Int.J.Curr.Microbiol.App.Sci (2017) *6*(7): 2466-2473

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 7 (2017) pp. 2466-2473**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.607.350

Evaluating the Efficiency of Microbes in Mung Bean by different Tests for Improving Crop Yields

N. Gowri Priya*, S. Triveni, R. Subhash Reddy, K. Kranthi Kumar and K. Manasa

Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad-500030, Telangana, India
**Corresponding author*

Keywords

PSB, Enzymes, Biosolubilization, Rhizosphere

Article Info

Accepted:
26 June 2017
Available Online:
10 July 2017

A B S T R A C T

A major focus in the coming decades would be on safe and eco-friendly methods by exploiting the beneficial micro-organisms for sustainable crop production. As mung bean is the major pulse crop in Telangana state, it was taken as a test crop in this study. A substantial number of microorganism species, mostly those associated with the plant rhizosphere have been isolated and characterized morphological, biochemical level and PGPR activities for their efficiency, rock phosphate biosolubilization, germination test, compatability studies, biofilm formation and enzyme synthesis(at different crop growth stages), for the organisms *Bacillus, Pseudomonas, Rhizobium* and *Trichoderma viridae.* For these above tests the organisms were highly responsive and more persistent and their by improved the yields.

Introduction

The rhizosphere is a narrow region of soil around the root that is directly influenced by root secretions and associated microbial activity. Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred as Plant Growth-Promoting Rhizobacteria (PGPR).

Isolation, identification and characterization of PSB (Phosphate solubilizing bacteria) isolated from economically important crop plants is more useful and the population level of PSB was higher in the rhizosphere soils (Tensingh *et al.*, 2015). Most efficient bacterial isolates were screened on the basis of their best performance of biochemical and PGPR activities. The above screened best phosphate solubilizing bacteria were selected for biofilm formation.

A biofilm is an aggregate of microorganisms in which cells are struck to each other and/or to a surface. *In vitro* screening of biofilms was carried out by different PGPR activities (Triveni *et al.*, 2015). The capacity of solubilization of rock phosphate by different combination of biofilms was carried out

(Gaur, 1990). And the population counts at different intervals of crop growth studies were carried out.

The nitrogen fixing ability was tested for the better nitrogen fixation in the cop root zone (Shrivastava, 2013).

These microorganisms occur in soils naturally, but their populations are often scanty.

In order to increase the crop yield, the desired microbes from rhizosphere are isolated and artificially cultured in adequate count and again introduced at root zone will improve the crop performance.

This plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility (Khan, 2006).

Materials and Methods

Isolation and characterization of PSB: Soil samples were collected from the Mungbean rhizosphere soils in NBPGR, Rajendranagar, Hyderabad (Vlassak *et al.,* 1992).

0.1ml of respective samples were spread on sterilized petri plates containing specific media media i.e., *Bacillus* on nutrient agar, *Pseudomonas* on Kings B, *Rhizobium* on Yeast extract mannitol agar with congo red and *Trichoderma* on Potato dextrose agar.

The petri plates were incubated at room temperatures (28°C ± 2°C) for 24 - 72 h.

Morphological characterization

All the eleven bacterial isolates cell morphology (shape, size, elevation, surface, margin, color, odour, pigmentation etc.,) and gram reaction (gram positive, gram negative) were recorded (Barthalomew and Mittewar, 1950) by using respective tests.

Biochemical and physiological characterization

Yeast Extract Mannitol Agar with Congo red test (YEMAC)

The isolates were streaked on YEMAC media plates and incubated at 28 ± 2°C for 48-72 h. *Rhizobial* colonies do not absorb color and remain white in color.

Hoffer's alkaline agar test

The isolates were streaked on Hoffer's alkaline media plates and incubated at 28 ± 2°C for 48 - 72 h. *Rhizobium* does not grow on the media plates (Vincent, 1970).

All the PGPR activities were tested for their efficiency to phosphate solubilization, siderophore production, HCN production, ammonia production etc.

Nitrogen fixation efficiency by Acetylene Reduction Assay (ARA)

The roots along with nodules were placed in a 100 ml conical flask. The flask was sealed with rubber septum (serum cap).

Ten percent (v / v) of the inert gas was removed from the flask with an air tight syringe. 10 ml of acetylene was injected into the flask and incubated for 24 h at room temperature. 1 ml gas mixture was removed from the flask with an airtight syringe. After incubation, 1 ml of gas sample was withdrawn and injected into the gas chromatograph (Agilent 7820 A, India) fitted with Porapak R column and Flame ionization detector (FID). The column temperature was maintained at 60 ^{o}C. Nitrogen gas was used as carrier gas at the flow rate of 30 ml min^{-1}). The acetylene and ethylene peaks were observed and ethylene peak height was measured (Bergersen, 1980).

The acetylene reduction activity of the isolates was calculated using the formula:

$$\frac{\text{Sample peak length of ethylene (mm) x Attenuation x Volume of gas phase of flask x 0.0006}}{\text{Incubation time (h) x Volume of gas sample injected into gas chromatograph (ml)}}$$

Compatability studies

To facilitate correct compatability between the bacteria and fungi attempts were carried out for biofilm formation. The isolates were selected and their compatibility with *Trichoderma* was investigated *in vitro* using the method Perpendicular streak assay. The best showed isolates were selected and streaked on 4 sides of *Trichoderma* inoculated in the centre of the plates. Then further studies were carried out for their best performance (Triveni *et al.,* 2012).

Biofilm formation

The screened best phosphate solubilizing bacteria were selected for biofilm formation. A biofilm is an aggregate of microorganisms in which cells are struck to each other and/or to a surface. The inocula used for the preparation of different biofilms were five days old culture of fungi (3 ml) and two days old culture of bacteria (5 ml) in 250 ml broth. Initially 5 ml of the bacterial culture was inoculated and then incubated for one day in a shaking incubator at 110 rpm and then inoculation of *Trichoderma viride* (5 ml). The flasks were incubated under static conditions at 30 ^{o}C for 16 days until a thick film of culture is observed on the surface of the liquid medium. The progressive growth of biofilm was observed under microscope. After 16 days of incubation the biofilm was harvested and prepared as a liquid suspension.

Results and Discussion

Bacteria exhibit a rich diversity of morphologies. Within this diversity, there is a uniformity of shape for each species that is replicated faithfully each generation, suggesting that bacterial shape is as selectable as any other biochemical adaptation.

Critical advances in studying bacterial efficiency in model bacteria like *Bacillus, Pseudomonas* and *Rhizobium* have identified and characterized by the fundamental tests responsible for increasing the efficiency. This expanding body of work revealed that *Bacillus* as rod shape stumpy Gram positive, *Pseudomonas* as small yellowish green Gram negative and small mucoid Gram negative in case of *Rhizobium*.

The identification of microorganisms including bacterial responses is essential for the advances in microbiology (for development of biofilms). During present experiment, a series of tests were conducted to identify bacterial responses. Upon compiling the data obtained from above analysis of bacteria, the results were generated as isolates showed a negative growth on Hofer‟s Alkaline medium because Rhizobium will not grow on Hoffer's alkaline medium, this is considered as useful means to distinguish between the two allied genera (Hofer 1935). Similarly all the nine isolates did not produce yellow coloration around their colonies indicating that Agrobacterium was absent and the isolate was identified as Rhizobium. Similarly isolate did not produce yellow coloration around their colonies indicating that Agrobacterium was absent and all of them were Rhizobium. The Rhizobium isolate grew well on yeast extract mannitol agar slants and Congored yeast extract mannitol agar plates but didn't show chromo genesis.

All the 5 *B. subtilis* individual isolates were able to form clear zone of phosphate solubilization on pikovaskaya's agar plate ranged from 11-18 mm. Among them GBC 1 of *B. subtilis* recorded the highest zone efficiency of 18 mm solubilization zone with 257.14% efficiency. Second best was GPS 4 with 171.42% solubilization efficiency followed by GBC 2 (170.00%), GPS 5 (166.66%) and GPS 3 (163.63%) respectively? The *Rhizobium leguminosarum* exhibited lowest phosphate solubilization efficiency i.e., 123.07 % with 16 mm solubilization zone.

Table.1 Serial dilutions used for viable plate count of different microorganisms

S. No.	Rhizobacteria	Dilutions
1	*Bacillus spp* (5 isolates)	10^{-3} to 10^{-6}
2	*Pseudomonas spp* (5 isolates)	10^{-3} to 10^{-6}
4	*Rhizobium spp* (1 isolate)	10^{-3} to 10^{-6}
5	*Trichoderma viride* (1 isolate)	10^{-3} to 10^{-5}

Table.2 Cultural and morphological characteristics of *Bacillus*, *Pseudomonas* and *Rhizobium* Isolates

Isolates	Cultural characteristics	Morphological characteristics
GBC1	Irregular, creamy whitish flat colony	Gram +ve, purple, rod shaped
GBC 2	Dull whitish, irregular colony	Gram +ve, purple, rod shaped
GBC 3	Creamy white, regular, medium colony	Gram +ve, purple, rod shaped
GBC 4	Light cream color, flat	Gram +ve, purple, rod shaped
GBC 5	Big, white, irregular colony	Gram +ve, purple, rod shaped
GPS 1	Yellowish green, round, fluorescent	Gram-ve, rod shaped, pink
GPS 2	Yellowish green, irregular, glistening, pigmented	Gram-ve, rod shaped, pink
GPS 3	Yellow colored, medium, spreaded	Gram-ve, rod shaped, pink
GPS 4	Yellowish, button shape small, round	Gram-ve, rod shaped, pink
GPS 5	Yellowish green, regular round	Gram-ve, rod shaped, pink
Rhizobium	Milky, mucoid, translucent, raised	Gram-ve, rod shaped, pink

Table.3 Study the phosphate solubilization efficiency of different bacterial isolates

Sl.No.	Isolates	Phosphate solubilization		
		Zone diameter		Solubilization efficiency (%)
		Solubulization zone (mm)	Culture diameter (mm)	
1	GBC 1	18.00	7.00	257.14
2	GBC2	17.00	10.00	170.00
3	GBC 3	15.00	12.00	125.00
4	GBC 4	14.00	9.00	155.55
5	GBC 5	11.00	8.00	137.50
6	GPS 1	0.00	7.00	0.00
7	GPS 2	-	8.00	-
8	GPS 3	18.00	11.00	163.63
9	GPS 4	12.00	7.00	171.42
10	GPS 5	15.00	9.00	166.66
11	RHIZO	16.00	13.00	123.07

Treatments: *Bacillus* strains (GBC 1, GBC 2, GBC 3, GBC 4, GBC 5), *Pseudomonas* strains (GPS 1, GPS 2, GPS 3, GPS 4, GPS 5), *Rhizobium* strain

Table.4 Influence of different biofilms and co-inoculations on nodule number and in nitrogenase activity

Treatments	µmol of Ethylene	Nodule no.
Control	53.33	7.00
T1 : *Trichoderma viride* + *Bacillus subtilis* (Biofilm)	70.34	9.00
T2 : *Trichoderma viride* + *Pseudomonas fluorescence* (Biofilm)	129.07	8.00
T3 : *Trichoderma viride* + *Rhizobium leguminosarum* (Biofilm)	211.13	10.00
T4 : *Trichoderma viride* + *Bacillus subtilis* + *P. fluorescence* + *R. leguminosarum* (Biofilm)	**219.07**	**11.00**
T5 : *Trichoderma viride* + *Bacillus subtilis* (Co-inoculation)	115.20	9.00
T6 : *Trichoderma viride* + *Pseudomonas fluorescence* (Co-inoculation)	152.30	8.00
T7 : *Trichoderma viride* + *Rhizobium leguminosarum* (Co-inoculation)	149.10	9.00
T8 : *Trichoderma viride* + *Bacillus subtilis* + *Pseudomonas fluorescence* + *Rhizobium leguminosarum* (Co-inoculation)	131.10	7.00
CD	0.42	0.99
SE(m)	0.14	0.33
CV	0.18	6.90

Fig.1 Bacterial biofilm SEM imagas

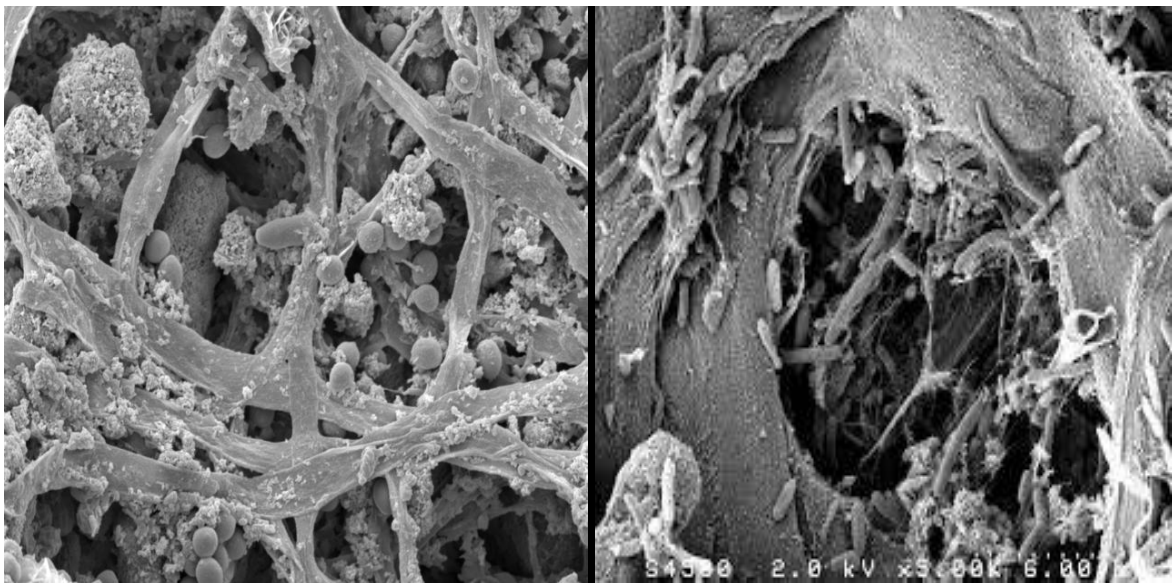


Fig.2 Microscopic image of *in vitro* developed Biofilms

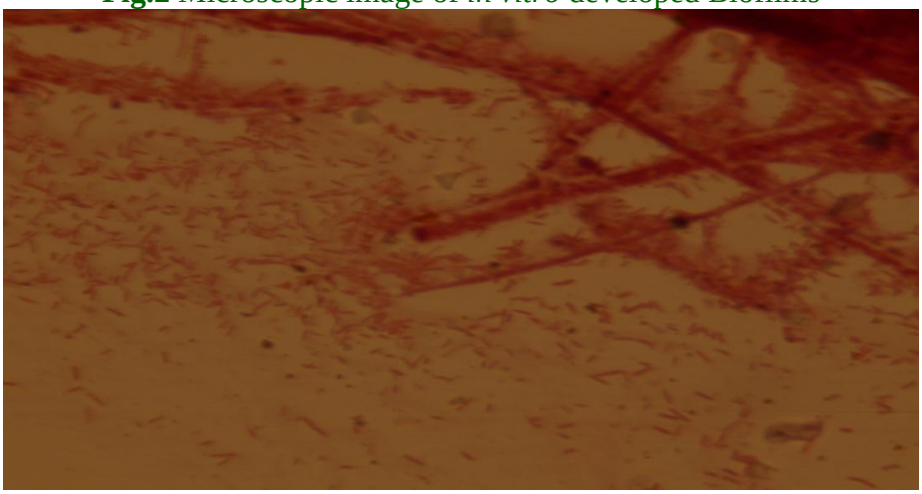
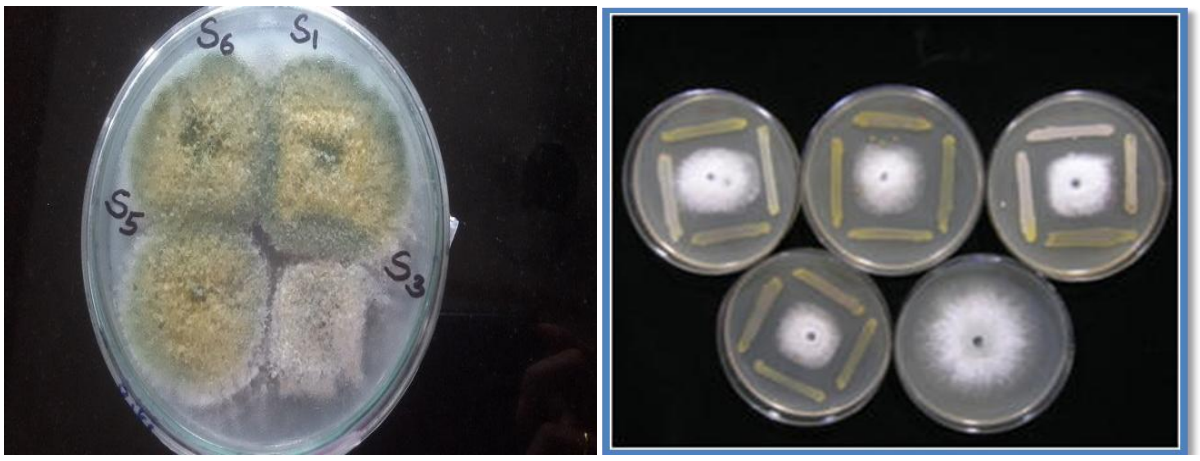


Fig.3 Compatibility studies



The high level of C_2H_4 produced by the plants might adversely affect plant growth and N fixation. ARA test provides an extremely simple and apparently accurate assay of N-fixing activity when applied to pot-grown plants. In our study all the treatments have shown positive results. T4 (*Trichoderma viride* + *Bacillus subtilis* + *Pseudomonas fluorescence* + *Rhizobium leguminosarum* - Biofilm) showed highest nitrogen fixing ability (219.00 µmoles C_2H_4) followed by T3 (*Rhizobium leguminosarum* + *Trichoderma viride* – Biofilm) (211.00 µmoles C_2H_4), and least was observed in T1 (*Trichoderma viride* + *Bacillus subtilis*) (70.34 µmoles C_2H_4).

All the bacterial isolates were tested for their compatability with the fungus *Trichoderma viridae* for the development of novel biofilmed biofertilizers. The degree of inhibition of bacterial isolates by the fungi (*Trichoderma*) was very less and the feasibility of combining *Trichoderma* with bacterial isolates and the degree of intimacy between the two partners is very impressive. This compatability was observed effectively at different days.

Successfully the in vitro biofilm mats were developed with different microbial consortium. They were depicted as different treatments based on the microorganisms present in that particular biofilm. This in vitro developed biofilms were observed under Scanning Electron Microscope (SEM) for the better developed partnership. Then the 16 days old biofilm suspension were used for the seed treatment (Tables 14;).

On the basis of results obtained from the experiments of in-vitro finally concluded as the better ability of these isolates and biofilms was due to its higher nitrogen fixing ability, PGPR activity, strongest interaction between bacteria and fungi (High compatablity), and resistant to different environmental conditions will influence the crop yields.

References

Barthalomew, J.W and Mittewer, T. 1950. A simplified bacterial strain. *Stain Technology*. 25: 153.

Bergerson, F.J. 1980. Methods for evaluating biological nitrogen fixation. John wiley and sons, New York. 702.

Gaur, A.C. 1990. Phosphate solubilizing microorganisms as biofertilizer. New Delhi: *Omega Scientific Publishers.*

Hofer AW (1935) Method for distinguishing between legume bacteria and their most common contaminant. J Amer Soc Agron. 27:228-30.

Khan, M.S. 2006. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. 163: 173-181.

Shrivastava, U.P. 2013. Isolation and initial characterization of diazotrophic Plant Growth Promoting Rhizobacteria (PGPR) from rice rhizosphere of Parsa and Bara district of Nepal. *International Journal of Pharmacy & Life Sciences*. 4 (3): 2481-2488.

Tensingh Baliah, N and Jemeema Begum, P. 2015. Isolation, identification and characterization of phosphate solubilizing bacteria (PSB) isolated from economically important crop plants. *International Journal of Current Microbiology and Applied Science*, 4 (3): 915-924.

Triveni, S., Radha Prasanna., Arun Kumar., Ngangom Bidyarani., Rajendra Singh and Kumar Saxena. 2015. Evaluating the promise of *Trichoderma* and *Anabaena* based biofilmsas multifunctional agents in *Macrophomina phaseolina*-infected cotton crop. *Biocontrol Science and Technology*. 25 (6): 656-670.

Triveni, S., Radha Prasanna., Livleen Shukla and Anil Kumar Saxena. 2012. Evaluating the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth promoting inoculants. *Annals of Microbiology. DOI* 10.1007/s13213-012-0573-x.

Vincent, J.M. 1970. A manual for the practical study of the root nodule bacteria. Blackwell Scientific publications. *Oxford and Edinburgh*. 1-3.

Vlassak, K.L., Van, H and Duchateau, L. 1992. Isolation and characterization of *fluorescent Pseudomonas* associated with the roots of rice and banana grown in Sri Lanka. *Plant and soil*. 145: 51-63.

How to cite this article:

Gowri Priya, N., S. Triveni, R. Subhash Reddy, K. Kranthi Kumar and Manasa, K. 2017. Evaluating the Efficiency of Microbes in Mung Bean by different Tests for Improving Crop Yields. *Int.J.Curr.Microbiol.App.Sci.* 6(7): 2466-2473.
doi: https://doi.org/10.20546/ijcmas.2017.607.350