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# Biochemical Characterization of Plant Growth Promoting Rhizobacteria Colonizing Rhizoplane Soil of Rice

**D Prathyusha, AK Patibanda, V Prasanna kumari, P Madhusudan, K Jayalalitha and V Srinivasa Rao**
Department of Plant Pathology, Agricultural College, Bapatla, A.P

**ABSTRACT**

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can be found in the rhizosphere, in association with roots and which can enhance the growth of plants directly or indirectly. A large number of bacteria, including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Klebsiella*, *Rhizobium* and *Serratia* have been reported to enhance plant growth. Different biochemical tests *viz.*, catalase test, oxidase test, nitrate reduction test, methyl red test, vouges proskauer test, ammonia production, citrate utilization, urease activity, hydrogen sulfide production and gelatin liquefaction were performed for a set of 63 bacterial isolates that were obtained from rhizoplane soils of rice. Among the 63 bacterial isolates, all were tested positive for the catalase test, 37 were positive for the nitrate reduction test and the VP test, 48 found to be positive for the oxidase test, 43 isolates exhibited positive reactions for citrate utilization and urease activity, 42 were positive for the methyl red test, 46 exhibited ammonia production, 35 were able to liquefy gelatin and 10 were positive for the hydrogen sulphide test.

**Keywords:** *Biochemical characterization, PGPR, Rice.*

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and a major staple food for half of the world's human population (Singh and Singh, 2019). Its cultivation is the major activity and source of income for millions of households around the globe and the staple food for 17 countries in Asia. It provides 20% of the world's dietary energy supply, which is higher than wheat (19%) and maize (5%) (FAO, 2004). Rice production is geographically concentrated in Western and Eastern Asia, and Asia is the biggest rice producer, accounting for 90% of the world's production.

Kloepper and Schroth (1981) first used the term Plant Growth Promoting Rhizobacteria (PGPR) to demonstrate the microbial population in the rhizosphere that which is beneficial, colonizes the roots of plants and exhibits plant growth-promoting activity. The concept of rhizobacteria where the microbial population is accelerated by root activities was first given by Hiltner (1904) to detect the zone of soil that surrounds the roots.

PGPR colonize's rhizospere, which is the tightly adhered soil interface between the root surfaces (Kloepper *et al*., 1999). The rhizosphere is the portion of the soil that forms the complex habitat of plant roots and whose composition is altered by root activity (Lynch and Whipps, 1991). An important component of the rhizosphere is the actively growing microbial population, which thrives due to the provision of organic nutrients in root exudates. In turn, the microorganisms that colonize the rhizosphere profoundly affect root and plant biology in relation to nutrition, development and health (Sen, 2000). The concentration of bacteria found around the roots of plants is generally much greater than in bulk soil. Therefore, different soil microbes that have been reported as PGPR, belong to genera that exert a beneficial effect on the plant growth including *Pseudomonas*, *Bacillus*, *Azospirilium*, *Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Alcaligenes*, *Serratia*, *Rhizobium*, *Enterobacter*,

*Burkholderia*, *Beijerinckia*, *Klebsiella*, *Clostridium* and *Phyllobacterium* (Kalita *et al*., 2015). So these bacteria need to be characterization for understanding the bacteria and their activities. Biochemical characterized is one of the quickest method for microbe identification. In this present investigation, 63 bacterial isolates were characterized biochemically.

## MATERIALS AND METHODS

Different biochemical tests were performed and the protocols followed are briefly outlined below.

**Catalase test** (Rangaswami and Bhagyaraj, 1993)

This test was performed to study the presence of the catalase enzyme in bacterial colonies. Pure isolates (24 hours old) were taken on glass slides and one drop of H2O2 (30%) was added. The appearance of the gas bubble indicated the presence of the catalase enzyme.

**Nitrate Reduction test**

Nitrate broth was inoculated with a loopful of selected bacterial isolate and incubated at 28ºC for 7 days. Uninoculated nitrate broth was kept as a control. Control was also run without inoculation. After incubation, two drops of sulphanilic acid followed by two drops of napthylamine solution were added. The presence of nitrate was indicated by a pink, red or orange colour and the absence of colour change was considered nitrite negative.

**Oxidase test** (Collins and Lyne, 1970)

The test isolates were grown in the nutrient broth for 24 hrs 2-3 drops of freshly prepared 1% solution of tertramethyl-p-phenylene-diamine dihydrochloride were added to the bacterial broth. A test tube with sterile distilled water used as control. A positive reaction was indicated by an intense, deep purple hue that was developed within 5-10 seconds.

**Citrate utilization** (Macfaddin, 2000)

The isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± $2^0$C for 24 hours. The change in colour from green to blue indicated the positive reaction for citrate utilization.

**Methyl red test** (Crown and Gen, 1998)

Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2ºC for 48 hours. After incubation, five drops of methyl red indicator were added to each tube and gently shaken. Red colour development was taken as positive and yellow colour production was taken as negative for the test.

**Vogues Proskauer's test** (Macfaddin, 2000)

In the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at $37^0$C for 48 hours. After incubation, ten drops of Barritt's reagent-A were added and gently shaken, followed by the addition of 10 drops of Barritt's reagent-B. The development of brownish red colour in the broth was taken as positive for the test.

**Ammonia production** (Juanda, 2005)

The isolates were tested for ammonia production by inoculating the isolates into 10 ml of pre-sterilized peptone water in test tubes. The tubes were incubated for 48-72 hours at 36 ± 2oC. After that, Nessler's reagent (0.5 ml) was added to each tube. The change in colour of the medium from yellow to brown was taken as a positive test for ammonia production.

**Urease activity** (Aneja, 2006)

Test bacterial isolates were streaked on urea agar plates. The inoculated urea agar plates were then incubated at 37°C for 28 to 48 h. The colour change of the medium to pink indicates positive urease activity.

**Hydrogen sulfide test** (Beishir, 1991)

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 hours at 28 ± 2oC. The visualization of black along the line of inoculation indicated a positive reaction to the test.

**Gelatin liquefaction** (Macfaddin, 2000)

The overnight cultures of the test isolates were inoculated into sterilized nutrient gelatin deep tubes and incubated for 24 hours at 28 ± $2^0$ C. Then the tubes were kept in the refrigerator for 30 minutes at 4oC. The isolates showing liquefied gelatin were taken as positive and those that resulted in the solidification

of gelatin on refrigeration were recorded as negative for the test.

## RESULTS AND DISCUISSION

All the 63 bacterial isolates were tested for their biochemical characterization, *viz.*, Catalase test, Nitrate Reduction, Oxidase test, Citrate Utilization, Methyl red test, Vogues Proskauer test, Ammonia production, Urease test, Hydrogen sulphide test and Gelatine liquification test (Tables 4.11 and 4.12 and Plate 4.11).

All 63 bacterial isolates (100%) tested positive for catalase test, which was detected in the form of effervescence due to the production of oxygen from the breakdown of $H_2O_2$.

For the nitrate reduction test, 37 isolates (58.73%) were positive and 26 isolates (41.27%) were found to be negative.

Out of 63, 48 (76.19%) were tested positive by showing an intense deep purple colour for the oxidase test and the remaining 15 (23.81%) were found to be negative.

In the test of citrate utilization, 43 isolates (68.25%) among the 63 exhibited a positive reaction by a change in the colour of the medium from green to blue, while remaning 20 (31.75%) isolates were found to be negative without any colour change.

In the case of the methyl red test, 21 isolates (33.33%) were tested negative and 42 (66.66%) were positive.

For the test of Vogues Proskauer, out of 63 isolates, 37 (58.73%) were found to be positive exhibiting a pink colour and the remaining 26 (41.27%) showed negative reaction for the test.

For the test of ammonia production, 46 isolates (73.01%) turned the broth into brown after the addition of Nessler's reagent indicating a positive reaction, and the remaining 17 (26.98%) were found to be negative.

Among the 63 isolates that were tested for the presence of urease activity, 43 isolates (68.25%) were found to be positive by changing the colour of the medium to pink and the rest 20 (31.75%) were negative.

Except for ten isolates (15.87%), all were found to be negative for the hydrogen sulfide test as there was no visualization of black colour along the line of inoculation.

Out of 63 PGPR isolates, 35 (55.55%) were found to be positive by liquefying gelatin and the remaining 28 isolates (44.44%) were negative for the gelatin liquefication test.

Several research workers have performed different biochemical tests *viz*., Catalase test, Nitrate Reduction, Oxidase test, Citrate Utilization, Methyl red test, Vogues Proskauer test, Ammonia production, Urease test, Hydrogen sulphide test and Gelatine liquefication of different rhizobacterial strains isolated from various crops and found variability biochemically characterized 10 rhizospheric isolates obtained from wheat rhizosphere, of which nine were able positive for oxidase, catalase activity, citrate utilization and nitrate reduction 2 isolates were positive for hydrogen sulphide production and MR test, 5 were positive for urease and gelatin hydrolysis and none were positive for the VP test.

Among the 32 isolates evaluated biochemically, all were able to utilize citrate, twenty three were positive for the MR test, 19 were positive for the VP test, nine exhibited urease activity and six were found to be positive for the nitrate reduction test and gelatin liquefaction (Rani *et al*., 2012). All the rhizobacteria produced ammonia except strain AJ-RB13, while HCN and catalase test results were positive for all the bacterial strains (Hyder *et al*., 2020).

In the present study a total of 63 bacterial isolates were characterized biochemically by performing different biochemical tests. The 63 bacterial isolates that were obtained from rice rhizoplane showed wide variability regarding different biochemical characteristics. These findings suggest that the rhizobacterial strains isolated from various crops exhibit diverse biochemical characteristics. The ability of the isolates to produce hydrogen sulphide and hydrolyze gelatin may indicate their potential role in nutrient cycling and plant growth promotion.

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**Table 1. Biochemical characterization of test bacterial isolates obtained from rice rhizoplane**

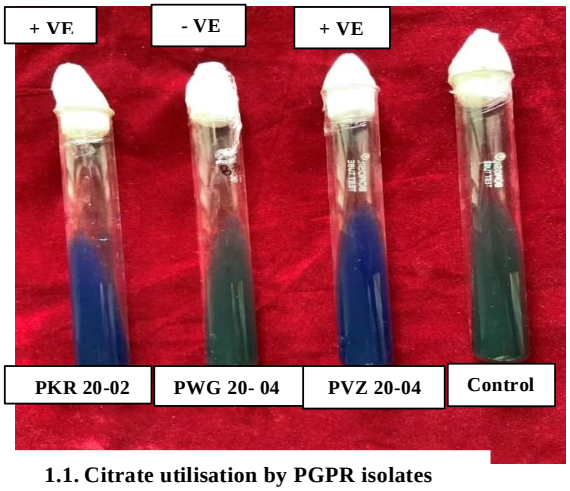
| S. No. | Bacterial Isolate Code | Motility | Catalase Test | Nitrate Reduction | Oxidase Test | Citrate Utilization | Methyl Red Test | Vogues Proskaeur Test | Ammonia Production | Urease Production | Hydrogen sulfide Production | Gelatin Liquefaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | PSK20-03 | + | + | + | + | + | - | + | + | + | - | + |
| 2 | PSK20-04 | + | + | + | + | + | - | + | + | - | - | + |
| 3 | PSK20-05 | + | + | - | + | - | + | - | + | + | - | + |
| 4 | PSK20-08 | + | + | - | + | + | - | + | + | - | - | - |
| 5 | PSK20-09 | + | + | + | + | + | - | + | + | + | - | + |
| 6 | PSK20-10 | + | + | + | - | - | - | + | + | + | - | + |
| 7 | PSK20-11 | + | + | - | + | + | - | + | - | + | + | - |
| 8 | PVP20-01 | + | + | + | + | + | + | - | - | + | - | - |
| 9 | PVP20-07 | + | + | - | + | + | - | - | - | + | - | + |
| 10 | PEG20-01 | + | + | + | - | - | + | - | - | - | - | + |
| 11 | PEG20-06 | + | + | + | + | + | - | + | + | + | - | + |
| 12 | PEG20-07 | + | + | + | - | + | - | + | + | + | - | - |
| 13 | PWG20-02 | + | + | - | + | + | + | - | + | + | + | - |
| 14 | PWG20-04 | + | + | + | + | + | - | - | + | + | - | + |
| 15 | PKR20-01 | + | + | - | + | - | + | - | - | + | - | - |
| 16 | PKR20-02 | + | + | + | + | + | - | + | + | + | - | + |
| 17 | PKR20-03 | + | + | - | + | + | - | + | + | + | - | + |
| 18 | PKR20-05 | + | + | - | + | + | - | + | + | - | - | - |
| 19 | PKR20-06 | + | + | + | + | + | - | + | + | + | - | + |
| 20 | PKR20-08 | + | + | + | + | - | - | - | + | - | - | - |
| 21 | PKR20-09 | + | + | + | + | - | + | - | - | - | + | - |
| 22 | PKR20-10 | + | + | - | + | - | + | - | - | + | + | - |
| 23 | PGN20-01 | + | + | + | + | + | - | + | + | + | - | - |
| 24 | PGN20-03 | + | + | + | + | - | - | + | - | + | - | - |
| 25 | PGN20-04 | + | + | - | + | + | - | + | + | + | - | + |
| 26 | PGN20-05 | + | + | + | + | + | + | - | - | + | - | + |
| 27 | PGN20-06 | + | + | + | + | + | - | + | + | + | - | + |
| 28 | PSK20-01 | + | + | + | - | - | - | + | + | + | - | + |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | PVZ20-03 | + | + | - | + | + | - | + | - | + | - | + |
| 30 | PGN20-07 | + | + | - | + | - | - | + | - | - | + | + |
| 31 | PVZ20-02 | + | + | - | + | + | + | - | - | + | - | - |
| 32 | PSK20-02 | + | + | - | - | + | + | - | + | + | - | + |
| 33 | PSK20-06 | + | + | - | + | - | + | - | + | - | - | + |
| 34 | PSK20-07 | + | + | - | + | - | - | + | + | - | + | - |
| 35 | PVZ20-01 | + | + | - | + | - | + | - | + | - | - | - |
| 36 | PVZ20-04 | + | + | + | + | + | + | + | + | + | - | + |
| 37 | PVZ20-05 | + | + | + | + | + | + | + | + | + | - | + |
| 38 | PVZ20-06 | + | + | + | + | + | - | + | - | + | - | + |
| 39 | PVP20-02 | + | + | + | + | + | + | + | + | + | - | + |
| 40 | PVP20-03 | + | + | + | + | - | + | - | + | + | - | + |
| 41 | PVP20-04 | + | + | + | + | - | + | - | + | + | - | + |
| 42 | PVP20-06 | + | + | + | + | + | - | - | - | + | - | - |
| 43 | PVP20-08 | + | + | + | - | + | - | + | + | + | - | + |
| 44 | PEG20-08 | + | + | - | + | + | - | + | + | - | - | - |
| 45 | PWG20-01 | + | + | - | + | + | + | - | + | + | - | - |
| 46 | PWG20-03 | + | + | + | - | - | - | + | + | + | - | + |
| 47 | PWG20-05 | + | + | + | - | - | - | + | - | + | + | + |
| 48 | PWG20-06 | + | + | - | - | + | - | + | - | + | - | + |
| 49 | PKR20-07 | + | + | - | + | - | + | - | + | + | + | - |
| 50 | PGN20-02 | + | + | + | - | + | - | + | + | + | - | - |
| 51 | PGN20-08 | + | + | + | + | + | - | + | + | + | - | + |
| 52 | PGN20-09 | + | + | + | + | + | - | - | + | + | - | + |
| 53 | PGN20-10 | + | + | - | + | + | - | + | - | - | - | - |
| 54 | PNL20-01 | + | + | + | - | + | - | + | + | + | - | - |
| 55 | PNL20-02 | + | + | + | + | - | - | + | + | + | - | + |
| 56 | PNL20-03 | + | + | + | + | + | - | + | + | - | - | - |
| 57 | PNL20-04 | + | + | + | + | + | - | + | + | - | + | - |
| 58 | PVP20-05 | - | + | - | - | - | + | - | + | - | + | - |
| 59 | PEG20-02 | - | + | + | - | + | - | + | + | - | - | + |
| 60 | PEG20-03 | - | + | - | + | + | + | - | + | - | - | - |
| 61 | PEG20-04 | - | + | - | - | + | - | - | + | - | - | + |
| 62 | PEG20-05 | - | + | + | - | + | - | - | + | - | - | - |
| 63 | PKR20-04 | - | + | - | + | + | - | - | + | - | - | - |

Table 2. Grouping of test bacterial isolates based on biochemical characters

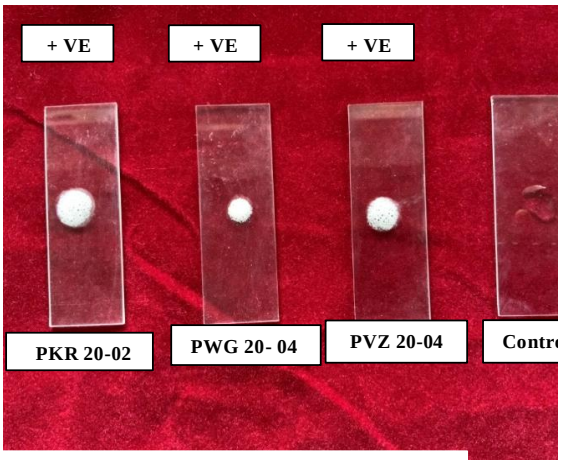
| S. No. | Biochemical Characters | Positive/Negative | Total isolates (%) |
|---|---|---|---|
| 01 | Catalase Test | +ve | 100% |
| | | -ve | - |
| 02 | Nitrate Reduction Test | +ve | 58.73% |
| | | -ve | 41.26% |
| 03 | Oxidase Test | +ve | 76.19% |
| | | -ve | 23.81% |
| 04 | Citrate Utilization Test | +ve | 68.25% |
| | | -ve | 36.50% |
| 05 | Methyl Red Test | +ve | 33.33% |
| | | -ve | 66.66% |
| 06 | Vogues proskaeur Test | +ve | 58.73% |
| | | -ve | 41.26% |
| 07 | Ammonia Production | +ve | 73.01% |
| | | -ve | 26.98% |
| 08 | Urease Test | +ve | 68.25% |
| | | -ve | 31.74% |
| 09 | Hydrogen sulphide production Test | +ve | 15.87% |
| | | -ve | 84.12% |
| 10 | Gelatin Liquefaction Test | +ve | 53.96% |
| | | -ve | 46.03% |

+ve= Positive;
-ve=Negative



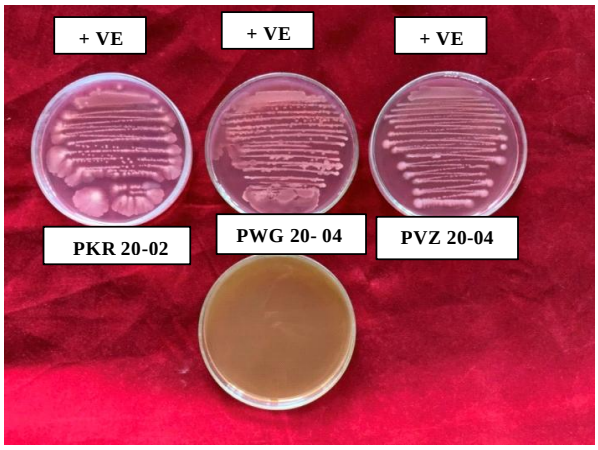

**1.1. Citrate utilisation by PGPR isolates**

Positive reaction indicated by change in colour from green to blue



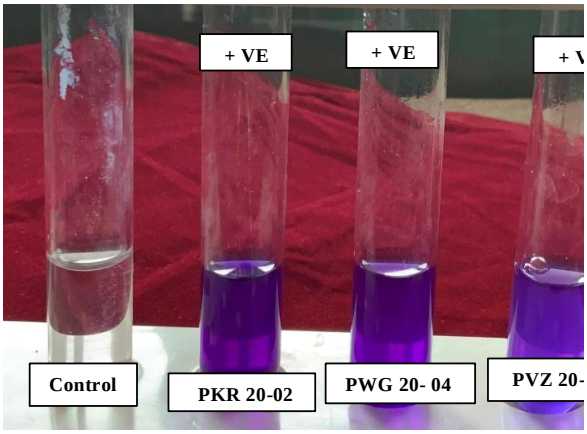

**1.2 Catalase activity by PGPR isolates**

Formation of effervescence indicates positive



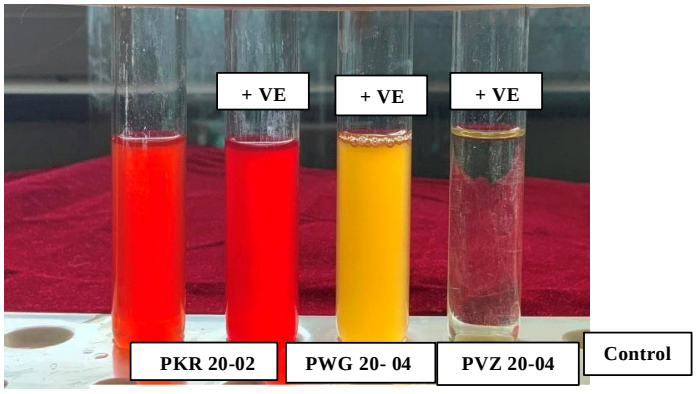

**1.3. Urease activity by PGPR**

Development of pink colour indicates positive



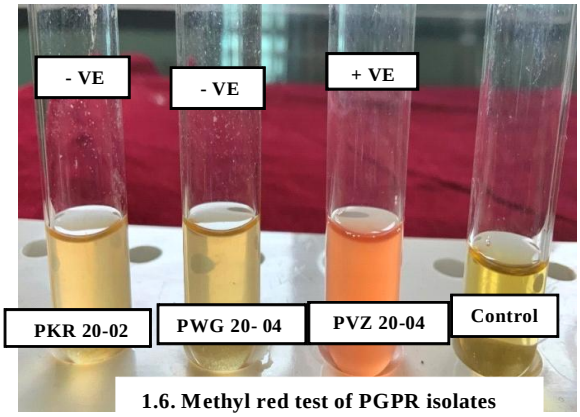

**1.4. Oxidase production by PGPR isolates**

Intense purple colour indicates positive for oxidase



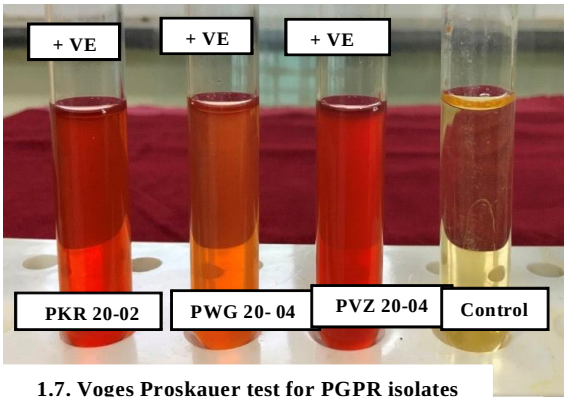

**1.5 Nitrate reduction test of PGPR isolates**

Change in colour to red or orange indicates positive



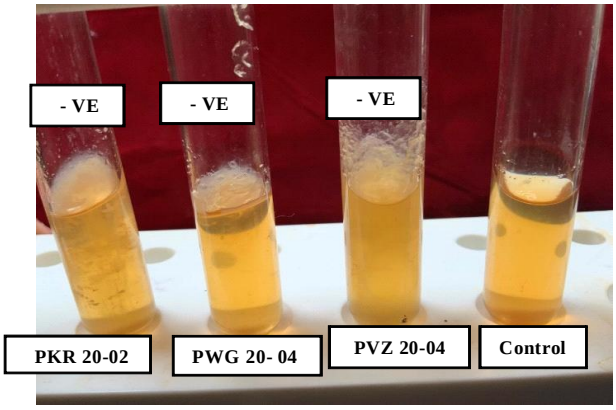

**1.6. Methyl red test of PGPR isolates**

Change of broth to red colour indicates positive



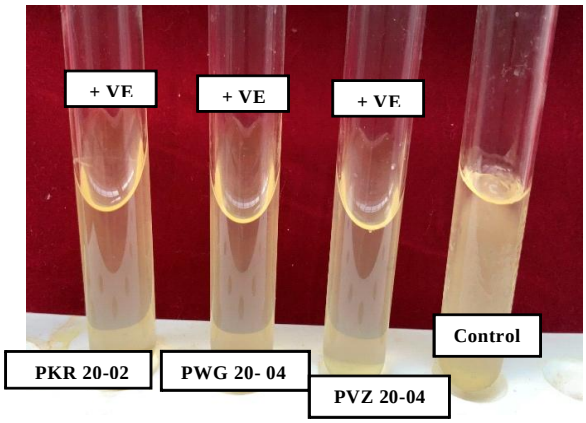

**1.7. Voges Proskauer test for PGPR isolates**

Change in colour to brownish red indicates positive



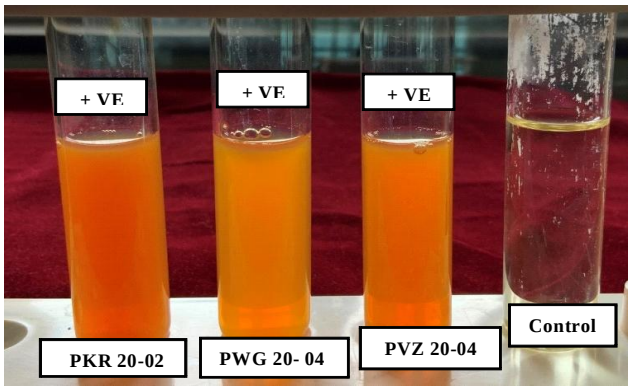

**1.8. $H_2S$ production by PGPR isolates**

visualization of black colour along the line of inoculation i




**1.9. Gelatin liquefaction by PGPR isolates**

Partial or total liquefaction of the inoculated tube at 40C indicates positive




**1.10. Ammonia production by PGPR**

Change in colour to brown indicates positive

**Plate 1. Biochemical characteristics of PGPR isolates**

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