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Evaluation of PGPR Activity of Cow Dung Rhizobacterial Isolates and Their Effect on Wheat Plant Growth

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Abstract:
Plant growth-promoting rhizobacteria (PGPR) are beneficial microbes residing in the rhizosphere that enhance plant growth by supplying essential nutrients and protecting against pathogenic organisms. This study aimed to identify and characterize PGPR strains native to cow dung in India and assess their plant growth-promoting potential.Ten bacterial isolates (designated KG1 to KG10) were isolated and subjected to in vitro screening for PGPR traits. Among these, isolate KG9 demonstrated superior performance. Phylogenetic analysis of 16S rRNA sequences revealed that these isolates represent novel strains of Klebsiella pneumoniae.Isolate KG9 exhibited high production levels of key growth-promoting compounds: indole-3-acetic acid (786 µg/mL), gibberellic acid (9.52 mg/mL), and ammonia (0.85 mg/L). The strain was applied to wheat seeds using various methods, including seed priming, bacterial broth application before and after germination, and encapsulation in beads both pre- and post-germination. These treatments were compared to untreated control seeds. The most significant enhancement in shoot length (1.48-fold increase) was observed with bacterial broth application prior to germination (KG9B), while the greatest improvement in root length (1.40-fold increase) occurred with bead encapsulation before and after germination (KG9D*). Additionally, treated plants showed increased chlorophyll and proline content. K. pneumoniae KG9 exhibits multiple beneficial traits that support wheat growth and development. This strain holds promise for use as a safe and effective bio inoculant in sustainable agriculture.

Keywords: Cow dung, Klebsiella pneumoniae, Plant growth-promoting rhizobacteria, Wheat growth promoter

1. Introduction:

For sustainable agriculture and ecosystem health, the rhizosphere plays a critical role. The area of soil that surrounds plant roots, known as the rhizosphere, has unique characteristics and is where intricate interactions between soil, plants, and microorganisms take place. Rhizosphere microorganisms, which include bacteria, fungi, and actinomycetes, thrive in this area because it is heavily flooded with nutrients and root exudates (Adeleke and Babalola 2021; Poria et al., 2018). These have not only been reported to provide essential phytohormones (Grover et al., 2021), organic acids, and nutrient availability (Kumar et al., 2022) for plant growth, but also help protect plants as biological control agents (Prisa et al., 2025). In literature, various PGPR microbes reported to provide insoluble potassium (Bechtaoui et al., 2019), phosphate (Lei et al., 2025; Bechtaoui et al., 2024) and zinc (Massod et al., 2021; Singh et al., 2024) in soluble form to plants, play important role in plant photosynthesis and respiration. Kundan et al (2015), describe the mechanism of release and action of auxin, gibberellin and cytokinin phytohormones released by PGPBs. In exchange plant provide the nutrients and reduced carbon for their growth and survival.

Plant growth and development faces challenges in many habitats, particularly in areas with extremely low soil nutrient availability, such as tropical regions. The introduction of PGPR as an inoculant in soil has been observed to enhance the availability of nutrient elements in soil, thereby reducing the use of chemical fertilizers, which are one of the cause of environmental pollution (Khatoon et al., 2024).

Cow dung has been utilised in agricultural field techniques since ancient times to enhance crop health. The cow's gut biome is inhabited by different bacterial and fungal microbes such as Bacillus , Pseudomonas, Klebsiella, Lactobacillus, Bifidobacterium and Saccharomyces cerevisiae (Sagar et al., 2025; Karnwal, 2023; Sharma and Aggarwal, 2021;) and providing the essential nutrients. These essential nutrients includes nitrogen, potassium, phosphorus, magnesium, and calcium (). During the passage of excreta, these microbes also washes off along with cow dung. Therefore, in this study cow dung has been used to isolate the PGPR strain and analyzed for different plant growth promoting properties. In addition, bio-formulation, further analysed for plant growth-promoting potential on wheat plant seed. Globally, wheat is a popular dietary staple food, followed by rice and corn fulfilling the core dietary energy needs (World health organization, 2022).

2. Methodology

2.1. Sample Collection and Isolation

Dung samples from Sahiwal cow were collected in sterile plastic bags from a local dairy farm in Bair Majra, Chandigarh, India. One gram of the dung sample was added to 9 mL of sterile saline solution (0.89%) and thoroughly mixed by vortexing. Serial dilutions were prepared up to 108, and 0.1 mL from each dilution was spread onto nutrient agar plates. The plates were then incubated at 28°C for 24 to 48 h. Distinct colonies that appeared were selected for further morphological and biochemical characterization.

2.2. Identification of bacterial strain

DNA was extracted using Xploregen gDNA Extraction kit and stored at −20◦ C. Further it is used as a template to amplify the 16S rRNA for phylogenetic analysis. For amplification, bacterial specific primers forward: GGATGAGCCCGCGGCCTA and reverse: CGGTGTGTACAAGGCCCGG were used. PCR amplification were performed with Big Dye Terminator Ready Reaction Mix: 4µL; Template (100ng/uL); 1µl Primer (10pmol) 2µl and Milli Q Water: 3µl. PCR amplification was carried out at an annealing temperature of 50oC (30 s), an initial denaturation temperature of 96oC (5 min), 31 amplification cycles with denaturation at 96oC (30 s) and extension at 72oC (90 s), followed by a final extension at 72oC (5 min). Purified PCR fragments were directly sequenced with Big Dye Terminator version 3.1 (Applied Biosystems, Forster City, CA, USA). The 16S rRNA gene sequencing method was used to identify the bacteria molecularly. Once the sequences were acquired, the NCBI database was searched using BLASTN to find sequence homology with the 16S rRNA gene. The phylogenetic tree was constructed using the Mega XI software.

2.3. Morphological and Biochemical Characterization of Isolated Strains

Haemolysis test

The pathogenicity of the bacteria was checked using haemolysis test. The bacteria was inoculated on the sheep blood agar and was incubated at 37°C for 48 h. The haemolytic activity can be detected by the presence of a definite, clear zone around a colony (Egwuatu et al. 2014).

Siderophore Production

Siderophore production by the isolated strain was assessed using the Chrome Azurol S (CAS) assay, as described by Payne (1993). A loopful of the bacterial culture was inoculated into Luria Bertani (LB) broth (100 mL) and incubated at 28°C for 48 h. The culture was then centrifuged at 10,000 rpm for 15 min at 4°C. 1 mL of the resulting supernatant was mixed with 1 mL of CAS reagent and incubated for 20 min at room temperature under dark conditions. A colour change from pale yellow to blue indicated the presence of siderophores. The optical density (OD) was measured at 630 nm using a spectrophotometer.

Phosphate solubilisation

Phosphate solubilization of isolated strain was determined using the spot inoculation method. The isolates were inoculated as a spot onto Pikovaskya agar plates and were incubated at 28°C for 72 h. Zone of clearance around the spot was observed, and the diameter was measured (Edi-Premono et al. 1996).

Zinc solubilization

For the estimation of zinc solubilization, the isolated strain was spot-inoculated on agar plate supplemented with 0.2% of each zinc salt, including zinc carbonate, zinc phosphate, zinc chloride and zinc oxide (Sharma et al. 2012) and was incubated at 28°C for 72 h. A zone of clearance around the spot was observed, and the diameter was recorded.

Potassium solubilisation

The potassium solubilization ability of the bacterial isolate was evaluated using the method described by Pikovskaya (1948). The strain was dot-inoculated onto Aleksandrovsk agar medium (50 mL) and incubated at 28°C for a period ranging from 48 to 168 h. The formation of a clear zone around the colony was considered indicative of positive potassium solubilization activity.

Cellulose hydrolysis test

Estimation of cellulose hydrolysis of isolated strain was evaluated by spot inoculation method. The bacteria was inoculated on carboxymethyl cellulose agar media (50 mL) supplemented with 0.02% congo red followed by incubation at 28°C for 48 hr. The halo zone formed around bacterial colony was considered as positive for cellulose hydrolysis (Sazci et al. 1986).

Nitrogen fixation

The nitrogen-fixing ability of the isolated strain was assessed using the spot method. The strain was inoculated onto Norris agar medium 100 mL supplemented with bromothymol blue (0.01%) and incubated at 28°C for 72 h. After incubation, the development of halo zones around the bacterial colonies were analyzed Norris (1965).
Indole acetic acid (IAA) production

A cow dung isolate was inoculated into autoclaved 100 mL tryptophan broth medium (TBM) and incubated at 28°C for 7 days at 120 rpm. Culture was taken out after an interval of 48h, 96h and 198h and were centrifuged at 11, 400g for 10 min at 4°C. 2 mL of Salkowski reagent (ferric chloride (FeCl3) - 0.5M containing 35% sulphuric acid (H2SO4) was added to the 1 mL culture supernatant, mixed properly, and incubated at room temperature in a dark condition for 30 min. The colour change was observed at an optical density (OD) of 530 nm using a spectrophotometer (Kamnev et al. 2001). IAA levels were assessed via a standard curve prepared from 20–140 µg/mL pure IAA.

Gibberellic acid production

The isolated strain was inoculated into Pikovskaya broth (100 mL) and incubation was done at 28°C for 198 h at constant agitation of 120 rpm. Centrifugation of the culture media was done at 10000 rpm for 15 min at 4°C. 0.28 mL of zinc acetate and potassium ferrocyanide solution (0.8 mL) was added to the supernatant and mixed well. The mixture was again centrifuged at 448g for 15min. 30% hydrochloric acid was added to the resultant supernatant and was incubated at 28°C for 75 mins. O.D was taken at 254nm using 5% hydrochloric acid as blank (Holbrook et al. 1961). Gibberellic acid levels were assessed via a standard curve prepared from 10–100 mg/mL pure gibberellic acid.

Ammonia Production

Ammonia production by the isolated strain was quantified following the method described by Dye et al. (1962). The strain was inoculated into peptone water (100 mL) and incubated at 28°C for 7 days. Post incubation, the culture was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected, and 50 µL of Nessler's reagent was added. The optical density (OD) was measured at 450 nm using a spectrophotometer to estimate ammonia concentration.

Microbead formation

The formation of beads from a polymeric solution was prepared with some slight modification to the method mentioned by Kaur et al (2023). The cell culture was centrifuged at 5000 rpm for 10 min and the cell pellets were collected. The polymeric solution was prepared by mixing Gum Arabic (2%.), rice brain (2.5%), tricalcium phosphate (2.5%) , tween (0.1%), and glycerol (5%.) in hot water (90°C). The solution was mixed with bacterial cell pellets and continuously stirred on a magnetic stirrer at 28°C for 1 h. After continuous stirring, the suspension was added dropwise into the chilled calcium chloride solution (0.5M) to form microbeads. Then beads were allowed to suspend in the calcium chloride solution overnight at 20°C. The beads were extracted from the solution and rinsed twice with distilled water, and were dried at room temperature for removal of water content from the beads. The beads and were stored in sterilized container.

Pot trial experiments

Wheat seeds were sterilized using 70% ethanol for 2 min and HgCl2 (0.05%) for 3-4 min. The seeds were then rinsed with distilled water and sown in sterilized soil comprising coco peat. The pots were provided with different treatments. The bacteria was suspended in nutrient broth and incubation was performed at 28°C for 24 to 48 h. The experiment was conducted in a complete randomized designed treatment (KG9C, KG9P, KG9B*, KG9B, KG9D*, KG9D) as showed in table 1. Seed priming was performed by dipping seeds in bacterial culture for 24h at room temperature. After incubation seeds were sown in pots. The plants were allowed to germinate for about 3-4 weeks. The plants were separated from the pots and roots were washed properly with distilled water to remove the soil from root surface. The shoot length and root length were measured after the proper growth of the plants. Each experiment was performed in triplicates and the data was collected and results were analysed statistically using one way anova.

Table 1: Set of experiments designed for determining the effects of PGPR

Control	Seed Priming	Broth before germination	Broth after germination	Beads before germination	Beads after germination
Set 1: KG9C1	KG9P1	KG9B1	KGB91*	KGD91	KGD91*
Set 2: KG9C2	KG9P2	KG9B2	KGB92*	KGD92	KGD92*
Set 3: KG9C3	KG9P3	KG9B3	KGB93*	KGD93	KGD93*

Chlorophyll Content

Extraction of chlorophyll involved grinding and homogenizing leaf tissue with 80% acetone solution. Filtration of the homogenate was done through Whatmann filter paper. The volume was adjusted to 25 mL using 80% acetone. Optical density (O.D.) was measured at 663 nm and 645 nm with acetone as a blank. The content of chlorophyll a, b and total chlorophyll was calculated using the following formula (Arnon, 1949).

Chl. Conc in µg/mL = [(OD645 × 20.2) + (OD663 × 8)] × 2

Proline Content

The leaf tissues were grinded using mortar pestle and homogenized with 10 mL of sulphosaliaylic acid. Homogenate was filtered through Whatmann filter paper. 2mL of ninhydrine solution and glacial acetic acid (2mL) was added into equal volume of collected filtrate. Incubation was performed at 100°C for 1h and then transferred to an ice bath. After 5mins of incubation in an ice bath 4mL of toluene was added and mixed well for 15-20 sec at room temperature. Toluene was aspirated from the aqueous phase and O.D was noted at 520nm using spectrophotometer (Sneha et al., 2013).

3. RESULT AND DISCUSSION

Microbial strains isolated from cow dung have demonstrated significant benefits for plant nutrition by synthesizing various phytohormones and contributing to pest management (Gupta et al., 2025). These isolates showed enhanced capabilities in phosphate solubilization, production of indole-3-acetic acid (IAA), ammonia, phosphate, and siderophores under both saline and non-saline conditions (Sarma and Deka, 2024). Furthermore, the strain was reported to produce IAA, siderophores, and hydrogen cyanide, along with exhibiting nitrogen-fixing potential. These attributes support its use as a biofertilizer and biocontrol agent, effectively mitigating root rot disease in sunflower (Komy et al. (2020). Thus in order to isolate a PGPR, Cow dung of Sahiwal breed was cultured on selective media to evaluate its functional traits relevant to plant growth promotion. PGPR colonizes both the outer surfaces and internal root tissues of plants, facilitating the supply of essential nutrients that support plant growth and development, either directly or indirectly (Asghar et al., 2023).

Morphological and Molecular Identification:

Morphologically, all isolates ranged from irregular to circular in shape, featured convex to smooth surfaces, and were rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences identified KG9 as a member of the genus Klebsiella sp. The sequences of the isolates KG9 showed 97.6 % similarity with different strain of Klebsiella pneumoniae using 16s rRNA gene sequencing and a phylogenetic tree was constructed as shown in Figure 1. Isolate KG9 was submitted to GenBank under accession number SUB15711558.

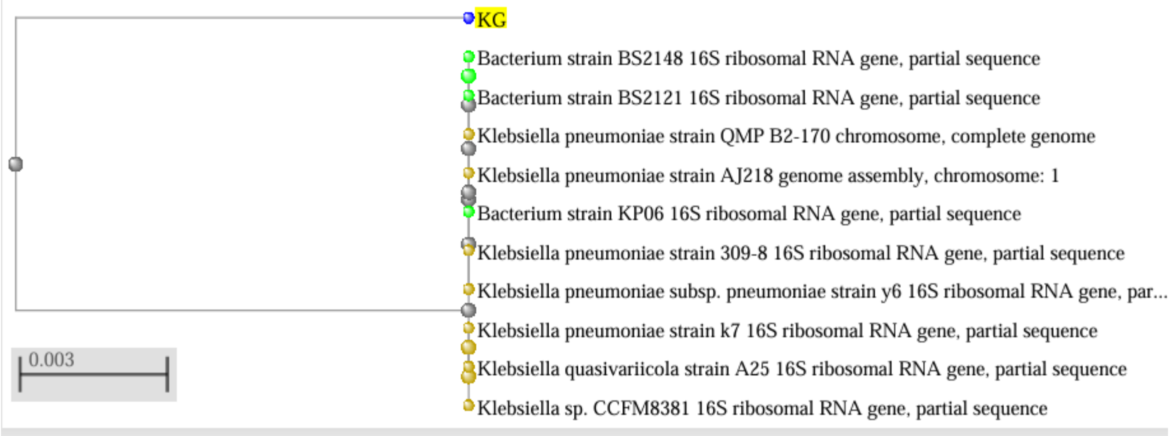

Figure 1: Phylogenetic tree of isolated strain KG9.

3.1. Primary screening of isolated strain

A total of 10 bacterial isolates designated as KG1 to KG10 and were evaluated for their functional traits, including phosphate solubilisation, potassium solubilisation, zinc solubilisation, nitrogen fixation, cellulose hydrolysis, and haemolytic activity as shown in Table 2. All these tests were performed on agar plates with specific substrate and zone of hydrolysis was determined. All of the stains were found non-haemolytic depicted the non- pathogenic nature of strain and used as a plant growth promoter without causing any human pathogenicity (Kumar et al., 2022). The production of siderophore is a defence strategy adopted by microbes involving the release of iron chelating molecules that enhance the availability of soluble iron to plants from the soil. This process simultaneously limits iron access to phytopathogens thereby protecting the plant from potential infections (Deb and Tatung, 2024). In this study, none of them exhibited siderophore production, which might be due to the low iron level in the microbial growth environment.

In this study, phosphate, zinc, and potassium solubilization were observed with varying zone formations: minimal in strain KG1, moderate in strains KG2 and KG3–7, 10, and extensive in strains KG8 and KG9. Among all, KG8 and KG9 showed superior mineral solubilization potential (Table 2). Since most minerals present in soil are naturally insoluble, their availability to plants is limited. Microbes play a crucial role in converting these minerals into soluble forms, thereby enhancing nutrient accessibility for plant uptake (Rafique et al., 2022). Several bacterial genera, including Bacillus sp. (Kashyap et al., 2019), Klebsiella sp. (Mazumdar et al., 2018), Pseudomonas sp. (Bakki et al., 2024), and Staphylococcus sp. (Javaid et al., 2023), have been reported to possess mineral solubilization capabilities, a characteristic of PGPR. Cellulolytic activity and nitrogen fixation potential were also assessed. It was found minimal in strains KG1, 2, and 5; moderate in strains KG3, 4, 6, 7, and 6, 7, 10; and wider in strains KG8 and KG9.

Table 2: Biochemical analysis of different isolated strains.

Isolate	Solubilisation		Nitrogen fixation	Siderophore production	Cellulose hydrolysis	Potassium solubilisation	Haemolysis test
	Phosphate	Zinc					
Halo size (mm)							
KG1	0.3	0.2	0.15	-	0.21	0.3	-
KG2	0.8	0.5	0.7	-	1.0	0.8	-
KG3	0.5	1.2	2.1	-	1.5	1.4	-
KG4	2-3	1.5	1-2	-	2.3	3.0	-
KG5	1.2	2.5	1.4	-	0.7	1.3	-
KG6	1.2	1.8	0.9	-	1.1	0.9	-
KG7	1.1	0.9	1.2	-	1.8	1.4	-
KG8	2-3	3.5	3.1	-	2.9	2.5	-
KG9	3-4	4-5	4-5	-	3.0	3-4	-
KG10	2-3	1.8	1.5	-	0.5	1.1	-

It was observed that, among all the isolates KG8 and KG9 formed a clear halo zone around colonies. The halo zone diameter of KG9 was found superior to KG8, indicating better abilities of mineral solubilisation, nitrogen fixation and cellulose hydrolysis. Thus, KG9 was chosen for further studies.

3.2. Secondary screening of the isolated strain

Further, KG1 to 10 were evaluated for the production of phytohormones such as IAA, gibberellic acid, and ammonia at different time interval from 48 to 240 h intervals and the maximal production of these all was achieved at 198 h of interval. Production of IAA helps in efficient nutrient uptake and root profusion. Among these K. pneumoniae KG9 has produced 786 µg/mL IAA (Figure 2), which is higher than others KG strains and the other reported studies. After 198 h of interval, IAA production starts decreasing. The K. varricola from wheat rhizosphere was reported to produce 78.45 ± 1.9 µg/mL IAA along with other anti- oxidants under saline conditions (Kusale et al., 2021). Other PGPR strains such as Pseudomonas sp. (Meliani et al., 2017), Bacillus sp. (Batista et al., 2021; Widawati et al., 2020) have been reported to produce plant root growth promoters.

Another phytohormone, gibberellic acid has been reported to promote plant growth under stress conditions by maintaining and enhancing the seed germination and stem elongation, and flowering (Rizza et al., 2019; Adeleke et al., 2021). Among KG1- 10, K. pneumoniae KG9 produced maximal 9.52 mg/mL of gibberellic acid at 198 h of interval (Figure 3). A 62 µg mL^{-1} of gibberellic acid of K. pneumoniae has been reported to enhance the shoot length and increase in dry biomass of wheat seedlings (Rana et al., 2019). Other than Klebsiella, Bacillus sp. PG-8 (Gohil et al., 2022) and Bacillus siamensis (Ambawade and Pathade, 2015) have been reported to produce 0.70 and 0.24 mg/mL of gibberellic acid after 72 and 96 h of interval, respectively.

Additionally, production of ammonia by plant growth-promoting bacteria is one of the common characteristic. This has been observed to suppress the growth of phytopathogens (Triantafyllou et al., 2023) and helps in improving the soil fertility and alkalinity (Alori et al., 2017). In this study, K. pneumoniae KG9 observed to produce 0.85 mg/L (Figure 4), which is lowered than the other reported findings. 7.75 ± 1.0 µg/mL of ammonia producing potential was exhibited by Klebsiella strain YNA12 (Kang et al., 2020). Biswas et al (2023) reported ammonia production 3.51 ± 0.15 and 2.92 ± 0.05 µg/mL by B. amyloliquefaciens and K. pneumoniae, respectively.

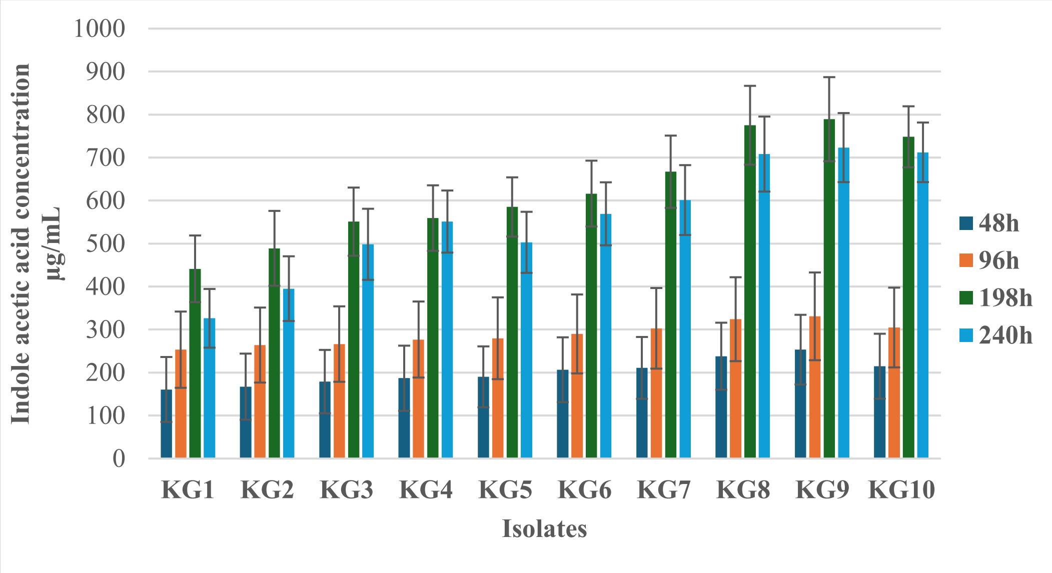

Figure 2: Production of indole acetic acid by K. pneumoniae KG9

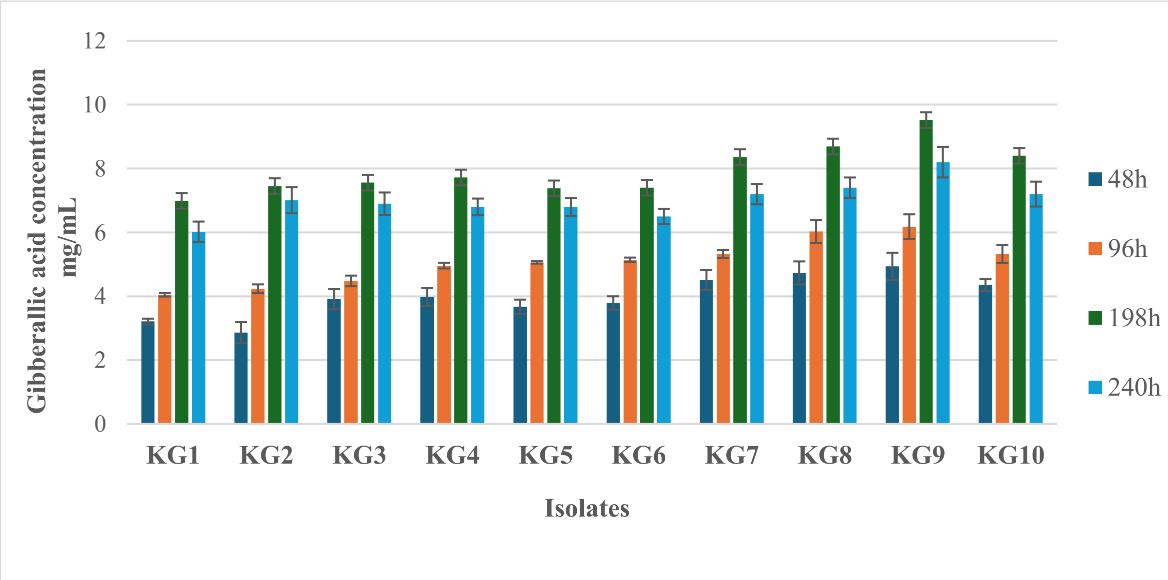

Figure 3: Production of Gibberellic acid by K. pneumoniae KG9

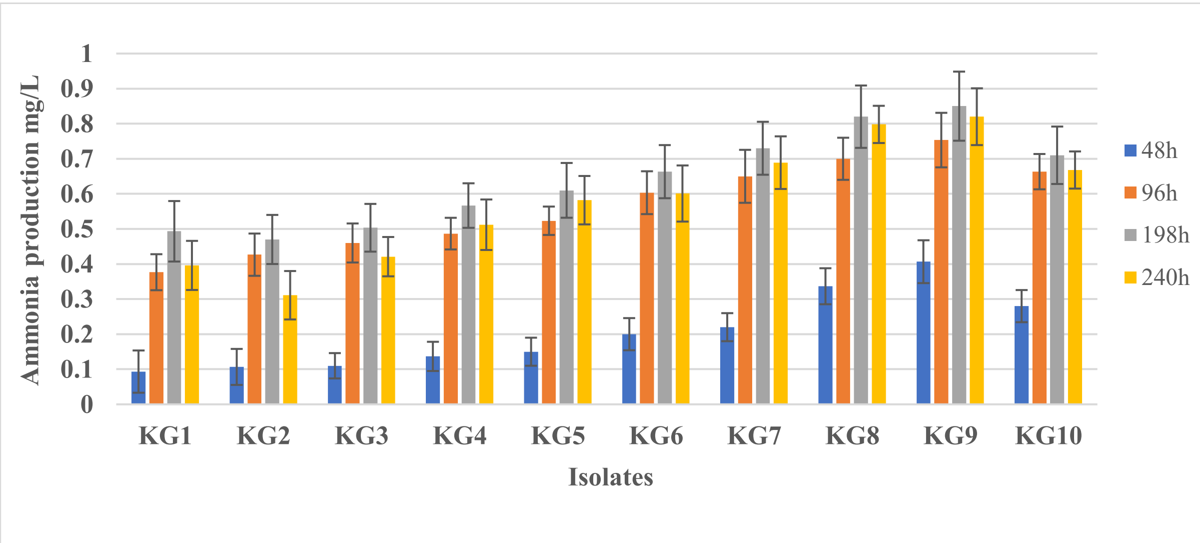

Figure 4: Ammonia production by K. pneumoniae KG9

3.3. Pot trail experiments

Following primary and secondary screening for plant growth-promoting potential, strain KG9 was selected from the ten isolates for pot trial experiments on wheat seeds. Various wheat seed types: KG9C, KG9P, KG9B, KG9B*, KG9D, and KG9D* (as showed in Table 1 were sown in pots in triplicate. Each pot was inoculated with 10 mL of K. pneumoniae KG9 culture. The seeds were allowed to germinate over a period of 3- 4 weeks, after which root and shoot development was assessed. All treated samples exhibited enhanced shoot growth, as depicted in Figure 5. Specifically, stem length increased by 1.25-fold in KG9P, 1.48-fold in KG9B, 1.42-fold in KG9B*, 1.39-fold in KG9D, and 1.34-fold in KG9D* compared to the untreated control (KG9C). Strain K. pneumoniae resulted 1.35 and 1.93, respectively fold increase in shoot and root length of Vigna unguiculata plant (Biswas et al., 2023). Similarly, Dey et al. (2019) reported that the endophytic strain K. pneumoniae HR1 significantly enhanced shoot and root lengths, as well as the dry biomass, in treated plants.

While, in case of root development, KG9P, KG9B, KG9D, and KG9D* exhibited 1.26-, 1.08-, 1.27-, and 1.40-fold increases in root length, respectively, when compared to the control sample (KG9C). Conversely, KG9B* showed a noticeable reduction in root length (Figure 5).

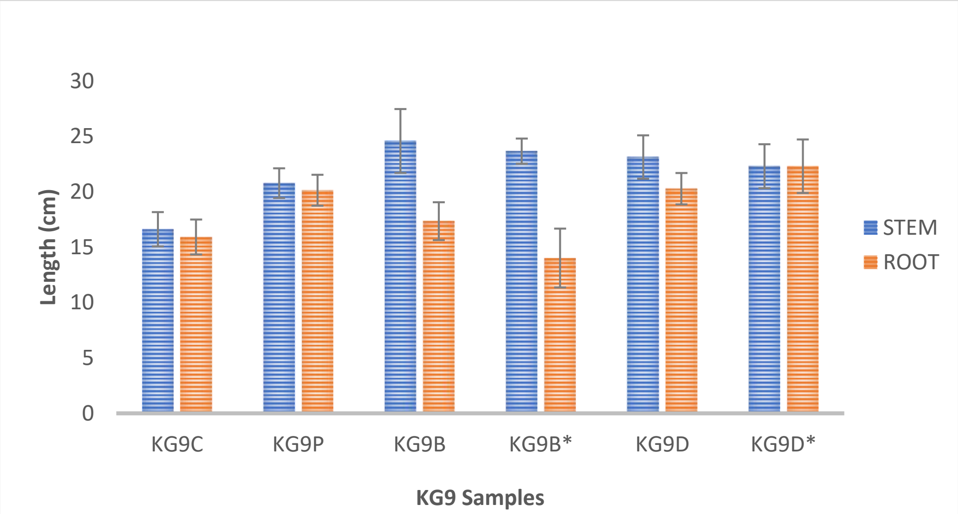

Figure 5: Effect on shoot and root length of plant after exposure of K. pneumoniae KG9 on wheat seed samples

Chlorophyll content serves as a direct indicator of a plant's physiological and metabolic state under diverse environmental conditions. Elevated chlorophyll levels are closely associated with improved growth performance, enhanced nutrient accumulation, and increased crop yield (Ahmad et al., 2022; Muhammad et al., 2022). Various studies have demonstrated that bacterial inoculation can enhance chlorophyll content in plants (Diniz et al., 2025; Soliman et al., 2025). Consistent with these findings, the present study showed that treatment of wheat seeds with strain KG9 led to increased chlorophyll a levels by 1.21-, 1.33-, and 1.31-fold improvements in KG9B*, KG9B, and KG9D samples, respectively. Similarly, chlorophyll b levels rose by 1.20-, 1.08-, 1.10-, and 1.15-fold in KG9B*, KG9B, KG9D*, and KG9D. Total chlorophyll content showed a corresponding increase of 1.33-, 1.17-, 1.02-, 1.21-, and 1.03-fold in KG9B*, KG9B, KG9D*, KG9D, and KG9P samples compared to the control (Figure 6).

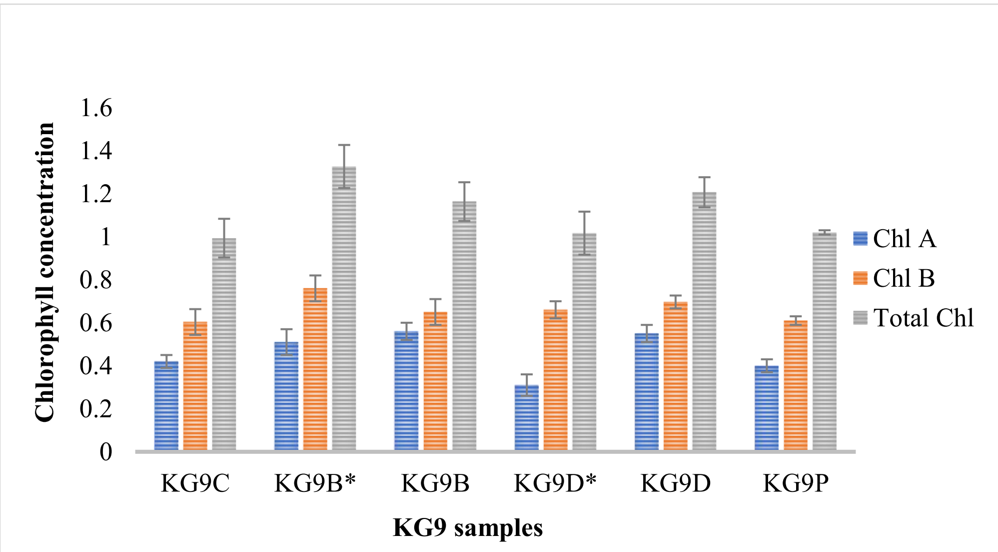

Figure 6: Yield of chlorophyll (Chl) content of K. pneumoniae KG9 treated wheat plants

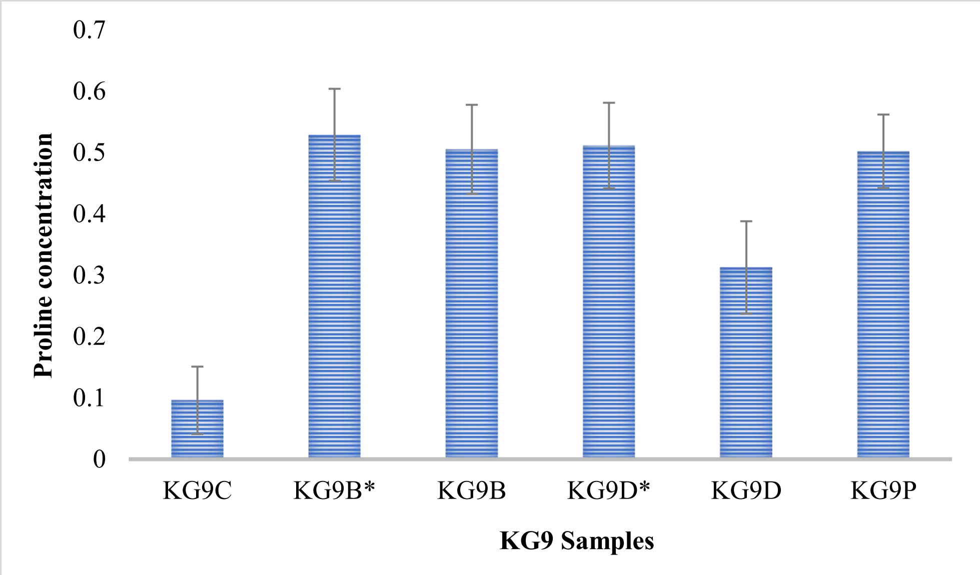

Figure 7: Yield of proline content of K. pneumoniae KG9 treated wheat plants

Improved nutrient uptake, resulting enhanced nitrogen assimilation, plays a crucial role in supporting amino acid metabolism and promoting proline synthesis in plants. Proline functions as a vital osmoprotectant, enabling plants to tolerate various stress conditions such as salinity, drought, and temperature fluctuations. It contributes to stress resilience by stabilizing membrane proteins and maintaining osmotic balance (Raza et al., 2023). In the present study, enhanced proline concentrations were reported in treated wheat samples, 5.51, 5.26, 5.32, 3.25, and 5.22 fold increase observed in KG9B*, KG9B, KG9D*, KG9D, and KG9P, respectively, compared to the control (Figure 7). Under drought or salinity stress conditions, wheat plants were inoculated with Klebsiella oxytoca (Ali et al., 2020) and K. pneumoniae (Egamberdieva et al., 2019), thus showed significantly higher proline content 1.5 to 2 times greater than uninoculated controls samples. This increase in proline is positively associated with enhanced chlorophyll stability and antioxidant enzyme activity, ultimately contributing to improved plant growth and productivity.

4. CONCLUSION:

In this study, cow dung-derived isolates were screened for plant growth-promoting bacteria. Among the ten isolates, the KG9 strain exhibited superior growth-promoting abilities, including mineral solubilization, siderophore production, cellulose hydrolysis, nitrogen fixation, and the synthesis of indole-3-acetic acid (IAA), gibberellic acid, and ammonia. Molecular characterization identified KG9 as Klebsiella pneumoniae. Pot trials were conducted using various wheat seed treatments such as seed priming, application of bacterial broth before and after germination, and encapsulation in beads before and after germination and compared against untreated controls. These treatments led to notable improvements in root and shoot length, chlorophyll content, and proline levels. Thus, K. pneumoniae KG9 demonstrates multiple plant growth-promoting capabilities and holds promise candidate as a bioinoculant and biofertilizer.

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