International Journal for Research in Applied Sciences and Biotechnology

ISSN: 2349-8889
Volume-8, Issue-6 (November 2021)

www.ijrasb.com

https://doi.org/10.31033/ijrasb.8.6.9

Isolation, Identification and Evaluation of Indigenous Plant Growth Promoting Bacterium *Klebsiella pneumoniae* PNE1

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ABSTRACT

Application of chemical fertilizer is an integral practice to optimize crop productivity, but the dominant use of chemical fertilizers contributes largely to the deterioration of the environment, leads to loss of soil fertility, increases pollution, and causes hazardous diseases. Hence, the chemical fertilizers, pesticides and other supplements are being replaced by the plant growth promoting bacteria (PGPB) due to their improved potency and environment friendly nature. Plant growth-promoting bacteria (PGPB) can enhance plant growth by a wide variety of mechanisms like Phosphate (P) solubilization, Potassium solubilisation, siderophore production, biological nitrogen fixation and Indole acetic acid (IAA) production. The *Klebsiella* species is also known to exhibit important PGP traits like solubilization of phosphate, phytohormone production and good germination potential. In present study the *Klebsiella pneumoniae* PNE1 was selected from the isolates obtained from vegetable waste collected from Kadi market. The isolate was selected on the basis of its ability for Nitogen fixation, Phosphate solubilization, Potassium solubilization, IAA production, EPS production and biopolymer degradation. The molecular identification through 16S rRNA gene sequence, confirmed the isolate as *Klebsiella pneumonia* PNE1. Quantitative analysis of ammonia production revealed that isolate *Klebsiella pneumonia* PNE1 produced 0.5 µg/ml of ammonia (NH3) on 6th day of incubation and produced 0.09 µg/ml Nitrite after 8th day of incubation. The Phosphate solubilisation Index (SI) of the isolate was 4.16 and the isolate released 177.50 µg/ml Phosphate. The qualitative estimation of Potassium solubilisation by the isolate Klebsiella *pneumoniae* PNE1 in terms of Potassium solubilisation zone was found to increase gradually from day 1 to 7 days and was maximum at 2nd day with a KSI of 3.6. The isolate *Klebsiella pneumoniae* PNE1 released 29.94 mg/l Potassium on 21th day of incubation respectively. The IAA production was found to be 94.96 µg/ml. The maximum the EPS yield was 11.3 mg/ml. The *Klebsiella pneumonia* PNE1 had capacity to degrade Cellulose, Pectin and Xylan i.e. all biopolymers tested. The antibiotic susceptibility test indicated that isolate was sensitive to all 22 antibiotics tested. The *Klebsiella pneumonia* PNE1 thus, shows important plant growth promoting traits and can be used in a bio-fertilizer formulation for sustainable agriculture.

Keywords- Biopolymer degradation, Plant growth promoting bacteria, EPS production, *Klebsiella Pneumoniae* PNE1, Nitrogen fixation, Phosphate and Potassium solubilization, IAA production.

I. INTRODUCTION

The agriculture sector is considered to be one of the major economy pillar in many developing countries [1]. Agriculture provides 80% of food security and occupation to over one-third of the people worldwide [2]. By 2025 India will have a population of 1403 million from the current population 1391 million (2020-2021). The population of world is also increasing, in this context, it will be impossible to feed the world's growing population without significant increase in the agricultural production. In order to fulfil the increasing demand of agricultural products for food, the chemical fertilization has been adopted as an effective method to improve crop yields for more than five decades [3], but the continual use of agrochemicals such as chemical fertilizers and pesticides in agricultural sector is harmful to human health and also causes ecological imbalance [4].The use of chemical fertilizer will also cause soil and ground water pollution resulting in eutrophication, hypoxia, harmful algal blooms . This practice also negatively affects the roots of the crops, making them unable to acquire nutrients. Additionally, the chemical fertilizers are expensive, affect the soil by reducing its water-holding capacity and fertility, cause imbalance in the soil nutrients, and result in unacceptable levels of water pollution [1]. On the other hand, biofertilizers are eco-friendly, cost-effective, non-toxic, and easy to apply; they help maintain soil structure and biodiversity of the agricultural land [5]. Thus, they serve as a good substitute for chemical fertilizers. Hence, the use of chemical fertilizers and pesticides needs to be replaced by environment friendly biofertilizer [6].

Biofertilizers are microbial formulations made from indigenous plant growth-promoting bacteria (PGPB), that can influence plant growth by synthesizing

plant hormone or facilitating uptake of nutrients from soil through different mechanisms [7] like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1- carboxylate deaminase (ACC), quorum sensing (QS) signal interference , EPS production, phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, interference with pathogen toxin production etc. The inoculation of plants with plant-growth-promoting bacteria (PGPB) has emerged as a great prospect for recovery of disturbed agricultural ecosystem. In past, several numbers of PGPB belonging to the genera *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been isolated and successfully used as biofertilizer [8].

Nitrogen plays a vital role in all living tissues of the plant. High nitrogen supply favors the conversion of carbohydrates into proteins, which in turn promotes the formation of protoplasm [9]. Nitrogen fixation can be considered as one of the most unique and important microbial activity as it makes the possible utilization of molecular nitrogen on earth [10]. The PGPR improve phosphate solubilization by secretion of organic acids and phosphatases which convert insoluble forms of P to soluble forms [11]. Potassium solubilizing bacteria (KSB) can solubilize K-bearing minerals and convert the insoluble K to soluble forms of K available to plant uptake [12]. The indole-3-acetic acid (IAA), produced by bacteria, plants and fungi, plays a central role in cell division, elongation, fruit development and senescence. The EPS-producing PGPR can significantly enhance the volume of soil macropores and the rhizosphere soil aggregation, resulting in increased water and fertilizer availability to inoculated plants. EPS-producing PGPR can also bind cations including Na+. Therefore, an increase in the population density of EPS-producing bacteria in the root zone is expected to decrease the content of Na+ available for plant uptake, and so improve salt stress in plants growing in saline environments [13]. Microorganisms that are involved in composting process excrete several enzymes viz, cellulase, protease, lipase, pectinase, xylanase and other enzymes that contribute in degradation of macro-molecules of organic wastes. *Klebsiella pneumoniae* produce some extracellular enzymes that specifically breakdown certain substrates like Cellulose, Pectin, Starch, Lignin and Xylan [14].

Klebsiella pneumoniae is a Gram negative, rod shaped, lactose fermenting and facultative anaerobic bacterium. *K. pneumoniae* is one of the most important members of *Enterobacteriaceae* family [15]. *Klebsiella* sp., are root-associated bacteria they play important role as plant growth promoting bacteria (PGPB) [15]. These bacteria are able to solubilize both organic and inorganic phosphates, fix nitrogen, and produce IAA growth hormone and ACC De aminase activity and can be effectively used biofertilizer [8]. In present study, *Klebsiella pneumoniae* PNE1 isolated from the vegetable market wastes has been used for the characterization of its plant growth promoting potential (in terms of Nitrogen fixation, Phosphate solubilisation, Potassium solubilisation, IAA production, EPS production) and antibiotic sensitivity. On the basis of results obtained we propose the use of indigenous isolate *Klebsiella pneumonia* PNE1 for the development of Biofertilizer.

II. MATERIALS AND METHODS

Isolation of strain:

Isolation was carried out from vegetable market waste. Vegetable market waste was collected from Kadi vegetable market. Separation of solid and liquid component was carried out by shredding and dewatering process [16]. 1 gm of separated solid component was added in 10 ml sterilized water and then serially diluted up to 10^{-6}. The serially diluted samples were then spread into Ashby's Mannitol agar and the plates were incubated at 37 ± 2^0C for 48 h. The Ashby's Mannitol agar is nitrogen free medium used for isolation of free-living nitrogen fixing bacteria. The colonies developing on Ashby's Mannitol agar are presumed have ability of nitrogen fixation. The colonies showing gummy consistency and exhibiting clear zone were isolated and purified for further study.

Biochemical characterization:

Biochemical characterization of selected isolate was carried out using HI-Assorted biochemical test kit (KB003) of HI-media Laboratories, Mumbai, India.

Molecular identification:

For molecular identification, DNA was isolated from the isolate (PNE1). Quality of DNA was evaluated on 1.0% Agarose Gel, Fragment of 16S rRNA gene was amplified by PCR. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with 357F & 1391R primers using BDT v3.1 Cycle. Sequencing Kit on ABI 3500xl Genetic Analyzer. The 16S rRNA sequence was used to carry out BLAST with the database of NCBI GenBank. Based on maximum identity score first fifteen sequences were selected and aligned using multiple sequence alignment software programs [6].

Phylogenetic Analysis:

The evolutionary history was inferred using the Neighbor-Joining method [17]. The evolutionary distances were computed using the p-distance method [18] and are in the units of the number of base differences per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of

918 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [19].

Antibiotic Susceptibility test:

Antibiotic susceptibility test of the isolate (*Klebsiella pneumoniae* PNE1) was conducted using disc diffusion method. The method involved spreading the 0.1ml of overnight grown culture (set the O.D 0.5 at 540 nm) [20] on Nutrient agar plate and placing antibiotic multidisc on the surface of the plate. Plate was incubated at 37°C for 24 hrs. Antibiotic susceptibility test was conducted using combined multidisc (Combi Disc) for Gram Negative bacteria from Hi Media Laboratories. The zone of inhibition was measured [6].

Determination of ammonia nitrogen and nitrite nitrogen:

Quantitative determination of ammonia nitrogen and nitrite nitrogen was carried out by estimation ammonia nitrogen and nitrite nitrogen production in nitrogen fixing pathway. 3 ml of 24 hrs old culture O.D adjusted to 0.5 was inoculated in 100 ml of Ashby's N-free liquid medium.The inoculated flasks were incubated at room temperature in static condition. Approximately 2ml broth was harvested at the interval of 24 hrs. After the respective time of incubation the culture broth was centrifuged at 10000 rpm for 10 min. Ammonia nitrogen and nitrite nitrogen production in the broth was examined at interval of 24 hrs of incubation.Ammonia production ability of the isolate was estimated by the indophenol blue method [21]. Nitrite production ability of the isolate *Klebsiella pneumonia* PNE1 was estimated by standard method [22].

Determination of Phosphate Solubilisation activity:

Qualitative determination of phosphate solubilization activity was carried out on Pikovskya's agar medium. The plates were spot inoculated with the isolate (*Klebsiella pneumonia* PNE1) and incubated at 37±2°C for 48 hrs. The halo zone formation around the growing colony was considered as positive and solubilization index was measured [6].

Phosphate solubilization index = (colony diameter + halo zone) / colony diameter

Quantitative estimation of phosphate solubilisation was carried out by estimation of P released from Tri Calcium Phosphate (TCP). Three ml of 24 hrs old culture O.D adjusted to 0.5 was inoculated in Pikovskya's broth supplemented with 0.5%Tricalcium phosphate (TCP) [23]. The flasks were incubated for at 37±2°C on environmental shaker at 120 rpm. At the interval of 24 hrs broth was harvested. This was followed by centrifugation at 10000 rpm for 10 min. The amount of P released in the broth was estimated from supernatant by Vanadomolybdo phosphoric acid method [24]. A simultaneous change in the pH was also recorded from the supernatant by eqiptronic Digital pH meter [25].

Determination of Potassium Solubilization activity:

Qualitative determination of Potassium Solubilisation was carried out by using modified Aleksandrow agar medium + bromothymol blue. The plates were spot inoculated with the isolate *(Klebsiella pneumonia* PNE1) and incubated at 37±2°C for 24 hrs. Appearance of clear halos around the colony was measured by Khandeparkar's selection ratio method [26].

D/d =Diameter of zone of hydrolysis / Diameter of growth

Quantitative estimation of Potassium Solubilization activity:

Quantitative determination of potassium solubilisation was carried out by estimation of K released from broth supplemented with 0.5 % Feldspar. Three ml of 24 hrs old culture O.D adjusted to 0.5 was inoculated in 100 ml of GYF (Glucose Yeast extract feldspar) broth [27]. The inoculated flasks were incubated in environmental shaker at 120 rpm. The amount of K released in the broth was examined at interval of 7, 15 and 21 days of incubation [28]. Approximately 5ml broth was harvested at the interval of 7 days. The broth cultures were centrifuged at 10,000 rpm for 10 min in the centrifuge to separate the supernatant from the cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometric method [29].

Determination of Indole acetic acid (IAA) production:

Qualitative determination for the IAA production was detected using Luria agar supplemented with 0.06% sodium dodecyl sulphate and 1% glycerol. The overnight grown culture of the isolate was spot inoculated in each plate. The plates were layered immediately with sterile disc of Whatman No.1 filter paper. After 48hrs, the filter paper disc was removed from the plates and treated with Salkowaski's reagent (2% of 0.5M $FeCl_3$ in 35% per chloric acid) by soaking in a petridish containing the reagent. The reaction was allowed to proceed until adequate colour was developed [30].

Quantitative estimation IAA production:

Overnight grown bacterial suspension adjusted to optical density 0.5 at 540nm was inoculated in 100 ml LB broth (With 0.1% tryptophan and without tryptophan). The flasks were incubated in shaking incubator (37±2 ºC, 120 rpm) for 7 days. At the internal of 24 hrs, 1.5 ml broth was harvested. After centrifugation of harvested broth at 10000 rpm for 10 min, supernatant (1ml) was determined for IAA content using Salkowski's reagent (2ml) [31].

Quantitative estimation of EPS production:

Ability of the isolate (*Klebsiella pneumonia* PNE1*)* for production of EPS was measured using standard method. For quantitative estimation, the overnight grown culture the isolate was inoculated in

yeast extract medium supplemented with the 5% sucrose and incubated at 37±2°C for 5 days on environmental shaker at 120 rpm. At the interval of 24 hrs, 10 ml broth was collected and centrifuged at 10,000 RPM for 10 min.Thrice the volume of chilled acetone was added. EPS was separated from the mixture in the form of a slimy precipitate. Precipitates were collected on a pre-dried filter paper. The precipitates were allowed to dry overnight at 50±2°C. The dried filter paper was reweighed after overnight drying. The increase in the weight of filter paper was recorded which gives the measure of EPS produced [32] [33].

Determination of Potential for biopolymer degradation:

Determination of the potential of the isolate for degradation of cellulose, pectin, and xylan was carried out on solid medium containing 1% CMC, 1% Pectin, and 1% beech wood Xylan respectively as substrate with pH 6.8. Plated were spot inoculated with the isolate (*Klebsiella pneumonia* PNE1) and further incubated at 37±2ºC till the development of colonies. After adequate growth, plates were flooded with Gram's iodine solution [34]. Zone of hydrolysis was measured in mm. Zone ratio was determined using Khandeparkar's method [26].

D/d =Diameter of zone of hydrolysis / Diameter of growth

III. RESULT AND DISCUSSION

Isolation of strain Klebsiella pneumoniae PNE1:

The strain was isolated from solid content of vegetable market waste. The isolate was selected from Ashby's Mannitol agar. The isolate was purified and maintained on the same medium. The ability of isolate to grow on Nitrogen free medium indicated its ability to fix atmospheric nitrogen **(Figure-1).**

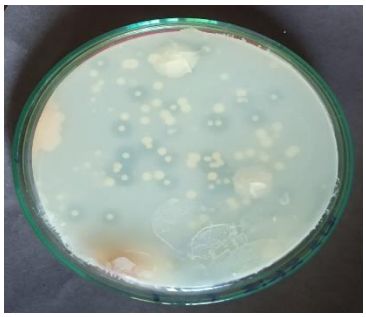

Figure 1: Initial isolation from Ashby's Mannitol agar

Cultural and Morphological study:

Figure 2 depicts the data of cultural characteristics on Ashby's Mannitol agar medium and Morphological characteristics of the selected isolate. On Ashby's Mannitol agar, isolate formed non pigmented, medium, round, entire glossy, mucoid, opaque convex colony. Morphologically, the isolate was found to be Gram negative short rod and capsulated in nature.

Capsule staining **Gram staining**

Figure 2: Cultural and morphological characteristics of isolate PNE1.

Biochemical characterization:

Results depicted in Table-1 show the data of biochemical characterization of the selected isolate PNE1. Out of twenty-five biochemical tests performed, the isolate shows positive result for 16 tests and negative result for 9 tests. Out of 13 sugars the isolate fermented 10 sugars. Only 3 sugars such as Rhamnose, Raffinose, and Sorbitol are not utilized by the isolate. The isolate also gave positive results for ONPG, Urease production, Nitrate reduction, Citrate utilization, Voges Proskauer test and Esculin hydrolysis indicating its metabolic diversity. Mazumdar *et a*l (2018) reported positive results for VP, Citrate utilization, Nitrate reduction and utilisation of 11 sugars such as Arabinose, Cellobiose, Glucose, Glycerol, Lactose, Maltose, Mannitol, Mannose, Sorbitol, Sucrose, Xylose for the isolate *Klebsilla pneumoniae* rs26 [35].

Table 1: Biochemical characterization of the Isolate PNE1.

No	Biochemical Test	Result
1	ONPG (O-Nitrophenyl-β-D-Galactopyranoside)	+
2	Lysine	-
3	Ornithine utilization	-
4	Urease	+
5	Phenylalanine	-
6	Nitrate reduction	+
7	H_2S production	-
8	Citrate utilization	+
9	VP (Voges Proskauer)	+
10	MR (Methyl Red)	-
11	Indole	-
12	Esculin hydrolysis	+
13	Arabinose	+
14	Xylose	+
15	Adonitol	+
16	Rhamnose	-
17	Cellobiose	+
18	Melibiose	+
19	Saccharose	+
20	Raffinose	-
21	Trehalose	+
22	Glucose	+
23	Lactose	+
24	Sorbitol	-
25	Malonate	+

Molecular identification of the PNE1:

Based on the 16S rRNA partial gene sequence result and on nucleotide homology analysis, the isolate PNE1 has been identified as *Klebsiella pneumoniae* PNE1. The isolate showed 99.78% sequence similarity with *Klebsiella pneumoniae* strain DSM 30104 (NCBI Accession no. NR 117s683.1). Phylogenetic tree for the same is depicted in figure 3. Partial 16S rRNA gene sequence of *Klebsiella pneumonia* PNE1 deposited at the NCBI Gene bank is accessible by Accession numbers MZ948809.1

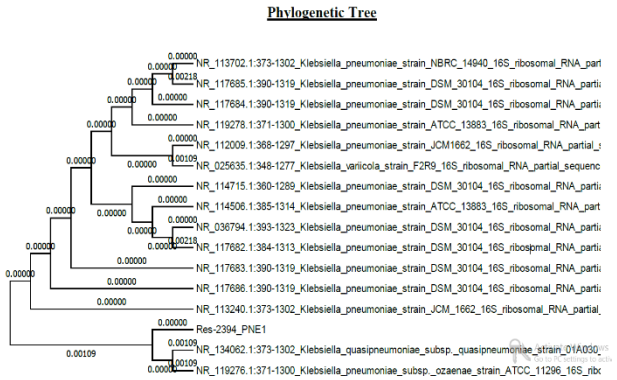

Figure-3: Phylogenetic Tree of *Klebsiella pneumoniae* PNE1

Antibiotic susceptibility test:

As presented in Table-2, the isolate *Klebsiella pneumoniae* PNE1 was found to be highly susceptible against 22 antibiotics tested. The isolate is highly susceptible to Ciprofloxacin (35 mm) followed by Co-Trimazine, Ofloxacin and Moxifloxacin as compared to other antibiotics. Ciprofloxacin, Ofloxacin and Moxifloxacin inhibit DNA replication by inhibiting the enzymes DNA gyrase and DNA topoisomerase [36]. The isolate *Klebsiella pneumoniae* PNE1 also shows good susceptibility to inhibitors of protein synthesis (Streptomycin, Carbenicillin, Gentamicin, Netllin, Tetracycline, Amikacin and Kanamycin) and antibiotics which inhibit cell wall synthesis (Ceftriaxone, Ceftazidime, Cefepime, Cephalothin, Cefotaxime, Imipenem, Meropenem) [37]. According to Bhardwaj *et al.*, (2017) the isolate *Klebsiella pneumoniae* VRE36 is highly susceptible to Ciprofloxacin (27 mm) [6].

Table 2: Antibiotic Susceptibility test of *Klebsiella pneumoniae* PNE1

Antibiotic Disk	Concentration µg/ml	Zone of inhibition (mm)
Tetracycline (TE)	30	10
Streptomycin (S)	10	20
Nitrofurantoin (NIT)	300	8
Kanamycin (K)	30	13
Co-Trimazine (CM)	25	25
Carbenicillin (CB)	100	20
Amikacin (AK)	30	16
Ofloxacin (OF)	25	23
Co-Trimoxazole (COT)	25	10
Gentamicin (GEN)	10	12
Ceftriaxone (CTR)	30	18
Ceftazidime (CAZ)	30	18
Cefepime (CPM)	30	13
Cephalothin (CEP)	30	15
Cefotaxime (CTX)	30	20
Imipenem (IPM)	10	20
Meropenem (MRP)	10	20
Ciprofloxacin (CIP)	5	35
Tobramycin (TOB)	10	10
Moxifloxacin (MO)	5	25
Sparfloxacin (SPA)	5	10
Netllin (NET)	30	20

Determination of ammonia nitrogen and nitrite nitrogen:

Table 3: Determination of ammonia nitrogen and nitrite nitrogen by *Klebsiella pneumoniae* PNE1

	***Klebsiella pneumoniae* PNE1**		
	6th day	**8th day**	**9th day**
Ammonia	0.5 µg/ml	-	-
Nitrite	-	0.09 µg/ml	0.08 µg/ml

The isolates *Klebsiella pneumoniae* PNE1 was found to grow well in N-free Ashby's Mannitol media indicating their ability to fix N to ammonia and nitrite. The result Table 3 show that isolate *Klebsiella pneumoniae* PNE1 produced 0.5 µg/ml of ammonia after 6th day of incubation and produced 0.09 µg/ml nitrite after 8th day of incubation. Mazumdar *et a*l (2018) reported that the isolate *Klebsiella pneumoniae* rs26 produced 10.13 µg/ml of ammonia after 24 h of incubation [35].

Phosphate Solubilization activity:

The isolate *Klebsiella pneumoniae* PNE1 was found to be potent phosphate solubilizer showing clear halo zone around its colony on Pikovaskys medium. The Phosphate solubilization index recorded was 4.16.

The quantitative estimation of phosphate solubilsation by *Klebsiella pneumoniae* PNE1 was assessed by determining the amount of P released from tri-calcium phosphate (TCP). As shown in Figure-5, the amount of P released increases with increasing incubation time till 6th day after which it decreases. The range of inorganic phosphate solubilization varied from 97.09 µg/ml to 177.50µg/ml. The maximum solubilization of 35.5 % (177.50µg/ml) was achieved on 6th day of incubation by isolate *Klebsiella pneumoniae* PNE1. The P solubilisation is accompanied with decrease in the pH of broth. The pH of broth decreased

from pH 7 to pH 5 on 8th Day of incubation. This result suggests that the isolate *Klebsiella pneumoniae* PNE1 has promising potential for phosphate solubilisation and solubilisation of phosphate is by secreting some organic acids in the media which is reflected by the decreased medium pH. Organic acids such as citric, propionic, gluconic, succinic and lactic acids are reported to be the most common in phosphate solubilisation [38]. Several scientific reports suggested that the *Klebsiella sp* can solubilise the inorganic Phosphates such as *Klebsiella sp.* Br1, *Klebsiella pneumoniae* Fr1 [39]. The *Klebsiella pneumoniae* VRE36 from the rhizosphere of *Saccharum officinarum* was found to solubilise 17.4 µg/ml of inorganic Phosphate [6]. The solubilisation of the Phosphate by *Klebsiella pneumoniae* strain rs26 increased gradually from 2 to 7 days (10-29 µg/ml) [35]. The P solubilisation by *Klebsiella pneumoniae* strain HB3 was reported to be 14.23µg/ml [40]. The activity of PSB is crucial for crop production under condition where P is a limiting factor as well as for mobilising bound phosphates in soil.

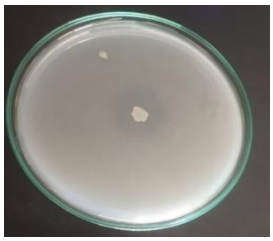

Figure - 4: Zone of Phoshphate solubilization by *Klebsiella pneumoniae* PNE1

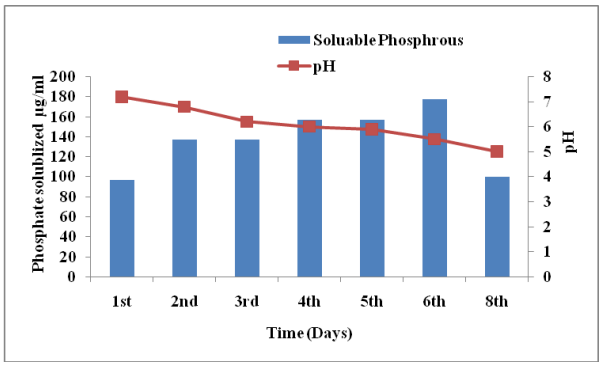

Figure 5: Quantitative estimation of soluble Phosphorus by *Klebsiella pneumoniae* PNE1

Potassium Solubilization activity:

Table 4: Qualitative determination of soluble Potassium by *Klebsiella pneumoniae* PNE1.

Days	Colony size (mm)	Zone size (mm)	Zone ratio (mm)
1	6	15	2.5
2	8	29	3.6
4	10	32	3.2
7	20	35	1.75

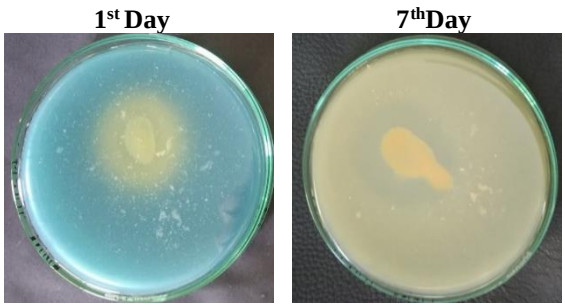

Figure 6: Acid production by Bacterial isolate *Klebsiella pneumoniae* PNE1 on Aleksandrov medium + Bromothymol blue

Table 4 showed Potassium solubilization index of isolate *Klebsiella pneumoniae* PNE1 which ranges from 1.75 to 3.6 on Aleksandrov medium + BTB within 7th days of incubation. The Potassium solubilisation zone was maximum (35 mm) at day 7th, but KSI ratio was found to be low on 7th day, the maximum KSI was detected on 2nd day. The isolate not only exhibited clear zone of potassium solubilization but also showed yellow color formation around the growth in Aleksandrov medium + BTB (figure 6).The change in the colour of BTB indicates acid production by the isolate. The potassium solubilastion by isolate *Klebsiella pneumoniae* PNE1 may be through the organic acid production. Wang, J *et al* (2020) reportd that the KSI index of *Klebsiella pneumoniae* K6 was 1.57 and KSI index of various *Klebsiella varicoala* ranged from 1.39 to 2.78 [41].

The quantitative estimation amount of Potassium released from feldspar revealed the Potassium solubilisation ability of isolate *Klebsiella pneumoniae* PNE1 (figure:7). The range of inorganic Potassium solubilization varied from 19.08 mg/l to 29.94 mg/l. The maximum solubilization of 29.94 mg/l was achieved on 21th day of incubation by isolate *Klebsiella pneumoniae* PNE1. The K solubilisation is accompanied with decrease in the pH of broth. Wang, J *et al* (2020) reportd that the Potassium solubilsation by *Klebsilla pneumoniae* K6 to be 38.55 ± 2.82 mg/l and the Potassium solubilsation by *Klebsiella variicola* K5 to be 64.16 ± 2.19mg/l [41].

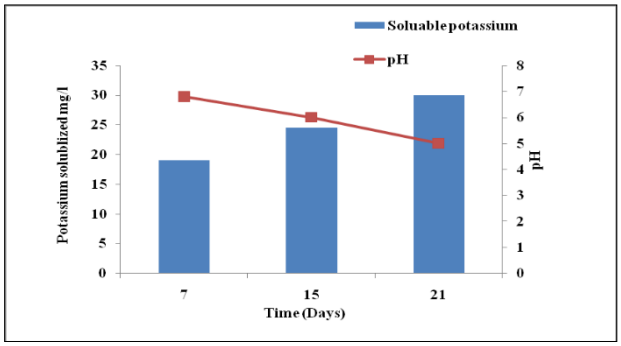

Figure 7: Potassium solubilization potential of isolate *Klebsiella pneumoniae* PNE1.

ISSN: 2349-8889
Volume-8, Issue-6 (November 2021)

https://doi.org/10.31033/ijrasb.8.6.9

Indole acetic acid (IAA) production:

IAA is a metabolite derived from Tryptophan by many Tryptophan dependant and Tryptophan independent pathways in plants and bacteria [42]. Tryptophan is considered an efficient physiological precursor of IAA production, and its application to soils has been shown to influence plant growth and development positively [43]. Figure-8 shows the qualitative determination of IAA production by the isolate *Klebsiella pneumoniae* PNE1. Based on the development of red colour on the filter paper after the 72 hrs incubation (Qualitative method), the isolate was considered as positive for IAA. As shown in Figure-9, *Klebsiella pneumoniae* PNE1 produced a substantial amount of IAA both in the absence and presence of tryptophan. In presence of tryptophan, IAA production ranges from 20.57-94.9 µg/ml. Highest 94.96 µg/ml IAA production was recorded in presence of tryptophan after 120 h. In absence of tryptophan, highest production 43.09µg/ml of IAA was recorded at 72 Hrs. The *Klebsiella pneumonia* VRE36 was reported to produce 45.32 µg/ml of IAA after 96 h of incubation in the presence of tryptophan [6]. The IAA production for *K pneumoniae* K42 was reported to be 11.12 µg/ml respectively after 72 h of incubation [20]. The *Klebsiella pneumonia* K8 produced maximum IAA 27.5 µg/ml in presence of tryptophan at 72 h incubation [20].

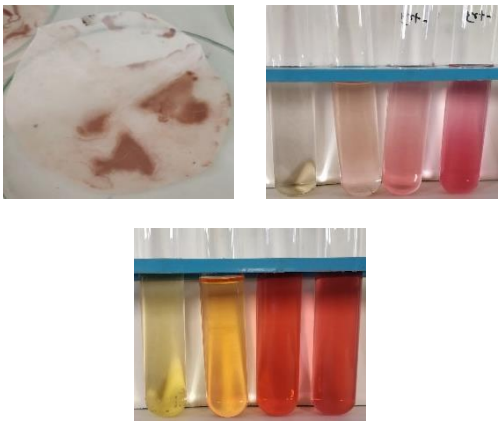

Figure 8: Qualitative determination of Indole acetic acid production by *Klebsiella pneumoniae* PNE1.

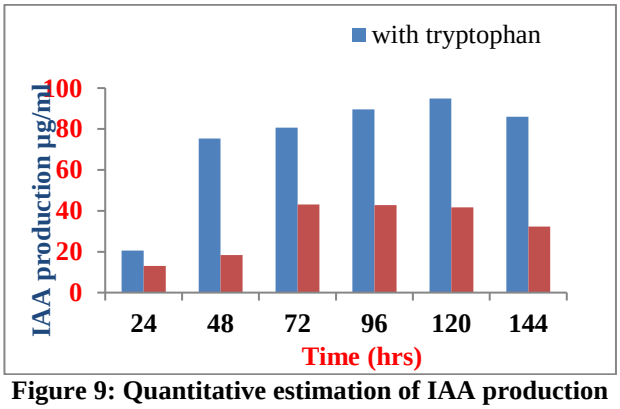

Figure 9: Quantitative estimation of IAA production by *Klebsiella pneumoniae* PNE1.

Quantitative estimation of EPS production:

Data presented in figure-10 depicts EPS Production by *Klebsiella pneumoniae* PNE 1 using 5% sucrose as substrate. Amount of EPS produced ranged from 9 g/l to 11.3 g/l. The EPS production increased till the second day and there after it continuously decreased. Thus, maximum production could have occurred around 48 hrs. The decrease in amount of EPS after 72 hrs may be due to utilization of own EPS as substrate by the producing organism or EPS yield may probably decrease because of the action of glycohydrolases produced in the culture that catalyzed the degradation of polysaccharides, resulting in decreased EPS yields [9]. According to Hayet *et al.*, (2021) the yield of EPS obtained by isolate *Klebsiella oxytoca* KY498625 was 4.6 g/l [44]. Ram. *et al* (2004) reported 18.1 g/l of EPS yield by *Klebsiella pneumoniae* K63 after the 22 hrs incubation [45].

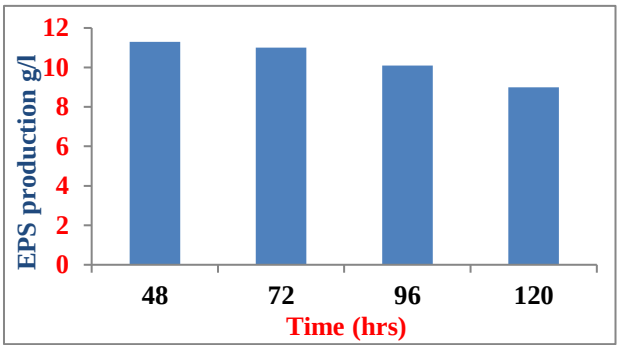

Figure-10: EPS Production by *Klebsiella pneumoniae* PNE1 using 5% sucrose.

Determination of Potential for biopolymer degradation:

Figure 11: Zone of biopolymer hydrolysis by bacterial isolate *Klebsiella pneumoniae* PNE1.

Table 5: Potential for biopolymer degradation:

Zone of hydrolysis (mm)								
Cellulose			Pectin			Xylan		
Colony size	Zone size	Zone ratio	Colony size	Zone size	Zone ratio	Colony size	Zone size	Zone ratio
10	55	5.5	7	65	9.2	12	20	1.6

As shown in table 5, the isolate *Klebsiella pneumoniae* PNE1 was found to have good ability for degradation of cellulose, xylan and pectin. Based on the

zone ratio the degradative ability was best for pectin, good for cellulose and was least for xylan.

IV. CONCLUSION

Based on 16s rRNA sequencing and molecular identification and phylogenic analysis, the isolate is identified as *Klebsiella pneumoniae* PNE1.The isolated *Klebsiella pneumoniae* PNE1 has promising ability for Nitrogen fixation, Phosphorus and Potassium solubilsation from unavailable to available form through biological processes. So, the isolate can prove to be useful for improving nutrient status in terms for NPK availability for improving plant growth. Additionally, the isolate *Klebsiella pneumoniae* PNE1 also produces significant amount of phytohormones (Indole acetic acid), produces significant amount of exopolysaccharide and has extended polymer degradation capability. The production of phytohormones. Indole acetic acid in plants is beneficial as this phytohormone plays a central role in cell division, elongation, fruit development and growth. The EPS production ability of microorganism is of concern as the EPS improves soil texture by enhancing the volume of soil macropores and the rhizosphere soil aggregation, resulting in increased water and fertilizer availability to inoculated plants and also preventing soil erosion. The extended potential for polymer degradation by *Klebsiella pneumoniae* PNE1 ensures survival and activity of the isolate in diverse environments having complex biopolymers. The Nitrogen fixation ability, Phosphate and Potassium solubilisation ability, production of plant growth promoting hormones, Exopolysaccharide production ability and biopolymer degradation ability of the isolate *Klebsiella pneumoniae* PNE1 makes it an ideal candidate as Plant growth promoting bacteria and we propose its use for development of biofertilizer.

ACKNOWLEDGEMENT

We are thankful to Gujarat State Biotechnology Mission, Government of Gujarat for providing fund for the research work as Major Research Project grant. We are also thankful to Pramukh Swami Science and H.D. Patel Arts College, Kadi for all the facilities and support.

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ISSN: 2349-8889
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