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ORIGINAL ARTICLE

Isolation and Biochemical Characterization of Plant Growth-Promoting Microbes and Their Influence on Enhancing Germination of Bajra (*Pennisetum glaucum* [L.]) in the YSR Kadapa District, Andhra Pradesh, India

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ABSTRACT
This study aimed to develop a consortium of plant growth-promoting microbes (PGPMs) targeting early-stage diseases in Bajra (Pennisetum glaucum [L.]) in YSR District-Kadapa, Andhra Pradesh, India. Dominant PGPM strains were selected based on diverse plant growth and protection attributes, characterized, and grouped according to compatibility to formulate a PGPM consortium. In in vitro experiments, the PGPM consortium significantly enhanced germination rates and root induction compared to control, particularly against Aspergillus niger and Fusarium oxysporum. Furthermore, PGPMs exhibited superior efficacy in stimulating and safeguarding plant growth compared to the control. The developed PGPM consortia displayed multiple beneficial plant growth traits, including phosphate solubilization, production of hydrogen cyanide (HCN), and indole acetic acid, coupled with broad-spectrum antagonism against tested phytopathogens.

Keywords: *Plant growth-promoting microbes (PGPMs); Bajra (Pennisetum glaucum L.); Consortium; Early-stage diseases; Phytopathogens.*

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INTRODUCTION

Plant growth-promoting microbes (PGPMs) constitute a diverse array of microorganisms, including bacteria, fungi, and archaea, crucially involved in augmenting the growth and development of plants through intricate physiological and biochemical processes (14). These microorganisms colonize the rhizosphere and rhizoplane of plants, establishing symbiotic relationships that significantly contribute to plant health and productivity (10). By employing various mechanisms, PGPMs enhance overall plant vigor and yield (7). Key mechanisms include nitrogen fixation, whereby certain bacteria such as rhizobia and diazotrophic bacteria convert atmospheric nitrogen into plant-usable forms, enriching the soil with this vital nutrient (17). Additionally, phosphate solubilization by PGPMs, particularly phosphate-solubilizing bacteria (PSB), enhances phosphorus availability to plants, essential for various physiological processes including energy transfer and root development (8).Moreover, PGPMs produce phytohormones like indole acetic acid (IAA), regulating fundamental aspects of plant growth and development such as cell elongation and root initiation (6). By synthesizing IAA, PGPMs stimulate root growth, thereby improving nutrient and water uptake efficiency (12). Furthermore, PGPMs secrete hydrolytic enzymes like cellulases, pectinases, and chitinases, which facilitate the decomposition of organic matter in the soil, releasing essential nutrients and promoting soil fertility (1). The decomposition of organic matter enhances nutrient cycling within the soil-plant system (15).

Bajra (*Pennisetum glaucum* [L.]), commonly known as pearl millet, is a staple cereal crop cultivated extensively in arid and semi-arid regions, notably in the YSR Kadapa District of Andhra Pradesh, India.

Despite its inherent resilience to adverse environmental conditions, bajra productivity often encounters constraints imposed by biotic and abiotic stressors, including nutrient deficiencies and soil-borne pathogens such as *Aspergillus niger* and *Fusarium oxysporum* (1). These pathogens significantly affect bajra cultivation, leading to yield losses and economic hardships for farmers. *A.niger* is a prevalent seed-borne fungal pathogen causing various diseases in bajra, including seed rot, root rot, and damping-off, consequently reducing germination rates, seedling vigor, and overall plant growth. Similarly, *F.oxysporum* induces Fusarium wilt, characterized by wilting, leaf yellowing, and vascular discoloration, resulting in severe yield losses and crop failure (5). Given these challenges, harnessing the potential of PGPMs emerges as a promising strategy to enhance bajra growth and development, mitigate the detrimental effects of fungal pathogens, and promote sustainable agriculture in arid and semi-arid regions. The aim of this research is to investigate the isolation and biochemical characterization of PGPMs and evaluate their influence on enhancing the germination and root development of bajra in the YSR Kadapa District, Andhra Pradesh, India. Specifically, we aim to examine early control strategies utilizing PGPMs to alleviate the effects of *A. niger* and *F. oxysporum* on bajra cultivation and discuss the efficacy of a PGPMs consortium in enhancing plant growth while combating fungal pathogens. Our objective is to provide sustainable solutions for improving bajra production and securing food resources in the region.

MATERIAL AND METHODS

Chemicals, Phytopathogens, and Bajra Crops

Sources Fungal phytopathogens *A. niger* and *F. oxysporum* were obtained from the National Collection of Industrial Microorganisms (NCIM) in Pune, India. Bajra cultivars (APFB-2 variety) used in the experiments were sourced from commercial vendors in the YSR Kadapa district. All media and chemicals were obtained from HiMedia Laboratories, Mumbai, India. The chemicals and reagents used in this study were of analytical grade and were used without any further purification.

Isolation of Potential PGPMs from Bajra Crop Fields

The isolation of potential PGPMs from Bajra crop fields involved conducting a survey of various bajra fields in YSR-Kadapa, Andhra Pradesh, with the aim of assessing the presence of plant growth-promoting microorganisms (PGPMs) among different bajra cultivars. Soil samples, each weighing approximately 100 g, were collected using a Random Block Design (RBD) from both the rhizosphere and non-rhizosphere zones of bajra fields at a depth of 15 cm across multiple locations, including the Lingala and Kamalapuram areas of the YSR-Kadapa district. These samples were then combined to form composite samples for storage and subsequent analysis. Additionally, physicochemical parameters were measured to characterize the abiotic conditions prevailing at the sampled sites, as outlined in Table 1.

A 10 g soil suspension (prepared by suspending 10 g of soil in 100 ml of double-distilled water and stirring it at 250 rpm for 2 hours) was subjected to serial dilution, and the resulting dilutions were used to inoculate various culture media, including Nutrient agar medium [NAM], Pikovskaya's agar, and King's B agar. For NAM, 10 grams of peptone, 5 grams of beef extract, 15 grams of agar, and optionally 10 grams of sodium chloride were dissolved in 1000 ml of distilled water. The pH was adjusted to around 7.0, and the mixture was then autoclaved at 121°C for 15 minutes to sterilize it. Pikovskaya's agar was prepared by dissolving 10 grams of soluble starch, 0.5 grams of calcium carbonate, 0.5 grams of potassium dihydrogen phosphate, 0.2 grams of magnesium sulfate, 0.1 grams of ammonium sulfate, and 0.001 grams of manganese sulfate in 1000 ml of distilled water. The pH was adjusted to 7.0, and the medium was autoclaved at 121°C for 15 minutes to sterilize it. King's B agar was prepared by dissolving 20 grams of proteose peptone, 10 grams of glycerol, and 1.5 grams of K_2HPO_4 in 1000 ml of distilled water. The pH was adjusted to 7.0 using 1 M NaOH or 1 M HCl, and the medium was autoclaved at 121°C for 15 minutes to sterilize it. The microbial populations obtained from these media were subsequently assessed for plant growth-promoting traits, such as PO_4 solubilization, HCN production, IAA production, NH_3 production, as well as hydrolytic enzymes, including Cellulase, Pectinase, and Chitinase. Standard protocols, as outlined in the Supplementary Information, were followed for the assessment of these traits. Colonies exhibiting positive results were sub-cultured for further analysis to obtain pure cultures.

Selection of Dominant PGPMs

The selection of dominant PGPMs commenced with evaluating their antagonistic activity against selected pathogens of the bajra crop, namely *A. niger* and *F. oxysporum*, through in vitro assessments utilizing the standard dual culture method. Isolates demonstrating significant inhibition against these pathogens were subsequently subjected to qualitative evaluation for various plant growth-promoting traits. Those isolates exhibiting substantial antagonistic activity coupled with positive plant growth promotion traits were identified as dominant PGPMs.

Development of PGPM Consortium

The development of the PGPM Consortium involved several steps. First, the dominant isolates were evaluated for compatibility using a modified dual-culture technique. Each isolate underwent a 24-hour growth period at 30°C in nutrient broth (for Bacillus and Acinetobacter isolates) and King's B medium (for Pseudomonas isolates). Subsequently, each isolate was streaked onto nutrient agar plates alongside three other isolates to assess compatibility. Isolates showing no antagonism towards proximal isolates were deemed compatible and grouped accordingly into the PGPMs consortium.

Growth interactions within Group 1 and Group 2 were examined following the methods outlined by Shanmugam et al. (16). Culture filtrates devoid of bacteria were obtained after 72 hours and added to fresh sterile nutrient broth. To this, 0.5 ml of compatible isolate was added, and the mixture was incubated at 150 rpm and 30°C for 24 hours. Growth was measured as optical density at 610 nm using a spectrophotometer against a blank and control (without broth filtrate). The developed consortia then underwent further assays to assess their potential for plant growth promotion and biological control. All experiments were conducted in triplicates, and the data are presented as mean values.

In Vitro Seed Germination Promotion Assay

In the *in vitro* seed germination promotion assay, the potential of PGPM consortia G1 and G2 to promote seed germination was evaluated. Bajra seeds of the APFB-2 variety were first surface sterilized using $HgCl_2$ (0.02% w/v) and then washed thrice to eliminate any residual $HgCl_2$. Subsequently, the seeds were immersed in the PGPM consortia suspended in potassium phosphate buffer containing 20 mM glucose and 0.5% CMC, with an approximate bacterial density of 1×10^6cfu/ml. After immersion, the seeds were air-dried and placed in a germination chamber with a 16-hour cycle of light and dark for seven days. During the incubation period, the percentage of germination and the induction of roots were recorded for both treatments, as well as for the control group.

Statistical Analysis

The experiment was conducted in triplicates, and the data presented represent the mean values along with the standard deviation.

RESULTS AND DISCUSSION

Isolation of Potential PGPMs from Bajra Crop Fields

In the isolation process of potential PGPMs from bajra crop fields, cultivable microbial populations from composite samples (both rhizosphere and non-rhizosphere) were grown (Figure 1).

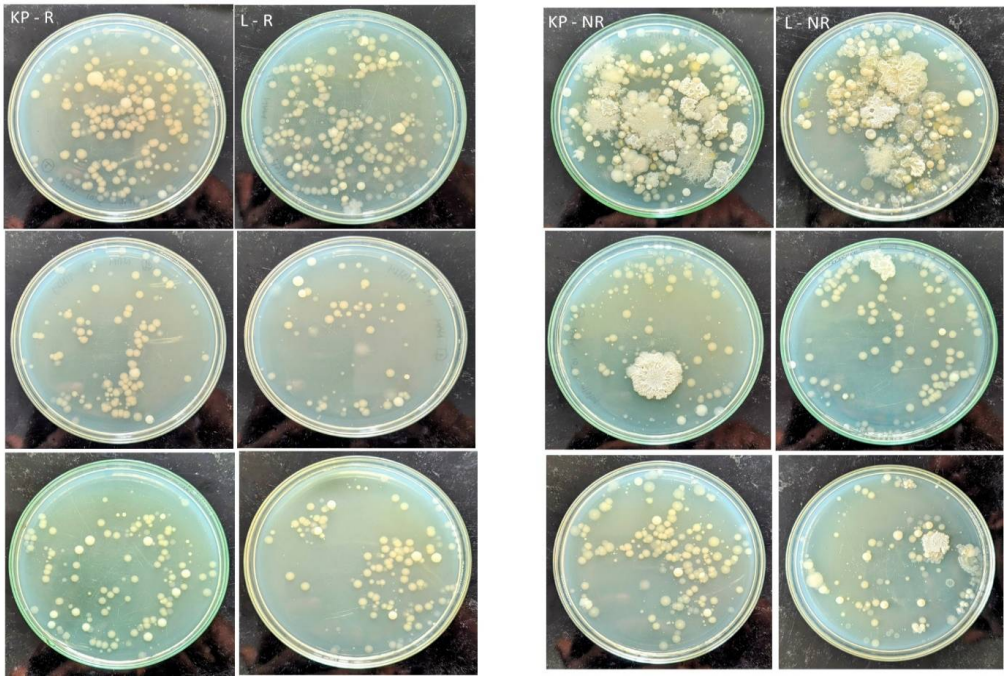

Figure 1. Isolated bacterial colonies from Bajra Crop Fields (KP-R = Kamalapuram Area Rhizosphere; KP-NR = Kamalapuram Area Non-Rhizosphere; L-R = Lingala Area Rhizosphere; L-NR = Lingala Area Non-Rhizosphere).

Initially, 36 different colonies were identified and marked for further screening regarding plant growth promotion traits. Subsequently, isolates demonstrating multiple PGPMs traits such as plant hormone production (IAA), antibiosis (HCN), and nutrient solubilization (P) were selected. These selected isolates were named as KP-1 to KP-7 and L-1 to L-10, and further assays were conducted to identify the dominant PGPMs.

Selection of Dominant PGPRs

The selection of dominant PGPRs was based on thorough screening of potential isolates for their abilities to promote plant growth and provide protection against fungal pathogens in vitro. From this screening, isolates demonstrating significant plant growth promotion traits (Table 2) while also effectively suppressing fungal pathogens were identified. These selected isolates, namely KP-1, KP-3, KP-4, KP-7, L-2, L-3, L-5, L-6, and L-9, were deemed promising candidates and thus subjected to further characterization studies. Table 3 provides detailed information regarding the morphology, Gram stain results, and IMViC test outcomes of these potent isolates. Notably, all of these isolates were sourced from the rhizosphere of bajra crops, indicating their close association with the plant root environment. This observation underscores the significance of rhizosphere microorganisms in influencing plant health and productivity.

The selection of these dominant PGPRs holds considerable promise for agricultural applications, as they exhibit dual functionality by enhancing plant growth and offering protection against fungal pathogens (2). Further characterization studies will enable a deeper understanding of their mechanisms of action and potential applications in sustainable agriculture practices. Additionally, the identification of these potent isolates contributes to the ongoing efforts aimed at harnessing the beneficial interactions between microorganisms and plants to optimize crop yields and mitigate disease incidence in agricultural systems.

Table 1. Physiochemical properties of soil used in this study (Values are the means of triplicates and are represented as means ± S.E).

Sample site Area	Lingala areas of Kadapa District		Kamalapuram areas of Kadapa District	
Parameter	R	NR	R	NR
Sand (%)	41 ± 2.7	47 ± 4.6	34 ± 3.1	45 ± 3.43
Silt (%)	34 ± 3.1	38 ± 1.7	42 ± 5.3	36 ± 2.45
Clay (%)	26 ± 2.4	16 ± 2.3	25 ± 2.1	19 ± 1.55
pH	7.9 ± 0.23 (SA)	8.2 ± 0.15 (MA)	7.51 ± 0.5 (SA)	7.72 ± 0.6 (SA)
Water holding capacity (ml/ g^{-1} soil)	0.84 ± 0.02	1.21 ± 0.01	1.27 ± 0.03	1.43 ± 0.03
Electrical conductivity	1.132 ± 0.001 (N)	1.321 ± 0.04 (N)	0.453 ± 0.07 (N)	0.951 ± 0.005 (N)
Organic matter (%)	2.32 ± 0.02 (H)	1.84 ± 0.02 (H)	1.92 ± 0.03 (H)	1.45 ± 0.07(H)
Available N (kg/ha)	181 ± 4.2 (M)	164 ± 1.5 (M)	236 ± 11.4 (M)	205 ± 4(M)
Available P (kg/ha)	764 ± 12.7 (H)	643 ± 5.4 (H)	423 ± 16.54 (H)	380 ± 5.1 (H)
Available S (PPM)	1.54 ± 0.01 (D)	1.36 ± 0.04 (D)	1.21 ± 0.03 (D)	0.91 ± 0.03 (D)
Ca Cmol (P^+)/kg	6.9 ± 0.3 (D)	6.3 ± 0.4 (D)	7.2 ± 0.2 (D)	7.8 ± 0.1 (D)
Mg Cmol (P^+)/kg	14.1 ± 0.4 (S)	9.1 ± 0.3 (D)	12.4 ± 0.5 (S)	9.4 ± 0.2 (D)
Zn ppm	4.0 ± 0.2 (S)	1.83 ± 0.4 (S)	3.5 ± 0.2 (S)	2.14 ± 0.3 (S)
Fe ppm	32.0 ± 0.4 (S)	27.5 ± 1.7 (S)	14.6 ± 0.2 (S)	09.3 ± 0.1 (D)
Cu ppm	1.7 ± 0.1 (S)	2.7 ± 0.4 (S)	1.65 ± 0.2 (S)	2.4 ± 0.1 (S)
Mn ppm	8.1 ± 0.21 (S)	6.9 ± 0.15 (S)	9.5 ± 0.4 (S)	8.1 ± 0.3 (S)

A: Acidic	SA: Slightly Alkaline	MA: Moderately Alkaline	HA: Highly Alkaline	N: Neutral	N: Normal
L: Low	M: Medium	H: High	S: Sufficient	D: Deficient	
(the values in parenthesis are Standard Deviation of mean triplicates).					

Table 2. The plant growth promoting characteristics displayed by the potent isolates (in vitro assays).

Isolate	PGPM traits									
	PO_4 Solubilisation	HCN Production	NH_3 Production	IAA Production	Hydrolytic enzyme production (Zone of substrate hydrolysis in mm)			Induction of germination (% higher than control)	Antagonism against fungal pathogens (Inhibition zone mm)	
					Cellulase	Pectinase	Chitinase		*A. niger*	*F. oxysporum*
KP-1	-	+	+	+	18±1.042	19±0.913	6±0.034	14±0.839	13±1.681	17±2.022
KP-3	+	+	+	-	20±0.402	21±1.051	8±1.021	16±1.025	8±2.412	16±1.021
KP-4	+	+	+	+	-	14±1.869	16±0.216	10±1.029	6±0.359	8±2.048
KP-7	+	+	-	-	10±0.056	-	5±1.011	8±1.028	12±0.492	15±0.892
L-2	-	-	+	+	-	20±2.914	4±0.052	16±0.937	17±0.621	14±1.235
L-3	+	-	+	+	16±1.084	-	10±0.085	10±1.982	10±1.263	19±2.048
L-5	+	+	+	-	-	15±2.014	14±1.432	8±2.074	12±0.651	13±1.870
L-6	+	+	-	-	22±1.065	-	8±1.036	18±1.089	14±0.927	20±1.853
L-9	-	+	-	+	17±2.024	-	10±2.471	14±2.091	10±2.061	8±1.051

-: negative/no activity detected; +: positive; Values presented here are the mean of triplicate observation

According to Table 2, the isolates demonstrate a diverse array of plant growth-promoting traits, which include phosphate solubilization, production of beneficial metabolites such as HCN, NH3, and IAA, secretion of hydrolytic enzymes, promotion of seed germination, and antagonism against fungal pathogens. These multifaceted traits collectively play pivotal roles in enhancing plant growth, facilitating nutrient uptake, and providing protection against pathogens. Phosphate solubilization enhances the availability of phosphorus to plants, an essential nutrient required for various physiological processes. Production of metabolites like HCN, NH_3, and IAA positively influences plant growth and development by stimulating root elongation, improving nutrient acquisition efficiency, and enhancing overall plant vigor (11).

The secretion of hydrolytic enzymes, including cellulases, pectinases, and chitinases, aids in organic matter decomposition, releasing essential nutrients for plant uptake and promoting soil fertility (13). Moreover, the ability to promote seed germination and antagonize fungal pathogens further highlights the potential of these isolates as effective agents for agricultural applications (3). By promoting seed germination, these isolates facilitate early plant establishment, while their antagonistic activity against fungal pathogens helps in disease suppression, ultimately leading to improved crop yields (9).

Table 3. Morphology, Gram stain, and IMViC test results of the potent isolates.

Isolate	Morphology & arrangement	Gram's reaction	IMViC tests				Isolate may be identified as	Molecular characterisation
			Indole	Methyl red	Vogues proskuer	Citrate utilisation		
KP-1	spiral-shaped or helical	-	-	-	-	+	*Azospirillum* sp.	Under process
KP-3	rod shaped	-	-	-	-	+	*Pseudomonas* sp.	
KP-4	spherical and rod shaped	-	-	-	-	+	*Acinetobacter* sp.	
KP-7	Rod shaped	+	-	+	-	+	*Bacillus* sp.	
L-2	filamentous	+	-	+	-	+	*Streptomyces* sp.	
L-3	Rod & single/chains	-	+	+	-	+	*Enterobacter* sp.	
L-5	Rod shpaed	+	-	-	-	-	*Lactobacillus* sp.	
L-6	Rod & chains	-	-	-	-	+	*Pseudomonas* sp.	
L-9	Slender rods & chains	+	-	+	-	+	*Bacillus* sp.	

The combination of these plant growth-promoting traits makes these isolates promising candidates for utilization as PGPMs in agricultural practices (4; 18). Their application has the potential to enhance crop productivity, improve nutrient utilization efficiency, and reduce reliance on chemical inputs, thereby contributing to sustainable and environmentally friendly agricultural systems.The findings depicted in Fig. 2 demonstrate a significant positive influence of PGPMs consortiums (C) and (D) on both germination and root development, in contrast to the control (A) and organic plant growth promoter (liquid fertilizer) (B). Germination, the initial stage of plant growth, and subsequent root development are pivotal processes in plant establishment and productivity. The observed enhancement in these parameters under the influence of PGPM consortiums underscores the potential of these microbial communities in promoting plant growth and development. These results suggest that the application of PGPM consortiums (C) and (D) may offer a promising avenue for sustainable agricultural practices, providing an alternative or complementary approach to traditional fertilization methods. Further investigation into the specific mechanisms by which PGPM consortiums facilitate germination and root development is warranted, offering insights into their broader applicability in enhancing crop yields and resilience to environmental stresses.

Figure 2. The germination and root development influenced by the PGPM consortiums (C) and (D) contrast with the Control (A) and Organic Plant Growth Promoter (Liquid Fertilizer) (B).

SUMMARY AND CONCLUSION

The study successfully isolated plant growth-promoting microorganisms from the rhizosphere soils of bajra crops, revealing their promising potential for agricultural applications in enhancing plant growth and combatting fungal pathogens. The investigation highlights the effectiveness of employing PGPMs as early control strategies to mitigate the impact of *A. niger* and *F. oxysporum* on Bajra cultivation in YSR Kadapa District. These findings underscore the importance of harnessing the beneficial properties of PGPMs in sustainable agricultural practices. Additionally, the analysis demonstrates the remarkable efficacy of a PGPMs consortium in significantly enhancing plant growth while effectively combating fungal pathogens, highlighting the potential of PGPMs consortia as a viable solution for improving crop productivity and resilience in agricultural systems. The study presents a significant advancement in understanding the role of PGPMs in agricultural systems. The findings emphasize the potential of PGPMs as effective tools for promoting plant health and combating fungal pathogens in bajra cultivation. Ongoing research endeavors will focus on identifying Plant Growth-Promoting Microorganisms (PGPMs) using advanced molecular techniques and conducting field studies to evaluate their impact on crop health, yield enhancement, and soil sustainability. These investigations will provide valuable insights into the mechanisms underlying the beneficial effects of PGPMs, thereby facilitating the development of sustainable agricultural practices aimed at enhancing food security and environmental sustainability.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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