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# Isolation and Characterization of Plant Growth-Promoting Rhizobacteria Associated with Okra Cultivation in the Haripur

**Shamsa Kanwal[1], Zahidullah[2], Zounaira[2], Kainat Fatima[3], Mehwish Shah[2], Haleema Sadia[4], Saud Aziz[2], Sayab Khan[2], Abdullah Jan[5], Muhammad Hassan Raza[6]**

[1]Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad, KP, Pakistan.
[2]Department of Microbiology, Hazara University, Mansehra, KP, Pakistan.
[3]Department of Microbiology, University of Haripur, KP, Pakistan.
[4]Department of Chemistry, Abbottabad University of Science and Technology, Abbottabad, KP, Pakistan.
[5]University of Wah, Wah Cantt, Pakistan.
[6]University of Lahore, Islamabad Campus, Pakistan.

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**Corresponding Author:** Shamsa Kanwal, Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad, KP, Pakistan.
Email: Kanwalabbasi750@gmail.com

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## ABSTRACT

Organic farming represents an ecologically sound and economically viable agricultural practice that enhances soil fertility and quality. However, as more complex agricultural techniques have been developed, organic farming has become increasingly limited worldwide. Culture-dependent plant growth-promoting bacterial isolates were isolated from the bulk and the rhizosphere. This study uses bacterial isolates from the Haripur region of KPK, Pakistan, as a plant growth promoter and alternative to chemical fertilizers due to the increasing environmental contamination caused by the deterioration of soil health and the use of chemical fertilizers to increase crop yield. In the region of Haripur, eleven bacteria were discovered, of which two, PGPR-2 and PGPR-8, were especially effective at promoting plant development by producing ammonia, indole, amylase, catalase, nitrate reduction, phosphate solubilization, and biological nitrogen fixation. Further investigation was conducted into the biochemical and extracellular enzymatic activities of PGPR-2 and PGPR-8. Using the Roll towel method, the germination rate, root length, and branch length of prospective vegetable isolates were measured.

## INTRODUCTION

The most important aspect of modern agriculture is the development and enrichment of agricultural productivity. The green revolution has resulted in remarkable achievements in this field and the lives of farmers. However, excessive habit of chemical fertilizers and pesticides in crop fields is a significant disadvantage to sustainable agriculture. This has had a negative influence on soil quality, accessible mineral nutrients, and groundwater. In this difficult situation, organic farming may be able to help improve things and reverse the ecosystem's degradation. Thus, bio-fertilizer, which is a mixture of growth-specific nutrients, represents a ray of hope for today's growth-oriented agriculture with sustainable crop production while safeguarding and maintaining the environment. The various bacterial genera are essential parts of soils. They participate in a variety of biotic activities that keep the soil ecosystem dynamic for nutrient cycling and resilient for crop production. Rhizobacteria, which live near or inside plant roots, are more adaptable than soil-derived nutrients in terms of converting, mobilizing, and solubilizing them. As a result, rhizobacteria play a key role in recycling the nutrients in the soil and are essential for soil fertility (GUPTA and Baig, 2001).

The majority of the isolates increase plant height, root length, and dry matter production of the shoot and root, making the use of PGPR an appealing alternative to chemical fertilizers, herbicides, and supplements. PGPR

aids in the eradication of plant diseases. Some PGPR can take hold of the crop's roots, particularly if they were injected into the seed beforehand. Biocontrol agents and cultural approaches of pest management work together in integrated pest management systems, of which PGPR is a part. Using this type of integrated system, vegetable transplants might be made that are more resistant to nematodes and other ailments for a few weeks after being placed in the field. The plant-mediated induced systemic resistance (ISR) response is effective against many different plant diseases because it is triggered by particular strains of beneficial PGPR. ISR is a plant-mediated process that, like conventional pathogen-induced resistance, increases the resistance to infection of previously uninfected parts of a plant that have been exposed to the pathogen (Spoel *et al*., 2003).

An ancient pulse crop called okra (Cicer arietinum L.) was initially cultivated in Turkey around 7,000 B.C. It has historically been grown in semiarid regions of India and the Middle East. Okra, a plant in the family Leguminosae, can fix a portion of the nitrogen it needs from soil air. The majority of the pods are developed at the top of the plant, which has an upright growth habit. Although seed size, shape, and color can vary, the typical seed has a beak and is wrinkled or ribbed. The exposed seedling root tip is known as the beak. Okra is divided into two commercial categories: desi and Kabuli. The desi kind features colored blossoms and a thick, colorful seed coat. In India, where it has a long history of manufacture, it is split and/or ground to create food products. The garbanzo bean variety known as Kabuli has white blooms and a thin, seed coat .It is mostly utilized in vegetable and salad mixes.

Okra plants grow between 30 and 70 cm (12 and 28 inches) tall. The majority of okra types are described as having fern-like leaves, with leaves about five centimeters (two inches) long and containing nine to fifteen leaflets Some Kabuli cultivars, as CDC Xena, have a single (unifoliate) leaf structure in place of leaflets. Okra has short, inflated pods with one or two seeds inside. Okra weighs 60 lbs. per bushel.

One of the most important pulse crops in Pakistan (Saxena and Singh, 1987), because of its multiple uses in traditional farming. It helps regulate soil fertility, especially in arid regions and is used as a human and animal food source. (SUZUKI *et al*., 1982). In 2006, it was grown on 1029,000 acres of land in Pakistan, with a yield of 480 kilograms per hectare.

It is a significant source of protein and is frequently consumed as a main food source by Indians. In India, okra is known as the "King of Pulses" because it is used to produce a range of dishes for everyday consumption and special occasions. It is rated third behind common bean (Phaseolus vulgaris L.) and pea (Pisum sativum L.).

The majority of terrestrial plants create a root system to delve into the ground and seek out nutrients to support growth. The root tip, the root meristem, the differentiation and elongation zones, and the growing lateral roots are all components of the root, which is a complex organ. Each of these zones performs a vital function. Root hairs are differentiated epidermal cells that are important for plant mineral nutrition, as determined by nutrient accumulation and gene expression studies. (Lauter *et al*., 1996, von Wirén *et al*., 2000). Additionally, root functional specialization is seen in plant-microbe interactions.

Orhan and four other scientists examined two PGPR strains, which are essentially bacillus strains, to determine how they affected raspberry plant yield, growth, and the nutritional makeup of the leaves between 2003 and 2005. Their findings demonstrated that strain treatment promotes plant growth and leads to a notable rise in plant output. Under organic growing circumstances, the addition of strains had some other notable impacts, including an increase in the Fe, Mn, N, P, and Ca contents of the leaves. 4 August 2006; 7 September 2007 (Ataturk University, Faculty of Agriculture, Department of Horticulture, 25240 Erzurm, Turkey) (Orhan et al., 2006).

## MATERIALS AND METHODS

### Graphical representation

**Figure 1**

*Graphical representation.*


### Gathering of a Soil Sample

The samples were taken from the rhizome of the okra plant.

### Sample Collection of Okra

The okra rhizoplane samples were gathered from Haripur, two of the four locations in Pakistan where okra is grown. When the okra plants were in the vegetative stage, the entire root system and adherent soil particles were uprooted along with it. The samples were obtained and stored at 4°C pending additional steps in aseptic bags.

**Removal of PGPR**
Using the serial dilution approach, the bacteria were isolated from the rhizoplane of okra (Rani, 2008).

**Rhizoplane Specimen**
The root was shed to get rid of the soil that was sticking to it while keeping the associated materials on the root. To create the dilution up to 10-6, one gram of root was dipped in 9 ml of sterilized distilled water and one milliliter of the dilution was collected from it.

**Serial Dilution Preparation**
About 9 McCartney bottles containing 9 ml of distilled water were autoclaved, given a number, and then placed into the laminar flow for additional processing for serial dilution. We marked each of the McCartney bottles in order from 1 to 9, added 1 g of soil sample to tube number 1, and then aggressively shook the McCartney bottles on an electric shaker. To ensure complete mixing of the soil sample and microorganisms, we transferred 1 ml of solution from McCartney bottle no. 1 into McCartney bottle no. 2, vigorously shook McCartney bottle no. 2, then transferred 1 ml of solution from McCartney bottle no. 2 into McCartney bottle no. 3, and repeated this process until the McCartney bottles no. 9. Before use, all glassware was autoclaved. An aliquot of 0.1 ml was collected through a micropipette from dilution number 10-4 to 10-6 and transferred to LB (Obukowicz et al., 1986) To perform PGPR isolation following serial dilution.

**Media Preparation**
**Table 1**
*Lauria Bertani (LB) medium (Maniatisal.1982).*

| Ingredients | Quantity |
|---|---|
| Yeast extract | 5g |
| NaCl | 10g |
| Tryptone | 10g |
| Agar | 15g |
| Distilled water | 1000Ml |
| Adjust pH to 7 and then autoclave | |

The previously listed **(Table 1)** substances were dissolved in a flask containing exactly 1000 ml of distilled water. Autoclaving the flask for 40 minutes at 121°C under 17 psi pressure was the next step. For this reason, we inverted the autoclaved Petri plates after the media had solidified and cooled for a brief while inside them. (Samasegaran *et al*., 1982).

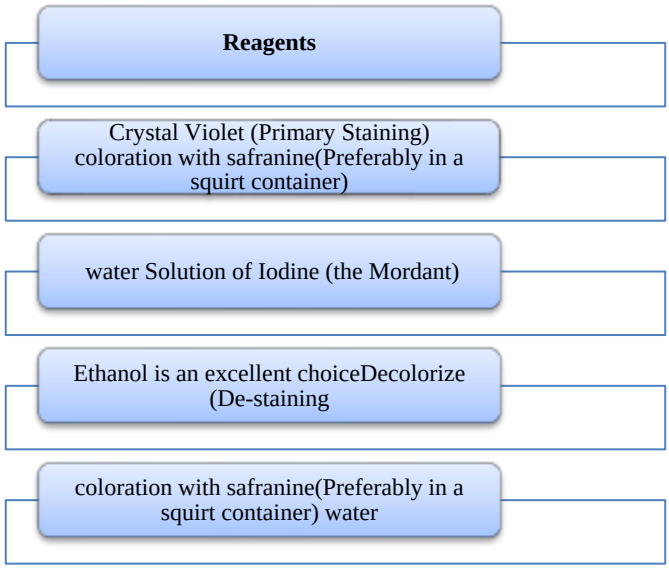
**Spreading**
With the identical tip, 0.1ml of each of the already-prepared dilutions in sequence 6, 5, and 4 were obtained, went down then dispersed on a media plate with a spreader, then marked. After each spread, the spreader is cleaned with spirit and disinfected with a flame. The resulting plates were spaced out and placed in an inverting incubator heated to 28 0 C.

**Incubation**
For one day, plates were incubated. Microscopic and colony characters were captured on tape.

**Streaking is a Method of Purifying Pure Culture**
After being streaked, the loop is sterilized by being rinsed with spirit. After streaking, these plates were put into the incubator at 28 0C to undergo the same purification process as the other cultures. From the growth plate no. 4 with the most clearly differentiated colonies, single colonies were picked using a sterile loop and streaked onto a media**.**

**Characterization of Morphology**
Bacterial cultures were raised on LB agar plates to examine the colony shape of these isolates. Each bacterial strain's single colony was selected, streaked, and cultured for 24 hours at 370°C. The morphology of the bacterial strains' overnight-grown colonies was examined.

**Gram Staining**



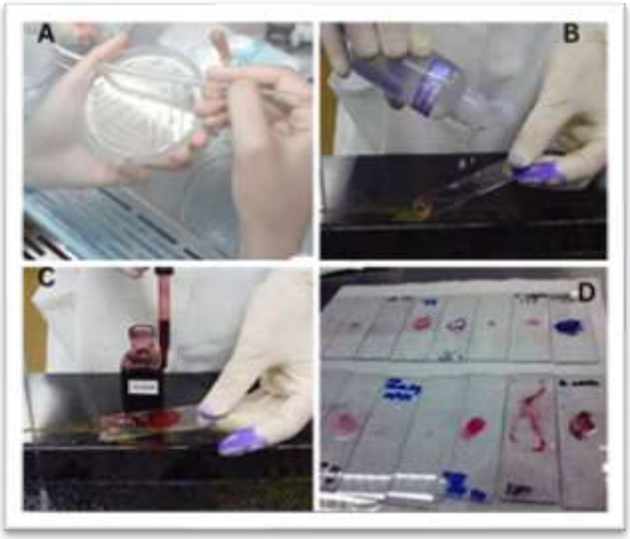

Gram staining is used to differentiate bacteria based on their forms, cellular morphologies, shapes and sizes, and gram responses. It is an asset in the medical microbiology laboratory for quickly detecting infectious pathogens and assessing the quality of clinical samples. Hans Christian S. Gram, a Danish bacteriologist, devised it in 1884. Gram stain splits all bacteria into 2 categories: those that retain the primary dye (gram-positive) as well as those that absorb the counterstain (gram-negative). Crystal violet comprises the primary dye, and Safranine is a supplementary dye. The steps are displayed in (**Fig 2 a, b c, d**).

**Procedure**
Pick a slide and place one drop of water that was distilled on it. Then, using a wire loop, take the colony off the pure culture and distribute it on the slide. Finally, heat fix the bacteria gently moving the spirit lamp. In the first step, slides containing heat-fixed bacteria have been colored using crystal violet dye to allow Gram-positive

bacteria if there were any to maintain their color. A drop of iodine was put to the slide, and it was left for one minute to allow crystal violet to fix to the Gram-positive bacteria's cell membrane. After that, the slide was cleaned utilizing alcohol decolorizer for removing any excess or unretained dye. Finally, safranin (a pink solution) dye was put to the slide to guarantee that the bacteria that were Gram-negative (if present) retained their pink hue. The slide was dried and cleaned after being subjected by safranine for 45 seconds, as indicated in (**Fig 2 a, b c, d**). Place the slide of film beneath the microscope and used one drop of oil for immerse. Gram-positive bacteria produce a purple tint, while Gram-negative bacteria produce a pink color.

**Figure 2**

*Gram staining: A. smear preparation B. fixation carry-out using iodine solution C. counter staining with safranine D.-stained slides(Denaro)*




**Test for phosphate solubilization**

Pikovskaya's medium **(Table 2)** (Pikovskaya, 1948), was employed for PSB separation. Pikovskaya's agar medium's composition is listed in Appendix ii (Pikovskaya's, 1948).

Placed a LOOP-full of colonies on a fresh glass slide after selecting them from a recent pure culture plate. Within 10 to 15 minutes, the result of adding one or two drops of 3% H2O2 could be seen.

**Table 2**

*Composition of Pikovskaya's agar medium (Pikovskaya,1948).*

| **Glucose** | **10.00 gram** |
|---|---|
| Ca3(PO4)2 | 5.00 gram |
| (NH4)2SO4 | 0.5 gram |
| NaCl | 0.2 gram |
| MgSO4.7H2O | 0.1 gram |
| KCL | 0.2 gram |
| Yeast Extract | 0.5 gram |
| MnSO4 | Trace |
| FeSO4.7H20 | Trace |
| Agar | 15 gram |
| Distilled Water | 1000ml |
| pH | 7.0 ± 0.2 |

**Test for Amylase Production**

The purpose of this experiment is to learn if the bacteria can grow on starch **(Table 3)** that was produced by fermenting glucose. The enzyme alpha-amylase aids in the breakdown of starch in the digestive system. A few colonies were selected from a pure culture plate using a sterile loop. A thin line of starch ran over the whole surface area of the plate. Multiple cultures can be studied at once on a single agar plate by drawing separate lines or by dividing the plate into four equal sections. 48 hours were spent incubating the plates at 370 C. The edges of colonies were treated with 2 to 3 drops of a 10% iodine solution. Ten to fifteen minutes later, the results were visible. A darkening of the medium was a good sign. However, areas near isolated colonies where amylase had digested starch seemed clear. Negative test result: Isolated colonies' edges were darkly colored by the media.

**Table 3**

*Composition of Amylase Medium*

| **Yeast extract** | **0.2g/200mL** |
|---|---|
| $MgSO_4.7H_2O$ | 0.02g/200mL |
| $K_2HPO_4$ | 1.4g/200mL |
| Agar | 4g/200mL |
| $KH_2PO_4$ | 0.4g/200mL |
| $(NH_4)2SO_4$ | 0.2g/200mL |
| NaCl | 1g/200mL |
| Starch | 1g/200mL |

**Test for Ammonia production**

To determine if these bacterial strains are creating ammonia or not, we do this test. Distilled water was combined with peptone water. Each test tube should contain 10 ml of solution listed in **(Table 4).** Autoclave each one. After the fluid had cooled, bacteria were added, and it was then incubated at 370°C for 48 hours. A few drops of the Nestler reagent were then added. A positive test would result in the color turning yellow. Negative test: No change in color.

**Table 4**

*Ammonia media composition (Bhatt et al., 2013).*

| **Peptone** | **1gram/1000ml** |
|---|---|
| NaCl | 0.5gram/1000ml |
| potassium nitrate | 0.5gram/1000ml |
| Nessler's reagent | 1ml/1000ml |

**Indole Production test**

The indole test employed tryptone broth, which is high in tryptophan (Table 5). Picked the colony, inoculated it into the suspension tubes, and incubated it for 48 hours at 37 degrees Celsius. A dark pink ring created while Kovac's reagent (P-dimethylaminobenzaldehyde) had been added to broth tubes indicated a favorable reaction. Whereas yellow or brown rings implied a poor outcome.

**Table 5**
*Indole Medium Composition.*

| Appendix v: indole medium | |
|---|---|
| Tryptone | 5grm/500ml |
| Yeast extract | 2.5grm/500ml |
| Tryptophane | 0.5grm/500ml |
| Distilled Water | 500ml |
| Ph. | 7 |

### Oxidase Production Test
This test determines whether bacteria have the enzyme cytochrome oxidase. To assess oxidase activity, a small amount to oxidase reagent was spread on filter paper, and colonies from plates were streaked onto the paper. If the reagent oxidized following 10-20 seconds, it showed the existence of oxidase in the bacterial isolate; otherwise, no color change indicated a negative outcome.

### Catalase Production Test
The catalase test is used in microbiology labs to distinguish between bacterial species that can produce catalase enzyme that hydrolyzes hydrogen peroxide. A hydrogen peroxide drop was placed on a slide to perform the test. Picked the colony with an applicator stick and then smeared the colony into the hydrogen peroxide drop. The organism is considered to be catalase-positive if bubbles form otherwise the organism is catalase-negative.

### Production of Indole Acetic Acid IAA
A qualitative assay was used to evaluate the bacterial isolates' synthesis of Indole-3-Acetic Acid (IAA). The isolates of bacteria were added to a nutritional broth that included 0.5% L-tryptophan, a precursor to the synthesis of IAA. To ensure enough aeration and bacterial development, the infected broth was shaken and maintained at 28–30°C for 48–72 hours. The broth that contained the culture was incubated, and the bacterial cells were separated by centrifuging it for 10 minutes at 10,000 rpm. The supernatant was then collected. Salkowski's reagent, which is a solution of 2% 0.5M ferric chloride and 35% perchloric acids, was combined with 1 mL of the supernatant and left in the dark for 30 minutes to detect IAA. The reaction mixture's transformation from pink to reddish, is shown in **(Table 6)**

**Table 6**
*Composition of IAA Medium.*

| Nutrient Broth | 13 g/L |
|---|---|
| L-Tryptophan | 5g/L |
| Distilled water | 1000mL |
| PH | 7 |

### HCN Production
The (TSA) supplemented with 4.4 g/L glycine to promote HCN generation shown in **(Table 7)** was used to investigate the hydrogen cyanide (HCN) production by bacterial isolates. A sheet of filter paper dipped in a 2% solution of sodium carbonate containing 0.5% picric acid was set on the Petri dish lid after the bacterial isolates had been spot-inoculated onto the set of agar plates, making sure the paper stayed floating on the agar surface without coming into direct contact with it. To ensure an airtight atmosphere, the plates were firmly sealed with parafilm and incubated for 48–72 hours at 28–30°C. The filter paper that was used was checked for color changes following incubation; a shift from yellow to red or orange would suggest the generation of HCN. But there was no discernible change in color.

**Table 7**
*Composition of HCN Medium.*

| Tryptic Soy Agar | 23g/L |
|---|---|
| Glycine | 4.4g/L |
| Distilled water | 1000mL |
| PH | 7 |

## RESULTS
### Isolation and selection of PGPR
The purpose of the study was to separate and analyze the PGPR extracted from the okra plant's rhizoplane. Seventy PGPR have been isolated through the rhizoplane within the okra plant. Out of 70 isolates, 11 PGPRs were selected for further study based on a range of features, as shown in (**Figures 3 & 4**).

**Figure 3**
*bacterial Colonies on Agar Plates.*

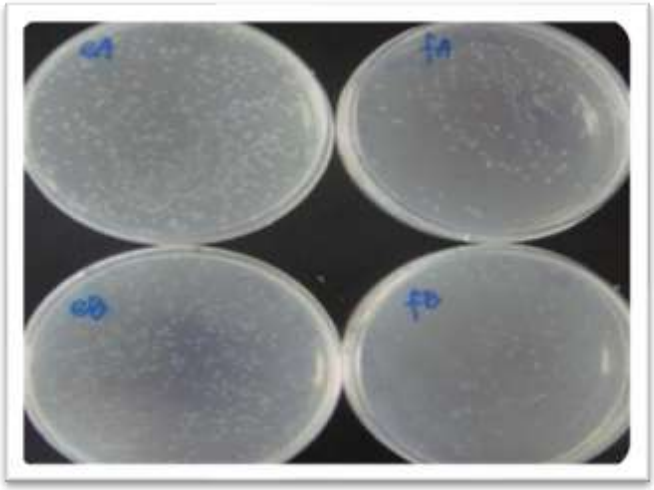


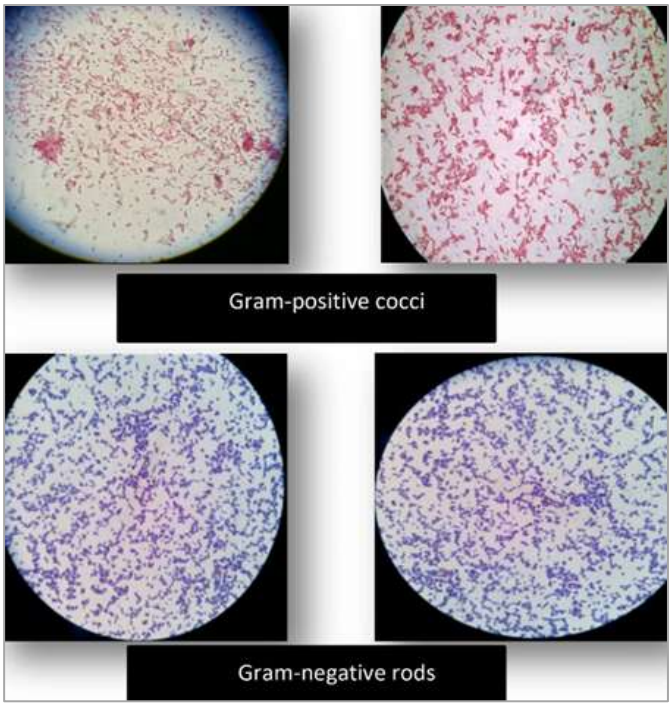
**Figure 4**
*streaking Plates.*

**Okra-isolated PGPR Bacterial Strains' Cell Morphology**

Following isolating & purification, Gram staining was utilized to examine cell morphology underneath a microscope. Bacterial isolates were prepared for microscopic inspection. Each waswere each smeared, prepped, stained, and examined using a microscope. Gram staining helped discriminate amongst gram-negative and gram-positive bacteria. Positive strains featuring thicker cell walls were shown in (**Fig 5**).

**Figure 5**

*A. Gram-positive cocci B. Gram-negative rods*




Gram staining revealed that 8 of the 11 isolates were Gram-positive rods (PGPR-1, PGPR-2, PGPR-3, PGPR-4, PGPR-6, PGPR-7, PGPR-8, and PGPR-9), whereas 3 isolates (PGPR-5, PGPR-10, and PGPR-11) were Gram-negative. This disparity in cell wall properties suggests potential differences in robustness and adaptability in the rhizosphere environment **(Table 8)**.

**Table 8**

*Cell Morphology of PGPR bacterial strains isolated from okra.*

| *Strain no.* | *Shape* | *Group* | *Color* | *Gram Reaction* |
|---|---|---|---|---|
| *Rp1(1)* | Coccus | Staphylococcus | Pink | Negative |
| *Rp 1(a)* | Bacillus | Streptobacillus | Pink | Negative |
| *Rp1(6)* | Coccus | staphylococcus | purple | Negative |
| *Rp2* | Bacillus | streptobacillus | Purple | Negative |
| *Rp2(4)* | Coccus | staphylococcus | purple | Negative |
| *Rp3(a)* | Coccus | streptobacillus | Pink | Negative |
| *Rp3(1)* | Bacillus | Monobacillus | Pink | Negative |
| *Rp3(2)* | Coccus | Diplococcus | Pink | Negative |
| *Rp3(3)* | Coccus | staphylococcus | Purple | Negative |
| *Rp4(2)* | Coccus | Monococcus | Pink | Negative |
| *Rp5* | Coccus | staphylococcus | Pink | Negative |
| *Rp5(1)* | Coccus | Tetrads | Pink | Negative |
| *Rp7* | Coccus | staphylococcus | Purple | Negative |
| *Rp7(a)* | Coccus | staphylococcus | Purple | Negative |
| *Rp7(b)* | Bacillus | Monobacillus | Pink | Negative |
| *Rp8(a)* | Coccus | staphylococcus | purple | Negative |
| *Rp8(1)* | Coccus | Diplococcus | Pink | Negative |
| *Rp9(a)* | Coccus | Staphylococcus | purple | Negative |
| *Rp9(b)* | Bacillus | Diplobacillus | Pink | Negative |
| *Rp10(a)* | Coccus | Tetrads | purple | Negative |

**Table 8** summarizes the morphological and gram-staining properties of 20 chosen PGPR isolates from the okra plant's rhizoplane. The isolates displayed different morphological traits and gram-staining results, providing insights into their possible taxonomic families and functional roles. Most of the 20 isolates (70%) were cocci, with the remaining 30% bacilli. Cocci were arranged in numerous ways, including staphylococcus, diplococcus, monococcus, and tetrads. Bacilli isolates had streptobacillus, monobacillus, and diplobacillus arrangements, showing a high level of structural diversity.

All isolates were Gram-negative in gram-staining reactions, as evidenced by their pink or purple hue. The color variation indicates changes in cell wall components, which may alter their interactions with the plant host and other microorganisms in the rhizosphere. Interestingly, the staphylococcus group was the most common among the cocci, with representatives including Rp1(1), Rp1(6), Rp3(3), Rp5, Rp7, and Rp9(a). The bacilli group contained isolates like Rp1(a), Rp3(1), and Rp9(b), indicating structural variation even within a single group.

The variations in shape, grouping, and color demonstrate the diversity of PGPR found in the okra rhizoplane. These kinds of plants may contribute to numerous plant growth-promoting characteristics such as nutrient solubilization, phytohormone synthesis, or pathogen control, necessitating further study into their functional capabilities.

**Biochemical Testing**

The biochemical properties of the bacterial isolates were investigated to better understand their enzymatic activity. All isolates showed oxidase and catalase activity, indicating that they are susceptible of active metabolic processes. Notably, every bacterial isolate tested positive for the catalase enzyme, indicating their ability to degrade hydrogen peroxide into water and oxygen, which is critical for survival under oxidative stress conditions.

**Catalase and Oxidase**

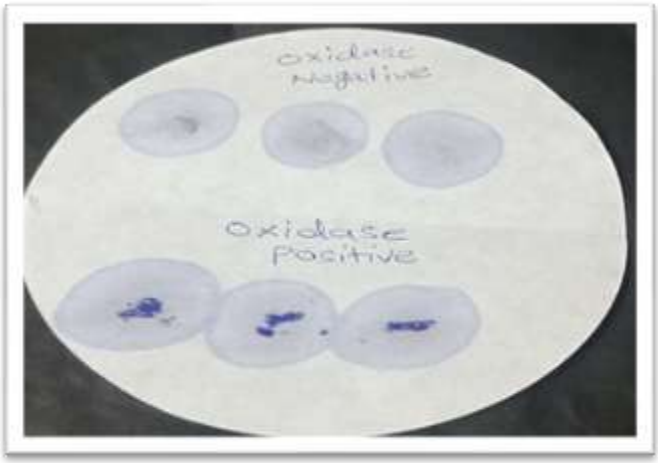
All bacterial isolates showed oxidase and catalase activity, It was discovered that all of the examined bacterial isolates tested positive for catalase enzyme. All gram-positive cocci-shaped bacteria as well as gram-negative rod-shaped bacteria received a catalase-positive test, as demonstrated in (**Fig 6**).

**Figure 6**
*Catalase Positive Results*


Catalase activity was consistently demonstrated among both Gram-positive cocci-shaped bacteria & Gram-negative rod-shaped bacteria, indicating a conserved feature across various morphological groups. This consistency in catalase activity amongst the isolates (**Fig. 6**) could indicate their adaptation to the the root zone the surroundings, where oxidative stress may be frequent due to interactions between plants and soil microbial competition.

To determine whether or not cytochrome oxidase is present, an oxidase test is used. All of these isolates tested oxidase-positive, and every single of these bacteria could modify the pigmentation of filter paper as demonstrated in (**Fig 7**). Indicating aerobic respiration and the ability to break down hydrogen peroxide.

**Figure 7**
*Oxidase results. Change of filter paper color indicated positive results and no color indicated negative results.*

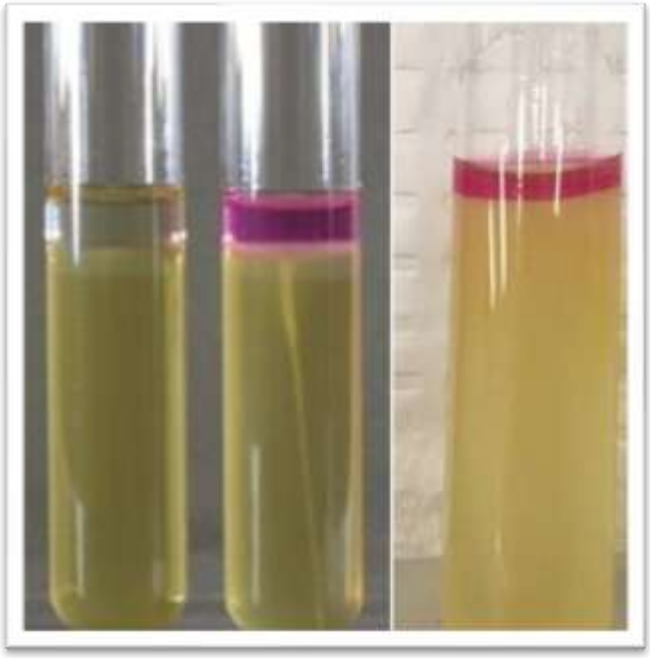



The existence of oxidase activity indicates the aerobic as well as facultative anaerobic character in these isolates, since the enzyme is involved in electron transfer during cellular respiration. These enzymatic capabilities highlight the PGPR isolates' potential functional diversity as well as their ability to thrive in a variety of environmental situations, all of which contribute to their involvement in plant growth and resilience.

**Indole Test Results**

The indole test measured the isolates' capacity to convert tryptophan to indole. Seven of the 11 isolates tested positive for indole synthesis, generating a pink ring after the use of Kovac's reagent. This shows that these strains may help with the soil nitrogen cycle by breaking down tryptophan shown in (**Fig 8**).

**Figure 8**
*The pink ring indicated positive indole test results while the brown ring indicated no indole production.*



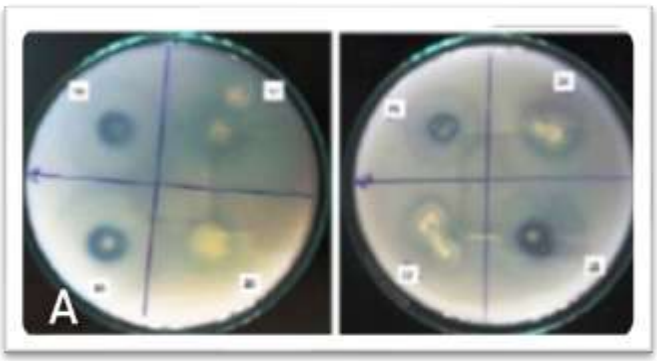
This finding implies that these isolates have the enzymatic machinery sufficient to convert tryptophan onto indole, pyruvate, and ammonia.Indole synthesis is an important feature in microbial interactions in the rhizosphere because it influences agricultural chemical signaling, microbial relationships, and nutrient cycling. The ability of these isolates to be beneficial to the soil cycles of nitrogen by processing tryptophan highlights their potential for improving sustainable agriculture practices.The strains of bacteria that proved positive for indole synthesis showed a variety of morphological and gram-staining features, indicating functional plasticity within the roots of plants microbiome. These findings emphasize the need of further investigating these strains to understand their distinct roles and uses in biofertilizer development.

**Table 9**
*Biochemical test results.*

| Strain no. | Amylase | Catalase | Indole | Ammonia | Psb |
|---|---|---|---|---|---|
| Rp1(1) | +++ | ++ | +++ | _ | +++ |
| Rp 1(a) | +++ | +++ | +++ | ++ | _ |
| Rp1(6) | ++ | +++ | ++ | + | ++ |
| Rp2 | + | +++ | _ | +++ | +++ |
| Rp2(1) | +++ | +++ | _ | +++ | +++ |
| Rp3(a) | +++ | ++ | +++ | +++ | +++ |
| Rp3(1) | +++ | ++ | + | +++ | ++ |
| Rp3(2) | +++ | + | _ | +++ | +++ |
| Rp3(3) | +++ | _ | +++ | _ | +++ |
| Rp4(2) | _ | +++ | +++ | _ | +++ |
| Rp5 | _ | +++ | ++ | +++ | +++ |
| Rp5(1) | +++ | +++ | ++ | +++ | +++ |
| Rp7 | +++ | +++ | +++ | +++ | +++ |
| Rp7(a) | +++ | +++ | +++ | +++ | _ |
| Rp7(b) | +++ | _ | +++ | ++ | +++ |
| Rp8(a) | +++ | +++ | +++ | ++ | +++ |
| Rp8(1) | +++ | +++ | ++ | +++ | +++ |
| Rp9(a) | +++ | +++ | + | +++ | _ |
| Rp9(b) | +++ | +++ | ++ | +++ | +++ |
| Rp10(a) | +++ | | +++ | +++ | +++ |

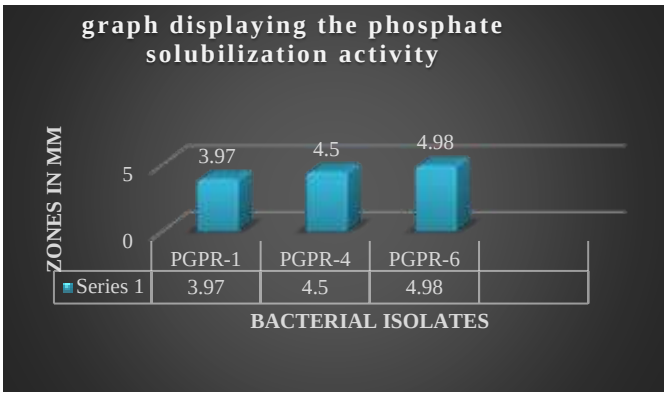
**Phosphate solubility and PSI values**
Three isolates, PGPR-1, PGPR-4, and PGPR-6, exhibit the ability to solubilize phosphate, as indicated by distinct halo zones on PVK agar plates. The isolates PGPR-1 ( 3.97±0.10), PGPR-4 ( 4.50±0.15), and PGPR-6 (4.98±0.12) . The phosphate solubilization index (PSI) readings ranged from 3.97 to 4.98, with PGPR-4 showing the highest solubility index. Based on their ability to produce clear zones around their colonies as a result of P solubilization in the surrounding medium. These clear zones served as indicators of the colonies' ability to solubilize phosphate on the Pikovskaya medium. Isolation and Characterization of Plant Growth Promoting Bacteria shown in (**Fig 9**).

**Figure 9**
*These Clear Zones Served as Indicators of the Colonies' Ability to Solubilize Phosphate on the Pikovskaya Medium.*




The PSI values ranged between 3.97 and 4.98, showing that the isolates' capability to solubilize phosphate varied. PGPR-4 had the greatest the dissolution index (4.98 ± 0.12), next to PGPR-6 (4.50 ± 0.15), and PGPR-1 (3.97 ± 0.10). These findings emphasize the isolates' potential to increase the level of phosphorus in the rhizosphere, which is a crucial nutrients for plant growth. PGPR-4's strong performance highlights its suitability as a possibility for biofertilizer applications aiming at increasing phosphorus uptake performance in crops. Further research will focus on improving environments for greatest solubilization and testing their efficacy in field experiments shown in (**Figure 10**).

**Figure 10**
*Graph Displaying the Phosphate Solubilization activity for PGPR-1, PGPR-4, and PGPR-6*




In **(Figure 10)** numerous isolates exhibited strong phosphate solubilization activity, with strains such as PGPR-1, PGPR-4, and PGPR-6 succeeding in this function, contributing to increased phosphorus solubility in the soil.

**Production of IAA and ammonia**
Bacterial isolates PGPR-2, PGPR-3, PGPR-7, and PGPR-8 generate IAA, which promotes plant growth. Seven isolates (PGPR-1, PGPR-2, PGPR-4, PGPR-6, PGPR-7, PGPR-8, and PGPR-9) demonstrated strains to produce large amounts of ammonia from peptone water shown in (**Fig 11 A and B**).

**Figure 11**
*AN IAA positive results B Ammonia production positive results*

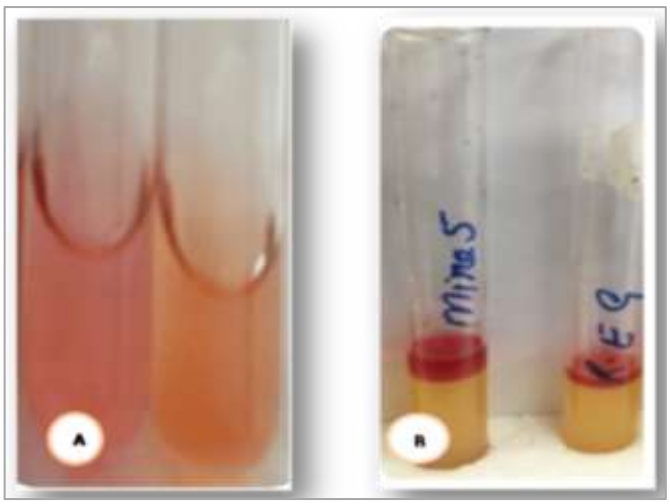
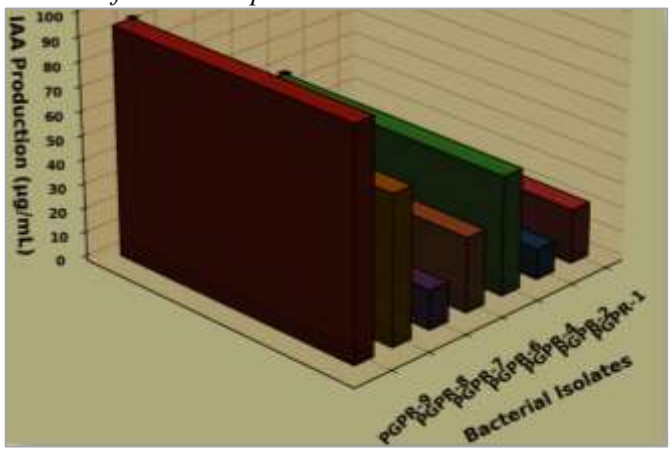



**Table 10**
*The potential of PGPR isolates to generate indole-3-acetic acid (IAA).*

| Sample | Media Conditions | IAA Concentration (µg/ml) | Observation (color Intensity) | Replicates (mean ± SD) |
|---|---|---|---|---|
| PGPR-1 | With tryptophan | 22.3 | Pink | 22.3±057 µg/mL |
| PGPR-2 | With tryptophan | 12.5 | Light Pink | 12.5±0.36 µg/mL |
| PGPR-4 | With tryptophan | 48.9 | Dark Pink | 48.9±0.63 µg/mL |
| PGPR-6 | With tryptophan | 30.7 | Pink | 30.7±0.45 µg/mL |
| PGPR-7 | With tryptophan | 16.2 | Light Pink | 16.2±0.51 µg/mL |
| PGPR-8 | With tryptophan | 61.4 | Dark Pink | 61.4±0.72 µg/mL |
| PGPR-9 | With tryptophan | 94.5 | Dark Pink | 94.5±0.79 µg/mL |

**Table 10** describes the potential of PGPR isolates to generate indole-3-acetic acid (IAA) in a medium containing tryptophan. IAA concentrations differed widely between isolates, spanning 16.2 µg/mL to 94.5 µg/mL. PGPR-9 The potential of PGPR isolates to generate indole-3-acetic acid (IAA) has been examined in a medium containing tryptophan. IAA concentrations differed widely between isolates, spanning 16.2 µg/mL to 94.5 µg/mL. PGPR-9 produced the most IAA (94.5 ± 0.79 µg/mL), afterwards PGPR-8 (61.4 ± 0.72 µg/mL) & PGPR-4 (48.9 ± 0.63 µg/mL). These isolates exhibited a dark pink hue, indicating significant IAA production levels.Isolates PGPR-7 and PGPR-1 showed lower IAA concentrations (16.2 ± 0.51 µg/mL and 22.3 ± 0.57 µg/mL, correspondingly) and lighter color intensities. This variance in IAA production shows that the isolates have different metabolic capabilities for using tryptophan during auxin biosynthesis. The ability to generate IAA, a major phytohormone that facilitates root elongation and cell division, demonstrates these isolates' ability to promote plant growth. For instance, PGPR-9, particularly the biggest IAA production, shows potential as a plant growth promoter. Future research is expected to concentrate on optimizing IAA production conditions and assessing their impact on crop performance in the field (**fig 12**).

**Figure 12**
*3D chart for the IAA production data.*




The generation of ammonia, a crucial feature of plant growth-promoting rhizobacteria, was examined in seven isolates. The isolates produced ammonia concentrations that fluctuated between 45.6 ± 1.2 µg/mL to 52.1 ± 1.4 µg/mL, demonstrating moderate heterogeneity between the pathogens. PGPR-6 produced the most ammonia (52.1 ± 1.4 µg/mL), closely followed by PGPR-2 (50.3 ± 1.5 µg/mL) as well as PGPR-8 (49.8 ± 1.2 µg/mL) described in (**Table 11**).

**Table 11**
*Results of bacterial strains' production of ammonia.*

| Bacterial strains | Production of ammonia (µg/ml) |
|---|---|
| PGPR-1 | 45.6±1.2 µg/mL |
| PGPR-2 | 50.3±1.5 µg/mL |
| PGPR-4 | 48.7±1.3 µg/mL |
| PGPR-6 | 52.1±1.4 µg/mL |
| PGPR-7 | 47.5±1.1 µg/mL |
| PGPR-8 | 49.8±1.2 µg/mL |
| PGPR-9 | 46.9±1.3 µg/mL |

These findings emphasize the possible ability of these isolates to improve the supply of nitrogen in the rhizosphere, as is critical for plant growth. Ammonia generation by PGPR promotes nitrogen absorption by plants and improves overall soil fertility. The isolated cells such as PGPR-6 and PGPR-2, which produce more ammonia, are especially intriguing candidates for bio Fertilizer formulations targeted at increasing agricultural output. The observed similarity in generation of ammonia across all isolates indicates a similar metabolic competence, which corresponds to their functions as successful rhizosphere colonizers. Future research will examine these stressors in field settings to confirm their effect on nitrogen absorption and agricultural production improvement.

### Amylase Production

PGPR-2 and PGPR-8 were the isolates that produced the most amylase, resulting in clear and noticeable halo

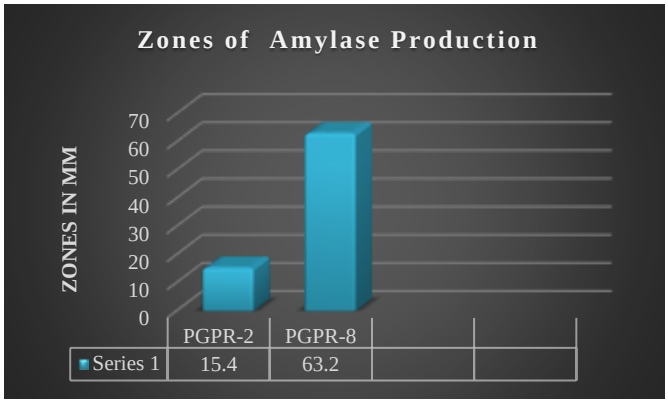
zones PGPR-2: 15.4 ± 0.43 µg/ml, PGPR-8: 63.2 ± 0.68 µg/ml. By monitoring the development of transparent halo zones on starch agar plates following the addition of iodine solution, the bacterial isolates' capacity to generate amylase was evaluated. These zones were a sign of amylase production and starch hydrolysis. According to these findings, these isolates may be plant growth-promoting bacteria (PGPR) because they exhibit considerable enzymatic activity for breaking down starch, as shown in (**Fig 13**).

**Figure 13**

*Amylase production results.*

**Figure 14**

*3d chart showing the estimated amylase production for PGPR-2 and PGPR-8. PGPR-8 produces a significantly higher amount of IAA compared to PGPR-2.*




In **Figure 14** shows PGPR-2 & PGPR-8 being bacterial isolates recognized for their high amylase production, as indicated by the clear and visible halo zones surrounding their colonies. PGPR-2 generated 15.4 ± 0.43 µg/ml of amylase, but PGPR-8 produced substantially more (63.2 ± 0.68 µg/ml). The appearance of translucent zones suggests starch hydrolysis in the medium, which demonstrates the isolates' enzymatic activity. The proportions and sharpness of these crown zones correspond with the amount of enzymatic produced, indicating that PGPR-8 is a more powerful amylase generator than PGPR2. These findings highlight the potential uses of these samples in applications requiring amylase activity, such as the foodstuff and biotechnology industries.

**HCN Production**

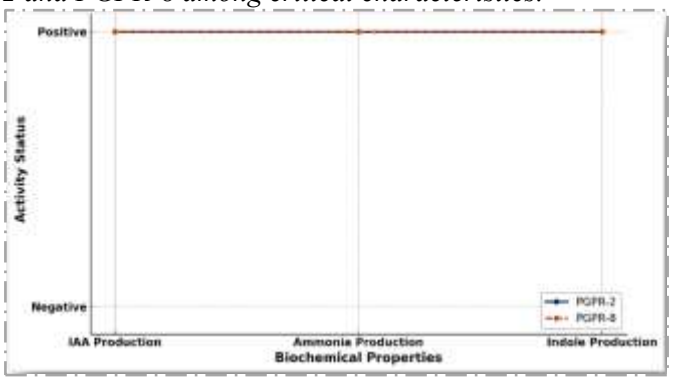
No HCN generation was observed in any isolates tested, indicating that they are non-toxic and suitable for plant application.

**Bacterial Strains having Strong Growth-promoting Properties**

PGPR-2 and PGPR-8 had considerable plant growth-promoting properties, as evidenced by favorable results in many biochemical experiments. Both strains were shown that they generate indole-3-acetic acid (IAA), ammonia, & indole, all of which indicate their ability to promote plant development. These molecules all play important roles in promoting plant growth, with IAA representing a natural plant hormonal that regulates numerous growth processes, ammonium contributing to soil growth, and indole synthesis related to positive microbial associations with plants. In addition to the aforementioned findings, PGPR-2 and PGPR-8 underwent additional biochemical and enzymatic tests, which revealed their high potential as growth-promoting bacteria such as r (PGPR). Based on these favorable findings, these strains were chosen for further investigation to determine their full capabilities in promoting plant growth and increasing agricultural production described in (**Fig 15**).

**Figure 15**

*This line chart depicts the chemical reactions of PGPR-2 and PGPR-8 among critical characteristics.*




- The x-axis represents biochemical parameters (IAA, ammonia, and indole production).
- The Y-axis indicates activity state (positive = 1, negative = 0).
- Line styles: PGPR-2 has a solid line, while PGPR-8 has a dashed line to indicate its activity.

Both strains exhibit consistent positive activity across various biochemical aspects, indicating that they might serve as plant growth-promoting bacteria.

**Keeping the Colonies Alive**

We created the appropriate media for the preservation of the microbial colonies, poured it into the slants, and then allowed it to cool and solidify. Then, we chose a microbial colony from a pure culture, streaked it in a slant, and similarly streaked the remaining colonies to indicate the slants shown in (**Fig 16**). These slants were

incubated for three days. These slants were kept after growth in the fridge.

**Figure 16**
*Slant preparation.*

## DISCUSSION

The purpose of this work was to extract and analyze plant growth-promoting rhizobacteria (PGPR) from the okra plant's rhizoplane in order to determine their potential agricultural applications. The effective extraction of 70 PGPR strains, 11 of which were chosen for a thorough examination, demonstrates the microbial richness of the okra rhizosphere, implying a diverse microbial ecology that could promote plant growth via multiple biochemical pathways.

### Cell Morphological and Gram Staining

The vast majority of the strains (8 out of 11) were Gram-positive, suggesting that their thick cell walls give structural integrity and protection from environmental pressures (Sumiati and Gunawan, 2007). The existence of a few isolates that are Gram-negative (3 out of 11) adds variety to the rhizosphere microbiome, potentially influencing interactions with other soil microorganisms and nutrient-cycling processes.

### Biochemical Analysis

The positive catalase and oxidase test findings in all selected isolates indicate aerobic metabolic capability, implying their possible efficiency in soil conditions with varied oxygen levels. (Sumiati and Gunawan, 2007). The synthesis of indole by 7 isolates is relevant since it has been linked to the breakdown of organic materials, which may improve nutrient cycling and stimulate plant growth (Carrique-Mas and Davies, 2008). Indole-positive strains may be effective biofertilizers by enhancing soil fertility, especially in nutrient-limited soils (Carrique-Mas and Davies, 2008).

### Phosphate Solubilization and Mineral Mobilization

Phosphate solubilization is an important feature of PGPR since phosphorus is an essential mineral for plant growth but is frequently immobilized in the soil. The isolates PGPR-1 ( 3.97±0.10), PGPR-4 ( 4.50±0.15), and PGPR-6 (4.98±0.12), exhibited significant phosphate solubilization capacity, with PSI values ranging from 3.97 to 4.98, showing their potential to enhance phosphate availability to plants. These findings are consistent with prior research by Ngomle et al. (2014)(Ambesh et al., 2014), who found that clean halo zones around colonies of bacteria on Pikovskaya's agar suggest effective phosphate solubilization. Thus, the isolates in this investigation, particularly those with a high PSI, show promise as biofertilizers for increasing okra growth and output.

### PGPR Compounds Production

Four isolates (PGPR-2, PGPR-3, PGPR-7, and PGPR-8) produce IAA, which appears to have a role in encouraging root growth and nutrient uptake. This hormone is known to promote root elongation, which improves a plant's access to water and nutrients in the soil. According to Arshad and Frankenberger (1991)(Arshad and Frankenberger Jr, 1997) and Pradhan and Mishra (2015)(Panda et al., 2015). In addition, seven isolates produced considerable amounts of ammonia, which could contribute to soil nitrogen enrichment and plant growth. Nitrogen is a limiting resource in many soils, and ammonia-producing bacteria can help maintain soil fertility, particularly in sustainable agriculture. (Ahmad et al., 2008).

None of the isolates produced HCN, indicating their potential for use in uses in agriculture without releasing harmful chemicals into the soil. This is consistent with the desirable attribute of non-pathogenicity in PGPR strains designed to be utilized as biofertilizers, enabling secure interactions with plants and the soil ecosystem.

### Significance and Future Utilization

The results emphasize the potential of isolates PGPR-2 and PGPR-8 as bio-fertilizers due to their remarkable plant growth-promoting properties. These strains exhibited several favorable features, including high IAA production, indole generation, phosphate solubilization, and ammonia production, making them promising candidates for future agricultural applications. Future research should include field trials to assess the efficacy of these isolates in improving okra growth and yield under a variety of soil conditions. Furthermore, the strains' long-term stability should be evaluated to establish their ability to survive and colonize in various agricultural contexts.

## CONCLUSION

Plant growth-promoting rhizobacteria (PGPR) were isolated and characterized from the rhizoplane of okra plants gathered in the Haripur area of KPK, Pakistan, for this study. Eleven bacterial isolates were examined for their ability to promote plant growth; isolates PGPR-2 and PGPR-8 showed particularly strong activity in a

variety of biochemical tests. These isolates specifically showed the ability to produce ammonia and indole, to produce amylase and catalase, to reduce nitrate, to solubilize phosphate, and to fix nitrogen biologically. Using the Roll Towel Method, their effects on plant growth were further assessed on a variety of vegetables, showing statistically significant improvements in germination rate, root length, and shoot length. According to these findings, PGPR-2 and PGPR-8 have a great deal of promise as biofertilizers, providing a sustainable agricultural method and an environmentally benign substitute for chemical fertilizers.

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