Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22 (2) : 2020 : 334-337

ISSN-0972-3005

DEVELOPMENT OF PLANT GROWTH PROMOTING MICROBIAL CONSORTIA WITH EFFICIENT ISOLATES

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(Received 5 December, 2019; accepted 3 January, 2020)

Key words: *Microbial consortia, Screening, Compatibility, PGPR Isolates.*

Abstract – Plant Growth Promoting Rhizobacteria were isolated from sorghum growing vertislos of different districts of Andhra Pradesh during Kharif 2016-17. The PGP bacteria like *Azotobacter*, *Azospirillium*, PSB, KRB, ZnSB and PGP Isolate with antagonistic activity were isolated. The isolated PGPRs were screened for their morphological, physiological, biochemical and PGPR characteristics. The isolates with good plant growth promoting traits like N_2 fixation, P-solubilization, K-releasing, Zn-solubilization and antagonistic activity were screened from a total of 60 isolates. The efficient isolates were tested for the compatibility on nutrient agar and tryptic soy agar. The efficient and compatible isolates viz., *Azotobacter* (KAA-1), *Azospirillium* (KAA-2), P-solubilizer (KAA-3), K-releaser (KNB-4), Zn-solubilizer (KPM-5) and PGP isolate (CPP-6) were utilized and three types of microbial consortia were developed.

INTRODUCTION

In a microbial consortia a group of different species of microorganisms act together as a community. Here the organisms work together in a complex system where all benefit from activities of others in the community. The microbial consortia are much more efficient than single strains of organisms with a diversity of metabolic capabilities and microorganisms interact with each other synergistically by providing nutrients, removing inhibitory products. Coinoculation, frequently increase growth and yield compared to single inoculation, provided the plants have balanced nutrition and improved absorption of nitrogen, phosphorus and mineral nutrients (Lakshmi, 2013).

The microbial consortium is developed for customized solution of soil health related problems such as with plant growth promoting properties including root and shoot length elongation, early and high germination rate, high yield, decrease in soil pathogenic load and increase soil micro and macronutrient status. These specifically designed polymicrobial formulations would further provide protection against plant pathogens lowering the need for nitrogen containing fertilizers, solubilize minerals, protect plants against pathogens, and make available to the plant valuable nutrients, such as phosphate, thus reducing and eliminating the need for using chemical fertilizers and chemical pesticides (Paikray, 2012).

MATERIALS AND METHODS

Efficient isolates of *Azotobacter*, *Azospirillium,* P-solubilizer, K-releaser, Zn-solubilizer and PGPR isolate were selected and microbial consortia were developed.

- Microbial Consortium 1 (*Azotobacter*, P-solubilizer, K-releaser, Zn-solubilizer and PGPR isolate).
- Microbial Consortium 2 (*Azospirillium*, P-solubilizer, K-releaser, Zn-solubilizer and PGPR isolate).
- Microbial Consortium 3 (*Azotobacter*, *Azospirillium,* P-solubilizer, K-releaser, Zn-solubilizer and PGPR isolate).

Compatibility of the isolates selected was carried so as to develop microbial consortia.

Compatibility test

Compatibility of the isolates was tested so as to develop the microbial consortia. Single bacterial strain was streaked as a straight line in the center of tryptic soya agar plate. Cultures to be tested were streaked perpendicularly across the initial culture and incubated at 28 ^{o}C for 48 to 96 hours. Lack of microbial growth (zone of inhibition) at the intersections was indicative of the antagonism of the cultures. While, the cultures growing in the close proximity were compatible to each other.

The microorganisms used in the experiment were tested for their compatibility using nutrient agar medium. Compatibility study was done by streaking dual inoculants on solidified nutrient agar medium to determine compatibility among the microorganisms.

The dual inoculants were

i) *Azotobacter* with *Azospirillium* / PSB / KRB / ZnSB /PGPR Isolate
ii) *Azospirillum* with *Azotobacter* / PSB/ KRB / ZnSB /PGPR Isolate
iii) PSB with *Azotobacter* / *Azospirillium* / KRB / ZnSB /PGPR Isolate
iv) KRB with *Azotobacter* / *Azospirillium* / PSB / ZnSB /PGPR Isolate
v) ZnSB with *Azotobacter* / *Azospirillium* / PSB / KRB /PGPR Isolate
vi) PGPR Isolate with *Azotobacter* / *Azospirillium* / PSB / KRB / ZnSB

Development of Microbial Consortia

Based on the compatibility tests, the isolates were confirmed to be compatible with one another. Isolates were cultured individually, using specific media then mixed and grown together in conical flask @ 10 mL/ isolate. The population of each PGPR organism in the consortia was monitored regularly in the conical flask and also in the soil to which they are applied for their screening on the host crop under green house conditions.

RESULTS AND DISCUSSION

Selection of efficient isolates to develop microbial consortia

The plant growth promoting rhizobacterial isolates were screened for their plant growth promoting traits and based on their efficiency to produce multiple plant growth promoting factors the isolates were screened and selected for the development of plant growth promoting microbial consortia.

Azotobacter isolate KAA-1, *Azospirillum* isolate KAA-2, P solubilizing bacteria isolate KAA-3, K releasing bacteria isolate KNB-4, Zinc solubilizing bacteria isolate KPM-5 and PGPR isolate (*Pseudomonas fluorescence*) CPP-6 were selected for development of different types of microbial consortia.

Efficient isolates of *Azotobacter*, *Azospirillium*, P-solubilizer, K-releaser, Zn-solubilizer and PGPR isolate were selected and microbial consortia were developed.

- Microbial Consortium 1 (*Azotobacter* (KAA-1), P-solubilizer (KAA-3), K-releaser (KNB-4), Zn-solubilizer (KPM-5) and PGPR isolate (CPP-6)).
- Microbial Consortium 2 (*Azospirillium* (KAA-2), P-solubilizer (KAA-3), K-releaser (KNB-4), Zn-solubilizer (KPM-5) and PGPR isolate (CPP-6)).
- Microbial Consortium 3 (*Azotobacter* (KAA-1), *Azospirillium* (KAA-2), P-solubilizer (KAA-3), K-releaser (KNB-4), Zn-solubilizer (KPM-5) and PGPR isolate (CPP-6)).

Marimuthu *et al.* (2013) selected AZ204 as N fixer and P solubilizer, Pf1 as biocontrol agent and prepared a consortium to apply on *Gossypium*

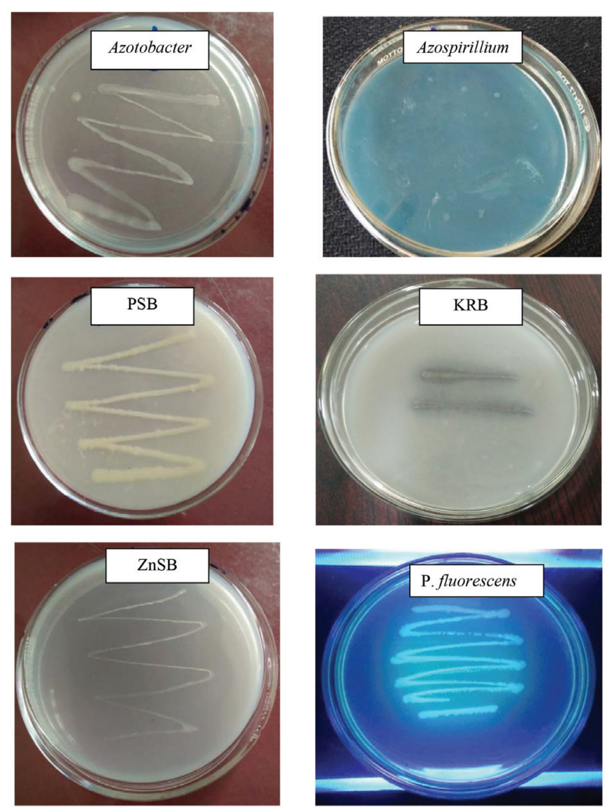

Plate 1. Different PGPR isolates used for preparation of microbial consortia

hirsutum. *Azospirillum* sp. AZ204 and *Pseudomonas fluorescens* Pf1 performed better and excluded *Rhizoctonia bataticola* more effectively compared to their individual inoculation. The consortium showed better plant growth promoting characteristics when applied as consortium so performed well.

Compatibility tests of the efficient isolates to develop microbial consortia

Compatibility of the isolates was tested so as to develop the microbial consortia. Single bacterial strain was streaked as a straight line in the center of tryptic soya agar plate. Cultures to be tested were streaked perpendicularly across the initial culture and incubated at 28 ^{o}C for 48 to 96 hours. Lack of microbial growth (zone of inhibition) at the intersections was indicative of the antagonism of the cultures but the cultures growing in the close proximity were compatible to each other.

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ii) *Azospirillum* with *Azotobacter* / PSB/ KRB / ZnSB /PGPR Isolate
ii) PSB with *Azotobacter* / *Azospirillium* / KRB / ZnSB /PGPR Isolate
iv) KRB with *Azotobacter* / *Azospirillium* / PSB / ZnSB /PGPR Isolate
v) ZnSB with *Azotobacter* / *Azospirillium* / PSB / KRB /PGPR Isolate
vi) PGPR Isolate with *Azotobacter* / *Azospirillium* / PSB / KRB / ZnSB

Deepa and Mathew (2017) carried compatibility studies of different microorganisms to prepare a consortium. The five bacterial isolates selected from *in planta* experiment were subjected to mutual compatibility test by cross streak method. No lysis was observed at the juncture of TRB-1 x VSB-1, TRB-1 x EkRB-1 and VSB-1 x EkRB-1 combination, which indicated the compatibility among the isolates.

Choure and Dubey (2012) developed plant growth promoting microbial consortium based on interaction studies. All strains were exposed to interact under *in vitro* conditions. Three strains viz. *Pseudomonas fluorescens* LPK2, *Streptomyces fredii* KCC5 and *Azotobacter chroococcum* AZK2 did not inhibit each other. *In vitro* dual culture studies on the interaction of one strain to another, revealed no mutual growth inhibition among *Streptomyces fredii* KCC5, *Pseudomonas fluorescens* and *Azotobacter chroococcum* AZK2. Spectrophotometric studies also showed that the individual growth of these strains was not affected in combined cultures, where strains were cultured together.

Singh *et al*. (2013) developed and investigated microbial consortium activity on *Cicer arietinum* against *Sclerotium rolfsii*. The organisms used in consortia preparation were *Pseudomonas aeruginosa* (PHU094), *Trichoderma harzianum* (THU0816), *Mesorhizobium sp.* (RL091). The reasons for the best action of microbial consortia compared to individual inoculation were due to activation of phenylpropanoid pathway, lignin deposition antioxidant mechanisms.

Development of microbial consortia with efficient isolates

Following the compatibility tests, the isolates were

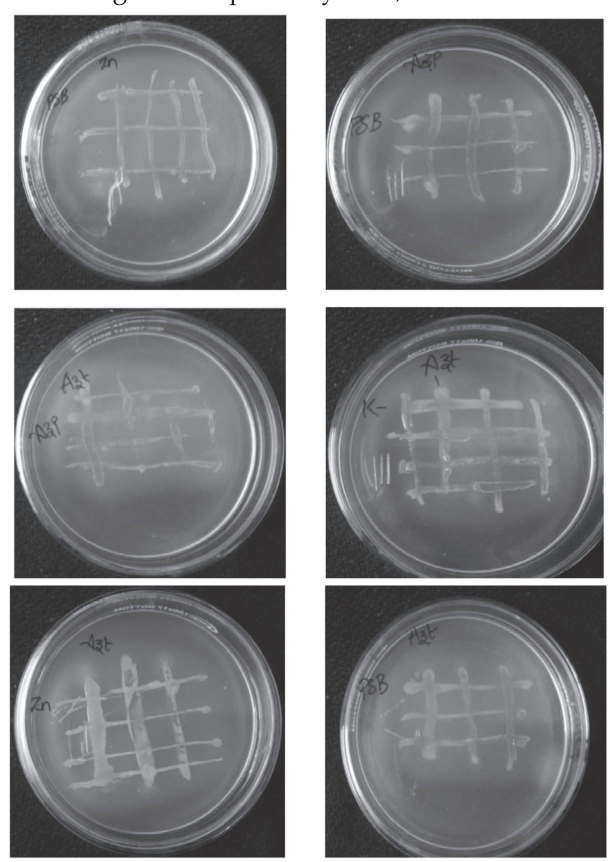

Plate 2. Compatibility tests for the PGPR isolates used in microbial consortia

confirmed to be compatible with one another. They were developed individually in their specific media then mixed and grown together in conical flask (10 mL : 10 mL : 10 mL : 10 mL : 10 mL : 10 mL). The count of each PGPR organism in the consortia was monitored regularly in the conical flask and tested purity (10^8 cells g^{-1}) prior to the time of sowing and also in the soil to which they are applied for their screening on the host crop under green house conditions. These microbial consortia are applied as soil application according to the treatments with recommended dosages.

Similar studies were conducted by Kundu and Gaur (1980b). They observed synergistic interaction between *Azotobacter* and phosphate solubilising bacteria when the two organisms were inoculated together in wheat. In the combined inoculation treatments, the population of both the organisms was enhanced. Kundu and Gaur (1984) observed synergistic interactions on plant growth by co-inoculation of PSB with N_2 fixers such as *Azotobacter* in rice.

Development of strain mixtures with non-competitive nature of the bacterial strains will have an additive effect in increasing the yield and growth. Strain mixtures of *Pseudomonads* in combinations with other bacteria were found effective than the application of individual organisms (Duijff *et al*., 1999).

CONCLUSION

A single microbial strain with specific activity provides soil and plant with that specific nutrient only. So, each nutrient supplement with specific PGPR organism will be of high cost and not economical to the farmers. Instead the microbial consortia helps farmers to provide with macro and micro nutrients along with disease resistance with antagonistic activity of PGPR. Thus a microbial consortia developed helps plants and soil by providing wide range of nutrients in a single formulation.

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