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ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING BACTERIA (PGPR) FROM RHIZOSPHERE OF OKRA

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Abstract
Plant growth promoting bacteria (PGPR) are a type of bacteria that inhabit the rhizosphere of plants and can aid in the growth of plants, either directly or indirectly. Many different bacterial species have been studied for their ability to promote plant growth, including *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Rhizobium*. The aim of the present study is to identify and isolate such bacteria from the rhizosphere of okra. The serial dilution approach was used in the current investigation to perform the isolation on LB-agar media. 15 strains were obtained for the characterization based on physical characteristics. Isolated isolates were identified using biochemical tests and morphological basis in vitro. 09 strains were gram positive while 05 strains were gram negative when stained it. In the current study, phosphate solublization was detected in seven bacterial isolates. Almost all of the bacterial isolates demonstrated IAA production. Twelve isolates tested positive for ammonia generation. All isolates were negative for HCN production. Fourteen isolates had nitrogen activity. Eleven isolates, nine isolates, and eleven isolates strains, respectively, demonstrated the activities of protease, pectinase, and amylase. To improve plant development, the potential of these PGPR isolates needs to be tested further.

Keyword: Plant growth promoting bacteria, Luria–Bertani agar media, hydrogen cyanide production, Indole acetic acid production.

1. INTRODUCTION

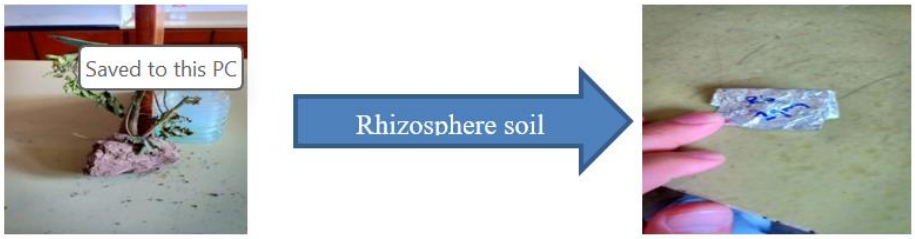
Soil is predominantly composed of organic matter derived from microorganisms such as bacteria, archaea, yeast, fungi, algae, and protozoa (KejelaeBraga et al., 2016; Kejela et al., 2016). Microbes can survive in extreme environments such as hot springs, deep beneath rocks, and under the ocean (Hongmei et al., 2005). Despite variation in bacterial numbers, their collective carbon mass is estimated to be trillions of tons (Rabaan et al., 2022; Rizvi et al., 2022). Bacteria, including Nitrosomonas, Nitrobacter, Thiobacillus, Rhizobium, Frankia, Bacillus, Clostridium, Caulobacter,

and Pseudomonas, are involved in important nutrient cycles in soil, such as nitrification, sulphur and iron oxidation, N2 fixation, carbon cycling, and manganese oxidation (Makhalanyane et al., 2015). These bacteria also produce various compounds used in biotechnology, such as antibiotics, fragrances, enzymes, ethanol, and acetone (Ahmed et al., 2022a; Assiry et al., 2023; Tariq et al., 2020). As many soil microbes remain unknown, researchers acknowledge their potential as sources of commercially significant products and novel metabolites (Goldstein et al., 2005; Saha and Santra, 2014). Rhizobacteria that colonize the rhizosphere are known as Plant Growth Promoting Rhizobacteria (PGPR) (Javed et al., 2022). PGPR can increase root growth, nutrient absorption, disease resistance, and soil fertility (Ahmad et al., 2008; Rahni, 2012; Wahyudi, 2009). The rhizosphere zone is the soil compartment impacted by the presence of living plant roots, and it supports a large and active microbial population that can positively, neutrally, or negatively impact plants (Hiltner, 1904). PGPR can promote plant growth through direct mechanisms, such as nitrogen fixation and synthesis of plant growth hormones, and indirect mechanisms, such as biological control of harmful bacteria and diseases (Ahmad et al., 2008; Nelson, 2004). One of the most popular and commonly used species in the Malvaceae family is okra (Naveed et al., 2009) is a vegetable crop that is produced on a large scale in tropical and subtropical regions of the world (Andras et al., 2005; Saifullah and Rabbani, 2009). Okra is also known by the Latin names Abelmoschus esculentus and Hibiscus esculentus (Kumar et al., 2010). Formerly, the okra plant and lady's finger belonged to the genus Hibiscus. Eventually, the name was changed to Abelmoschus, which is different from the genus Hibiscus (Adilakshmi et al., 2010). After that, in 1787, Medikus proposed giving Abelmoschus the rank of a distinct genus. Okra, which originated in Ethiopia, was being grown by the ancient Egyptians by the 12th century BC. Its cultivation extended widely over the Middle East and North Africa (Lamont, 1999). Okra is very nutritive and has been determined to be a necessary part of the human diet. Vitamins, minerals, carbs, proteins, lipids, and fiber are abundant in okra fruit (VarmuDy, 2011). Fully developed okra seeds are a fantastic source of protein, oil, and essential unsaturated fatty acids (like linoleic acid) for human nutrition. The paper industry uses the raw fiber from its ripening fruit and stem (Kumar et al., 2013). As can be seen from the geographical distribution of cultivated and wild varieties, okra is a plant that is grown all over the world, from tropical regions to the Mediterranean Sea (Qhureshi, 2007). Many nations, including India, Japan, Turkey, Western Africa, Bangladesh, Pakistan, Malaysia, Brazil, and Southern United States grow okra plants for commercial purposes (Benjawan et al., 2007). The amount of okra being grown in Pakistan has gradually increased. The okra crop is currently farmed on about 15500 hectares, yielding an average of 7.52 tons per hectare and producing 117900 tons in total (Khan and Rab, 2019). Nonetheless, Pakistan's average okra production is lower than that of several other nations worldwide (Rahman, 2012). Okra's growing area in Pakistan has changed significantly in recent years, although from 2002 to 2021, when it reached 263,448 tons, it tended to grow. India produced 6.47 million tons of okra in 2021, up from 1.24 million tons in 1972, expanding at an average yearly rate of 3.64%. The okra production of Asia increased from 1.26 million tons in 1972 to 7.12 million tons in 2021 growing at an average annual rate of 3.79% The aim of the present study was to isolates a bacteria from rhizosphere of okra, characterize on the basis of morphological and biochemical test to assess the potential of isolates bacteria in producing plant growth promotion. This research will have an effect on the identification process of microorganisms more easily and quickly, especially in identifying plant growth promoting rhizobacteria.

2. METHODOLOGY

2.1. Collection of sample

The sample of okra with rhizosphere soil was collected from the area of Peshawar, KPK. For the research purposes this sample was brought to microbiology lab of Attabak Pharmaceutical industries.



2.2. Isolation of plant growth promoting bacteria

Rhizosphere soil of okra was taken to isolate bacteria by serial dilution method on the Luria-Bertania (LB) agar plate. After incubation, to isolation of pure cultures of bacteria isolated colonies were further streaked on pre-sterilized LB-agar plates. This step was repeated up to 3 times for the isolation of the pure colony.

2.3. Morphological characterization

For morphological characterization, Gram staining was done for each isolated colony in accordance with the prescribed protocol (Ahmed et al., 2020; Ahmed et al., 2022b; Ahmed et al., 2022c). A smear of bacterial cells was used to create a clean glass slide using a moderate heat fixing. The heat-fixed smear was soaked with the crystal violet solution for one minute. After cleaning the smear with water, Gram's iodine was used as a mordant. The smear was decolored using 95% ethyl alcohol, followed by a water rinse. Finally, counter stains of safranin were employed for 60 to 80 seconds before being removed with water. Cells were then examined under a microscope (Ahmed et al., 2019).

2.4. Biochemical characterization

Phosphate solubilizing test

Phosphate solubilization refers to the process by which bacteria convert unavailable phosphorus into forms that plants may use. By cultivating bacterial isolates on plates using modified Pikovskaya's agar medium, the ability of the bacteria to solubilize phosphate was tested using the plate assay method. (Pikovskaya, 1948). Chemical composition was Glucose (10g/L), Calcium phosphate (5g/L), Ammonium sulfate (0.5g/L), Sodium chloride (0.2g/L), magnesium sulfate heptahydrate (0.1 g/L), potassium chloride (0.2 g/L), Yeast extract (0.5 g/L), MnSO4 (Trace), FeSO4.7H2O (Trace) and Agar (15g/L).

Ammonia production test

We performed this test to check that whether these bacterial strains are producing ammonia or not**.** Rhizobacterial strains were assayed for ammonia by using peptone water following the method of (Samuel and Muthukkaruppan, 2011). In test tubes containing 10 mL of peptone water, fresh rhizobacterial cultures were injected and then incubated for two days at 28°C. After that, 0.5 ml of Nessler's reagent was added to each test tube, and the appearance of a brown to orange color indicated that the corresponding rhizobacteria were producing ammonia.

Protease production test

Agar plates with 3% (w/v) powdered skim milk showed protease activity. A single bacterial colony of each strain was grown on this medium and kept in culture for up to 4 days at 30 °C.

Pectinase test

By introducing isolated isolates to pectin-modified M9 medium that had undergone sterilization, the ability of the strains to generate pectinase was assessed.. At 28°C, the Petri plates were inoculated for two days. Each Petri plate received 2M of HCL after the incubation period. Clear halo zones encircling the colonies served as a sign that pectinase was being produced.

Amylase production test

To test for amylase production, specific medium was used culture bacterial isolates. The isolates were streaked onto recently made plates, and they were then incubated at 35 °C.

NF production test

Nitrogen free media was prepared to check either microbial strain can fix the nitrogen or not. To grow pure bacterial cultures, Burk's modified nitrogen free medium plates were used. The medium was composed of the following ingradients per liter: sucrose 10.0 g; D1 malic acid 5g; K2HPO4· H2O, 0.1g; KH2PO4·H2O, 0.4g; NaCl 0.1g; CaCL2·H2O 0.02g; Na2MO4·2H2O 0.002g; FeCL3 0.01g and agar 15g. If visible colonies were observed on the agar, this was taken as a positive indication of nitrogen fixation.

Indole acetic acid production test (IAA)

A colorimetric technique called Salkowski's approach (Glickmann and Dessaux, 1995) was employed to ascertain whether the bacterial isolates were capable of generating indole acetic acid (IAA). Each bacterial culture was cultivated for three days at 30°C in nutrient broth medium that also contained 0.1 mg/mL l-tryptophan and 5% sodium chloride. Following incubation, 2 mL of Salkowski's reagent (2% ferric chloride in 35% Perchloric acid solution) were added to 1 mL of supernatant, which had been transferred to a fresh centrifuge tube. The samples were left in the dark for 25 minutes. At 530 nm, optical density was determined.

Hydrogen cyanide production test

A procedure suggested by Castric's (Castric, 1975) was used to ascertain whether the bacterial isolates were capable of creating hydrogen cyanide (HCN). By sprinkling rhizobacteria on LB agar supplemented with 4.4 g/L glycine, the generation of HCN was assessed. Each petri plate's upper lid was sealed with a filter paper strip that had been saturated with a picric acid (0.5%) and sodium carbonate (2.0%) solution. To prevent the gas emission, Petri plates were carefully sealed with parafilm. The filter paper's change in color from yellow to orange-brown after two days of incubation at 28°C showed a successful outcome.

2.5. Statistical Analysis

SPSS version 25.0 (on Windows 10) and Microsoft Office Excel 2018 (for statistical analysis) were used.

3. RESULTS

3.1. Morphological characterization of isolated bacterial strain

Visual inspection revealed that the isolated strain of bacteria from the rhizosphere of okra had morphologically unique colonies that were different in shape and color. The majority of the recovered bacterial strains had a rod or coccus form (Table 1). The colony was a pink to purple color. Colonies took the form of uneven, round, rounded colonies. Five strains were gram negative and displayed pink color at the end of the gram staining reaction, while nine strains were gram positive and displayed purple color (figure 1).

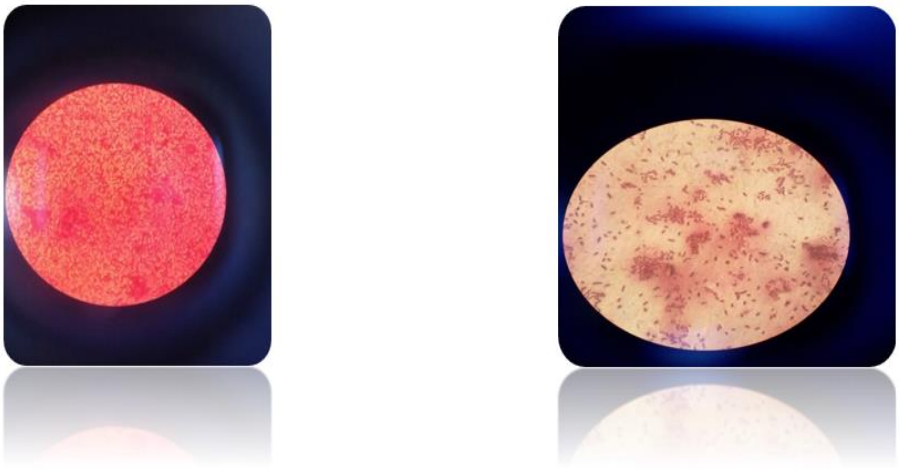


Figure1. Show the results of different isolated bacteria by using gram staining procedure.

Table 1: Gram staining of isolated strains results

S.no.	PGPR isolates Code	Source	Gram Staining	Observation	Shape
1	*ORS2 a*	Rhizosphere	Pink	Positive	*Rod*
2	*ORS2 b*	Rhizosphere	Purple	Negative	*Cocci*
3	*ORS2 c*	Rhizosphere	Purple	Negative	*Rod*
4	*ORS2 d*	Rhizosphere	Purple	Negative	*Cocci*
5	*ORS2 e*	Rhizosphere	Pink	Positive	*Cocci*
6	*ORS2 f*	Rhizosphere	Purple	Negative	*Rod*
7	*ORS2 g*	Rhizosphere	----	----	----
8	*ORS2 h*	Rhizosphere	Pink	Positive	*Cocci*
9	*ORS2 i*	Rhizosphere	Pink	Positive	*Cocci*
10	*ORS2 j*	Rhizosphere	Purple	Negative	*Cocci*
11	*ORS2 k*	Rhizosphere	Pink	Positive	*Cocci*
12	*ORS2 l*	Rhizosphere	Pink	Positive	*Cocci*
13	*ORS2 m*	Rhizosphere	Pink	Positive	*Rod*
14	*ORS2 n*	Rhizosphere	Pink	Positive	*Rod*
15	*ORS2 o*	Rhizosphere	Pink	Positive	*Cocci*

3.2. Biochemical characterization

Phosphate solubilizing test

In this 7 strain out of 15 strains are positive (table 2) and they show clear zone around the bacterial strain. This study also revealed quantitive estimation of PSB. After 7 days of incubation in pikovaskaya's broth medium (pH 7) absorbance was calculated. *ORS2a* and *ORS2j* represent high absorbance of phosphorus (table 3).

Table 2: Qualitative screening of isolated strains for phosphate solubilizing test

ID	RESULTS	ZD+CD	CD	SI	SE%
ORSS2 a	+++	0.7	0.4	1.75	75
ORSS2 b	-	-	-	-	-
ORSS2 c	+	0.55	3.25	0.16	83.07
ORSS2 d	-	-	-	-	-
ORSS2 e	-	-	-	-	-
ORSS2 f	-	-	-	-	-
ORSS2 e	-	-	-	-	-
ORSS2 h	-	-	-	-	-
ORSS2 i	+++	0.8	0.6	1.33	33.3
ORSS2 j	+	0.9	0.8	1.12	12.5
ORSS2 k	++	0.7	0.5	1.4	40
ORSS2 l	+	0.9	0.75	1.65	20
ORSS2 m	-	-	-	-	-
ORSS2 n	-	-	-	-	-
ORSS2 o	+++	0.75	0.5	1.5	50

Table 3: Quantitative screening of isolated strains for phosphate test

ID	y(ppm)
ORS2 i	18.93275
ORS2 o	15.99419
ORS2 c	15.44321
ORS2 l	16.17785
ORS2 k	16.42273
ORS2 j	19.3919
ORS2 a	20.12654

Ammonia production test

When the Nessler's reagent was added to peptone water that had been contaminated with bacteria in a falcon tube. The strains' capacity to create ammonia is shown by the formation of tubes with a yellow to brown tint. 12 strains were displayed in table 4 of ammonia production.

NF production test

Nitrogen free media was prepared to check either microbial strain can fix the nitrogen or not. Growth on medium showed the positive results. Except 1 strain all other bacterial strains showed positive results (Table 4).

Hydrogen cyanide production test

All isolated strains showed no change in color representing negative results. A complete result of HCN is showed in table 4.

Table 4: Qualitative screening of isolated strains for NF, HCN and Ammonia fixation test.

S.No	ID	Nitrogen fixation	Hydrogen cyanide	Ammonia fixation
01	ORS2 *a*	+	-	+++
02	ORS2 *b*	+++	-	+
03	ORS2 *c*	+	-	+
04	ORS2 *d*	+	-	+
05	ORS2 *e*	+	-	+++
06	ORS2 *f*	+	-	+++
07	ORS2 *g*	-	-	++
08	ORS2 *h*	++	-	-
09	ORS2 *i*	+	-	++
10	ORS2 *j*	+	-	++
11	ORS2 *k*	+++	-	-
12	ORS2 *l*	+++	-	+++
13	ORS2 *m*	+	-	-
14	ORS2 *n*	++	-	+
15	ORS2 *o*	+	-	++

Indole acetic acid production test

This study revealed quantitative estimation of indole production. Tryptone broth medium was used for quantitative estimation of indole production bacteria. After 3 days of incubation in Tryptone broth medium (pH 7) absorbance was recorded (Table 5). All isolated strains showed positive results.

Table 5: Quantitative screening of isolated strains for indole test

ID	y(ppm)
ORS2 a	82.00335
ORS2 b	24.90224
ORS2 c	61.8225
ORS2 d	93.8312
ORS2 e	41.00682
ORS2 f	16.41559
ORS2 g	20.72574
ORS2 h	17.18407
ORS2 i	85.2109
ORS2 j	84.91019
ORS2 k	21.1601
ORS2 l	148.0255
ORS2 m	17.15066
ORS2 n	12.13886
ORS2 o	44.64873

Enzyme production test

The potential of isolated isolates to generate proteases was tested on skimmed milk agar medium. After incubation, clear zones formed surrounding colonies, indicating the generation of proteases. The clear zone around the colonies was generated by 11 of the 15 isolated strains (Table 6), which is a positive indicator.

Table 6: Qualitative screening of isolated strains for protease test

ID	RESULTS	ZD+CD	CD	SI	SE%
ORS2a	+++	1.15	0.5	3.3	130
ORS2 b	-	-	-	-	-
ORS2 c	+	0.45	0.3	2.5	50
ORS2 d	+++	1.55	0.75	3.06	106
ORS2 e	+++	0.85	0.45	2.8	88.8
ORS2 f	++	1.6	0.6	3.6	166.6
ORS2 g	-	-	-	-	-
ORS2 h	-	-	-	-	-
ORS2 i	+++	1.5	0.45	4.3	133.3
ORS2 j	+++	1.25	0.6	3.9	108.3
ORS2 k	+++	1.45	0.65	3.23	123
ORS2 l	+++	1.55	0.5	4.1	210
ORS2m	+	0.5	0.25	3	100
ORS2 n	-	-	-	-	-
ORS2 o	++	0.95	0.35	3.7	171.4

The composition of M9 media include yeast extract0.1, ammonia sulphate0.2 g/100L, Na2HPo4 0.6 g/100L, KH2Po4 0.3 g/100L, Pectin 0.5 g/100L and agar 2g/100L they were used for the inoculation of bacteria to check the pectinolytic activity of isolated strains. After incubation, iodine drops were poured on inoculated bacteria which formed clear zone around colony showed pectinase production. Out of 15 strains 6 strains (ORS2 a, g, h, m, n and o) showed negative results while 9 strains showed positive results by the formation of clear zone around the colonies (Table 7).

Table 7: Qualitative screening of isolated strains for pectinase test

ID	RESULTS	ZD+CD	CD	SI	SE%
ORS2 a	-	-	-	-	-
ORS2 b	+++	1.65	0.65	2.53	153.8
ORS2 c	+++	1.6	0.8	2	100
ORS2 d	+	0.9	0.5	1.5	80
ORS2 e	+++	1.4	0.6	2.33	133.3
ORS2 f	+++	1.6	0.85	1.88	88.2
ORS2 g	-	-	-	-	-
ORS2 h	-	-	-	-	-
ORS2 i	++	0.85	0.45	1.88	88.8
ORS2 j	++	0.95	0.55	1.72	72.7
ORS2 k	++	0.95	0.55	1.72	72.7
ORS2 l	+++	1.2	0.55	2.18	118.1
ORS2 m	-	-	-		-
ORS2 n	-	-	-		-
ORS2 o	-	-	-		-

Amylase activity

Iodine drops were applied to the bacteria after incubation, forming a clear zone surrounding the bacterial colonies that demonstrated amylase production. Of 15 strains, 11 strains created clear zones surrounding the bacterial colonies, producing favourable results (Table 8), whereas the remaining two did not.

Table 8: Qualitative screening of isolated strains for amylase test

ID	RESULTS	ZD+CD	CD	SI	SE%
ORSS2 a	-	-	-	-	-
ORSS2 b	+++	1.85	0.6	3.08	208.3
ORSS2 c	+++	1.6	0.9	1.77	77.7
ORSS2 d	++	1.35	0.5	2.7	170
ORSS2 e	+++	1.65	0.45	3.66	266.6
ORSS2 f	+++	1.5	0.85	1.76	112
ORSS2 g	-	-	-	-	-
ORSS2h	-	-	-	-	-
ORSS2 i	+++	1.35	0.6	2.25	125
ORSS2 j	+++	1.55	0.6	2.58	158.3
ORSS2 k	+	0.85	0.6	1.41	41.6
ORSS2 l	++	1.3	0.65	2	30
ORSS2m	+	0.75	0.55	1.36	36.3
ORSS2n	+	0.7	0.5	1.4	40
ORSS2 o	-	-	-	-	-

4. Discussion

Organisms produce such enzymes and chemicals which are beneficial to the plant burgeoning. The favorable outcome of PGPR, which populates the rhizosphere of many plant species, include improved plant health and diminished vulnerability to illnesses brought over by nematodes, bacteria, viruses, and plant pathogenic fungi. We looked at the bacterial ecology in the rhizosphere of okra in this report. Sample was collected from the rhizosphere of okra of Peshawar city. The serial dilution method was initially used to isolate rhizosphere bacteria from the sample on LB-agar media. In this study neither fungi nor actinomycetes were investigated, and the focus was only on bacteria that formed visible colonies on LB-agar plates within 24 h. A bacterial colony's colour and form were used to identify it. A total of 15 single colonies were selected, grown on LB-agar media, and subjected to additional examination. However, there are few articles on okra that discuss the benefits and screening of PGPR from crop plants, specifically rice, maize, and sugar cane. There is not enough data available on okra screening and PGPR use. Gram staining and biochemical testing were used to identify the isolates of bacteria. It was discovered that 33% of the isolates were gram negative and displayed pink hue, whereas 60% were gram positive and displayed purple color. Some bacteria had coccus structure during microscopic inspection, whereas others had rod shape. As a macronutrient for plants, nitrogen is essential for the production of all proteins, enzymes, nucleic acids, and chlorophylls (Leghari et al., 2016). Despite the fact that N_2, an inert form of nitrogen that plants cannot directly absorb, is abundant in the environment, it is not used by plants. Only nitrate and ammonium, which are made available to plants by nitrogen-fixing organisms using nitrogenase, are the forms of nitrogen that plants can use. These organism can be either symbiotic or free-living and they increase the amount of nitrogen that is available to plants (Myrold and Posavatz, 2007). On modified Burk's medium without nitrogen, all isolated strains except one (ORS2 g) displayed prominent growth, indicating that they may have the ability to fix nitrogen dioxide. ORS2 *b*, ORS2 *k* and ORS2 *l* were produce clear zone on modified Burk's medium. After nitrogen, phosphorus is the second-most crucial macronutrient for plant growth. The majority of the phosphorus in the environment is insoluble, so plants cannot use it directly. Mineral phosphates can

be dissolved by specific soil bacteria and transformed into a form that plants can use, which indicates a potential way of promoting plant development in natural settings (Ashrafuzzaman et al., 2009). Owing to the creation of organic acids by PGPR, acidification is the major mechanism involved in the solubilization of phosphate (Puente et al., 2004). On Pikovskaya's agar plates, which also included calcium phosphate as an inorganic phosphate source, seven of the 15 isolates used in this experiment developed different zones around the colonies. This demonstrates that bacteria have dissolved inorganic phosphate. All of the isolates in the current investigation showed varied degrees of phosphate solubilization in the agar and broth assays, which was most likely caused by the production of organic acids. In both agar and broth culture experiments, our isolated strains consistently performed well in the solubilization of tricalcium phosphates; in the qualitative assessment of the phosphate solubilizing assay, ORS2 *a*, ORS2 I, and ORS2 o were most likely phosphate solubilizing strains. ORS2 *a* was used to quantify the optical density and solubilize the phosphate. The phosphate solubilization by PGPR produced consistent, comparable results, which were also seen by earlier researchers. (Nautiyal, 1999; Sarkar et al., 2012; Sarker et al., 2014). Ammonia generation is another significant PGPR characteristic that the organism demonstrates. In this process, the organisms break down complex nitrogen containing compounds and releases ammonia in the soil. Plant can then utilize this ammonia as a source of nutrients. However, when ammonia accumulate in the soil, it can create an alkaline environment that prevents the growth of certain types of fungi (Jha et al., 2012). In the current investigation, 12 isolated strains produced ammonia, effectively influencing plant growth (Samuel and Muthukkaruppan, 2011). The extremely good ammonia generation activity was demonstrated by the ORS2 a, ORS2 e, ORS2 f, and ORS2 I isolates. Ammonia results were combined with previous study (Nehra et al., 2016). Plant growth promoting bacteria that produce indole acetic acid can stimulate the growth and elongation of root, leading to an increase in the surface area of the root system. This, is turn, enhance the plant's ability to extract nutrients from the soil (Vessey, 2003). According to reports, the availability of substrate, culture conditions, growth stage, and different species and strains can all affect the IAA generation by PGPR. (Sajjad Mirza et al., 2001). The majority of the bacterial isolates in our study produced IAA, and these findings were consistent with other investigations (Kumar et al., 2012). It has been hypothesised that rhizobacteria's synthesis of HCN is crucial for the biological control of infections (Voisard et al., 1989). All isolated strains in the current study did not produce HCN. Under in vitro extra cellular activity, 11 isolates produce protease, amylase followed by 11 isolates and 10 produce pectinase production respectively. Our enzymatic activity result is consistent with other researchers (Moustaine et al., 2017; Praveen Kumar et al., 2012).

5. Conclusion

During the current study, 15 strains of bacteria were gathered from either the rhizosphere of okra. On the basis of morphological and biochemical tests, they were classified. Gram staining was used to determine their morphology; nine strains were gram positive and five strains were gram negative. These strains were subjected to biochemical tests to identify PGPR activity, which included phosphate solubilizing, nitrogen fixation, IAA, HCN, ammonia, and enzymatic activity (protease, amylase, pectinase).

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