%), *Peudomonas* (16%) and *Alcaligenes* (16%).

In maize, *Alcaligenes*was documented to be dominant population of zero day (40%). *Klebsiella* was the dominant rhizobacteria on 30th day (40%) while *Pseudomonas* and *Alcaligenes* were equally dominant on 60th day (30%). *Pseudomonas* was dominant on 90th day (60%) as well as 120th day (47%). In legume 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 30th day of cropping followed by *Alcaligenes* (25%), *Serratia* (25%) and *Bacillus* (17%). On 60th day of cropping *Pseudomonas* became a predominant population (50%) followed by *Bacillus* (25%) and *Alacligenes* (25%). On 90th day of cropping *Pseudomonas* and *Corynebacterium* were the dominant population (30%) followed by *Bacillus* and *Serratia* (20%).

3.1.2 Functional characterization of recovered rhizobacteria

The distributional of functional diversity amongst the recovered rhizobacteria is depicted in Fig. 7-8. Amongst wheat rhizobacteria maximum siderophore producers were from 30d sample; maximum protease producers and P-solubilizers were from 60d sample and maximum rhamnolipid producers were from 30d, 60d and 90d samples. In maize-legume cropping system 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.

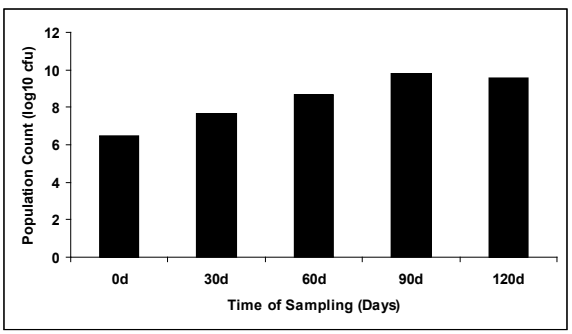


Fig. 1: Population structure of wheatrhizobacteria

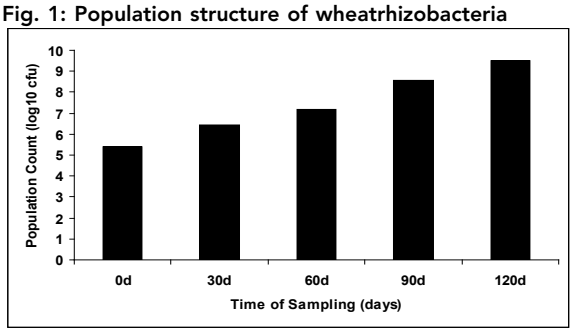


Fig. 2: Population structure of maizerhizobacteria

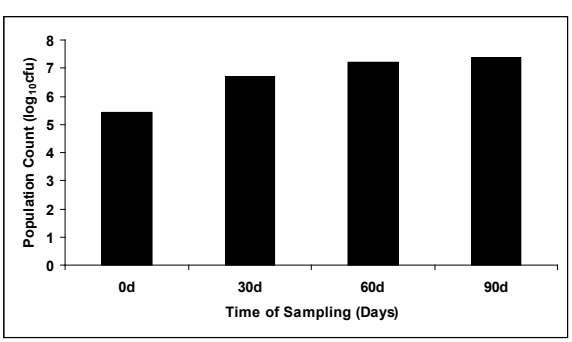
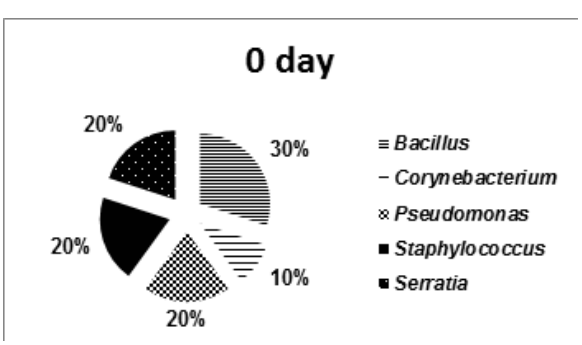


Fig. 3: Population structure of legumerhizobacteria



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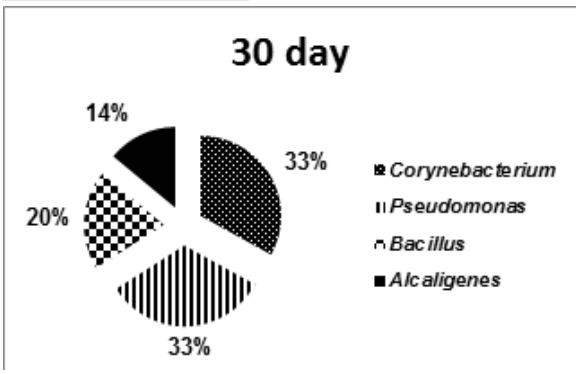
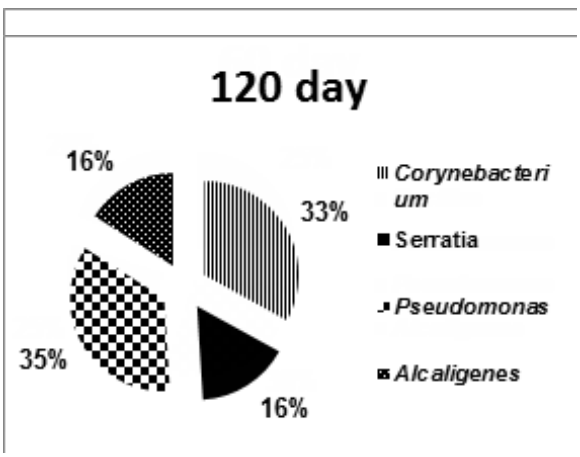




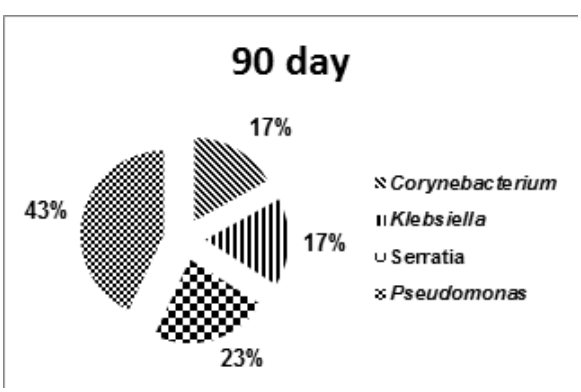
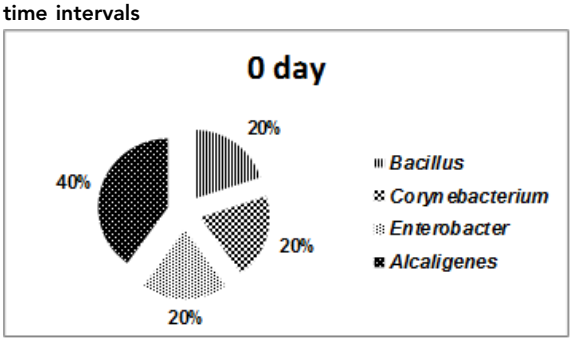
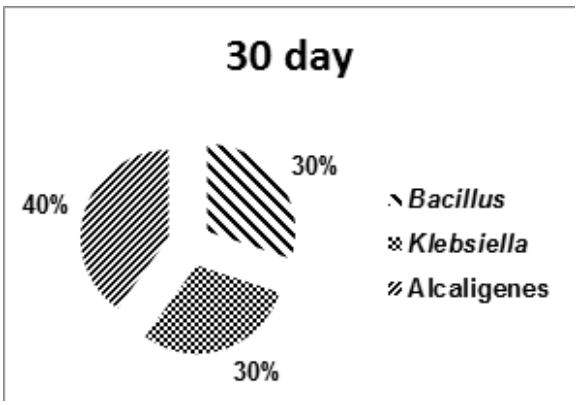
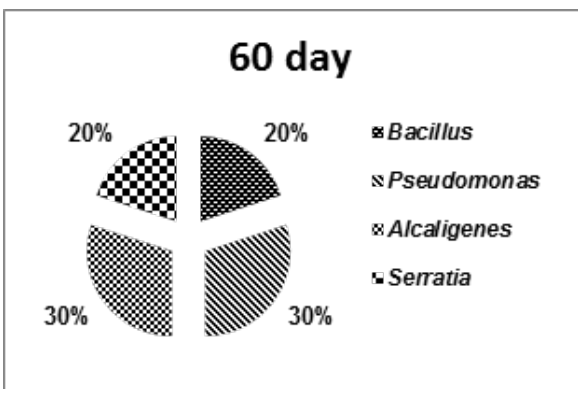
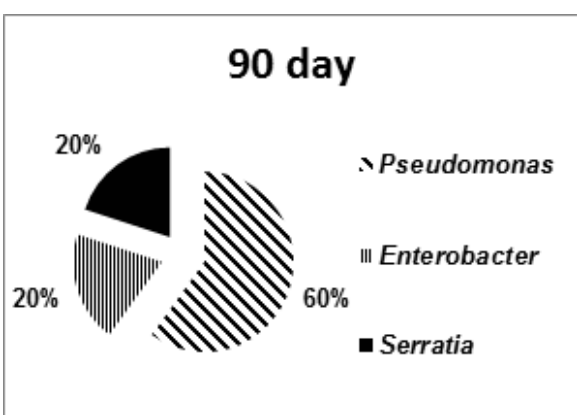


Fig.4: Distribution of wheat rhizobacteria at different time intervals









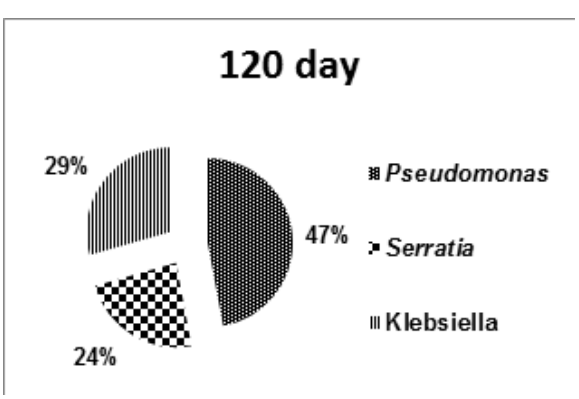


Fig. 5: Distribution of rhizobacteria in different days of maize crop

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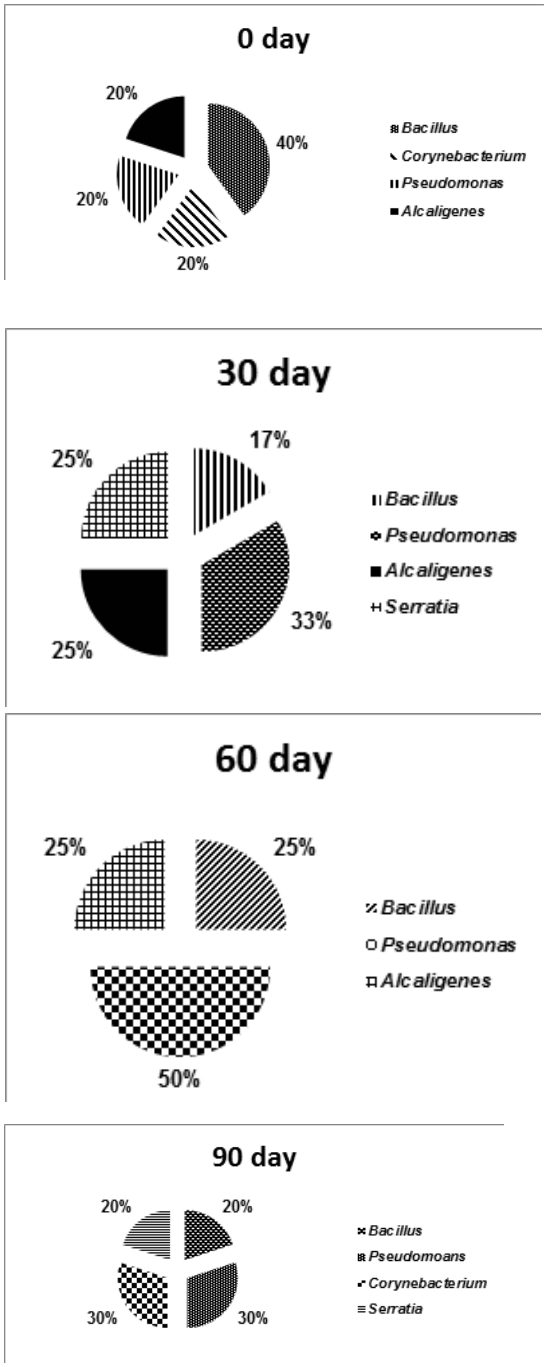

Fig.6: Distribution of legume rhizobacteria at different time intervals

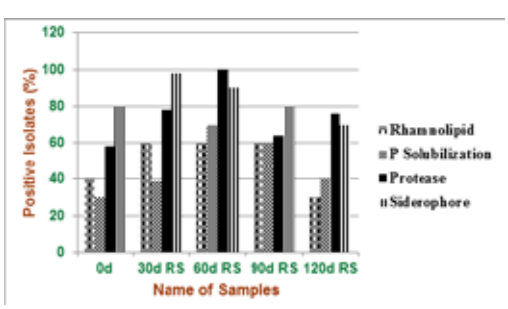

Fig. 7: Functional diversity of recovered rhizobacteriafrom wheat

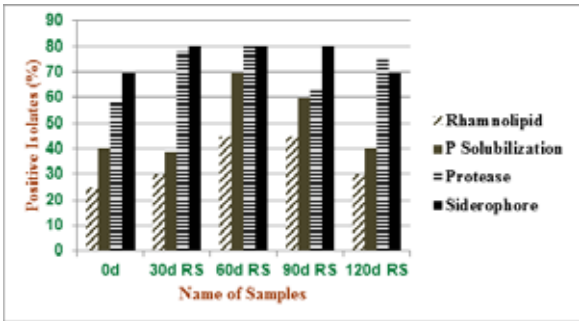

Fig. 8: Functional diversity of recovered rhizobacteria from maize and legume

4. Discussion

The study of microflora of cropping systems indicate the microbial succession with the change in the crop in the same field. This helps in understanding the interaction between plant and microflora. The rhizosphereharbors bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence, and in particular to favor plant growth. This in turn is beneficial to the whole rhizospheremicrobiota through the highly nutritive and energetically rhizo-depositions. Plant growth promotion and development can be facilitated both directly and indirectly. The present study characterized the rhizobacteria associated with the wheat-maize-legume cropping system. 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 wheat rhizobacterial population (log_{10}cfu) varied from 6.48 to 9.57. *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%). The maize rhizobacterial population (log_{10}cfu) varied from 5.4 to 9.5.*Alcaligenes*was documented to be dominant population of zero day (40%). *Klebsiella* was the dominnatrhizobacteria on 30th day (40%) while *Pseudomonas* and *Alcaligenes* were equally dominant on 60th day (30%). *Pseudomonas* was dominant on 90th day (60%) as well as 120th day (47%). The rhizobacterial population (log_{10}cfu) of legume 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.The functional diversity was also observed to be influenced with the age of crop as amongst wheat rhizobacteriamaximum siderophoreproducers were from 30d sample; maximum protease producers and P-solubilizers were from 60d sample and maximum rhamnolipid producers were from 30d, 60d and 90d samples. Amongst maize-legume rhizobacteria, 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 (Kloepper*et al.*, 1980; Marschner&Romheld, 1994).Maximum P

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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 (de Freitas*et al*., 1997; Rodriguez and Fraga, 1999; Nautiyal*et al*., 2000; Chen *et al*., 2006). Several researchers have consequently proven that PSB increase plant growth and yield (Griener& Larsson, 2001; Moura*et al*., 2001). 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(Sharma and Johri, 2002).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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