ISOLATION, IDENTIFICATION, AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM SUGARCANE (*SACCHARUM OFFICINARUM*) RHIZOSPHERE

ABSTRACT

The use of biofertilizers and biopesticides is the foundation of modern sustainable agricultural techniques. The rhizosphere of sugarcane *(Saccharum officinarum*) may provide rhizobacteria the ability to fertilize and repel pests. This research focused at the plant growth promotion (PGP) abilities of bacteria found in the rhizosphere of sugarcane. For example, how they break down phosphate, make IAA, make nitrogen, and help seeds sprout. We took sample from different types of sugarcane rhizosphere in the Ankleshwar area to obtain isolates. For further study and molecular identification using the 16S rRNA gene sequence, one strain of bacteria was picked because it could break down phosphate and make IAA. This confirms the isolate's identity as *Klebsiella pneumoniae.* The isolate had a phosphate solubilization index of 2.11, and the PKVK broth gave off 91.34±2 µg/ml of phosphate, which was calculated. After 72 hours of incubation at room temperature, 61.54±2 µg/ml of IAA without tryptophan was generated. The test on seed germination showed that treating plants with *Glycine max, Solanum melongena, Solanum lycopersicum, Capsicum annuum*, and *Oryza sativa* bacteria increased plant height, dry weight, and fresh weight more than the control group. *Solanum lycopersicum* exhibited the highest rate of germination (100%). The infected agricultural seeds' better seedling characteristics suggested that this isolate may be used to a biofertilizer formulation for environmentally friendly production.

Keywords: PGPR, *Klebsiella pneumoniae*, N_2 fixation, IAA, phosphate solubilisation

1. INTRODUCTION

In agriculture has used soil microorganisms used for many years to increase productivity. It is the job of these bacteria to provide nutrients to crops, help plants grow by doing things like making plant hormones, control or stop plant pathogen activity, improve soil structure, and bioaccumulate or microbially leach inorganics (Sengupta et al. 2015). In the age of sustainable crop production, the interactions between plants and

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microbes in the rhizosphere are very important. This is because they change, move, dissolve, and do other things with nutrients from a limited pool. This allows plants to absorb vital nutrients and reach their full genetic potential. In an integrated plant nutrient management system, biological methods are now gaining popularity as a supplement to chemical fertilizers for increasing crop production. Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that are beneficial for plant development. They may colonize the plant root and promote plant growth (Reddy & Reddy, 2013; Rizvi et al. 2017). The rhizosphere is an important place for microbes and plants to interact (Sureshbabu et al. 2016). It is home to PGPR, also known as nodule-promoting rhizobacteria (NPR) or plant health-promoting rhizobacteria (PHPR).

The study's aims were to separate PGPR from the sugarcane rhizosphere in a lab setting and choose the most potent isolate based on PGPR traits; to look at the isolate's biochemical properties, ammonia production, IAA production, and 16S rRNA sequence; and to test the isolate's PGP potency by planting cereal crops in a pot and observing the seeds germinatation.

2. MATERIAL AND METHODS

2.1. Bacterial strain isolation and screening

We took a rhizospheric soil sample from sugarcane (Cultivar: CoN 13072 (Gujarat Navsari Sugarcane-11)) to find bacteria that help plants grow. We serially diluted the samples up to 10^7 after adding them into a flask containing 90 ml of sterile distilled water. The plate was then filled with melting nutritional agar (NA) medium, and 100 µl of the previous three dilutions was used as an inoculum. Then plate was placed for the incubation for 24 hours at 37°C in an incubator. In order to see if phosphate (P) could be dissolved in Pikovskaya medium, nitrogen could be fixed in Jensen medium, and IAA could be made in LB broth with 0.1% tryptophan, the most diverse organisms were first put together. We selected the best nitrogen-fixing and phosphate-soluble bacteria (PSB) for further research (Bhardwaj et al. 2017; Mazumdar et al. 2018).

2.2. Morphological and Biochemical characterization

We performed different biochemical tests, including Gram staining, morphology, and biochemical traits, to identify the isolate. An example of these tests is the Voges-Proskauer test, which looks at how well something breaks down gelatine,

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amylase, casein, hydrogen sulphide, protease, citrate, HCN, ammonia, siderophore, and organic acid (Cappuccino and Sherman 2005; Bhardwaj et al. 2017). We incubated all the biochemical assays at 37°C.

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2.3. Phosphate solubilization activity

Pikovskaya's agar (Baghaee & Heidarzadeh, 2014; Sharath et al., 2021). We spot-inoculated plates with *K pneumoniae* culture and incubated them for four days at 30°C to measure the phosphate solubilization. You could see that the bacteria were able to dissolve phosphate because there were clear halos around them, and the solubilization index was found (Karmakar et al. 2018).

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Phosphate solubilization index (PSI) = $\frac{\text{colony diameter + halo zone}}{\text{colony diameter}}$

To find out how much soluble phosphate was in the broth, 150 ml Erlenmeyer flasks were filled with 25 ml of NBRIP medium, which had 10 g of glucose per liter. We used 5 grams of $MgCl_2.6H_2O$, 0.25 grams of $MgSO_4.7H_2O$, 0.2 grams of KCl, and 0.1 grams of $(NH_4)_2SO_4$. The bacterial strain *K. pneumoniae* was inoculated in triplicate in autoclaved broth, whereas the uninoculated medium was used as a control. We set the incubator shaker to 180 rpm and incubated the flasks for 96 hours at 30°C. We used centrifugation for 10 minutes at 10,000 rpm to collect the cultures. We assessed the amount of phosphorus present in the cell-free culture supernatant. Mix 10 ml of the supernatant with 50 ml of Olsen reagent (Olsen, 1954). Then, add 5 drops of p-nitrophenol. Keep adding H_2SO_4 until the yellow color goes away. Then, add 40 ml of distilled water. Finally, add 5 ml of L-ascorbic acid solution. Shake well and keep it at RT for 30 minutes. After 30 minutes, record the OD at 880 nm by using the spectrophotometer, and the standard curve was used to extrapolate the quantity of P dissolved.

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2.4. N_2 Fixation

We put *Klebsiella pneumoniae* on Ashby's N-free agar plates that had 20 grams of mannitol, 0.2 grams of $MgSO_4 \cdot 7H_2O$, 0.2 grams of K_2HPO_4, 0.2 grams of NaCl, 0.1 grams of K_2SO_4, 5.0 grams of $CaCO_3$, and 20 grams of agar-agar. We kept the plates at 37°C for 24 hours. We calculated the isolate's ammonia production capacity using the methodologies outlined by Bhatt et al. (2020) and Goswami et al. (2014).

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Klebsiella pneumoniae was put into four separate 100 ml Erlenmeyer flasks and left to grow at 37 °C for 24, 48, 72, and 96 hours in 50 ml of Asbhy's N-free liquid medium. Following the appropriate incubation period, the culture broth was centrifuged for 10 minutes at 10,000 rpm, and 200 µl of the supernatant was added together with 1000 µl of Nessler's reagent. The total volume was then increased to 8500 µl by adding twice d/w. We incubated the mixture at RT for 30 minutes. The mixture developed from brown to yellow, indicating ammonia generation. A standard curve made with 0.1 to 10 µmol ammonium sulphate was used to compare the optical density at 450 nm to find out how much ammonia was there.

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2.5. Indole acetic acid (IAA) production

We assessed IAA production using Duca & Glick's (2020) approach. After infecting *Klebsiella pneumoniae* and incubating it in LB broth at 37°C for 96 hours, tryptophan (1 mg/ml) was either present or absent. We centrifuged the culture for 10 minutes at 10,000 rpm after the allotted incubation period. Salkowski's reagent, which is 50 ml of 35% $HClO_4$ and 1 ml of 0.5 M $FeCl_3 \cdot 6H_2O$, was mixed with 2 ml of the culture supernatant and left to sit for 30 minutes at room temperature. The creation of a pink color showed that IAA had been made. To find out how much IAA was in the mixture, the optical density at 530 nm was measured using a standard curve made with 6–100 µg/ml of standard IAA.

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2.6. Seed germination assay

We inoculated a single colony of *Klebsiella pneumoniae* into nutritional broth and incubated it for 24 hours at 150 rpm in an orbital shaker. To get a final concentration of 10^8 colony-forming units (CFU/ml), the culture was centrifuged in 15 ml sterile plastic tubes at 6000 rpm for 15 minutes. The pellets were then re-suspended in sterile distilled water. After soaking in a 2% NaOCl solution for ten minutes, the seeds of brinjal, tomato, chili, black gram, and soybean were sterilized and then rinsed three times with sterile distilled water. There was a laminar airflow environment where the seeds were dried for 30 minutes after being dipped in the inoculant (10^8 CFU/ml). They were then put in a container with 5 kg of twice autoclaved soil (pH 7.03). We treated the seeds with sterile distilled water as a control. The experiments used a totally randomized block design, and after five days, we assessed the growth parameters.

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2.7. PCR amplification of 16S rRNA

We amplified the 16S rRNA gene by PCR using the genomic DNA of *Klebsiella pneumoniae* as a template. The reaction mixture was made up of 60 microliters: 25.6 microliters of nuclease-free water, 30 microliters of 2X PCR buffer, 1.2 microliters of forward primer (0.2 µM) 27 F (5' AGAGTTTGATCCTGGCTCAG 3'), 1.2 microliters of reverse primer (0.2 µM) 1492R (5' CGGTTACCTTGTTACGACTT 3'), and 2 microliters of template DNA. A 5-minute denaturation phase at 95 ºC was the first step in the PCR process. There were then 35 cycles of denaturation at 95 ºC for 30 seconds, annealing at 50 ºC for 30 seconds, extension at 72 ºC for 1 minute and 45 seconds, and a final extension at 72 ºC for 7 minutes. The PCR product was separated on a 1.7% agarose TAE gel and then cut off of the gel. The SLS Research PCR Clean-up Kit (Cat: #SCMR009) was used to remove and clean the product. We used the Sanger dideoxy chain termination technique to sequence the purified PCR product. The Big Dye TM Terminator V3.1 kit was used to set up the sequencing PCR reaction in the Applied Biosystems TM MiniAmp TM plus Thermal Cycler.

2.8. Phylogenetic Analysis

You can find out how the strain Klebsiella pneumoniae fits into the family tree by using the BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) to match the 16S rRNA sequence with sequences from the NCBI GeneBank database (Altschul et al., 1990). Tamura et al. (2021) used the neighbor-joining approach in MEGA 11 to build the tree.

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2.9. Tolerance for salt

Adding different amounts of NaCl to nutritional broth (NB) medium and keeping the mix at 37 °C for 24 to 72 hours showed how well the isolates could handle salt (Sharma et al. 2021).

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2.10. Analysis of statistics

We used the NCBI BLAST program and MEGA software (11.0) to look at bioinformatics data and make a phylogenetic tree using evolutionary connection analysis.

3. RESULT AND DISCUSSION

These are free-living bacteria in the soil that aggressively colonize plant roots. When applied to seeds or crops, they help the plants grow and produce more (Kumar et al. 2014). The purpose of the present research was to describe sugarcane PGPR *K. pneumoniae's* capacity to promote plant development through its biochemical activity. Over 90 different organisms were found on nutrient agar plates. We chose strain K pneumoniae for further study due to its ability to dissolve P, fix nitrogen, and produce IAA. Figure 1 shows the study sites where we collected the soil for further analysis.

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Figure 1 Location map indicating the study sites.

3.1. Characterization by morphology and biochemistry

Gram test morphological characterization of the isolate showed that the bacterium was rod-shaped and gram-negative. A nutrient agar plate with colony characteristics shows that the isolate was clear, growing quickly, and had a small, spherical colony with a smooth surface and a high elevation. The isolate yielded favorable results in thirteen of the sixteen biochemical assays conducted on it. Table 1 gives the details of the sugarcane root rhizosphere isolate. Table 2 gives the scientific classification of the isolated PGPR bacterial strain *K. pneumoniae*. Table 3 summarizes the findings of the biochemical analysis.

Table 1: Details of Sugarcane root rhizosphere isolate

Identified by 16s rRNA	***Host cultivar***	***Place of collection***	***Accession no***	***IAA production µg/ml***	***Phosphate solubilization index***	***Phosphate solubilization µg/ml***
K pneumoniae	Sugarcane	Ankleshwar	PQ358414	61.54	2.11	91.34

Table 2: Scientific classification of *Klebsiella pneumoniae*

Domain	***:***	***Bacteria***
Phylum	:	Pseudomonadota
Class	:	Gammaproteobacteria
Order	:	Enterobacterales
Family	:	Enterobacteriaceae
Genus	:	*Klebsiella*
Species	:	*K. ~~pneumoniae~~pneumonia*

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Table 3: Biochemical properties of *K pneumoniae*

Biochemical test	**Results**
Gram's stain	Negative
UV Fluorescent	Green Pigment
Catalase test	Positive
Urease test	Positive
Organic acid	Positive
Starch hydrolysis	Positive
MR test	Positive
VP test	Positive
Gelatine hydrolysis test	Negative
Amylase production test	Positive
Casein hydrolysis	Positive
Hydrogen sulphide production test	Negative
Protease test	Positive
Citrate utilization test	Positive
HCN	Positive
Siderophore	Positive

3.2. Phosphate solubilization activity

It was possible to find out how well and how much the isolated strain could break down inorganic phosphate by growing it in PKVK agar medium and broth medium for 24, 48, 72, and 96 hours, in that order. Figure 2 displays pictures that show how much phosphate was broken down by the single strain. This was done by measuring the phosphate solubilization zone and the phosphate solubilization index (PSI). The PSI went up slowly from 24 hours to 96 hours, reaching its highest point at 96 hours with a value of 2.11. The phosphate solubilization zone reached its maximum (15.2 mm) on day 4 (after 96 hours), but the PSI ratio was 2.11. That same year, Kerketta et al. 2025 said that the *Klebsiella variicola PSEG-1* strain could break down phosphate well in Pikovskaya's medium, with an index of 1.6. Additionally, the result indicated a positive correlation (r=0.91) between the diameter of the strain's spot inoculants.

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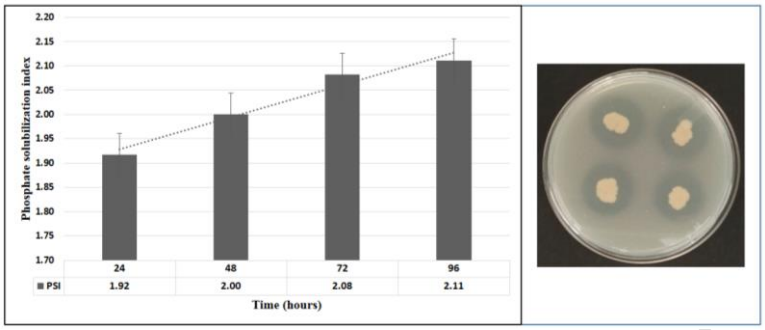

Figure: 2 A look at the phosphate solubilization index (PSI) that *K. pneumoniae* made on PKVK agar medium after 24, 48, 72, and 96 hours of incubation, as well as the zone on the PKVK plate.

Quantitative tests showed that adding more incubation time decreased the amount of phosphate that was soluble. *K. pneumoniae* broke down the phosphate over 96 hours (0–91 µg/ml). It was found by Glick et al. in 1998 that gluconic, succinic, propionic, and lactic acids were the most common organic acids in the process of breaking down phosphate. *Klebsiella* species, such as *Klebsiella sp. Br1*, *Klebsiella pneumoniae Fr1* (Kaun et al., 2016), *Klebsiella pneumoniae VRE36* (Bhardwaj et al., 2017), and *K. pneumoniae* (Biswas et al. 2023), can break down inorganic phosphates, according to many research papers.

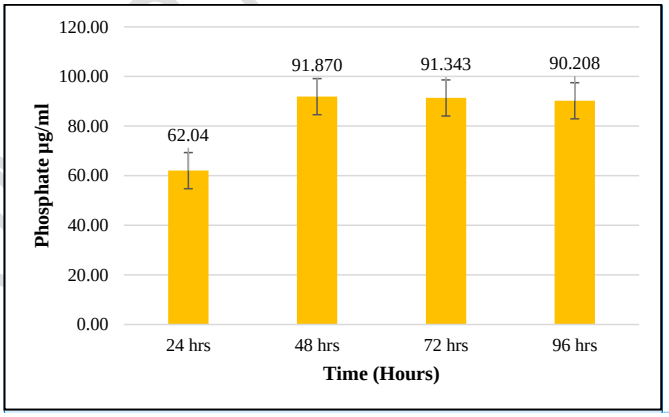

Figure 3: The study looked at how much soluble phosphorus *K. pneumoniae* could produce on Pikovskaya's broth medium after 24, 48, 72, and 96 hours of incubation.

3.3. Production of IAA

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Many bacteria make IAA, a crucial PGP hormone, via both independent and tryptophan-dependent mechanisms. The phytohormone IAA is very important for plant growth because it helps cells divide, grow, form lateral and adventitious roots, make fruit, and age. It also acts as a signaling molecule (Duca et al. 2014). Bacterial IAA improves the plants' ability to absorb nutrients by weakening the cell wall and increasing the length and surface area of the roots. Several reports have shown that certain strains of plant growth-promoting bacteria (PGPB) can make IAA. These strains include *Bacillus species*, *Klebsiella species*, *Azotobacter species*, *Agrobacterium species*, *Pseudomonas species*, *Streptomyces species*, and *Burkholderia* species (Glick, 2012). According to the findings in Figure 4, *K. pneumoniae* produced IAA with or without tryptophan. Additionally, the synthesis increased when the amino acid was absent. At 72 hours of incubation, the highest levels of IAA generation were 46.28 and 61.54 µg/ml, respectively, in the presence and absence of tryptophan. After 96 hours of incubation, the production dropped to 3.2 µg/ml without tryptophan and 1.13 µg/ml with it. A new study (Kumar et al. 2021) says that *K. pneumonia strain M6*, which was found in the rhizosphere of the mango plant, produced 35.53 ± 0.2 µg/ml of IAA in tryptic soy broth (TSB) that had 0.5% tryptophan added to it. It was reported by Jasim et al. (2013) that PGP endophytic *Klebsiella* and *Enterobacter* species were found in *Piper nigrum* and were able to make IAA. Sachdev et al. (2009) say that six strains of *K. pneumoniae* found in the rhizosphere of wheat produced IAA in the lab. The *K8* strain produced the most, at 27.5 µg/ml. Bharadwaj et al. (2017) say that after 96 hours of growth at 37 °C, the *K. pneumoniae VRE36* strain made 45.32 ± 2.46 µg/ml of IAA. After 72 hours of incubation at 37 °C, our research shows that *K. pneumoniae* produces a very high amount of IAA (61.54 ± 1.13 µg/ml). Additionally, the optimization procedure improved the generation of IAA.

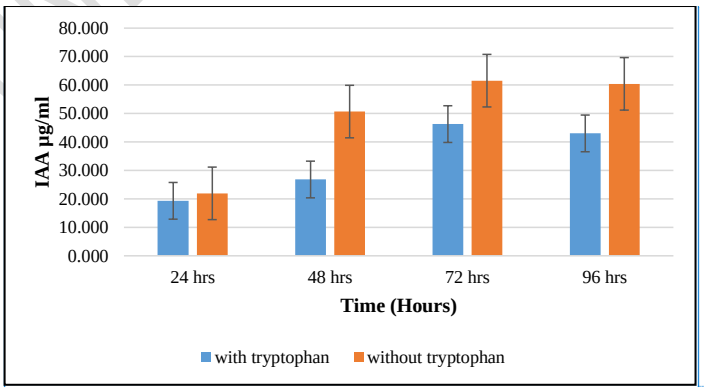

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Figure 4: *K. pneumonia* produces IAA on LB broth medium with or without tryptophan. Three duplicates of the mean ± SD were used to represent the data.

3.4. *K. pneumoniae's* fixation of N_2 and generation of ammonia

By making it easier for plants to take in nitrogen, microorganisms fix nitrogen in a way that is either symbiotic or non-symbiotic (Goswami et al., 2014). Our investigation revealed that the isolated strain *K. pneumoniae* could fix nitrogen to ammonia since it grew effectively on N-free Ashby's medium. *K. pneumoniae* produced 53.5 g/ml of ammonia after 24 hours of incubation, and after 72 hours, we measured the strain's highest ammonia production at 106.9. Figure 5 displays these data. Since the strain also produced a pellicle in the Nfb semisolid N-free medium, we classified it as a free-living N fixer. We have already identified and isolated various diazotrophic bacteria. They are members of the *Enterobacter, Klebsiella, Zoogloea, Azospirillum,* and *Azoarcus* genera. (Malik et al., 1994; Bilal and Malik, 1987). Isolating and characterizing isolates with nif-lac fusions has helped a lot with understanding how nitrogen fixation works in *Klebsiella pneumonia* (Mazumdar et al., 2018).

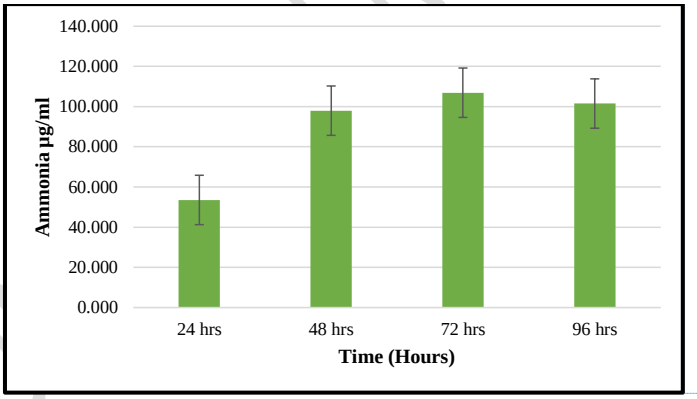

Figure 5: Ammonia generation by *K. pneumoniae* in N-free Asbhy's nitrogen medium. Data were given as triplicate of mean ± SD.

3.5. Molecular identification and phylogeny

We subsequently identified the isolates for their species using the partial 16S rDNA sequencing technique. The results of BLAST showed that the isolate had 99.74% of the same genetic material as *K. pneumoniae* isolate 10. We added the 16S rDNA gene

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sequences of *K. pneumoniae* found in this study to the GenBank database at (https://www.ncbi.nlm.nih.gov/) with the accession number PQ358414. The evolutionary history was inferred using the Neighbor-Joining technique (Saitou and Nei, 1987). Researchers believe that the bootstrap consensus tree, comprising 500 replicates, demonstrates the evolution of the studied species over time. Collapsed branches are those that correspond to partitions that were replicated in fewer than 50% of bootstrap replicates. According to Tamura et al. (2004), the percentage of trees with 500 replicates along the branches shows how related taxa are grouped in the bootstrap test. We found the evolutionary distances using the Maximum Composite Likelihood method (Tamura et al. 2021). We give the distances in terms of the number of base substitutions per site. We analyzed eleven nucleotide sequences. Codon positions covered were 1st + 2nd + 3rd + noncoding. We removed all ambiguous positions for each sequence pair using the pairwise deletion option. There were a total of 1530 locations in the final dataset. We conducted evolutionary analyses in MEGA11 (Felsenstein, 1985). We conducted evolutionary studies in MEGA 11.0. *Klebsiella pneumoniae* strain phylogenetic tree According to Arihant, the strain is found on a distinct clade, suggesting that these *Klebsiella* strains belonged to distinct phylotypes.

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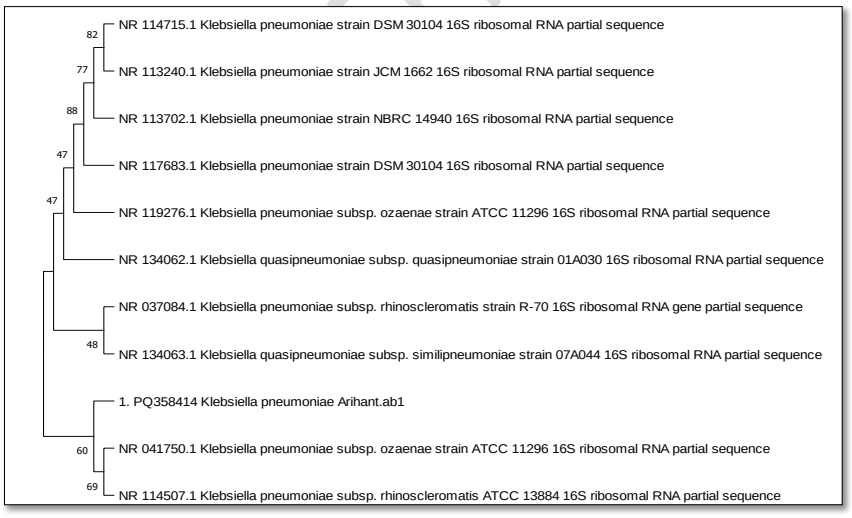

Figure 6*:* *Klebsiella pneumoniae* strain Figure uses a phylogenetic tree based on 16S rRNA gene sequences to show Arihant's position in relation to other *Klebsiella* species. The bar displays one nucleotide change per base. Numbers at nodes represent bootstrap values. The far left lists each strain's accession number.

3.6. Assay for seed germination

The seeds, the plant's reproductive organs, should produce healthy plants. Plant height, fresh weight, and dry weight of sterilized soil control should be greater than those without treatment, as PGPR application should encourage shoot and root development. The use of *K. pneumoniae* in this investigation supported higher germination rates and other growth characteristics (Figure 7). These bacteria could also be useful in farming, as shown by research on *Klebsiella* strains that help wheat plants grow in axenic conditions (Sachdev et al. 2009; Bhardwaj et al. 2017; Gupta et al. 2021). According to our study, *Solanum lycopersicum* had the highest percentage of germination (GP) at 100%. Significant differences existed between the control and treated seeds in terms of plant height, dry weight, and fresh weight (Table 4).

The production of the growth hormone IAA may be the reason why PGPR-treated seeds germinate and grow better than untreated seeds (Hayat et al. 2010; Amara et al. 2015; Batool et al. 2016). The treated plants' heights went from 26 cm for *Glycine max*, 13 cm for *Solanum melongena*, 14.5 cm for *Solanum lycopersicum*, 10.1 cm for *Capsicum annuum*, and 19.2 cm for *Oryza sativa* to 31.3 cm, 14 cm, 23.2 cm, 21 cm, and 27.5 cm (Table 4). In comparison to the control (26 cm), treated *Glycine max* seeds had the greatest plant height (31.3 cm).

Bacterial treatments were much greater than control in every pot experiment. Since it made more IAA and could dissolve phosphate, the isolate in this study may have had an effect on seed germination and plant growth, either directly or indirectly.

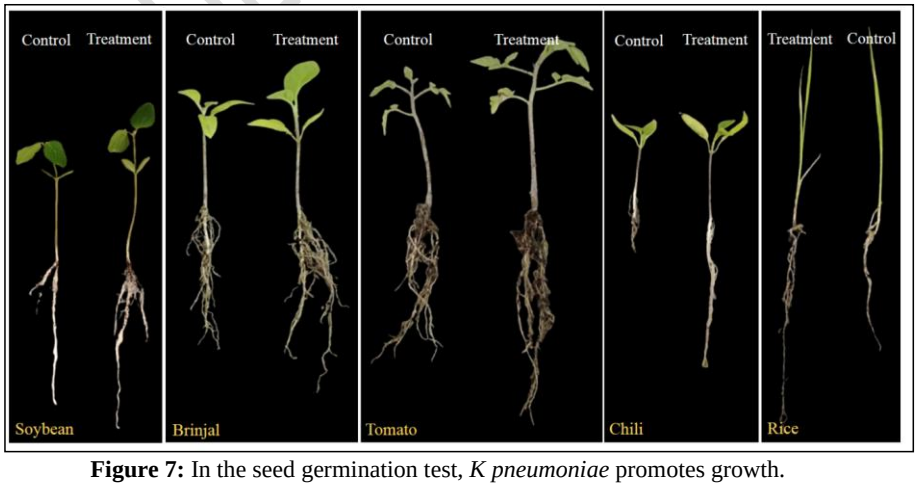

Figure 7: In the seed germination test, *K pneumoniae* promotes growth.

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Table: 4 Effects *K pneumoniae* on plant growth parameters in seed germination assay. Data represents Mean (n=10) ± Standard Deviation. Germination percentage (GP), Plant Height (PH), Leaf width (LW), Leaf length (LL), Shoot Length (SL), Root length (RL), Fresh Weight of plant (FWP), Dry weight of plant (DWP).

Seeds		GP (%)	PH(Cm)	LW(Cm)	LL(Cm)	SL(Cm)	RL(Cm)	FWP(Gm)	DWP(Gm)
***Glycine max* (Soybean)**	Control	72	26	2.1	3.7	14	12	--	--
	Treatment	86	31.3	2.8	4.2	14.3	17	--	--
***Solanum melongena* (Brinjal)**	Control	77	13	0.6	1.3	7	6	0.230	0.047
	Treatment	98	14	1.7	2	6.5	7.5	0.359	0.050
***Solanum lycopersicum* (Tomato)**	Control	88	14.5	1	1.6	7.5	7	0.579	0.077
	Treatment	100	23.2	1.8	1.8	9.7	13.5	0.892	0.090
***Capsicum annuum* (Chilli)**	Control	66	10.1	0.5	1.3	4.1	6	0.474	0.014
	Treatment	91	21	0.6	1.5	5.5	15.5	0.497	0.016
***Oryza sativa* (Rice)**	Control	--	19.2	--	4.9	11	8.2	0.161	0.027
	Treatment	--	27.5	--	7.5	13.5	14	0.171	0.030

3.7 Salt tolerance

We tested the isolates for salt tolerance at different salt concentrations (Figure 8). *Klebsiella pneumoniae* isolates have shown tolerance to a concentration of 8% NaCl.

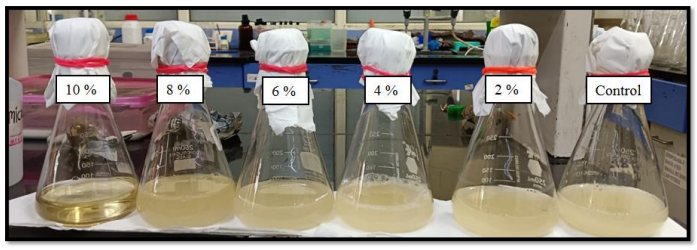

Figure 8: *Klebsiella pneumoniae* strains found in the rhizosphere of sugarcane grow on nutrient broth media that has 2–10% NaCl added to it.

Recently, it was found that three endophytes from the genus *Pseudomonas* that were taken from sorghum can survive up to 7.2% NaCl without harm. This is much greater than the plant's degree of salt tolerance (Gamalero et al. 2020). According to other research, *Salicornia bigelovii* seedlings showed enhanced plant growth and physiological activity in vitro after being inoculated with PGP rhizobacteria *K. pneumoniae* at a high salt concentration of 0.25 M (1.45%) (Rueda-Puente et al. 2013). A study by Kumar et al. (2016) found that the three *Bacillus spp.* bacterial endophytes that were taken from

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Curcuma longa L. and showed PGP characteristics and antimicrobial activity could survive up to an 8% NaCl concentration. The endophytic strain *K. pneumoniae* has strong PGP characteristics and can withstand a NaCl concentration of up to 8%, according to our current investigation. In agriculture, their capacity to provide plants with salt resistance may have enormous promise for raising crop yields in salinity-stressed environments.

Our results suggest that the stem isolate *K. pneumoniae* possesses multiple PGP traits that directly help in plant growth. Therefore, we can use these isolates to enhance crop yield in sustainable agriculture.

4. CONCLUSION:

In this investigation, we identified a powerful strain from the sugarcane plant's rhizosphere. The isolated strain of Klebsiella pneumoniae showed promising results in plant development. It enhanced its potential by breaking down phosphate, producing IAA, ammonia, and more. By converting insoluble phosphorus into a form that plants can absorb, this bacteria improves soil health and reduces environmental pollution, offering a sustainable alternative to artificial fertilizers. Farmers may face certain challenges in field experiments, despite the fact that using this strain to meet soil phosphorus demands produces satisfying results in laboratory or greenhouse conditions. To completely comprehend the advantages of the *K. pneumoniae* strain, including how to use them in different agricultural settings and how they interact with other soil microorganisms, further study is required.

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ABBREVIATIONS

PGP	:	Plant Growth Promotion
%	:	Percentage
°C	:	Degree Celsius
$CaCO_3$	:	Calcium Carbonate
CFU	:	Colony Forming Unit
d/w	:	Distilled Water
DNA	:	Deoxyribonucleic Acid
F Primer	:	Forward Primer
gm	:	Gram
h	:	Hour
HClO4	:	Perchloric Acid
HCN	:	Hydrogen Cyanide

IAA	:	Indole Acetic Acid
K_2HPO_4	:	Dipotassium Phosphate
K_2SO_4	:	Potassium Sulphate.
KCl	:	Potassium Chloride
LB broth	:	Luria Britani Broth
Min	:	Minute
Ml	:	Millilitre
MR test	:	Methyl Red Test
N_2	:	Nitrogen
NA	:	Nutrient Agar
NaCl	:	Sodium Chloride
NaOCl	:	Sodium Hypochlorite
NBRIP	:	National Botanical Research Institute's Phosphate Growth Medium
NCBI	:	National Centre For Biotechnology Information
Nm	:	Nano Meter
NPR	:	Nodule Promoting Rhizobacteria
P	:	Phosphorus
PCR	:	Polymerase Chain Reaction
PGPR	:	Plant Growth Promoting Rhizobacteria
PHPR	:	Plant Health Promoting Rhizobacteria
PKVK broth	:	Pikovskaya's Broth
PSB	:	Phosphate Solubilising Bacteria
PSI	:	Phosphate Solubilization Index
R Primer	:	Reverse Primer
Rpm	:	Rotation Per Minute
rRNA	:	Ribosomal Ribonucleic Acid
RT	:	Room Temperature
TAE gel	:	Tris-Acetate-EDTA Gel
VP test	:	Voges-Proskauer Test
w/v	:	Weight/Volume
Mg	:	Microgram
$FeCl_3.6H_2O$	:	Ferric Chloride Hexahydrate
$MgCl_2.6H_2O$	:	Magnesium Chloride Hexahydrate

$MgSO_4.7H_2O$: Magnesium Sulphate Heptahydrate

$(NH_4)_2SO_4$: Ammonium Sulphate

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