



**“Multigeneric Microbial Coaggregates” - Effect of different bioformulations of PGPR cells on the enhancement of PGPR characteristics and biocontrol against *Xanthomonas oryzae* pv. *oryzae* in rice grown under lowland condition**

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**ABSTRACT**

*The application effect of different bioformulations viz., single strain inoculation, co-inoculation and coaggregates application (natural and artificial), of PGPR cells viz., Azospirillum (AZ-3), Pseudomonas (PF-3) and Methylobacterium (MB-3), on the enhancement of PGPR characteristics and biocontrol against Xanthomonas oryzae pv. oryzae was studied under in vitro condition in lowland rice cv.BPT-5804. It was observed that the application of “Multigeneric microbial coaggregates, (natural)”, consisting of Azospirillum, Pseudomonas and Methylobacterium enhanced the PGPR characteristics, viz., seed vigour index, adherence to rice roots and reduction in the incidence of Xanthomonas oryzae to a higher level followed by multigeneric microbial coaggregates (artificial) application, coinoculation and individual cell application. Among the different coinoculation treatments, the coinoculation of all the PGPR cells viz., Azospirillum, Pseudomonas and Methylobacterium enhanced the above said parameters to a higher level followed by coinoculation of any two PGPR isolates. Regarding the individual cell application, the application of Pseudomonas recorded higher values for PGPR characteristics and biocontrol against Xanthomonas oryzae followed by individual cell application of Azospirillum and Methylobacterium. It was concluded that the application of PGPR cells viz., Azospirillum, Pseudomonas and Methylobacterium, as natural coaggregates, augmented the PGPR characteristics and biocontrol against Xanthomonas oryzae pv. oryzae to a higher level when compared to other formulations of PGPR cells.*

**Keywords:** PGPR, bioformulations, “Multigeneric microbial coaggregates”, growth and biocontrol, lowland rice.

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**INTRODUCTION**

Rice (*Oryza sativa* L.) is one of the most important staple food for over two billion people in Asia and America. Among the various rice growing countries in the world, India has the largest area under rice crop and ranked second in the production, next to China. Among the different rice production systems of India, the irrigated lowland system is the first and foremost one in terms of area (6.3 Mha) and production (5.1

MT) but with least productivity ( $0.8 \text{ t ha}^{-1}$ ). Of the several biotic and abiotic constraints, low soil fertility and incidence of diseases are considered to be the major constraints that eventually lead to the low productivity in lowland rice. Hence, the lowland rice productivity must be greatly increased by providing additional nutrient inputs and through effective control of phytopathogens.

Nitrogen is one of the key nutrient and an expensive input that most frequently limits rice production. Lowland rice grows in an environment easily prone to 'N' solubilization and run-off. Moreover, the incidence of bacterial leaf blight disease, the most distractive bacterial disease caused by *Xanthomonas oryzae*, causing a yield loss upto 90 per cent [1]. The development and use of plant growth promoting rhizobacteria (PGPR), as biological approach, might be an alternative strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemical fertilizers and pesticides.

Rhizospheric bacteria that favourably affect the plant growth and yield of commercially important crops are now dominated as Plant growth promoting rhizobacteria 'PGPR'. They can cause plant growth promotion directly by producing and secreting plant growth regulators or by eliciting root metabolic activities by supplying biologically fixed 'N' [2]. The well known PGPR, include bacteria belonging to the genera *Azotobacter*, *Azospirillum*, *Azoarcus*, *Klebsiella*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Methylobacterium* and *Rhizobium* on non-legumes and widely used as agricultural bioinoculant for the enhancement of growth and yield in lowland rice [3].

Fluorescent *Pseudomonas* has emerged as the largest, potentially most promising group of PGPR, possessing traits also involved in the biocontrol of phytopathogens, due to the production of secondary metabolites such as siderophore, antibiotics and phytohormones production for plant growth development (Suslow, 1982; Kloepper *et al.*, 1980). *Methylobacterium*, as plant symbionts, utilize the methanol emitted from plants and in turn, impart beneficial effects on plant growth through one or more mechanisms that include production of phytohormones (Basile *et al.*, 1985; Trotsenko *et al.*, 2001). Neyra *et al.*, (1995) proposed to the use of "EPS mediated *Azospirillum* bioflocs" as a delivery system, for the enhancement of growth and yield in different commercially important field crops.

However, the introduced bacteria exhibited poor performance in natural environments and in the rhizosphere of crop plants due to lack of stress tolerance and survivability in soils. [4] reported the importance of physiological status of microorganisms in any agricultural bioinoculant preparation rather than their cell numbers to ensure more stress tolerance and survival in soil. [5] suggested that instead of trying single strain with single trait, as agricultural bioinoculant, trying to use microbial consortia for harnessing multiple benefits. [6] proposed the concept of "Multigeneric microbial coaggregates" for the production of multipurpose agricultural bioinoculant with multiple benefits. The development and use of natural bioflocs of *Azospirillum*, as agricultural bioinoculant, has been reported by many authors [7, 8, 3]. In our laboratory, the development of artificial bioflocs of *Azospirillum*, using plant seed flocculants, has been reported by [9].

On the basis of [6] and [9] concepts, the development of "Multigeneric microbial coaggregates", of PGPR cells and use the same, as agricultural bioinoculant, for the enhancement of PGPR characteristics and biocontrol, against *Xanthomonas oryzae* might be a suitable strategy in order to increase the productivity of lowland rice. Hence, the present research work has been undertaken with an aim to evaluate the bioinoculation effect of different formulations *viz.*, single strain inoculation, coinoculation and multigeneric microbial coaggregates application of PGPR cells *viz.*, *Azospirillum*, *Pseudomonas* and *Methylobacterium* on the enhancement of growth and yield and biocontrol against bacterial leaf blight disease in lowland rice under pot culture condition.

## MATERIALS AND METHODS

**Strains used:** Strains of *Azospirillum brasilense* (AZ-3) *Pseudomonas fluorescens* (PF-3) and *Methylobacterium phyllosphaerae* (MB-3), isolated from the rhizosphere and phyllosphere of rice cv. BPT-5804, were maintained in Nfb, King's 'B' and MMS medium, respectively at 35°C with monthly transfer and used throughout the study. *Xanthomonas oryzae* pv. *oryzae* (AU099), obtained from Department of Plant Pathology, Faculty of Agriculture, Annamalai University, was used as reference strain for the biocontrol study and the same was maintained in Peptone sucrose agar (PSA) slants and examined periodically for its virulence.

**Preparation of inoculum:** Strains of *Azospirillum brasilense* (AZ-3), *Pseudomonas fluorescens* (PF-3) and *Methylobacterium phyllosphaerae* (MB-3), were grown in Nfb, King's 'B' and MMS broth, respectively in a shaking bath at 30 ± 2°C for 5 days. Then, the media were centrifuged, separately, at 5000 x g for 10 min to get stationary phase cells and the pellets, obtained after centrifugation, washed three times in 0.1 M phosphate buffer (pH 6.8), finally, the cells were resuspended, separately, in the same buffer to a cell concentration of 1 x 10<sup>7</sup> CFU / mL by measuring the OD at 420 nm and used as inoculum.

**Preparation of Multigeneric microbial coaggregates (natural):** The preparation of Co-Ag buffer was done according to the specification of [10]. The Multigeneric microbial coaggregates was prepared according to [11], as stated herewith. One ml aliquot of each PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium* was mixed together with ten ml of Co-Ag buffer, vortexed for 10sec., shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 24 h. After the incubation period, the coaggregates settled at bottom of the tube, obtained after decanting the buffer.

**Preparation of Multigeneric microbial coaggregates (artificial):** One ml aliquot of each PGPR cells at 1 x 10<sup>7</sup> cells / mL inoculum level was mixed together in 10 ml of Co-Ag buffer [10] together with the addition of one ml of individual plant seed extract viz., *Moringa oleifera*, *Strychnos potatorum*, *Allium cepa*, *Sappindus emarginatus* and *Asteracantha longifolia*. The mixture was vortexed for 10 sec., shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 1h. After the inoculation period, the coaggregates, settled at the bottom of the tube, obtained after decanting the buffer.

**Effect of different bioformulations of PGPR cells on seed vigour index of rice:** Rice (*Oryza sativa* L.) cv. BPT-5804 seeds were surface sterilized by immersion in 95 per cent ethanol for 1 min, followed by 20 min in 1 per cent NaOCl. After rinsing three times with sterile distilled water, the sterilized seeds were placed on the surface of 1 per cent water agar in petriplates (9 cm dia, at five seeds per plate). Then, they were incubated in an inverted position for 3 days at room temperature to allow germination. The plates were sealed with wax to avoid agar dryness during germination. Then, the germinated rice seeds were subjected to the following treatments viz., T<sub>1</sub>-Control, T<sub>2</sub>-*Azospirillum*, T<sub>3</sub>-*Pseudomonas*, T<sub>4</sub>-*Methylobacterium*, T<sub>5</sub>- *Azospirillum* + *Pseudomonas* co-inoculation, T<sub>6</sub>- *Azospirillum* + *Methylobacterium* co-inoculation, T<sub>7</sub>- *Methylobacterium* + *Pseudomonas* co-inoculation, T<sub>8</sub>- *Azospirillum* + *Pseudomonas* + *Methylobacterium* co-inoculation, T<sub>9</sub>- *Azospirillum* + *Pseudomonas* + *Methylobacterium* coaggregates (natural), T<sub>10</sub>- *Azospirillum* + *Pseudomonas* + *Methylobacterium* coaggregates (artificial), dried in shade for 30 min. Then, the inoculated rice seeds were arranged in two rows on a sheet of blotting paper dipped in sterile water. Then, they were covered with another blotting paper dipped in sterile water, rolled and placed vertically in a moist chamber at 20°C. After the incubation for 5 days, each roll was opened and the vigour indices of germinated rice seeds, from each treatment, were calculated by the method [12]. The experiment was arranged in Randomised block design with three replications.

Vigour index = Germination % x Total length of seedling (mm) (Root and shoot length)

**Effect of different bioformulations of PGPR cells on adhesion to rice roots:** The preparation of different formulations of PGPR cells was done as stated elsewhere in the text. Rice seeds were surface

sterilized and germinated as detailed earlier. After the germination, the three day old sterile seedlings were transferred to slopes of Fahraeu's solution [13], solidified with 1.5 per cent agar in test tubes. Sterile Fahraeu's solution was added to fill the empty portion of the agar slopes and the tubes were incubated for three more days (24°C day / 22°C night). After the incubation period, the roots were collected from each tube, separately, washed first with sterile water and later three times in 0.1M phosphate buffer (pH 6.8), cut into 5 cm pieces and used in the adsorption experiments. The adsorption experiment was carried out according to [14] with the abovesaid 10 treatments. The experiment was arranged in Randomised block design (RBD) and three replications were maintained for each treatment.

### **Effect of different bioformulations of PGPR cells on the enhancement of growth and yield in lowland rice :**

A pot culture experiment was conducted to study the effect of different formulations viz., single strain inoculation, co-inoculation and co-aggregates application of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium* together with challenge inoculation of *Xanthomonas oryzae* on the enhancement of growth and yield parameters in lowland rice with special emphasis to biocontrol against bacterial leaf blight disease (*Xanthomonas oryzae* pv. *oryzae*). The study was conducted during Rabi season (Sep to Jan, 2012-13) with rice cultivar BPT-5804 at the polyhouse of Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, India. Rectangular cement pots with 18"x12"x12" size were filled with 45 kg of paddy field soil, flooded with water for 2 days and brought into fine puddle condition. Seeds of the rice variety BPT-5804 were loosely packed separately in small gunny bag and soaked in water for 12 hr. Then, the bags were kept in dark place, after covering with wet gunny bags, to ensure optimum condition for germination. The pre-germinated seeds of rice (cv. BPT-5804) were subjected to the following treatments viz., T<sub>1</sub>-Control, T<sub>2</sub>-*Azospirillum*, T<sub>3</sub>-*Pseudomonas*, T<sub>4</sub>-*Methylobacterium*, T<sub>5</sub>-*Azospirillum* + *Pseudomonas* co-inoculation, T<sub>6</sub>- *Azospirillum* + *Methylobacterium* co-inoculation, T<sub>7</sub>-*Methylobacterium* +*Pseudomonas* co-inoculation, T<sub>8</sub>- *Azospirillum* +*Pseudomonas* +*Methylobacterium* co-inoculation, T<sub>9</sub>- *Azospirillum* +*Pseudomonas* +*Methylobacterium* coaggregates (natural), T<sub>10</sub>- *Azospirillum* +*Pseudomonas* +*Methylobacterium* coaggregates (artificial) and sown as rows in pots, separately. On the 5th day of sowing, the seedlings were thinned out to get 50 numbers of seedlings per pot. The age of the seedlings were counted from the time of sowing. The experiment was arranged in randomized block design (RBD) with three replications. The rice plants were challenge inoculated with *Xanthomonas oryzae* pv. *oryzae* by spraying the spore suspension of the same at 50,000 spores mL<sup>-1</sup> inoculum level on 10<sup>th</sup> DAS with an atomizer whereas the control plant was sprayed with distilled water only. High humidity was created by sprinkling the water frequently in the polyhouse. The bacterial leaf blight disease incidence was enumerated with a score chart of 0 to 9 grades devised by International Rice Research Institute (1980). The plant height, shoot and root dry weight and chlorophyll content was estimated according to [15] whereas the Indole acetic acid content of rice roots was estimated according to [16]. The total nitrogen and phosphorous content was estimated according to [17,18], respectively while the grain yield of lowland rice was recorded during the time of harvest.

**Statistical analysis:** The experimental results were statistically analysed in Randomized block design (RBD) and in Duncan's Multiple Range Test (DMRT) as per the procedure described by [19].

## **RESULTS AND DISCUSSION**

The application effect of different bioformulations, viz., single strain inoculation, co-inoculation and coaggregates application of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium* on the enhancement of rice root adhesion and seed vigour index of rice cv.BPT-5804 was studied under *in vitro* condition and the results presented in table-1.

**Table 1.** Effect of different formulations of PGPR cells on the enhancement of seed vigour index and root adherence in lowland rice cv.BPT-5804

Treatment	Seed vigour index <sup>a</sup>	Statistics <sup>b</sup>	No. of adhered cells (10 <sup>4</sup> /g dry wt. of root/h) <sup>a</sup>	Statistics <sup>b</sup>
Control	11000 ± 8.76	j	-	j
<i>Azospirillum</i> (AZ-3)*	12905 ± 6.80	h	268.2 ± 5.67 <sup>h</sup>	h
<i>Pseudomonas</i> (PF-3)*	13067 ± 7.12	g	243.4 ± 4.78 <sup>g</sup>	g
<i>Methylobacterium</i> (MB-3)*	12665 ± 9.10	i	210.0 ± 6.89 <sup>i</sup>	i
AZ-3 + PF-3 Co-I**	14887 ± 5.87	d	301.5 ± 9.41 <sup>d</sup>	d
AZ-3 + MB-3 Co-I**	14466 ± 9.56	f	297.5 ± 3.65 <sup>f</sup>	f
MB-3 + PF-3 Co-I**	14708 ± 9.07	e	279.2 ± 1.89 <sup>e</sup>	e
AZ-3 + PF-3 + MB-3 Co-I**	15958 ± 7.45	c	312.0 ± 6.00 <sup>c</sup>	c
AZ-3 + PF-3 + MB-3 Co-AG (N)	17014 ± 2.50	a	399.5 ± 8.56 <sup>a</sup>	a
AZ-3 + PF-3 + MB-3 Co-AG(A)	16445 ± 5.56	b	349.4 ± 7.12 <sup>b</sup>	b

a-Values are mean of three replications ± SD

b-Values followed by different letters are significantly differed at 5% level according to student 't' test.

Among the single strain inoculation treatments, the application of wild strains of *Pseudomonas* augmented the rice root adhesion and seed vigour index of rice to a higher level followed by application of *Azospirillum* and *Methylobacterium*. But, the effect was more pronounced when the wild strains (both *Pseudomonas* and *Azospirillum*) were applied as coinoculants followed by the coinoculation of *Azospirillum* and *Methylobacterium* and *Methylobacterium* and *Pseudomonas*. However, the highest rice root adhesion and seed vigour indices were recorded when the PGPR cells (*Azospirillum*, *Pseudomonas* and *Methylobacterium*) were applied as coaggregates (natural). The highest seed vigour index of rice (17014) and highest rice root adhesion (399.5 10<sup>4</sup>/g dry weight of root/h) were recorded during the application of strains of *Azospirillum*, *Pseudomonas* and *Methylobacterium*, as coaggregates (natural), followed by coaggregates (artificial), coinoculation of PGPR strains and single strain inoculation. The phytostimulatory and adhesion mechanism of *Pseudomonas* cells with many crop plants have already been reported [20-24]. [25] reported the poor adhesion of floc negative mutant strain of *Azospirillum* to wheat roots and emphasized the positive role of EPS in the adhesion processes. [7] reported the improved adhesiveness of *Azospirillum* bioflocs with plant roots and suggested the positive role of EPS in the early events of adhesion to plant roots. [6] described the phytostimulatory effect of "Intergeneric coaggregates" viz., *Azospirillum* and *Rhizobium*, on the enhancement of growth parameters in faba bean. Moreover, the phytostimulatory effect of bacterial EPS has been demonstrated in *Azospirillum* [3]. In the present study the application of EPS rich, PGPR coaggregates (natural), consisting of *Azospirillum*, *Pseudomonas* and *Methylobacterium* augmented the rice root adhesion and seed vigour index of rice to a higher level followed by the application of PGPR coaggregates (artificial), co-inoculation of three PGPR strains, co-inoculation of any two PGPR



strains and individual application of PGPR cells and the results of the present study are in conformity with the earlier findings of [6,3,9].

The effect of different bioformulations viz., single strain inoculation, co-inoculation and coaggregates application of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium* together with challenge inoculation of *Xanthomonas oryzae* pv. *oryzae* on the enhancement of various growth parameters viz., height, dry weight of root and shoot, 'N' and 'P' content of plant, IAA and chlorophyll content, grain yield and incidence of bacterial leaf blight disease (*Xanthomonas oryzae* pv. *oryzae*) was studied under pot culture condition and the results presented in Table-2.

**Table-2.** Effect of different bioformulations of PGPR cells and challenge inoculation of *Xanthomonas oryzae* pv. *oryzae* on the enhancement of growth and yield parameters and biocontrol of bacterial blight disease in lowland rice cv. BPT-5804

Treatment <sup>a</sup>	Plant height (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Nitrogen content (%)	Phosphorous content (%)	Indole acetic acid content (mg/g)	Chlorophyll content (mg/g of leaf)	Grain yield (t ha <sup>-1</sup> )	Percentage of disease incidence <sup>c</sup>
Control	51.79 <sup>j</sup>	0.266 <sup>j</sup>	1.023 <sup>j</sup>	0.84 <sup>j</sup>	0.39 <sup>j</sup>	10.30 <sup>j</sup>	2.16 <sup>j</sup>	5.32 <sup>j</sup>	90.5 ± 1.32 <sup>j</sup>
<i>Azospirillum</i> (AZ-3)*	61.12 <sup>h</sup>	0.215 <sup>h</sup>	1.199 <sup>h</sup>	1.98 <sup>h</sup>	0.62 <sup>h</sup>	13.95 <sup>h</sup>	2.34 <sup>h</sup>	5.55 <sup>h</sup>	44.0 ± 0.98 <sup>h</sup>
<i>Pseudomonas</i> (PF-3)*	63.95 <sup>g</sup>	0.322 <sup>g</sup>	1.302 <sup>g</sup>	2.49 <sup>g</sup>	0.78 <sup>g</sup>	14.98 <sup>g</sup>	2.46 <sup>g</sup>	5.73 <sup>g</sup>	40.4 ± 1.78 <sup>g</sup>
<i>Methylobacterium</i> (MB-3)*	59.18 <sup>i</sup>	0.294 <sup>i</sup>	1.128 <sup>i</sup>	1.38 <sup>i</sup>	0.50 <sup>i</sup>	12.50 <sup>i</sup>	2.25 <sup>i</sup>	5.43 <sup>i</sup>	48.2 ± 2.65 <sup>i</sup>
AZ-3 + PF-3 Co-I**	68.54 <sup>d</sup>	0.424 <sup>d</sup>	1.465 <sup>d</sup>	3.45 <sup>d</sup>	1.18 <sup>d</sup>	17.75 <sup>d</sup>	2.78 <sup>d</sup>	6.13 <sup>d</sup>	21.4 ± 1.05 <sup>d</sup>
AZ-3 + MB-3 Co-I**	66.03 <sup>f</sup>	0.388 <sup>f</sup>	1.338 <sup>f</sup>	2.70 <sup>f</sup>	0.89 <sup>f</sup>	15.93 <sup>f</sup>	2.56 <sup>f</sup>	5.99 <sup>f</sup>	33.1 ± 0.57 <sup>f</sup>
MB-3 + PF-3 Co-I**	67.14 <sup>e</sup>	0.364 <sup>e</sup>	1.396 <sup>e</sup>	3.08 <sup>e</sup>	1.06 <sup>e</sup>	16.97 <sup>e</sup>	2.66 <sup>e</sup>	5.88 <sup>e</sup>	28.2 ± 2.08 <sup>e</sup>
AZ-3 + PF-3 + MB-3 Co-I**	70.85 <sup>c</sup>	0.447 <sup>c</sup>	1.543 <sup>c</sup>	4.02 <sup>c</sup>	1.43 <sup>c</sup>	19.45 <sup>c</sup>	2.87 <sup>c</sup>	6.29 <sup>c</sup>	17.2 ± 1.54 <sup>c</sup>
AZ-3 + PF-3 + MB-3 Co-AG (N)	73.06 <sup>a</sup>	0.492 <sup>a</sup>	1.652 <sup>a</sup>	4.96 <sup>a</sup>	1.99 <sup>a</sup>	21.96 <sup>a</sup>	3.11 <sup>a</sup>	6.58 <sup>a</sup>	10.5 ± 1.82 <sup>a</sup>
AZ-3 + PF-3 + MB-3 Co-AG(A)	71.93 <sup>b</sup>	0.469 <sup>b</sup>	1.597 <sup>b</sup>	4.44 <sup>b</sup>	1.72 <sup>b</sup>	20.23 <sup>b</sup>	2.98 <sup>b</sup>	6.41 <sup>b</sup>	14.2 ± 1.32 <sup>b</sup>
LSD (P = 0.05)	1.05	0.004	0.005	0.06	0.03	0.03	0.07	0.07	-

a - Average of three replication

b - Values are mean of three replications ±SD

\* - Individual application of *Azospirillum* (AZ-3), *Pseudomonas* (PF-3) and *Methylobacterium* (MB-3) isolates at  $1 \times 10^9$  CFU/mL inoculum level.

\*\* - Co-inoculation of PGPR isolates at  $1 \times 10^9$  CFU/mL inoculum level.

N - Natural coaggregates; A- Artificial coaggregates of PGPR isolates.

The effect of *Pseudomonas* inoculation on the augmentation of growth parameters of rice has been reported by many authors [26-29]. The positive effect of *Azospirillum* in augmenting the growth and yield parameters of rice has already been reported [30-33]. The augmenting role of *Methylobacterium* bioinoculation on the enhancement of growth and yield in lowland rice has been well established [34,35]. The co-inoculation effect of *Azospirillum* and *Methylobacterium* has also been reported by [36].

In the present study, single strain inoculation of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium*, could augment the growth and yield parameters of lowland rice to a higher level when compared to control. But the effect was more pronounced when any two PGPR cells viz., *Azospirillum* and *Pseudomonas*, *Azospirillum* and *Methylobacterium* or *Pseudomonas* and *Methylobacterium* were coinoculated. However, the effect was found to be the highest when the PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium* were applied as "Multigeneric microbial coaggregates". Regarding

the single strain application, the application of *Pseudomonas* was found to enhance the growth and yield parameters of rice to a higher level followed by *Azospirillum* and *Methylobacterium* inoculation. Interestingly, the co-inoculation of PGPR cells recorded the highest response than the single strain application but not with coaggregates application. However, the response was found to be the maximum when the PGPR cells were applied as “Multigeneric microbial coaggregates” (natural). Greater plant height of rice due to the bioinoculation of *Pseudomonas* and *Azospirillum* has already been reported [37]. Increase in dry matter production of rice with *Pseudomonas* inoculation has been well established by many authors [38,27,29]. The increase in the total ‘N’ and ‘P’ content of rice due to the inoculation of *Pseudomonas* and *Azospirillum* has already been reported [39,40,16]. The increase in chlorophyll content of rice leaves due to bioinoculation of *Azospirillum* has been reported by [41]. The production of IAA by different isolates of *Pseudomonas* has already been reported [20,42]. The augmentation of grain yield due to *Pseudomonas* inoculation has been reported by [43]. The co-inoculation effect of *Pseudomonas* and *Azospirillum* on the enhancement of growth and yield parameters of different cereal crops has been reported [44].

Interestingly, the bioinoculation of “Multigeneric microbial coaggregates” (natural), consisting of *Azospirillum*, *Pseudomonas* and *Methylobacterium* also reduced the bacterial leaf blight disease incidence (10.5 per cent) of rice to a higher level followed by the application of “Multigeneric microbial coaggregates” (artificial), co-inoculation of *Azospirillum*, *Pseudomonas* and *Methylobacterium* co-inoculation of any two PGPR cell and individual application of PGPR cells. [45] reported the positive effect of bioinoculation of *Pseudomonas fluorescens* and challenge inoculation of *Xanthomonas oryzae* on the enhancement of growth and yield and reduced incidence of bacterial blight disease in rice. Bioinoculation of *Pseudomonas fluorescens* and concomitant Induction of systemic resistance during endophytic colonisation of rice has been reported by many authors [46,47].

In our laboratory, the positive bioinoculation effect of EPS rich, Intergeneric coaggregates of PGPR cells, consisting of *Pseudomonas* and *Paenibacillus*, or *Azospirillum* and *Paenibacillus* on the enhancement of growth and yield in upland rice and maize has been reported by [48], [49] respectively. Rubiya[50] reported the positive bioinoculation by effect of “Multigeneric microbial coaggregates”, consisting of *Azospirillum*, *Azotobacter* and *Rhizobium*, on the augmentation of growth and yield parameters in lowland rice. In the present study also, the application of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium* as “Multigeneric microbial coaggregates” (natural) augmented the growth and yield parameters and biocontrol against bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) in lowland rice when compared to other formulations and the results of the present study are in conformity with the earlier findings of [6,48-50].

## APPLICATIONS

Bioinoculation of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium*, as natural coaggregates, augmented the plant growth stimulation, yield and biocontrol against *Xanthomonas oryzae* pv. *oryzae* in lowland rice which lead to the enhancement of rice productivity.

## CONCLUSIONS

It was concluded that the application of PGPR cells viz., *Azospirillum*, *Pseudomonas* and *Methylobacterium*, as EPS rich, natural coaggregates, augmented the PGPR characteristics and biocontrol against bacterial leaf blight disease, caused by *Xanthomonas oryzae* pv. *oryzae*, to a higher level which ultimately lead to the enhancement of crop productivity in lowland rice.

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