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Formulating rhizosphere consortia: Harnessing plant growth-promoting rhizobacteria for enhanced agricultural sustainability

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Abstract
The development of efficient rhizosphere consortia using Plant Growth Promoting Rhizobacteria (PGPR) plays a significant role in enhancing plant growth and agricultural sustainability. This study focuses on formulating a rhizosphere consortium by utilizing eight different PGPR strains, including *Bradyrhizobium japonicum* (SB-120), *Pseudomonas striata* (PSB), *Bacillus* sp. (KSB), Zinc solubilizing bacterium (ZnSB), Silica solubilizing bacterium (SiSB), Actinobacterium (JK-16), Pink pigmented facultative methylotroph (PPFM-33) and Lactic acid bacterium (LAB-75). These strains were characterized based on their plant growth-promoting traits such as nitrogen fixation, indole acetic acid production, and solubilization of essential nutrients like phosphorus and potassium. Various combinations were formulated using a common growth medium, Luria Bertani, amended with additives like polyethylene glycol and glycerol. The formulation demonstrated that six PGPR strains, when combined, maintained high efficacy and offered a viable alternative to single-strain inoculants.

Keywords: PGPR, rhizosphere consortia, nitrogen fixation, indole acetic acid, phosphorus solubilization

Introduction
Microorganisms play a vital role in various ecosystem processes, especially in enhancing soil fertility. They facilitate biogeochemical cycling, making essential soil mineral nutrients like nitrogen, phosphorus and potassium available to plants, thus promoting plant growth when inoculated Agriculturally beneficial microorganisms such as bacteria and fungi, establish mutualistic relationships with plants. They contribute to nutrient availability, actively participate in organic matter decomposition, produce phytohormones, aid in phytoremediation, and act as biocontrol agents against plant pests and diseases (Hussain *et al.*, 2020) [1].

Co-inoculation of soybean plants with *Bacillus amyloliquefaciens* LL2012 and the natural symbiont *Bradyrhizobium japonicum* resulted in improved plant growth, enhanced nodulation and increased colonization of plant roots by *B. japonicum* (Masciarelli *et al.*, 2014) [2]. Improved nodulation is favored by production of flavonoid like compounds or by stimulating the host legume to produce flavonoid signal molecule (Pankaj *et al.*, 2009) [3]. Phosphate solubilizing microorganisms, solubilization is achieved through the production of inorganic acids (carbonic and sulfuric), as well as organic acids like citric, butyric, oxalic, malonic, lactic, etc., and phosphatase enzymes (Whitelaw *et al.*, 1997 and Sundara *et al.*, 2001) [4, 5]. Potassium solubilizing bacteria show higher mobilization of potassium from waste mica, acting as a source of potassium for plant growth (Singh *et al.*, 2010) [6].

Introduction of beneficial microorganisms such as actinobacteria, PPFM (pink pigmented facultative methylotrophs) and lactic acid bacteria (LAB) into soil as microbial inoculants is reported to enhance plant growth, health and yield by providing or mobilizing nutrients, production of growth promoting substances and reducing the population of harmful bacteria occurring on plant parts. Many produce HCN and siderophores that have potential application in control of diseases**.** A "microbial consortium" is a collection of several microorganisms, wherein various kinds of microorganisms are employed, acting as a community and interacting in a synergistic manner.

Liquid bioinoculants are unique formulations containing the desired microorganisms, their nutrients and special cell protectants which promote extended shelf life and tolerance to adverse conditions (Vora *et al.*, 2008) [7]. Compared to peat-based inoculants, liquid bioinoculants are reported to have a longer survival period, with 10 to 100 times

higher viable cell numbers (Susewee, 1994) [8]. Therefore efforts were made to develop seventeen consortia comprising of eight PGPR strains in liquid formulations, with an aim of reducing costs on mass production and turnaround times without compromising their effectiveness.

Materials and Methods

The experiment was conducted in 2022-23 in the Institute of Organic Farming, Dharwad. The PGPR strains consisted of *Bradyrhizobium japonicum* (SB-120), *Pseudomonas striata* (PSB), *Bacillus* sp. (KSB), Zinc solubilizing bacterium (ZnSB), Silica solubilizing bacterium (SiSB), Actinobacterium (JK-16), Pink pigmented facultative methylotroph (PPFM-33) and Lactic acid bacterium (LAB-75) were collected from the Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad. Morphological characterization, Biochemical characterization and functional characterization were carried out. Compatibility between the strains was tested. A single common culture medium was standardized. A total of 32 Liquid formulations of 17 consortia were on a common medium (LB- luria bertani agar) was amended with standardised concentrations of additives such as Polyethylene Glycol (PEG), Glycerol and Ascorbic acid (Table-1).

Results and Discussion

All the PGPR strains were grown on luria bertani broth. This study focuses on formulating a rhizosphere consortium by utilizing eight different PGPR strains, including *Bradyrhizobium japonicum* (SB-120), *Pseudomonas striata* (PSB), *Bacillus* sp. (KSB), Zinc solubilizing bacterium (ZnSB), Silica solubilizing bacterium (SiSB), Actinobacterium (JK-16), Pink pigmented facultative methylotroph (PPFM-33) and Lactic acid bacterium (LAB-75).

These eight PGPR strains were mixed to obtain seventeen different consortia. The method adopted and the ratio set clearly helped to achieve formulations each with equal populations and to avoid any differences that could have arisen due to variation in their populations and differential growth rates. To accomplish this, all the eight strains were inoculated in a staggered system so as to get their maximum populations coinciding with the mass production schedule.

Table 1: Different additives, adjuvant and antioxidant and their concentrations found optimal and used in developing liquid formulation of PGPR strains

Amendments		Concentrations of amendments							
		SB-120 (*Bradyrhizobium japonicum*)	**PSB (*Pseudomonas striata*)**	**KSB (*Bacillus* sp.)**	**ZnSB (Zinc solubilizing bacterium)**	**SiSB (Silica solubilizing bacterium)**	**JK-16 (Actinobacterium)**	**PPFM-33 (Pink pigmented methylotrophs)**	**LAB-75 (Lactic acid bacterium)**
Additives	Poly Ethylene Glycol (%)	0.5	0.5	0.5	0.4	0.4	0.4	0.3	0.5
	Glycerol (%)	0.5	0.5	0.5	0.6	0.6	0.6	0.7	0.5
Adjuvant	Gum arabica (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Antioxidant	Ascorbic acid (Molar)	-	-	-	-	-	-	0.02	0.02

Table 2: Composition of different PGPR consortia formulated based on equal population of PGPR strains

PGPR consortia	Treatments	Volume of PGPR inoculants in 100 ml consortia								Final volume (ml)
		SB-120	PSB	KSB	ZnSB	SiSB	JK-16	PPFM-33	LAB-75	
MC 1	SB-120 + PSB + KSB	20.00	16.87	62.50	-	-	-	-	-	99.37
MC 2	SB-120 + PSB + KSB + ZnSB	15.84	13.36	49.5	21.28	-	-	-	-	99.98
MC 3	SB-120 + PSB + KSB + SiSB	13.11	11.06	40.96	-	35.24	-	-	-	100.39
MC 4	SB-120 + PSB + KSB + PPFM-33	16.75	14.13	52.35	-	-	-	16.75	-	99.98
MC 5	SB-120 + PSB + KSB + LAB-75	17.58	14.80	54.64	-	-	-	-	12.63	99.65
MC 6	SB-120 + PSB + KSB + JK-16	12.74	10.75	39.84	-	-	-	36.65	-	99.98
MC 7	SB120 + PSB + KSB + ZnSB + SiSB	11.14	9.40	34.84	14.98	29.96	-	-	-	100.32
MC 8	SB-120 + PSB + KSB + ZnSB + PPFM-33	13.67	11.53	42.73	18.37	-	-	13.67	-	99.97
MC 9	SB-120 + PSB + KSB + ZnSB + LAB-75	14.22	12.00	44.44	19.11	-	-	-	10.22	99.99
MC 10	SB-120 + PSB + KSB + ZnSB + JK-16	10.88	9.18	34.01	14.62	-	31.29	-	-	99.98
MC 11	SB-120 + PSB + KSB + SiSB + PPFM-33	11.59	9.78	36.23	31.15	-	-	11.59	-	100.34
MC 12	SB-120 + PSB + KSB + SiSB + LAB 75	11.98	10.11	37.45	-	32.20	-	-	8.61	100.35
MC 13	SB-120 + PSB + KSB + SiSB + JK-16	9.52	8.03	29.76	-	25.59	27.38	-	-	100.28
MC 14	SB-120 + PSB + KSB + ZnSB + SiSB + PPFM-33	10.03	8.46	31.34	13.47	26.95	-	10.03	-	100.28
MC 15	SB-120 + PSB + KSB + ZnSB + SiSB + LAB-75	10.32	8.70	32.25	13.87	27.74	-	-	7.41	100.29
MC 16	SB-120 + PSB + KSB + ZnSB + SiSB + JK-16	8.44	7.12	26.38	11.34	22.69	24.27	-	-	100.24
MC 17	SB-120 + PSB + KSB + ZnSB + SiSB + PPFM-33 + LAB-75 + JK-16	7.37	6.22	23.04	9.90	19.81	7.37	21.19	5.29	100.19

The results were in accordance with the findings of Parvati and Patil (2018) [9] conducted a comprehensive study on the growth of *Pseudomonas striata* in liquid formulations with different concentrations of additives, adjuvants, and surfactants. Their research revealed that formulation-18 (Pikovskaya's broth with 10 mM glycerol, 0.5% PEG, 0.05% CMC, 0.15% gum arabica, and 250 ppm polysorbate-20), containing Pikovskaya's broth with specific additives, significantly enhanced the population of *Pseudomonas striata*, reaching 203.33×10^{10} at 72 hours after inoculation. The formulated liquid was then applied to maize and sorghum seeds at varying concentrations, resulting in higher viable populations in treated seeds compared to the lignite-based formulation. This study showed the potential of formulation-18 as an effective bioinoculant for enhancing plant growth and offered valuable insights for the development of cost-effective and efficient liquid formulations for agricultural applications.

Suman *et al.* (2016) [10] investigated the bioefficacy effect of three formulations of hydrogel based bioinoculant

consortium of *Azotobacter chroococcum*, *Pseudomonas fluorescence* and *Trichoderma viride* on growth of wheat. The results showed that the consortium positively enhanced plant growth with a multiple synergistic mechanisms of consortium of inoculants over liquid and lignite based microbial consortia.

Vijaykumar and Brahmaprakash (2018) [11] evaluated the effect of Agriculturally Important Microorganisms (AIMs) *viz*., *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* in four different formulations *viz*., alginate based, fluid bed dryer based, lignite and liquid based formulations on growth and nutrient uptake of green gram (*Vigna radiata* L.). They observed that plants treated with triple inoculants in liquid formulation recorded higher plant height of 38.93 cm, the maximum number of leaves of 9.67, total chlorophyll content of 2.54 mg/g of leaf, total nitrogen uptake of 103.56 mg/ plant, total phosphorus uptake of 63.11 mg/plant and total biomass content of 12.87 g/plant as compared to the other test formulations.

Santhosh *et al.* (2015) [12] formulated liquid biofertilizer for efficient biofertilizer strains of the HK region using various cell protectants and nutrients in liquid broth. The cell protectants tested included glycerol (0.5%), polyvinyl pyrrolidone (PVP, 0.5%), polyethylene glycol (PEG, 0.5%), gum arabic (GA, 0.5%), and sodium alginate (SA, 0.1%). Additionally, treatments without cell protectants (only broth) and a carrier (lignite) based formulation were included as controls. These formulated liquid biofertilizers of *Rhizobium*, *Azotobacter*, *Azospirillum*, and *Bacillus megaterium* were then stored at 28±2 °C in a BOD incubator for 180 days, with the viable cell count determined monthly. Among the tested cell protectants, the combination of PVP and glycerol at 0.5% each showed the highest retention of viable cells in all strains, followed by PEG, GA, and SA.

Suman *et al.* (2016) [10] investigated the effectiveness of three formulations of a microbial consortium consisting of *Azotobacter chroococcum*, *Pseudomonas fluorescence*, and *Trichoderma viride* in maintaining growth and shelf-life stability. The shelf-life studies of lignite, liquid, and hydrogel based bioinoculant formulations were conducted for up to 90 days at room temperature. The results indicated that the formulations showed varying population counts over the storage period. The nitrogen fixer *Azotobacter chroococcum* had population counts of 1.2×10^7, 1.4×10^8, and 3.5×10^9 CFU mL^{-1} in lignite, liquid, and hydrogel formulations, respectively. For *Pseudomonas fluorescence*, the population counts were 2.2×10^7, 2.4×10^8, and 4.5×10^9 CFU mL^{-1} in lignite, liquid, and hydrogel formulations, respectively. The phyto-stimulator *Trichoderma viride* had population counts of 1.4×10^6, 2.8×10^7, and 2.5×10^8 CFU mL^{-1} in lignite, liquid, and hydrogel formulations, respectively.

Miljakovic *et al.* (2022) [13] evaluated the effectiveness of *Bradyrhizobium japonicum* and newly isolated *Bacillus megaterium* strains as single inoculants and co-inoculant during seed bio-priming for two soybean cultivars. *Bacillus megaterium* significantly improved seed germination, shoot and root growth, and seedling vigor in various tests compared to the control. Co-inoculation further showed significant improvements in several parameters.

Fatharani *et al.* (2018) [14] conducted a survey to isolate and characterize rhizobacteria from the paddy rhizosphere that have the potential to dissolve mineral feldspar as a potassium source. Seven bacterial isolates, namely LJK 1, LJK 2, LJK 4, LBK 4, LBK 5, PSUK 4, and PSIK 6, were identified for their ability to solubilize potassium. The potassium-solubilizing index indicated that all seven isolates exhibited the capacity to solubilize feldspar in Alexandrov solid medium, with LJK 2 being the most effective isolate in this regard. These isolates were further subjected to morphological and physiological-biochemical tests, involving a total of 39 characteristics.

Hussain *et al.* (2019) [1] investigated the production and implications of bio-activated organic fertilizers (BOZ) enriched with zinc-solubilizing bacteria (ZSB) to enhance maize (*Zea mays* L.) production and biofortification. They tested various formulations of BOZ with ZnO and $ZnSO_4$ in field conditions over two cropping seasons. The results showed that BOZ4 (with a composition of 6:4 ZnO to orange peel waste) had the most significant positive impact on plant growth, yield, physiology, and zinc content in maize. BOZ4 also improved grain quality by increasing fat, crude protein, and mineral contents. Overall, bio-activation of ZnO with ZSB proved to be an efficient and economical approach to enhance maize growth and nutritional quality.

Conclusion

This study successfully formulated a rhizosphere consortium utilizing eight distinct PGPR strains, highlighting the synergistic potential of these microorganisms to enhance plant growth and promote agricultural sustainability. The formulation of liquid bioinoculants with three strains inoculants, four, five, six and eight strain inoculants were effective in comparison to uninoculated control. It demonstrated that six PGPR strains could effectively maintain high efficacy levels, offering a viable alternative to traditional single-strain inoculants. This approach not only addresses the limitations associated with single-strain applications but also enhances nutrient solubilization and overall plant health. The findings indicate that the combination of diverse PGPR strains can lead to improved plant growth parameters through enhanced nitrogen fixation, phosphorus and potassium solubilization, and phytohormone production. Moreover, the study shows the importance of optimizing their formulations for effective field application. By reducing production costs and turnaround times without compromising efficacy, this research paves the way for the development of sustainable agricultural practices.

Conflict of Interest

Not available

Financial Support

Not available

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