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Isolation, Identification, and Characterization of Plant Growth-Promoting Rhizobacteria from Sugarcane (*Saccharum Officinarum*) Rhizosphere

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DV and BP drafted the experimental design and performed the experiments, as well as the initial draft of the manuscript text. Authors AP, ST, LM and NP helped in data collection and data analysis. All authors read and approved the final manuscript.

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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 solubilize phosphate, produce IAA, produce nitrogen, and help seeds sprout. Soil samples were taken 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 solubilize phosphate and produce 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.

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/NB	*:*	*Nutrient Agar/Nutrient Broth*
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*
µg	:	*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*

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 & Gunri, 2015). In the age of sustainable crop production, the interactions between plants and 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 cultivation of sugarcane is a significant tropical crop that is used in the manufacturing of sugar, biofuels, and electricity. The cultivable bacteria in the sugarcane rhizosphere under different levels of drought stress were characterized and evaluated for their plant growth-promoting activities, as reported by Pereira et al., (2019). The researchers suggested that the microorganisms that were isolated from the *Saccharum officinarum* root surface could be investigated for potential applications in agriculture, such as biocontrol agents or biofertilizers. Taulé et al., (2012) found the organisms and figured out how certain bacteria connected to plants can help non-legume crops like sugarcane grow better in Uruguay.

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 germination.

2. MATERIALS AND METHODS

2.1 Bacterial Strain Isolation and Screening

Rhizopheric soil samples were taken by us from sugarcane (Cultivar: CoN 13072 (Gujarat Navsari Sugarcane-11)) to find bacteria that help plants grow. Samples were serially diluted up to 10^7 after adding them into a flask containing 90 ml of sterile distilled water. The plate was then filled with melting nutrient 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. The best nitrogen-fixing bacteria and phosphate-soluble bacteria (PSB) were selected by us for further research (Bhardwaj et al., 2017; Mazumdar et al., 2018).

2.2 Morphological and Biochemical Characterization

The different biochemical tests, including Gram staining, morphology, and biochemical traits were performed by us to identify the isolate. An

example of these tests is the Voges-Proskauer test, which looks at how well something solubilize gelatine, amylase, casein, hydrogen sulphide, protease, citrate, HCN, ammonia, siderophore, and organic acid (Cappuccino & Sherman, 2005; Bhardwaj et al., 2017). All the biochemical assays were incubated by us at 37°C.

2.3 Phosphate Solubilization Activity

Plates of Pikovskaya's agar were inoculated with a culture of *K. pneumoniae* (Baghaee & Heidarzadeh, 2014; Sharath et al., 2021), and then they were incubated at a temperature of 30 degrees Celsius for a period of four days. According to Karmakar et al., (2018), the presence of distinct halos surrounding the bacterial colony was indicative of the capability to solubilize phosphate, and the solubilization index was measured.

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

There were 150 ml Erlenmeyer flasks made with 25 ml of NBRIP medium, which included 10 g of glucose, 5 g of $MgCl_2{\cdot}6H_2O$, 0.25 g of $MgSO_4{\cdot}7H_2O$, 0.2 g of KCl, and 0.1 g of $(NH_4)_2SO_4$. The purpose of this preparation was to evaluate the concentration of soluble phosphate in the broth. The bacterial strain *K. pneumoniae* was injected in triplicate in autoclaved broth, whereas the medium that had not been infected served as the control. Under conditions of 30 degrees Celsius and 180 rpm, the flasks were incubated for a total of 96 hours. Centrifugation at 10,000 rpm for ten minutes was used to collect the cultures. An analysis was performed on the supernatant of the cell-free culture to determine the content of phosphorus. After combining ten milliliters of the supernatant with fifty milliliters of Olsen reagent, the results were as described by Olsen, (1954). After that, put in five drops of p-nitrophenol. You should continue to add H_2SO_4 until the yellow hue disappears. After that, forty milliliters of water that had been distilled was added. At long last, five milliliters of a solution of L-ascorbic acid was added. Give it a good shake, and then let it sit at room temperature for half an hour. After thirty minutes, the results were recorded using the spectrophotometer at a wavelength of 880 nm. These results were then extrapolated using the standard curve to determine the amount of phosphorus that was dissolved.

2.4 N_2 Fixation

Klebsiella pneumoniae was cultured on Asbhy's N-free agar plates containing (g/l): 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 at 37ºC for 24 h. The approach that was provided by Bhatt et al., (2020) and Goswami et al., (2014) was used in order to determine the ammonia generation capacity of the isolate studied. An inoculum of *Klebsiella pneumoniae* was placed in separate 100 ml Erlenmeyer Flasks, and the bacteria was then incubated at 37 degrees Celsius for 24, 48, 72, and 96 hours in 50 milliliters of Asbhy's N-free liquid medium. Following the specified duration of incubation, the culture broth was subjected to centrifugation at a speed of 10,000 rpm for a duration of ten minutes. Following this, 200 µl of the supernatant was added along with 1000 µl of Nessler's reagent. The total volume was then increased to 8500 µl by adding twice distilled water. Thirty minutes were spent incubating the mixture at 37 degrees Celsius. The presence of a brown to yellow color was observed, which is indicative of the production of ammonia. The concentration of ammonia was determined by comparing the optical density at 450 nm to a standard curve that was prepared using 20-200 ppm of ammonium sulfate.

2.5 Indole Acetic Acid (IAA) Production

The production of IAA by *K. pneumoniae* was identified using the modified method outlined by Duca & Glick, (2020). A 24-hour grown culture was inoculated into 10 ml of sterile LB supplemented with 0.1% tryptophan. After incubation at 37 °C for 96 hours, the amount of IAA produced was determined in a 2 ml culture supernatant using 4 ml of Salkowski reagent (50 ml of 35% $HClO_4$ and 1 ml of 0.5 M $FeCl_3{\cdot}6H_2O$) and the IAA concentration was extrapolated from the standard curve. Optical density was assessed at 530 nm using a Rexford uv-vis double beam-spectrophotometer model no. LI-2600.

2.6 Seed Germination Assay

The *K. pneumoniae* culture was created by inoculating a single colony into NB and then incubating it for twenty-four hours at a speed of two hundred rpm in an orbital shaker. A final concentration of 10^8 colony-forming units (CFU/ml) was achieved by centrifuging the

culture in a 2 ml Eppendorf tube at 8,000 rpm for ten minutes. This was done in order to acquire the desired concentration. Following that, the pellets that were formed were re-suspended in distilled water that had been sterilized. The seeds of brinjal, tomato, chili, black gram, and soybean were sterilized by soaking them in a solution of 2% sodium hypochlorite for ten minutes. Following this, the seeds were washed three times with sterile distilled water on three separate occasions. The seeds were dried in a laminar air flow atmosphere for a period of thirty minutes after being dipped in the inoculant, which contained 10^8 colony-forming units per milliliter. After that, they were placed in a container that contained five kilograms of soils that had been autoclaved twice and had a pH of 7.03. For the purpose of serving as a control, the seeds were treated with sterile distilled water. The studies were conducted using a block design that was fully random, and we evaluated the growth parameters at regular intervals of five days.

2.7 PCR Amplification of 16S rRNA

Genomic DNA of *Klebsiella pneumoniae* was used as template for PCR amplification of 16S rRNA gene. The reaction mixture in total volume of 60 µl contained; 25.6 microliters 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. PCR condition was initial denaturation step at 95^{o}C for 5 min followed by 35 cycles of denaturation at 95^{o}C for 30 sec, annealing at 50^{o}C for 30 sec and extension at 72^{o}C for 1 min 45 seconds and then a final extension at 72^{o}C for 7 min. The PCR product was separated on 1.7 % agarose TAE gel, cut from the gel. The SLS Research PCR Clean-up Kit (Cat: #SCMR009) was used to remove and clean the product. The Sanger dideoxy chain termination technique was used 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

In order to determine the phylogenetic relationship of the strain *Klebsiella pneumoniae*, a comparison was made between the 16S rRNA sequence and the sequences that were retrieved from the Gen Bank database of the NCBI using the BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) (Altschul et al., 1990). According to Tamura et al., (2021), the tree was built by employing the neighbor joining method with the MEGA 11 software.

2.9 Tolerance for Salt

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

2.10 Analysis of Statistics

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

3. RESULTS 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. Fig. 1 shows the study sites where we collected the soil for further analysis.

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 favourable 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.

Fig. 1. Location map of the study sites

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*

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

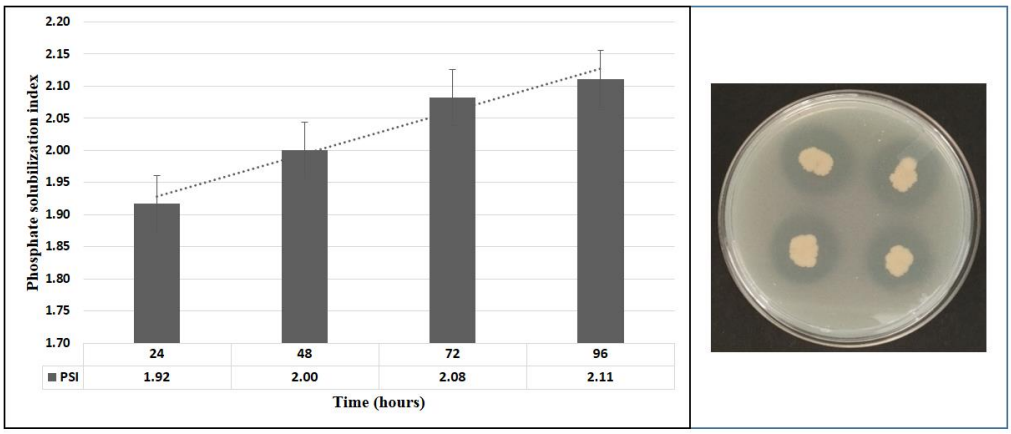

Fig. 2. The phosphate solubilization index (PSI) (where, *K. pneumoniae* made on PKVK agar medium after 24, 48, 72, and 96 hours of incubation, as well as the zone on the PKVK plate)

3.2 Phosphate Solubilization Activity

It was possible to find out how well and how much the isolated strain could solubilize inorganic phosphate by growing it in PKVK agar medium and broth medium for 24, 48, 72, and 96 hours, in that order. Fig. 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

solubilize 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.

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

In Fig. 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

Many bacteria produce 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 signalling 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 produce 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 Fig. 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. pneumoniae 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 produce 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. Bhardwaj 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.

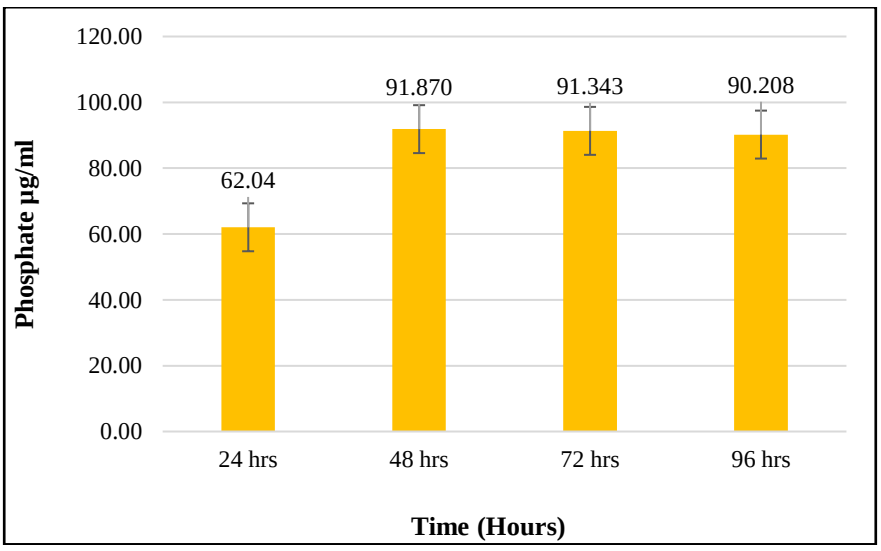

Fig. 3. Graph of soluble phosphorus poduced by *K. pneumoniae* during incubation

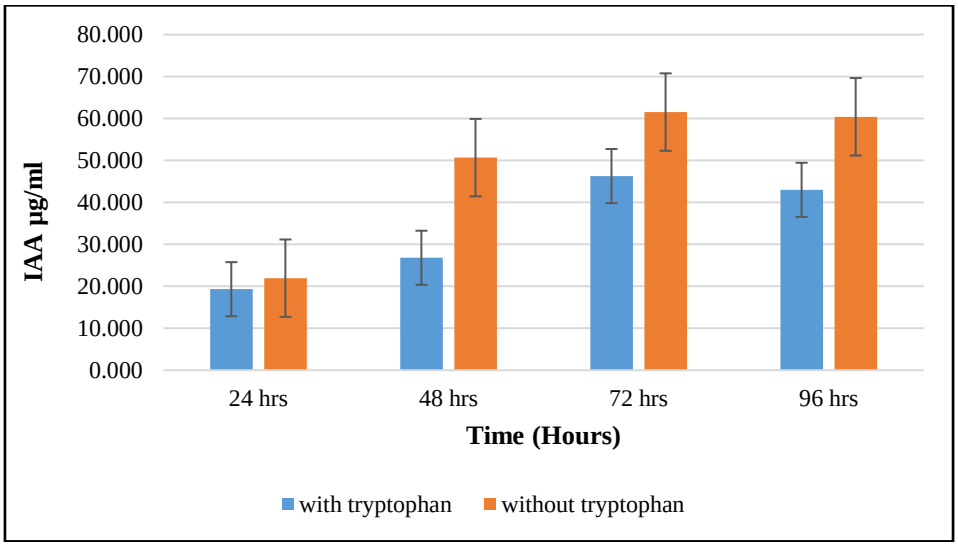

Fig. 4. The graph of production of IAA by *K. pneumoniae* with or without tryptophan

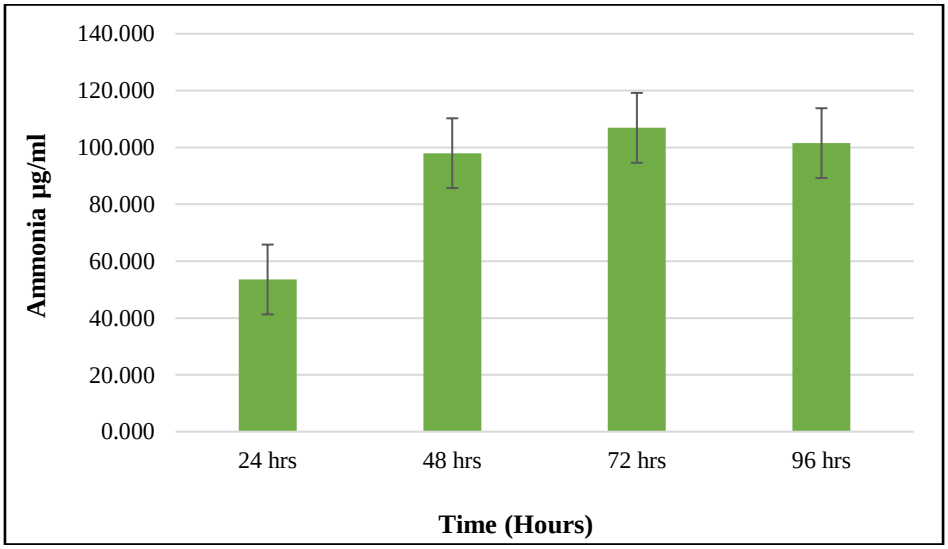

Fig. 5. Ammonia generation by *K. pneumoniae* in N-free Asbhy's nitrogen medium. Data were given as triplicate of mean ± SD

In Fig. 4 *K. pneumoniae* 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. Fig. 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 & Malik, 1987). Isolating and characterizing isolates with nif-lac fusions has helped a lot with understanding how nitrogen fixation works in *Klebsiella pneumoniae* (Mazumdar et al., 2018).

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 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 & 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 analysed 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.

In Fig. 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 (Fig. 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.

3.7 Salt Tolerance

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

In Fig. 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 *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.

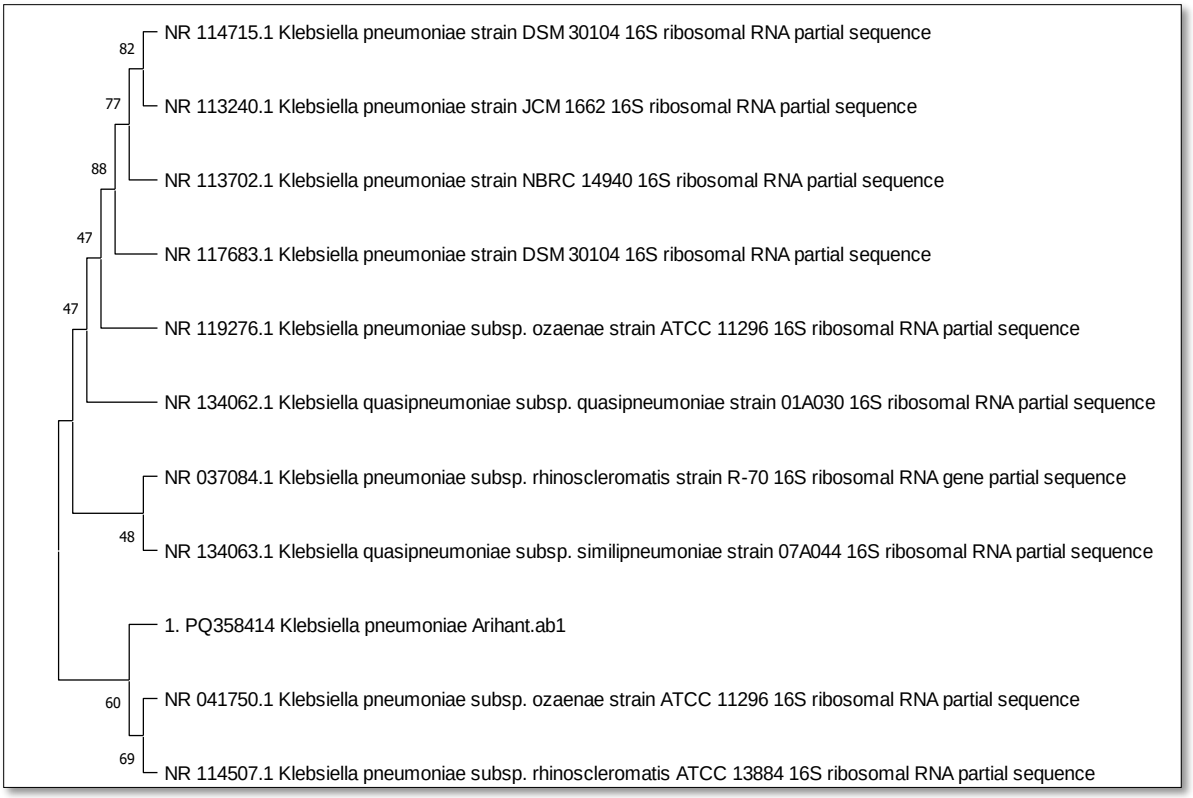

Fig. 6. A phylogenetic tree based on 16S rRNA gene sequences

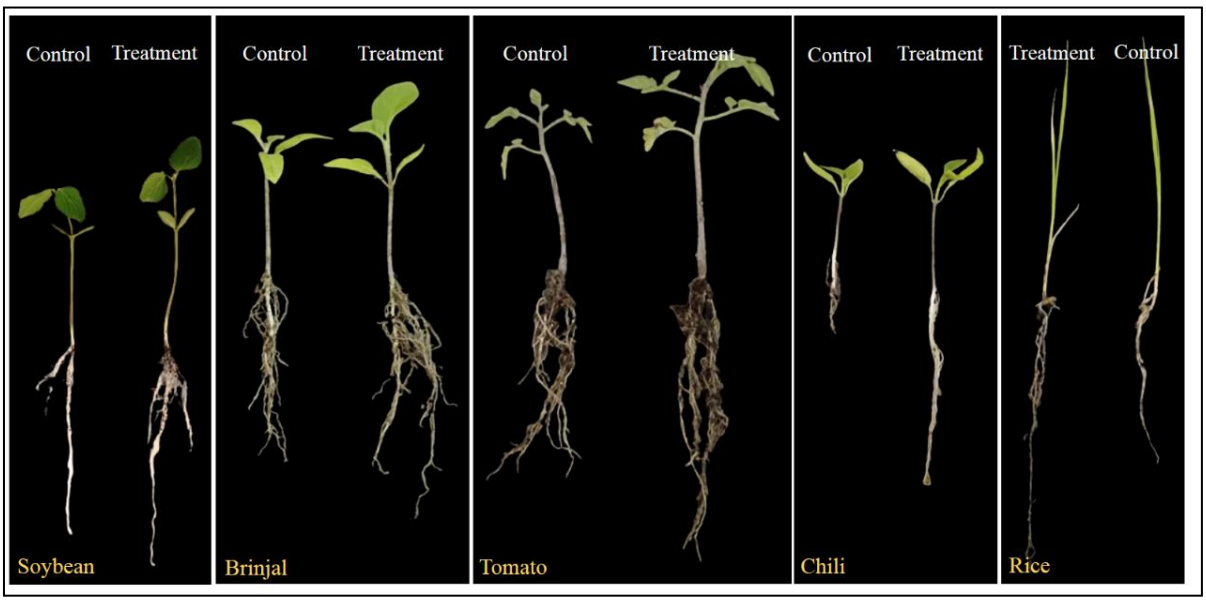

Fig. 7. The seed germination test, *K. pneumoniae* promotes growth

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

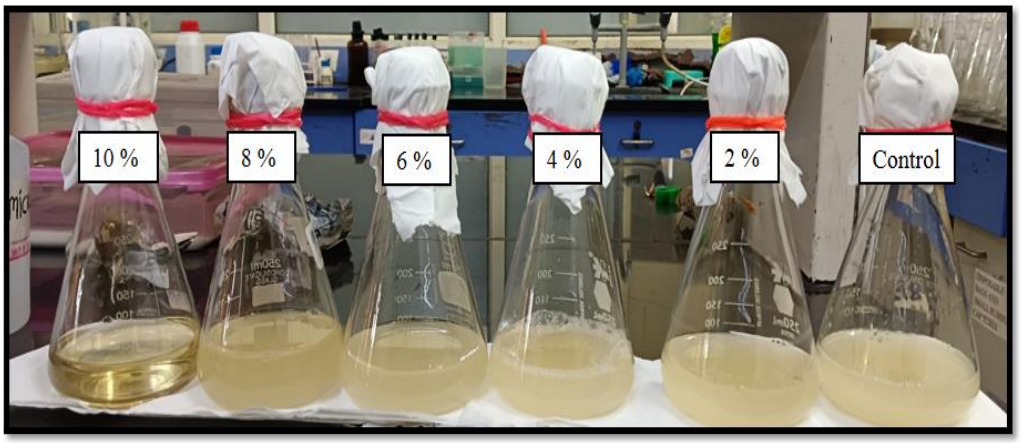

Fig. 8. *Klebsiella pneumoniae* strains

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 solubilize 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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