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Evaluation of the compatibility between native rhizobacteria isolates

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ABSTRACT

Compatibility between the Plant Growth Promoting Rhizobacteria (PGPR) microbes to colonize the root system without inhibiting each other is a pre-requisite for successful benefit of using multiple microbes in a crop field. Taking this into account, the present investigation was conducted to test the compatibility between different native rhizobacterial isolates *in-vitro* in the Plant Bacteriological Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during 2021-22. All the efficient and native rhizobacterial isolates tested were compatible with each other under *in-vitro* conditions except BRB 88 and BRB 89, BRB 35, BRB 42, BRB 52, PR 19; BRB 89 and BRB 35, SM 9, SM 14; BRB 35 and PR 19; BRB 42 and PR 18; BRB 52 and SM 9; CK2LPP and CK2LP12, GP2, GP8, G11SP37, K11SP4, SS2LP; CK2LP12 and GP2; GP8 and K11SP4; SS2PLP and SS2PP; GP2 and G1SP37, SS2PP; G11SP37 and K11SP4; SS2PP and S21SP14; BRB 88 and CK2LPP, GP2; PR 16 and SS2PP, S21SP14; PR 20 and GP8; BRB 52 and CK2LPP, CK2LP8, GP2, GP8; BRB 56 and GP2, GP8; SM 9 and CK2LPP, CK2LP8, CK2LP12, G11SP37.

Key words: *Compatibility, Native plant growth-promoting rhizobacteria (PGPR), West Bengal*

Introduction

Plant growth-promoting rhizobacteria (PGPR), also termed as plant health-promoting rhizobacteria (PHPR), yield-increasing bacteria (YIB) or nodule promoting rhizobacteria (NPR), are the important bio control agents (Wu *et al.,* 2016) and effective in reducing both abiotic and biotic stresses (Tewari and Arora 2016). PGPRs, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Pantoea*, *Bacillus*, *Serratia* and *Rhizobium*,have shown an ability to improve plant growth (Verma *et al.,* 2019). Among these, species of *Bacillus* and *Pseudomonas* are predominant because of their distinctive plant growth promoting characteristics (Karnwal, 2017). Biofertilization, phytostimulation and biological control are diverse traits of heterogeneous PGPR (Chandler *et al.,* 2008) and can be exploited to develop formulations for management of several phytopathogens, enhancement of yield and food production by using fewer resources and less reliance on the chemical fertilizers and pesticides (Grover *et al.,* 2011). Because of the broad-host range of pest and pathogens, changing climates, high prices of agrochemicals and ecological crises, devising multi-purpose bio-formulations will be a more practical strategy for integrated pest and nutrient management. They also reduce the application of chemical fertilizers which would be economically feasible and eco-friendly for lower

production cost as well as recognize the best management practices of soil and crop to achieve more sustainable agriculture as well as fertility of the soil. PGPR are subjected to variation in soil characteristics, soil temperature, pH, rainfall pattern, affecting the number of viable microorganisms available in or near root zone. Because of these limitations, variations in the PGPR are bound to occur in different agro- climatic regions. Some native strains may not be compatible with each other. There is a need to check the compatibility between native strains to optimize the benefit. Moreover, there are no report on compatibility of different efficient rhizobacteria with native species in this agroclimatic zone. Keeping this in mind, the present investigation was undertaken to evaluate the compatibility between different native rhizobacterial isolates.

Materials and Methods

The investigation was carried out *in-vitro* in Plant Bacteriological Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal during 2021-22. The preparation of media, sterilization, isolation and maintenance of microbial cultures, *etc*., were done following the method developed by Nene and Thapliyal (1993) with slight modification.

Collection of native plant growth promoting rhizobacteria: Thirteen isolates of fluorescent pseudomonads and thirteen isolates of *Bacillus* sp. were obtained from the Plant Bacteriological Laboratory, Department of Plant Pathology, BCKV and these strains were maintained by frequent sub-culturing after 30 days interval and stored at 4 ^{o}C in the test tube slants of Nutrient Agar (NA) media. The isolates with their identified species are listed in the Table 1.

Test of compatibility of different efficient rhizobacterial isolates against native rhizobacterial isolates: The rhizobacterial isolates were tested for their compatibility between them by a cross-streak assay method (Al- Hussini *et al.,* 2019). One isolate of antagonistic bacteria was streaked in the centre of Petri dish containing NA medium in parallel lines. Other test bacterial isolates were streaked at right angle to the first bacterial isolate. The plates were incubated at room temperature 27±1 ^{0}C for 48 hrs and the growth of the bacteria at the intersection was observed for possible merger of bacteria. The zone of inhibition was analysed and recorded as incompatible (IC) for the presence of inhibition zone, whereas compatible (C) for the absence of the inhibition zone after 48 hrs of incubation at 27 ±1 ^{0}C.

Results and Discussion

Compatibility studies between different native *Bacillus* isolates (Table 2) indicated that there was a zone of inhibition recorded between (BRB 88 and BRB 89, BRB 35, BRB 42, BRB 52, PR 19); (BRB 89 and BRB 35, SM 9, SM 14); (BRB 35 and PR 19); (BRB 42 and PR 18); (BRB 52 and SM 9) exhibiting incompatible. Whereas there was no zone of inhibition recorded between the other isolates exhibiting compatible.

When compatibility studies were performed between fluorescent pseudomonads isolates, it was

Table 1. List of native rhizobacterial isolates

Native *Bacillus* isolates	Name of Rhizobacteria	Native Fluorescent Pseudomonads	Name of Rhizobacteria
BRB 88	*Bacillus subtilis* subsp.*subtilis*	BCLP4	*Pseudomonas fluorescens*
BRB 89	*Bacillus pumilus*	CK2LPP	——-
BRB 35	*Bacillus altitudinis*	CK2LP8	——-
BRB 42	*Bacillus rugosus*	CK2LP12	——-
BRB 52	*Bacillus pumilus*	GP2	*Pseudomonas aeruginosa*
BRB 56	*Bacillusamyloliquefaciens*	GP8	*Pseudomonas aeruginosa*
BRB 74	*Bacillus subtilis*	G11SP37	*Pseudomonas geniculata*
PR 16	*Bacillus australimaris*	G15SP38	*Pseudomonas putida*
PR 18	——-	K11SP4	*Pseudomonas baetica*
PR 19	*Bacillus subtilis* subsp.*subtilis*	K22SP8	*Pseudomonas fluorescens*
PR 20	*Bacillus subtilis*	SS2PP	——-
SM 9	——-	SS2LP	——-
SM 14	——-	S21SP14	*Pseudomonas putida*

concluded (Table 3) that there was a zone of inhibition recorded between (CK2LPP and CK2LP12, GP2, GP8, G11SP37, K11SP4, SS2LP); (CK2LP12 and GP2); (GP8 and K11SP4, SS2PLP and SS2PP); (GP2 and G1SP37, SS2PP); (G11SP37 and K11SP4);(SS2PP and S21SP14) exhibiting incompatible. Whereas there was no zone of inhibition recorded between the other isolates exhibiting compatible.

Compatibility studies between different native *Bacillus* and fluorescent pseudomonads isolates (Table 4) indicated that there was a zone of inhibition recorded between (BRB 88 and CK2LPP, GP2); (PR 16 and SS2PP, S21SP14); (PR 20 and GP8); (BRB 52 and CK2LPP, CK2LP8, GP2, GP8); (BRB 56 and GP2, GP8); (SM 9 and CK2LPP, CK2LP8, CK2LP12, G11SP37) exhibited incompatible. Whereas there was no zone of inhibition recorded between the other isolates exhibiting compatible.

The isolates, which were compatible with each other under *in-vitro* conditions, could be attributed to the existence of synergism between the metabolites produced by the isolates and may be exploited for the preparation of microbial consortia. Combining compatible biocontrol agents is a strategic approach to control plant disease. The dual inoculation of compatible biocontrol agents reduced plant disease severity more than mono inoculation of the potential antagonistic rhizobacterial isolates (Mota *et al.,* 2017).

Conclusion

Most of the isolates were compatible with each other under *in-vitro* conditions could be attributed to the

Table 2. Compatibility test between isolates of native *Bacillus* sp.

	Bacillus												
Isolates	BRB 88	BRB 89	BRB 35	BRB 42	BRB 52	BRB 56	BRB 74	PR 16	PR 18	PR 19	PR 20	SM 9	SM 14
BRB 88	C	IC	IC	IC	IC	C	C	C	C	C	IC	C	C
BRB 89	IC	IC	IC	C	C	C	C	C	C	C	C	IC	IC
BRB 35	IC	C	C	C	C	C	C	C	C	C	IC	C	C
BRB 42	IC	C	C	C	C	C	C	C	IC	C	C	C	C
BRB 52	IC	C	C	C	C	C	C	C	C	C	C	IC	C
BRB 56	C	C	C	C	C	C	C	C	C	C	C	C	C
BRB 74	C	C	C	C	C	C	C	C	C	C	C	C	C
PR 16	C	C	C	C	C	C	C	C	C	C	C	C	C
PR 18	C	C	C	IC	C	C	C	C	C	C	C	C	C
PR 19	IC	C	IC	C	C	C	C	C	C	C	C	C	C
PR 20	C	C	C	C	C	C	C	C	C	C	C	C	C
SM 9	C	IC	C	C	IC	C	C	C	C	C	C	C	C
SM 14	C	IC	C	C	C	C	C	C	C	C	C	C	C

Table 3. Compatibility test between isolates of native fluorescent pseudomonads

Isolates	BCLP4	CK2 LPP	CK2 LP8	CK2L P12	GP2	GP8	G11S P37	G15S P38	K11S P4	K22 SP8	SS2PP	SS2 LP	21S P14
BCLP4	C	C	C	C	C	C	C	C	C	C	C	C	C
CK2LPP	C	C	C	IC	IC	IC	IC	C	IC	C	C	IC	C
CK2LP8	C	C	C	C	C	C	IC	C	C	C	C	C	C
CK2LP12	C	IC	C	C	IC	C	C	C	C	C	C	C	C
GP2	C	IC	C	IC	C	C	IC	C	C	C	IC	C	C
GP8	C	IC	C	C	C	C	C	C	IC	C	IC	IC	C
G11SP37	C	IC	IC	IC	IC	C	C	C	IC	C	C	C	C
G15SP38	C	C	C	C	C	C	C	C	C	C	C	C	C
K11SP4	C	IC	C	C	C	IC	IC	C	C	C	C	C	C
K22SP8	C	C	C	C	C	C	C	C	C	C	C	C	C
SS2PP	C	C	C	C	IC	IC	C	C	C	C	C	C	IC
SS2LP	C	IC	C	C	C	IC	C	C	C	C	C	C	C
S21SP14	C	C	C	C	C	C	C	C	C	C	IC	C	C

Table 4. Compatibility test between isolates of native *Bacillus* sp. and fluorescent pseudomonads

Isolates	BCLP4	CK2 LPP	CK2 LP8	CK2L P12	GP2	GP8	G11S P37	G15S P38q	K11S P4	K22 SP8	SS2PP	SS2LP	S21S P14
BRB 88	C	IC	C	C	IC	C	C	C	C	C	C	C	C
BRB 89	C	C	C	C	C	C	C	C	C	C	C	C	C
BRB 35	C	C	C	C	C	C	C	C	C	C	C	C	C
BRB 42	C	C	C	C	C	C	C	C	C	C	C	C	C
BRB 52	C	IC	IC	C	IC	IC	C	C	C	C	C	C	C
BRB 56	C	C	C	C	IC	IC	C	C	C	C	C	C	C
BRB 74	C	C	C	C	C	C	C	C	C	C	C	C	C
PR 16	C	C	C	C	C	C	C	C	C	C	IC	C	IC
PR 18	C	C	C	C	C	C	C	C	C	C	C	C	C
PR 19	C	C	C	C	C	C	C	C	C	C	C	C	C
PR 20	C	C	C	C	C	IC	C	C	C	C	C	C	C
SM 9	C	IC	IC	IC	C	C	IC	C	C	C	C	C	C
SM 14	C	C	C	C	C	C	C	C	C	C	C	C	C

existence of synergism between the metabolites produced by the isolates. The phytopathogens, being a great threat for plant health and longevity, need to be controlled and restricted for invasion. The use of eco-friendly indigenous soil micro-organisms can be of great benefit to combat against these potential pathogens. The deliberate administration of rhizobacteria in soil can be of worth importance as their intricate symbiotic and antagonistic relationships with plants and plant pathogens respectively, are vital to plant growth and survival. However, prior to incorporation, the safety aspects should be considered and standards should be maintained.

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Conflict of Interest: There is no competing interest exists.

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