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Regulating Cotton Growth via *Rhizobium* Species

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Abstract

Unpredictable precipitation is a common problem for plant growth in India's Deccan plateau, which is known for its poor soil and frequent droughts. Critical to the regulation of plant diseases and the enhancement of plant growth are root-colonizing rhizobacteria like *Rhizobium*. Isolating productive *Rhizobium* species from soil around the Eturnagram region's cotton rhizosphere was the goal of a study carried out at Palamuru University. *Rhizobium* variant-5, currently known as *Rhizobium* sp. PKS [NCBI-OK663003, NCMR-MCC4960], was one of five different strains of *Rhizobium* isolated using the top layer method. It showed strong support for the growth of six different cotton cultivars. Out of the six cotton varieties tested, the Mahyco cultivar had the lowest proline levels while having higher amounts of IAA, proteins, chlorophyll, and sugars. The effectiveness of Mahyco was confirmed by experimental field testing conducted in four distinct cotton agricultural soils of Mahabubnagar District using *Rhizobium* sp. PKS [NCBI-OK663003, NCMR-MCC4960]. Deep black soil showed improved phytohormone synthesis and good biochemical alterations, whereas shallow black soil showed that the strains considerably enhanced plant development. Based on these results, the novel *Rhizobium* sp. PKS could be used as a bioinoculant in cotton fields on the Deccan plateau, which could improve agricultural yields despite the harsh conditions.

Keywords: Mahyco Cultivar, IAA (Indole acetic acid), Phytohormone, Bioinoculant

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INTRODUCTION

''Cotton, often called 'White Gold', is a very important economic crop for the country. An even distribution of nutrients is essential for its healthy development and growth. To reduce production costs and environmental concerns, microbial inoculants have the ability to replace mineral fertilizers, giving cotton a balanced diet.[1]

Cotton relies on nitrogen ''mini factories'' produced by microorganisms like *Rhizobium*, which cause nodule production. In symbiotic interactions with more than 20,000 members of the Fabaceae family, different *Rhizobium* species develop nodules on a variety of legumes.[2] This specificity is a hallmark of legume biology and is based on chemical signals exchanged between *Rhizobium* and the host legume. A large portion of the nitrogen in legumes comes from these mutualistic connections.

Beyond nitrogen fixation in legumes, *Rhizobium* injection has been used for more than a century in agricultural systems. Based on several studies,[3-6] *Rhizobium* can solubilize phosphorus through organic acid formation, synthesize growth hormones such as auxins and gibberellins, and operate as an antibiotic. "Research has shown that certain strains of *Rhizobium* can improve cotton roots' ability to absorb nutrients through the production of auxins.[7-9]

The rhizosphere is home to a varied range of bacteria that can influence plant growth in many ways.[10-12] In a direct manner, PGPR either facilitate nutrient intake or supply chemicals that promote growth. They fight phytopathogens indirectly. Some of the mechanisms involved have been studied in various studies.[13-18] These studies have focused on nitrogen fixation, modifying plant growth regulators, and phytopathogen antagonism through antibiotic and siderophore production.[5,19,20-22]

All state that rhizobia, in their PGPR form, can colonize non-legume plant rhizospheres, an essential first step in positive plant-bacteria interactions. Colonization improves plant growth[23] by facilitating nutrient uptake from the soil environment.[21,24] Nevertheless, owing to uneven crop responses, the effectiveness of microbial inoculants is typically only seen in minor production improvements under ideal conditions.[5]

Research shows that crops that are inoculated with rhizobia have higher nutritional levels compared to those that are not.[20] According to various studies,[2,5,20,22] the phosphorus solubilization capacity of Rhizobia enhances plant growth through nutrient mobilization, trace element availability, and the synthesis of growth-promoting compounds. In order to improve their adaptability in iron-deficient situations and their connections with both legume and non-legume plants, *Rhizobium* produce siderophores, which aid in the acquisition of iron, which is essential for bacterial metabolic processes.

A recently found *Rhizobium* species, *Rhizobium* sp. PKS, was the subject of this research investigation. It was isolated and studied from soil in the cotton rhizosphere in the Deccan plateau region. This research aimed to evaluate the biofertilizer potential by examining the plant growth-enhancing properties, specifically the capacity to fix nitrogen and boost plant growth. The study was conducted on four different types of cotton agricultural soils of Mahbubnagar District, which are typically associated with low plant growth potential.

MATERIALS AND METHODS

The trials were conducted at Palamuru University's Department of Microbiology, located in Mahabubnagar, within the state of Telangana, India.

Sample collection

The assessment of *Rhizobium* species began with the acquisition of cotton rhizosphere soil in the vicinity of Eturnagaram forest. The Top layer method was implemented for the isolation of *Rhizobium*. Soil underwent a series of dilutions, eventually leading to a 10^{-4} dilution that was cultured on Yeast Extract Mannitol Agar (YEMA) medium for subsequent analysis.

Preparation of standard inoculums of six variants of *Rhizobium*

The process involved in preparing the *Rhizobium* inoculants with seven isolates can be broken down into several steps.

1. **Broth culture preparation:** 150 ml of broth medium was inoculated into a 500 ml conical

flask. Incubated at 28°C under agitation at 100–150 rpm for three days until reaching an optical density of 0.5.

2. **Inoculation into Peat:** The broth culture of *Rhizobium* sp. was introduced into sterilized peat at a ratio of 100 ml per kg of peat. This peat-based *Rhizobium* inoculum was sterilized at 121°C and 15 psi pressure for one hour.
3. **Population Enhancement:** The *Rhizobium* inoculations containing roughly 10^8 MPN bacterial cells per gram of peat were used to coat cotton seeds. These coated seeds were then planted and maintained for a 60-day period in sterilized soil.

Preparation of sterilized soil

The soil preparation involved mixing sand and red soil in a 1:1 ratio, followed by thorough mixing to ensure proper integration. Subsequently, the mixture underwent sterilization in an autoclave at 121°C and 15 lbs pressure.

Genomic DNA extraction

The method for obtaining genomic DNA from bacterial cells was adapted from Sambrook.[25] Bacterial cells from pure culture were initially harvested by centrifugation (12,000 rpm for 2 minutes). The resulting cell pellets were mixed with 600 µl of lysis buffer [10 mM Tris–HCl, 1 mM EDTA (pH 7.5), 0.5% SDS, 100 g ml-1 proteinase C] and incubated at 37°C for 1 hour following the addition of 100 µl of 5 M NaCl and 80 µl CTAB/NaCl buffer. Subsequently, the samples were incubated at 65°C for 10 minutes, cooled to room temperature, and the aqueous phase was extracted with equal volumes of chloroform:isoamyl alcohol (24:11, v/v) and phenol:chloroform:isoamyl alcohol (25:24:1, v/v). This mixture underwent centrifugation at 12,000 rpm and 4°C for 10 minutes. Isopropanol (0.69) was then added to the aqueous phase, which was centrifuged at 12,000 rpm and 4°C for 10 minutes. Finally, the resulting DNA pellets were vacuum-dried and dissolved in Tris buffer [10 mM Tris–HCl, and 1 mM EDTA (pH 7.5)] for further use.

PCR analysis

Using 16S rRNA Universal primers, the small subunit rRNA gene of each sample's culture DNA was amplified during PCR analysis. Half a milliliter of bacterial DNA (about 200 ng), one microliter of Taq-DNA polymerase, five milliliters of Taq buffer, five milliliters of a 2 mM dNTP mix, five milliliters of forward primer (10 pM µl-1), and five milliliters of reverse primer (10 pM µl -1) made up the 50 µl PCR amplification reaction mixture. A 30-cycle amplification procedure was carried out in a Bio-Rad thermocycler. The denaturation, annealing, and extension phases of each cycle lasted 20–40 seconds at 94–48°C, respectively. The last extension was carried out for 5 minutes at 72°C after the 30 cycles were finished. The amplified DNA fragment that was produced, which was about 1,542 bp in size, was then purified using Qiagen spin columns after being separated on a 1% agarose gel.[26,27]

16S rRNA gene sequencing and phylogenetic tree construction

We used the Mo Bio microbial DNA isolation kit (Mo Bio Laboratories Inc., Solano Beach, CA, USA) to generate DNA for 16S rRNA gene sequencing, following the methods described by Lane.[28] We used BLAST sequence similarity search[29] and EzTaxon analysis[30] on the 1,502 nucleotides of the isolate's 16S rRNA gene sequence to find the closest taxa. All 16S rRNA genes associated with the ''Rhizobiaceae'' family were searched for in the database (http://www.ncbi.nlm.nih.gov). These sequences were aligned using the CLUSTAL_X algorithm,[31] and any necessary adjustments were done by hand. Then, two tree-making methods were used to construct phylogenetic trees: the Neighbor-Joining method in the PHYLIP package, version 3.5,[32] and maximum likelihood (ML) with the PhyML program.[33] The tree topologies that were produced were analyzed using bootstrap analysis with 1,000 resamplings, which was implemented using the SEQBOOT and CONSENSE tools inside the PHYLIP package. The evolutionary history was inferred using the Maximum Likelihood method. Evaluators looked into the MEGA X system's evolutionary history.

Phenotypic characterization of novel *Rhizobium* sp. PKS

To examine the cell shape and motility, a light microscope was employed. The TSA medium, which was used to evaluate motility, consisted of the following ingredients: agar (0.4 g), dipotassium hydrogen phosphate (2.5 g), dextrose (2.5 g),

sodium chloride (5 g), papaic digest of soyabean meal (3 g), and l-1 pancreatic digest of casein (17 g). Using established methodologies, we evaluated the culture's antibiotic resistance, temperature tolerance, metabolic traits, carbon absorption, and hydrogen sulfide generation.[34,35] We found that *Rhizobium* sp. PKS could grow on YEMA medium that had been pH-buffer-ed with citric acid-NaOH at 5 and 6, phosphate at 7 and 8, glycine-NaOH at 9 and 10, or Tris buffer at 11 and 12.

Production of IAA from *Rhizobium* sp. PKS

The evaluation was conducted based on the procedure described by Brick.[36] The emergence of a pink hue signified the occurrence of IAA synthesis.

Siderophore production from *Rhizobium* sp. PKS

Using blue agar plates supplemented with chrome azurol S dye, siderophore production was identified according to the method described by Schwyn and Neilands.[37] The presence of an orange halo surrounding the colony suggested the development of siderophores.

Phosphate solubilization of *Rhizobium* sp. PKS

In order to conduct the phosphate solubilization experiment, a pH 7 solution was utilized. In 1,000 milliliters of water, there were 2 grams of yeast extract, 20 grams of glucose, 2 grams of tricalcium phosphate, 60 milligrams of actidione, and 15 grams of agar. A small amount of this combination was added to petri plates that already contained *Rhizobium* sp. PKS strain for inoculation. After that, the dishes were kept in an incubator set at 28°C for a duration of 5 days. Rosas *et al*.[38] reported that phosphate solubilizers were found in bacterial colonies with clearly defined zones.

Biochemical analysis of cotton plants inoculated with *Rhizobium* sp. PKS

Extraction and analysis of total protein

In order to extract total protein, half a gram of plant tissue was homogenized in ten milliliters of 0.2 M perchloric acid. Pellet extraction was performed twice using a solvent mixture of ethanol, ether, and chloroform in a ratio of 2:2:1 (v/v/v), following centrifugation at 5,000 g for 10 minutes at 24°C. Once the treatment was complete, the residue was let to stand overnight with 0.2 M NaOH. The total protein was estimated using the supernatant that was produced.[39]

Estimation of sugars

According to Mahadevan and Shridhar,[40] a UV-VIS spectrophotometer (Spectronic D20) was used to detect the optical density at 625 nm after heating 1 g of plant tissue with 0.2% anthrone reagent.

Estimation of proline

It was Bates *et al*.[41] that laid the groundwork for proline estimation. To begin, 5 milliliters of 3% (w/v) sulphosalicylic acid was used to homogenize 0.5 gram of fresh leaf tissue. Whatman #2 filter paper was used to filter the supernatant after centrifugation at 5,000 rpm for 10 minutes to remove the residue. After that, the filtrate was mixed with ninhydrin and glacial acetic acid in equal volumes and left to incubate at 95°C for an hour. After cooling the combination in an ice bath for about half an hour, we extracted 4 milliliters of toluene by vigorously mixing the ingredients for 15 seconds to stop the reaction. After gathering the toluene phase that contained the chromophore, it was left to warm up to room temperature for 10 minutes. Then, the concentration of proline was measured using colorimetry and quantified as mg g^{-1}.

Estimation and extraction of chlorophyll

Using 80% acetone, we followed Harborne's method[42] to extract chlorophyll pigment from 1 gram of cotton leaves inoculated with *Rhizobium* sp. PKS. Dark circumstances were used for the filtering of the resultant extracts. The filtrate's optical density (OD) values were measured at two wavelengths 650 nm and 663 nm using a UV-VIS spectrophotometer. Using Arnon's formula, the total chlorophyll content was determined.

Phytohormone production from cotton plants inoculated with *Rhizobium* sp. PKS

Quantification of Indole-3-Acetic Acid

One gram of leaf material was crushed with one milliliter of phosphate buffer to prepare

the samples. For the Salkowski reagent, which is 2% 0.5 M $FeCl_3$ in 35% perchloric acid, two drops of perchloric acid were added to the supernatant after centrifugation, bringing the volume to 2 ml. Using a UV-VIS spectrophotometer, the optical density (OD) values were recorded at 530 nm after 25 minutes. The concentration of IAA in µg ml^{-1} was plotted against the optical density at 530 nm to create a standard graph.

Sampling soil from various cotton agricultural fields

The cotton fields in Mahabubnagar District were sampled for their soil types, which included sandy soil from Narayanpet, deep black soil from Kalwakurthy, red soil from Makthal, and shallow black soil from Malleboinpally.

Physico-Chemical Characteristics of Cotton Agricultural Field Soil Samples

Subbiah and Asija[43] used the alkaline potassium permanganate method to evaluate the soil's available nitrogen,[44] examined the soil's available phosphorus, and Jackson[45] measured potassium levels using flame photometry.

RESULTS

Novel Isolation of *Rhizobium* sp. PKS as a Plant Growth-Promoting Rhizobacteria

Finding the most effective and powerful *Rhizobium* species in the soil of cotton plants' rhizospheres close to the Eturnagram forest is the primary goal of this research. Using the top layer approach and forest soil, seventeen frequently grown cotton cultivars in the Mahabubnagar area were planted and observed for 60 days. As shown in Table 1, six of these cultivars showed considerable plant growth over 35 cm. The phytohormone and biochemical production of

Table 1. Plant growth characteristics of 17 cotton cultivars (aged 60 days) by top layer method

Variant /seed variety	Top layer method (Eturnagram Soil)
Mahyco	+++
Ajeet	++
Rashi	+++
Tulasi	++
Marvel	+++
Bunni	++
PCH-	++
Nusun	+++
Kaveri	+++
Raj Seeds	+++
Super seeds	+
Veda	++
Brahmaputra	+
S99Bt	++
Obama	++
Bunni Seeds	++
Sunny (NCS-108)	±

plant growth above 35 cm: +++, plant growth below 25 cm: ++, plant growth below 20 cm: +, plant growth below 15 cm: ±

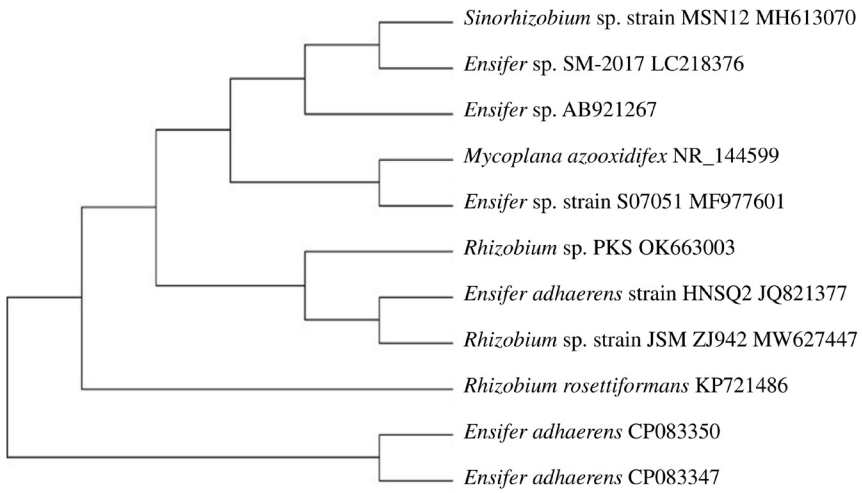

Figure. Evolutionary analysis by Maximum Likelihood method

Table 2. Phytohormone and biochemical synthesis of Six cotton cultivars (aged 60 days) grown by Top layer method provided with Eturnagaram forest soil

Cultivar	IAA (µg/gm)	Proteins (mg/gm)	Sugars (mg/gm)	Chlorophyll (mg/gm)	Proline (mg/gm)
Mahyco	872.33 ± 2.51	212.73 ± 2.57	134.3 ± 1.73	184.43 ± 1.15	0.05 ± 0
Rashi	800 ± 2	188.26 ± 1.15	120.63 ± 0.8	170.46 ± 1.35	0.06 ± 0
Marvel	774 ± 1	147.5 ± 0.98	104.4 ± 0.81	161.5 ± 0.98	0.06 ± 0
Nusun	686 ± 1	125.06 ± 0.25	98.46 ± 0.45	95.36 ± 0.22	0.05 ± 0
Kaveri	553 ± 1	115.46 ± 0.35	86.53 ± 0.23	78.34 ± 0.3	0.16 ± 0
Raj seeds	482 ± 1	93.4 ± 0.26	72.4 ± 0.23	65.45 ± 0.17	0.22 ± 0

Table 3. Plant growth parameters and Nitrogen content of Mahyco cultivar inoculated with 5 strains of *Rhizobium* sp.

Isolates	Height of the plant		Plant fresh weight		Plant dry weight		N2 Content (%)
	Shoot	Root	Shoot	Root	Shoot	Root	
Strain-1	17.66 ± 0.96	9.16 ± 0.65	1.67 ± 0.35	0.84 ± 0.08	0.37 ± 0.04	0.07 ± 0.01	0.77 ± 0.06
Strain-2	23.8 ± 1.05	10.16 ± 0.4	2.12 ± 0.12	1.21 ± 0.02	0.6 ± 0.02	0.15 ± 0	1.2 ± 0.1
Strain-3	21 ± 0.65	10.1 ± 0.6	2.1 ± 0.3	1.09 ± 0.06	0.53 ± 0.07	0.12 ± 0.01	1.13 ± 0.05
Strain-4	17.9 ± 0.6	8.23 ± 0.35	1.52 ± 0.13	0.71 ± 0.08	0.24 ± 0.03	0.06 ± 0.01	0.76 ± 0.05
Strain-5	28.93 ± 0.55	12.23 ± 0.76	3.62 ± 0.25	2.46 ± 0.34	0.85 ± 0.05	0.25 ± 0.03	1.56 ± 0.14

Table 4. Cultural Characteristics of *Rhizobium* sp. PKS

Characteristics	*Rhizobium* sp. PKS
Cell morphology	Rod
Gram's nature	Negative
Cell size (µm)	1.0–1.4 x 3.0–5.4
Temperature range (°C)	23 to 30
pH range	6.0–7.5
Salinity Tolerance	0–2.5
Oxidase	+
Catalase	+
Chitinase	-
lipase	-
coagulase	-
amylase	-
urease	-

these six plants was further investigated, as shown in Table 2. Due to its superior performance in biochemical and phytohormonal synthesis among six cultivars, the Mahyco cultivar was selected for further study. This cultivar demonstrated the highest values across all parameters. Therefore, it was essential to isolate *Rhizobium* from the

Table 5. Plant growth improving traits in new strain of *Rhizobium* sp. PKS

Traits	*Rhizobium* sp. PKS
Test for Phosphate Solubilization	+
Test for IAA production	+
Test for Siderophore production	+

Table 6. Characteristics of four agricultural soils in Mahabubnagar District: Assessment of physico-chemical properties and available sulphur and micronutrient content

Location	Soil type	pH	N	P	K	S	Fe	Mn	Zn	Cu
Malleboinpally	Shallow black soil	8.0	225.14	93.72	115.84	9.1	2.48	26.24	0.30	0.29
Chitteboinpally	Red soil	7.0	192.68	92.15	99.69	12.1	4.98	27.31	1.05	0.27
Kalwakurthy	Deep black	8.0	251.23	105.58	141.12	8.0	2.19	35.21	0.41	0.60
Divtipally	Sandy soil	8.0	189.87	75.92	95.75	3.5	0.18	15.25	0.38	0.05

Table 7. Growth parameters of Mahyco Cultivar inoculated with *Rhizobium* sp. PKS assessed across four diverse agricultural soils in Mahabubnagar District

Soil type	Location	Combination	Height of the plant (cm)		Plant fresh weight (g)		Plant dry weight (g)	
			Shoot	Root	Shoot	Root	Shoot	Root
Shallow Balck Soil	Malleboinpally	Control	24.1± 1.17	14.3± 0.1	5.62± 0.15	2.13± 0.03	0.82± 0.03	0.81± 0.03
		M+Rhi	58.16± 0.25	24.36± 1.49	7.23± 0.3	3.3± 0.12	2.77± 0.18	1.77± 0.1
Red Soil	Chitteboinpally	Control	25.63± 1.2	16.3± 0.52	5.58± 0.54	1.86± 0.04	0.88± 0.04	0.84± 0.05
		M+Rhi	51.6± 0.81	28± 0.45	6.3± 0.55	3.17± 0.03	2.07± 0.09	1.69± 0.07
Deep Balck Soil	Kalwakurthy	Control	29.3± 1.22	17.23± 0.55	6.13± 0.15	2.6± 0.02	1.31± 0.05	1.2± 0.04
		M+Rhi	59.13± 1.25	31.23± 0.35	12.06± 0.51	6.5± 0.2	3.83± 0.08	1.77± 0.09
Sandy Soil	Divtipally	Control	20.53± 0.92	15.93± 0.55	5.03± 0.2	2.06± 0.06	0.53± 0.05	0.62± 0.05
		M+Rhi	50.4± 0.85	27.76± 0.51	7.12± 0.12	2.78± 0.19	1.83± 0.11	1.65± 0.05

M-Mahyco Cultivar, Rhi-*Rhizobium* sp. PKS

Table 8. Exploring the phytohormone production and biochemical features in the Mahyco cotton cultivar across diverse agricultural soils of Mahabubnagar district. *Rhizobium* sp. PKS [NCBI-OK663003, NCMR-MCC4960]

Soil type	Location	Combination	IAA (µg/gm)	Proteins (mg/gm)	Sugars (mg/gm)	Chlorophyll (mg/gm)	Proline (µg/gm)
Shallow Balck Soil	Malleboinpally	Control	488 ± 1	70 ± 1	80.33 ± 2.08	66.23 ± 1.15	0.04 ± 0
		M+Rhi	841 ± 1	196.33 ± 1.52	147 ± 1	157 ± 1	0.03 ± 0
Red Soil	Chitteboinpally	Control	548.66± 1.52	73± 1	79.33± 1.52	70.3± 0.45	0.05± 0
		M+Rhi	778 ± 2	150.33 ± 1.52	117.33 ± 1.52	123 ± 1	0.04 ± 0
Deep Balck Soil	Kalwakurthy	Control	564 ± 1	74 ± 1	91 ± 1	78.16 ± 0.3	0.03 ± 0
		M+Rhi	873.66 ± 1.52	207 ± 1	156.66 ± 1.52	173.66 ± 1.52	0.03 ± 0
Sandy Soil	Divtipally	Control	511 ± 1	60.66 ± 1.52	71 ± 1	59.3 ± 0.36	0.06 ± 0
		M+Rhi	719 ± 1	110.33 ± 2.08	99.66 ± 1.52	115.33 ± 1.52	0.05 ± 0

M-Mahyco cultivar, Rhi- *Rhizobium* sp. PKS

rhizosphere soil of Mahyco using YEMA media. This process was crucial to understand the unique characteristics of this cultivar. The liquid inoculum of five separate colonies was serially diluted until it reached a concentration of 10^8 CFU ml^{-1}. Following the steps indicated in Table 3, this inoculum was applied to Mahyco seeds and left to germinate in sterile soil for 60 days.

Plant height, dry weight, and nitrogen content were all metrics by which Strain-5 stood out among the five isolates. After examining its 16S rRNA gene sequence and plant growth-improving properties (Table 4 and 5), this isolated strain was subsequently named *Rhizobium* sp. PKS.

The efficiency of *Rhizobium* sp. PKS as a potent PGPR

The results shown above make it clear that *Rhizobium* sp. PKS could be a useful PGPR for bioformulations that try to improve the fertility of low-quality soils by increasing plant growth. We analyzed four soil samples for NPK and trace element content (Table 6). These samples were chosen because of their low plant growth capability, as described in the 'Materials and Methods' section. Subsequently, *Rhizobium* sp. PKS was either introduced into these soil samples or they were kept uninoculated for control purposes. After that, the Mahyco cultivar was left to its own devices for 60 days in each of these four soil types to see how well the isolate performed.

In every type of soil, the Mahyco cultivar exhibited positive growth in terms of its physical parameters, phytohormone production, and biochemical traits. However, red soil showed intermediate development, sandy soil showed the least, and deep black soil and shallow black soil showed the greatest.

In addition, four distinct soil types in the Mahabubnagar District were tested for *Rhizobium* sp. PKS (Table 6). In terms of supporting plant characteristics and phytohormonal biochemical productions, the results showed that deep black soil was the best (Table 7 & 8), followed by shallow black soil. In contrast, sandy soil provided the worst circumstances for plant growth, while red soil performed marginally better than black soil.

Description of novel *Rhizobium* sp. PKS

Bashan *et al*.[46] and Pini *et al*.[47] found that different species of *Rhizobium* have different motility patterns, metabolic traits, growth circumstances, and morphology of their colonies and cells. The color of colonies might be opaque, cream, or pink. They usually have spherical shapes with smooth borders and can be tiny to medium in size.[46] The rod-shaped (bacilli) or irregularly shaped (NKS) cells of *Rhizobium* sp. can be found alone, in pairs, or in short chains, and they are typically small to medium-sized.[46,47] Table 4 shows that *Rhizobium* sp. PKS thrives in temperatures between 25 and 30 degrees Celsius, with an ideal pH between 6.0 and 7.5, according to Bashan *et al*.[46] and Pini *et al*.[47] Most *rhizobium* species may move about in wet environments or liquid media thanks to their polar or peritrichous flagella.[46,47] Catalase, urease, phosphatase, and oxidase assays frequently show positive findings for *Rhizobium* sp. PKS species. The 16S rRNA gene sequence analysis showed that *Rhizobium* sp. PKS [NCBI-OK663003, NCMR-MCC4960], a member of the Rhizobiaceae family in the Proteobacteria phylum, has a 99.13% sequence similarity with *Ensifer adhaerens* strain HNSQ2 and a 98.77% sequence similarity with *Rhizobium* sp. JSM ZJ942 as shown in Figure.

The plant growth-promoting characteristics of *Rhizobium* sp. PKS were tested (Table 2). After that, the biochemical characteristics and phytohormone production of six cultivars that showed the best growth with *Rhizobium* sp. PKS were examined. Out of all the cotton varieties tested, Mahyco has the highest concentrations of IAA, sugars, proteins, and chlorophyll, with the lowest concentrations of proline. Table 3 shows that Sunny (NCS-108) had higher quantities of proline and lower levels of IAA, proteins, carbohydrates, and chlorophyll.

DISCUSSION

Martinez-Viveros *et al*.[48] states that the nitrogen fixation process relies on the bacteria *rhizobium*, which is well-known for its symbiotic relationship, and that leguminous plants depend on it. Much like *Pseudomonas* and *Bacillus*, *Rhizobium* is a PGPR that has been extensively studied and found to play an important role in the rhizosphere.[49] Studies conducted by Broadbent *et al*., Pal, Sudha *et al*., Cammakci *et al*., Sahin *et al*., and Aslantas *et al*.[50-54] have demonstrated that

some *Rhizobium* species can enhance the yield of certain crops.

According to Wang *et al*.,[55] *Rhizobium* can improve the yield of legumes including beans, lentils,[56] peas, chickpeas, and soybeans. *Rhizobium* can be found in the rhizosphere, endosphere, and phyllosphere of non-leguminous crops, according to Dɒez Mꞁndez and Menꞁndez.[57] Regardless, cotton *Rhizobium* inoculation has been seldom documented in the literature. Romero-Perdomo *et al*.[58] found that *Rhizobium* was the sole bacterium that positively impacted cotton development in soils deficient in phosphorus. Rhizobia thrive in soil or other mediums with good air circulation and a broad variety of metabolic capabilities. Nitrogen fixation, the conversion of atmospheric nitrogen to ammonia, is one of their several impressive characteristics. [46,47]

Research carried out by Hafeez *et al*.[7] proved that Rhizobial inoculation significantly enhances cotton's nitrogen absorption, general growth, and seedling emergence. Their process includes the generation of phytohormones like IAA and the solubilization of phosphorus.[59] Researchers have isolated bacteria capable of producing IAA from a variety of soils in the rhizosphere. Genera *Aeromonas, Azotobacter, Bacillus, Burkholderia, Enterobacter, Pseudomonas*, and *Rhizobium* include these bacteria. The study used *Rhizobium* sp. 16S rRNA gene sequence analysis confirmed that PKS enhanced plant growth by promoting the solubilization of phosphate and creation of siderophores, among other beneficial characteristics.

Improving crop yields depends on PGPRs' capacity to convert insoluble phosphorus (P) into a form plants can use. Some bacteria can dissolve phosphatic compounds, according to Zaidi *et al*.[60] This suggests that microbial solubilization of phosphates in soil could be possible. Rhizobacteria play a crucial role in the rhizosphere by dissolving mineral P and making it accessible to plants. One possible explanation for the dissolution of mineral P is the production of organic acids such citric, acetic, malic, gluconic, oxalic, and succinic acids. Our current understanding is that *Rhizobium* sp. PKS shows promise as a phosphate solubilizer.

Beneficial and efficient *Rhizobium* sp. along with higher quantities of IAA, proteins, chlorophyll, and proline, PKS has demonstrated remarkable growth when hybridized with the Mahyco cultivar, particularly in extremely dark soil, PKS showed substantial benefits in plant development. These findings suggest that this novel bacterial isolate has potential as a bioinoculant for cotton crops. The fact that it works well on a wide range of soil types and is adaptable makes it a promising solution to agricultural problems caused by low soil quality and erratic rainfall, especially in regions like the Deccan plateau, where droughts are common and soil isn't always perfect.

Field experiments conducted validated the efficacy of *Rhizobium* sp. PKS., especially in deep black soil, showcasing substantial plant growth enhancements. These findings suggest that this novel bacterial isolate could serve as a beneficial bioinoculant for cotton fields. Moreover, its adaptability and effectiveness across different soil types, especially in regions like the Deccan plateau characterized by periodic droughts and suboptimal soil conditions, underscore its potential to address agricultural challenges related to soil quality and irregular rainfall.

CONCLUSION

The *Rhizobium* sp. PKS [NCBI-OK663003, NCMR-MCC4960] isolate, known for its innovation and efficiency, has shown significant improvement in the development of cotton plants. This includes enhancements in biochemical characteristics, physical parameters, and phytohormones. Interestingly, it has demonstrated adaptability to both deep and shallow dark soils. Given its strong plant growth-promoting abilities, it has great potential as a biofertilizer, making it an ideal bioinoculant for cotton fields.

Furthermore, *Rhizobium* sp. PKS has exhibited potential as a biofertilizer for cotton cultivation on the Deccan plateau, as per the study. This presents an opportunity to increase agricultural productivity in Mahabubnagar areas where the soil quality is subpar and rainfall patterns are inconsistent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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