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Research Article

PLANT GROWTH PROMOTION OF CHILLI (*Capsicum annum* L.) USING ACC DEAMINASE POSITIVE PGPR ISOLATES

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Abstract- Use of plant growth promoting rhizobacteria (PGPR) is recently being studied for their ability to enhance growth of various plant species. Native bacteria with multiple PGPR attributes and ACC deaminase activity could be added advantage for making an isolate more potential PGPR. Five isolates with multiple PGPR traits and ACC deaminase activity were evaluated for their ability to show increase in plant growth. Results indicate that isolates IS-7 and IS-74 identified as *Bacillus halotolerans* (Accession No. OR593309) and *Enterobacter hormaechei* respectively were efficient compared to control plants and other isolates. Also, ability of these isolates in accumulation of total soluble sugars, proline and increased relative water content (RWC) of chilli indicate that they could be potential agents for alleviation of stress conditions.

Keywords- *Capsicum annum, PGPR, ACC deaminase, Bacillus halotolerans, Enterobacter hormaechei*

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Introduction

Change in climatic conditions, growing population, and decrease in land for agriculture is a major challenge to food demand and supply and impacts the economies of countries around the globe. According to an estimate, there is a 51-82% crop yield loss across the globe due to abiotic stresses, majorly with staple foods, cereals, vegetables, and fruits [1]. To meet food requirements across the world, the farmers and stakeholders of countries are encouraged to extensive application of chemical fertilizers and pesticides. As every coin has two faces, we are at saturation face in the use of such application which results in toxic effects on land, water, and air. Environmental protection agencies laud at all countries for encouraging sustainable agriculture to keep the globe viable for a long time.

According to research sources, there are viable methodologies designed by researchers for sustainable, toxic-free agriculture. The best one is the use of plant root-associated microorganisms as growth promoters because they can release phytohormones, (Auxins, Gibberlic acid, Cytokinins), fix nitrogen, solubilize phosphate, other metals, induce systemic resistance (ISR) and modulate ethylene production [2-3], ACC-Deaminase production [4]. Hence, they are referred to as plant growth promoters (PGP). The bacterial species reported to be associated with PGP include *Enterobacter, Klebsiella, Arthrobacter, Acinetobacter, Frankia, Mesorhizobium, Phyllobactum, Azotobacter, Azospirillum, Rhizobium, Bacillus, Pseudomonas, Xanthomonas, Bradyrhizobium, Streptomyces, Serratia, Thiobacillus, Flavobacterium, and Clostridium, Burkholderia* [5-6].

Chili, originally cultivated in South America, notably in Mexico around 6000 BCE, experienced widespread dissemination during the colonial era, becoming a favoured ingredient globally. This culinary journey was propelled by the peppers appealing taste, high vitamin C content, and antioxidant properties. Thriving in warm to hot climates with temperatures ranging from 21 to 32 degrees celsius, chilli peppers prefer sandy loam or loamy soils. Typically grown in rain-fed conditions, insufficient moisture levels can adversely affect both crop yield and quality. This experimental study has explored the potential benefits of residential Plant Growth-Promoting Rhizobacteria (PGPR) in enhancing chilli growth.

Material and Methods

Collection and Physicochemical analysis of soil

The soil samples were collected from chilli rhizosphere from five different places of Jadcherla Taluka, Mahabubnagar district of Telangana State, India. The soil sample are analysed for pH, EC and chemical properties by using WD–XRF (Axios- Mos Panalytical) at National Mineral Development Corporation, Hyderabad.

Isolation of pure cultures and analysis of biochemical properties

The selected soils samples serially diluted and a 10^{-7} dilution was taken and plated on to the nutrient agar plates and incubated at 32-35°C / 24 hrs. Colony counts were expressed in CFU/gram of soil [7]. The selected individual colony from each plate was inoculated on Congo-red YEMA media, Jensen's media and Kings-B media for obtaining pure cultures and subsequently subjected for preservation at 4°C. The preserved pure cultures were analysed for staining (Gram's) nature and other biochemical properties following standard procedures as per bergey's manual systematic bacteriology [8].

Determination of PGP properties

Plant growth promotion abilities associated with selected bacterial isolates were determined by various standard protocols. Indole acetic acid (IAA) was estimated by salkowsky method [9], siderophore activity detected by orange halo zone around the isolate colony on Chrom azurol S media (CAS) media [10], Phosphate solubilisation was measured using Pikovskaya agar medium [11-12], Gibberellic acid is estimated by method developed by Graham and Thomas (1961) [13], HCN production is estimated by Lorck method [14], nitrogen fixation can be assessed by growing isolates in nitrogen free medium and ACC deaminase Production assed by using Dworkin and Foster (DF) salt minimal medium with ACC as a sole Nitrogen source [15].

Table-1 Chemical Characteristics of the collected soil samples

SN	Soil No.	Latitude	Longitude	pH	EC (dS/m)	N (g/100g)	P%	K%	Na%	Cl%	Fe%	Cu%	Zn%	Mn%	Mg%
1	Avancha	16.713200"N	78.247365"E	7.87	0.2	0.046	0.109	4.734	1.474	0.018	10.001	0.011	0.016	0.090	0.385
2	BRR Degree college	16.764298"N	78.146078"E	7.66	0.27	0.068	0.105	5.784	1.237	0.021	7.242	0.011	0.012	0.083	0.380
3	Macharam	16.783334"N	78.136457"E	8.05	0.22	0.087	0.221	6.106	1.594	0.027	6.322	0.110	0.014	0.097	0.326
4	Rangareddyguda	16.899162"N	78.176492"E	7.85	0.23	0.026	0.076	4.98	1.678	0.076	7.389	0.019	0.008	0.068	0.297
5	Yasayakunta Thanda	16.742461"N	78.115973"E	7.79	0.21	0.112	0.12	5.877	1.405	0.017	11.237	0.016	0.016	0.078	0.564

Table-2 Biochemical characteristics of selected isolates from three different media Capsicum annum

SN	Area	Viable count (10^4 cfu/g)	Media	Isolates No.	Colony morphology	Gram staining	Indole	Methyl red	Voges proskauer	Citrate	Catalase	Urease	Oxidase	H_2S	Gelatin Hydrolysis
1	Avancha	48.4	YEMA	IS-7	Pale yellow	+	--	--	--	--	+	--	+	+	--
			Jensens	IS-8	Viscous, milky	--	+	+	--	--	+	--	+	--	+
			King's B	IS-9	White &medium turns green	--	--	--	--	+	+	--	+	--	+
2	BRR Degree college	87.9	YEMA	IS-13	Convex, White gummy	--	+	+	+	--	+	--	+	--	--
			Jensens	IS-14	Opaque, white colour	--	+	+	--	--	+	--	+	--	+
			King's B	IS-15	White &medium turns green	--	--	--	--	+	+	--	+	--	+
3	Macharam	49.6	YEMA	IS-43	Mucilaginous, gummy	--	+	+	--	--	+	--	+	--	--
			Jensens	IS-44	Gummy, dull to cream white	--	+	+	--	--	+	--	+	--	+
			King's B	IS-45	White &medium turns green	--	--	--	--	+	+	--	+	--	+
4	Rangareddyguda	54.9	YEMA	IS-73	Mucilaginous, gummy	--	+	+	+	--	+	--	+	--	--
			Jensens	IS-74	Gummy, dull to cream white	--	+	+	+	+	+	--	--	--	--
			King's B	IS-75	Creamy, off white	--	--	--	--	+	+	--	+	--	+
5	Yasayakunta Thanda	95.4	YEMA	IS-85	Mucilaginous, gummy	--	+	+	+	--	+	--	+	--	--
			Jensens	IS-86	Gummy, dull to cream white	--	+	+	--	--	+	--	+	--	+
			King's B	IS-87	White &medium turns green	--	--	--	--	+	+	--	+	--	+

Table-3 Characterization of PGPR traits of selected isolates from Capsicum annum. L

SN	Isolates No.	IAA	Siderophore	N_2-Fixation	P' solubilization	HCN	GA	ACC deaminase
1	IS-7	+	+	--	+	+	--	+
2	IS-8	--	+	--	--	--	--	+
3	IS-9	+	+	--	+	--	--	--
4	IS-13	--	--	--	--	--	--	--
5	IS-14	--	--	+	--	--	--	--
6	IS-15	--	--	--	--	--	--	--
7	IS-43	+	+	--	+	--	--	+
8	IS-44	--	--	+	--	--	--	--
9	IS-45	+	+	--	+	--	--	+
10	IS-73	+	+	--	+	--	--	+
11	IS-74	+	+	+	+	-	--	+
12	IS-75	+	+	--	--	--	--	--
13	IS-85	+	--	--	--	--	--	--
14	IS-86	--	--	+	--	--	--	--
15	IS-87	--	--	--	--	--	--	+

Table-4 Effect of selected PGPR isolates for plant growth promotion in Capsicum annum. L (40DAS)

SN	Isolate No	No. of Leaves (Plt^{-1})	Leaf area (mm^2)	Shoot height (mm)	Root length (mm)	Shoot Wt. Wgt (gm)	Shoot Dry Wt.(gm)	Root Wt Wgt (gm)	Root Dry Wgt (gm)	Root Volume (cm3)	Root Shoot Ratio
1	IS-7	43.94^{a}	7426.22^{a}	50.46^{a}	17.62^{a}	70.34^{a}	27.42^{a}	10.69^{ab}	4.96^{a}	1.219^{b}	$0.18(25.1)^{a}$
2	IS-43	41.67^{a}	6527.45^{a}	48.28^{a}	16.26^{a}	62.13^{c}	26.41^{bc}	10.28^{ab}	4.51^{b}	1.201^{c}	$0.17(24.35)^{ab}$
3	IS-45	40.33^{a}	6356.72^{a}	47.73^{a}	15.82^{a}	61.36^{bc}	26.15^{c}	10.19^{b}	3.96^{c}	1.218^{b}	$0.16(23.57)^{bc}$
4	IS-73	42.61^{a}	7254.86^{a}	48.55^{a}	16.37^{a}	61.73^{bc}	26.34^{bc}	10.36^{ab}	4.27^{bc}	1.219^{b}	$0.17(24.35)^{ab}$
5	IS-74	45.69^{a}	8104.84^{a}	49.84^{a}	16.42^{a}	64.86^{b}	26.88^{b}	11.46^{a}	4.22^{bc}	1.246^{a}	$0.15(22.79)^{cd}$
6	Control	28.22^{b}	3046.44^{b}	29.64^{b}	11.64^{b}	60.82^{c}	26.72^{b}	7.92^{c}	3.86^{c}	1.228^{ab}	$0.14(21.97)^{d}$
7	C.D at 0.05	6.28	1793.55	7.99	2.08	3.63	0.47	1.20	0.40	0.016	1.16
8	C.D at 0.01	8.69	2480.33	11.06	2.88	5.02	0.64	1.65	0.56	0.023	1.61

Table-5 Effect of selected PGPR isolates on biochemical parameters in Capsicum annum. L(40DAS)

SN	Isolate No.	Chlorophyll A (mg g^{-1} FW)	Chlorophyll B (mg g-1 FW)	Total chlorophyll (mg g-1 FW)	Total soluble carbohydrates (mg g-1 DW)	Relative water content (RWC)	Total Proline (mMg^{-1})
1	IS-7	19.84^{a}	1.96^{ab}	52.64^{b}	0.42^{a}	90.46^{a}	4.28^{a}
2	IS-43	18.61^{a}	1.63^{cd}	51.59^{bc}	0.36^{b}	89.36^{bc}	4.16^{bc}
3	IS-45	18.33^{a}	1.59^{d}	51.46^{bc}	0.31^{b}	89.21^{c}	4.09^{c}
4	IS-73	19.17^{a}	1.78^{bc}	52.39^{b}	0.35^{bc}	89.46^{bc}	4.13^{bc}
5	IS-74	21.62^{a}	2.23^{a}	54.44^{a}	0.39^{ab}	89.88^{ab}	4.24^{ab}
6	Control	8.96^{b}	1.49^{d}	50.62^{c}	0.34^{c}	88.68^{bc}	3.96^{d}
7	C.D at 0.05	4.49	0.28	1.32	0.04	0.61	0.11
8	C.D at 0.01	6.20	0.38	1.83	0.05	0.84	0.16

Pot Experiment

Chilli seeds were subjected to sterilization process involving a one-minute exposure to 75% ethyl alcohol, succeeded by a 30-second treatment with 1% sodium hypochlorite, followed by thorough washing in sterile water (5-6 times). Subsequently, selected bacterial isolates are cultured in a nutrient broth to attain a bacterial broth concentration of 5 x 10^6 CFU/gram. The sterilized seeds were incubated for 20-minute in the bacterial broth and 1% CMC, followed by air-drying. Finally, these treated seeds are planted in pots containing sterile soil [16].

Measurement of morphological characters

Plants were carefully excavated after 40 days of sowing. Standard processes were employed for measuring morphological traits, including the number of leaves, leaf area, shoot length, root length, leaf count, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, and root morphology. For obtaining dry weights, harvested material was carefully placed in a hot air oven (70°C, 48 hr) until constant weight is obtained.

Estimation of Biochemical Parameters

Estimation of Chlorophyll

One gram of fresh chilli leaves was carefully cut into small discs and placed in a test tube containing 5 mL of dimethyl sulfoxide (DMSO). Following an incubation period, absorbance was measured using a spectrophotometer at wavelengths 645 nm and 663 nm, with DMSO serving as the blank [17].

Chlorophyll content was subsequently performed using specific formulas

Chlorophyll a (mg/g) = (12.7 × A663) - (2.59 × A645)

Chlorophyll b (mg/g) = (22.9 × A645) - (4.7 × A663)

Total chlorophyll (mg/g) = (8.2 × A663) + (20.2 × A645)

Relative water content (RWC)

For the assessment of physiological parameters, such as relative water content (RWC), one gram of finely cut fresh leaves were immersed in 25 mL of distilled water for a 6-hour period in a clean Petri plate. Following gentle blotting and weighing, the leaves were oven-dried at 70°C for 24 hours, and the final weight was recorded [18].

RWC= (Fresh Weight - Dry Weight) / (Saturated Weight - Dry Weight) × 100.

Soluble carbohydrates

One gram of fresh leaves was homogenized in a mixture of ethanol, chloroform, and water (60:25:15 v/v), followed by incubation and centrifugation. The supernatant was adjusted, combined with phenol and sulphuric acid, and soluble carbohydrates were determined against a glucose standard curve at 490 nm, according to the methodology given by Maness [19].

Total Proline

Total proline content was evaluated by homogenizing one gram of fresh leaves in 3% sulfosalicylic acid, followed by centrifugation. The resulting supernatant was treated with Ninhydrin and glacial acetic acid, incubated, and then assessed at 520 nm against a proline standard curve [20].

Statistical Analysis

All experiments were repeated twice with at least three replicates for each treatment. The data pertaining was subjected to statistical analysis using Fischer's one-way ANOVA at P> 0.05. Values in the data were compared with each other using LSD at p< 0.05.

Results and discussion

Five rhizosphere soil samples were carefully collected and subjected to a comprehensive analysis of their physiochemical properties, as delineated in [Table-1]. The observed pH levels, ranging from 7.66 to 8.05, unequivocally indicate an alkaline nature of the soils. Impressively, the electrical conductivity (EC) values, spanning from 0.20 to 0.27, signify an optimal soil quality conducive to crop cultivation. The mineral profiling has revealed percentages within the normal range, further affirming the soil's suitability for agricultural purposes.

Cultivation efforts on CR-YEMA media, Jensen's media, and Kings-B media yielded a diverse array of 15 bacterial isolates. Notably, Gram-positive characteristics were exhibited by a singular isolate, while the remaining isolates displayed Gram-negative nature. Subsequent characterization efforts, as elucidated in [Table-2], paved the way for a focused investigation into the plant growth promotion abilities of these isolates.

The five isolates (IS-7, IS-43, IS-45, IS-73, and IS-74) exhibited the remarkable ability to produce ACC deaminase, along with other PGP traits as shown in [Table-3] setting them apart. These isolates were chosen for an in-depth exploration of their impact on chilli plant growth. Isolates with more PGPR traits was taken as criterion for selection of isolates. In addition to this ability of isolate to produce ACC deaminase will be added advantage for alleviation of stress conditions [21].

A meticulous 40-day experiment ensued, wherein the selected PGP-capable organisms were introduced to chilli plants, alongside a control group. The resulting morphological and biochemical assessments, detailed in [Table-4] and [Table-5], revealed substantial growth promotion in plants exposed to the bacterial isolates and this was in correlation to the findings of Hyder *et al.* [22]. Inoculation benefits of PGPR were reported well recently in chilli. It was reported that PGPR could lower the usage of nitrogen fertilizer by 25% [23]. Significant increase in number of leaves, area, shoot and root fresh and dry weights root volume and root shoot ratio was observed compared to un inoculated control plants. Increase in number of fruits, fruit weight and yield in chilli plants were reported using native Bacillus and Streptomyces species in field conditions [24]. Notably, IS-7 and IS-74 are found more efficient compared to control plants and rest of the isolates. These findings were particularly significant across all parameters, indicating the potential of these isolates in fostering plant development.

[Table-5] further highlighted the enhanced chlorophyll content (a, b, and total), total soluble carbohydrates, relative water content, and total proline in plants treated with the PGP bacteria. This compelling dataset unequivocally points towards a pronounced growth promotion in chilli plants using *Bacillus sonorensis* a novel PGPR [25]. The pure cultures of IS-7 and IS-74 subjected for genomic DNA isolation for 16s r RNA characterization and sequence data was obtained with the help of universal primers. The obtained sequences are subjected for BLAST analysis in NCBI website and established phylogenetic relationship IS-7 as *Bacillus halotolerans* (Accession No.OR593309) and IS-74 as *Enterobacter hormaechei* (Accession No. OR593312). Earlier research indicates that in chilli plants PGPR could induce resistance to anthracnose disease and *Phytopthora capsici* infestation using B. *amyloliquefaciens* and *Pseudomonas putida, P. libanensis, P. aeruginosa, B.subtilis B. megaterium, B. cereus* respectively [26,22]. The identified strains IS-7 (*Bacillus halotolerans*) and IS-74 (*Enterobacter hormaechei*) were observed to show significant increase in the chlorophylls, soluble carbohydrates, relative water content and proline compared to control plants and other isolates. Alleviation of salinity stress in wheat was recently being studied using *B. halotolerans* KKD1 indicating physiological and metabolic enhancement [27]. Similarly, enhanced yield was observed due to *Enterobacter hormaechei* (MF957335) in tomato subjected to salinity stress [28]. In the present study, enhanced parameters in [Table-5] due to IS-7 and IS-74 indicate possible intervention of these isolates for alleviation of stress conditions.

Conclusion

Plant growth promotion was found clearly enhanced using the isolates tested. Presence of multiple PGPR traits might be the reason for enhanced performance. Isolates IS-7 and IS-74 are observed to be promising agents for plant growth promotion. Presence of ACC deamiase activity could be the added advantage for these to alleviate stress conditions as evidenced from increased proline, RWC and total soluble sugars. Further evaluation of these isolates for identifying their capability to withstand stress conditions is needed. Also, dual inoculation could provide a better understanding of their possible applications in agriculture. In conclusion, it can be inferred that both the isolates IS-7 and IS-74 could be regarded as potential agents for pant growth promotion of chilli.

Application of research: Evaluated isolates can be used as inoculants for plant growth promotion in chilli plants

Research Category: Agricultural microbiology

Abbreviations: PGPR-Plant growth promoting rhizobacteria, ACC-1-amino cyclopropane-1-carboxylate

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Study area / Sample Collection: Palamuru University, Mahabubnagar, 509001

Cultivar / Variety / Breed name: *Capsicum annum* L.

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