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# Isolation & Biochemical Characterization of Halotolerant Plant Growth Promoting Rhizobacteria

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***Abstract:***
*Rhizobacteria possessing multiple plant growth promoting activities. Halotolerant rhizobacteria were isolated from the groundnut soil sample with salinity 0.7mS/cm in Jodiya region of saurashtra. 48 bacterial isolates were obtained among which 45 isolates showed halotolerancy upto 12% NaCl concentration and different tests were performed to find plant growth promoting traits at 2% NaCl concentration. like NITROGEN FIXATION by bacteria in which all the bacteria has shown us that capacity. By performing the PHOSPHATE SOLUBILIZING TEST 24 isolates were positive. AMMONIA TEST and in IAA PRODUCTION TEST all isolates had given positive results and the maximum indole production was 62.18µg/ml. And in ANTIFUNGAL TEST 4 isolates had given positive result. Further several qualitative enzymatic test are performed like protease, catalase, amylase, cellulase, lipase. After that biochemical tests like MR test, VP test, H2S production test, $H_2O_2$ test, nitrate reduction test, TSI agar test, urea hydrolysis, citrate utilization test were peformed.*

**1. Introduction**

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Geetha et al.,2014). Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them (Teaumroong et al., 2010). Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization. In the rhizosphere, only 1-2% of bacteria promote plant growth. ( Antoun and Kloepper, 2001).

In saline soil the plant cannot grow properly. Due to high concentration of salt the toxicity increases in plant. It also affects the sodium-potassium ion channel pump in plants.Due to high concentration of salt rate of transpiration also decreases indirectly. High salt causes formation of ash of salt due to which there is difficulty in uptake of nutrients & water.

Halotolerant PGPR (plant growth promoting rhizobacteria ) helps to promote plant growth in even saline conditions. Halophiles are extremophile organisms that thrive in environments with very high concentrations of salt. The name comes from Greek word “salt-loving”. Halophiles are distinguished by their requirement of hypersaline conditions for growth. They may be classified according to their salt requirement: slight halophiles grow optimally at 0.2– 0.85 molL21 (2–5%) NaCl; moderate halophiles grow optimally at 0.85–3.4 molL21 (5–20%) NaCl; and extreme halophiles grow optimally above 3.4–5.1 molL21 (20–30%) NaCl. In contrast, nonhalophiles grow optimally at less than 0.2 molL21 NaCl. Halotolerant organisms can grow both in high salinity and in the absence of a high concentration of salts.

We can provide these microorganisms as a biofertilizer and augmentation to the plants for the better crop production even in the saline soil. In Gujarat, ground nut is one of the main crops, accounting for nearly 13.5 per cent of the total value of the agricultural output in the state. Saurashtra being known as the "groundnut bowl" of India.
The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (Biofertilizers), or phytohormone production (Biostimulants). Inoculant development has been most successful to deliver biological control agents of plant disease i.e. organisms capable of killing other organisms pathogenic or disease causing to crops.

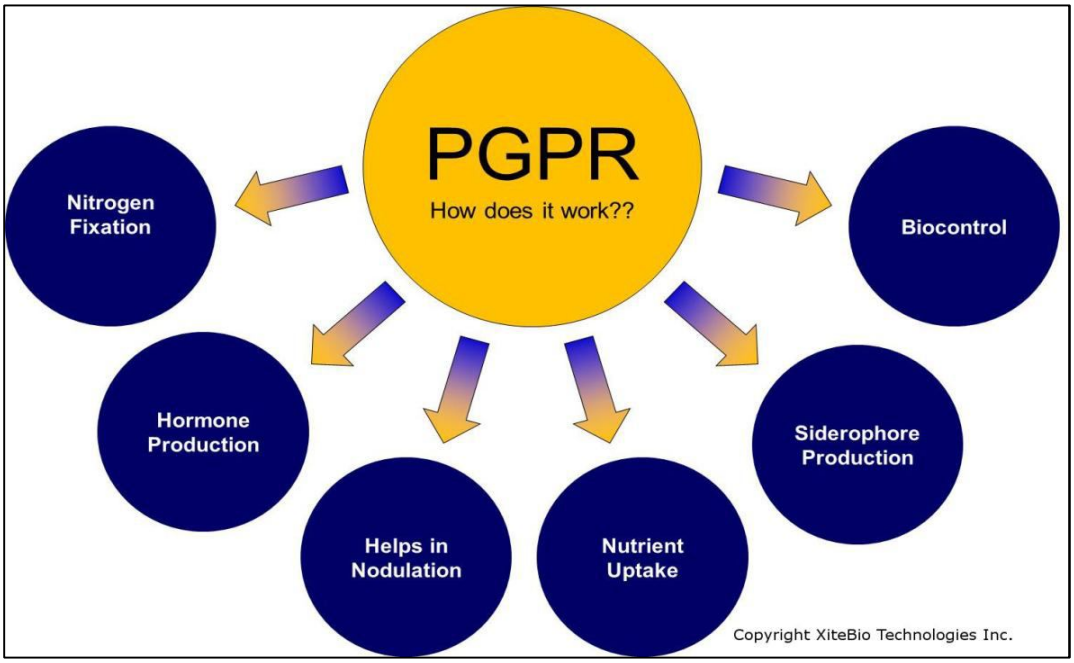


*Figure 1: Application of PGPR*

**2. Materials and Methods**

*2.1. Collection of Soil Samples*
The Soil samples were collected from Rhizosphere from soils of Jodiya,Jamnagar district saurashtra of Gujarat. The soil sample were collected in the polybags and stored at4°C in the Laboratory. Then the soil samples were dried under sunlight and sieved by 2mm sieve to obtain the fine soil.

*2.2 Chemical Analysis*
The soil samples were prepared by soil:water dilution of 1:2 by Dellavella Method, and the salinity of the soil was measured as 0.7mS/cm.

*2.3. Isolation of Bacteria*
The bacteria were isolated by serial dilution method in which 1 g soil was added in 9ml of D/W and diluted from $10^{-1}$ to $10^{-6}$ . From $10^{-4}$,$10^{-5}$& $10^{-6}$ sample was streaked on 2% salt containing N-agar plate and incubated at 37ºC for 24 hrs and further pure isolates were obtained from it.

*2.4. Halotolerancy*
After that to check the halotolerancy of microorganisms, the isolates were grown on different salt concentration such as 4%,6%,8%,10% and 12% which were streaked on N-agar plate & incubated at 37ºC for 24 hrs

*2.5. Preparation Glycerol Stock*
The isolates were cultured in N –broth and incubated at 37ºC for 24 hrs till log phase. 0.5ml of culture from broth was transfer to sterile appendrof under aseptic condition and than 0.5ml glycerol was added which was prepared by adding 48 ml of glycerol in 2 ml distill water and than preserved at 4ºC.

*2.6. Microscopic Observation and Morphological Characterization*
The obtained PGPR isolates were examined for their morphological features. These morphological characteristics like the colour, shape, size, surface and gram staining etc. were recorded.

*2.7. Screening of PGPR for Multiple Plant Growth Promoting Activities*

Further all the 48 isolates were screened for their plant growth promoting activities such as Nitrogen Fixation, HCN Production, Phosphate solubilization, IAA production, Ammonia production, Antifungal activity. Hydrolytic enzyme production are like Catalase, Lipase**,** Cellulase, Protease, Urea hydrolysis, Amylase, Nitrate reductase were performed.

2.7.1. HCN Production

HCN production was checked by method of Bakker and Schipper (1987) on N-agar medium. These isolates were streak on N-agar medium having an extra component that is 4.4 g per liter of glycine. A Whatman no.1 filter paper disc (9 cm in diameter) was soaked in 2% picric acid in 2%sodium carbonate prepared in autoclaved D/W. Soaked disc was placed in the lid of each petriplate. Petriplates were sealed with parafilm& incubate at 37ºC for 7 days. An uninoculated medium with the soaked filter paper was kept as control for comparison of results (Lorck, 1948).

2.7.2. Phosphate Solubilisation

The isolates were streaked on NBRIP (National Botonical Research Institute's Phosphate) agar medium that contains Glucose 10.0 g/l, Tricalcium phosphate 5.0 g/l, Magnesium chloride hexahydrate 5.0 g/l, Magnesium sulphateheptahydrate 0.25 g/l, Potassium chloride 0.2 g/l, Ammonium sulphate 0.1 g/l, 1.5% agar, Distilled water 1000ml, pH 7 and incubate at 37ºC for 14 days. The tricalcium phosphate which is present in medium on acidification gives clear zone around colony (Nautiyal et.al).

2.7.3. IAA Production

Indole Acetic Acid (IAA) production was detected as described by Brick et al. (1991) using Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Here loopful of culture was inoculated in N-broth, Media amended with 50µg/ml tryptophan. 1ml of reagent was added to the aliquot. Sample was incubated for 25 minutes at room temperature. Absorbance was measured at 535 nm. Auxin quantification values were recorded by preparing calibration curve made by using IAA standard in the range of 10-100µg/ml. Standard graph of IAA concentration was plotted against O.D. at 530nm. Development of pink colour indicates IAA production (Gordon and Weber).

2.7.4. Ammonia Production

Bacterial isolates were tested for the production of ammonia as follows: Freshly grown culture were inoculated in 10 ml nutrient broth and incubated at 30°C for 48 h in a rotator shaker after that 0.5 ml of Nessler's reagent was added to each tube. The positive reaction for ammonia production had shown the development of brown to yellow colour.

2.7.5. Nitrogen Fixation

Jensen Medium is recommended for detection and cultivation of nitrogen fixing bacteria. Freshly inoculated overnight grown culture in jensen's broth was pelleted down washed with saline and then spotted on the jensen medium plate and the colonies developed were observed.

2.7.6. Anti-Fungal Activity

Freshly grown PGPR isolates were streaked on four ends of potato dextrose agar the plate and spores of fungal species *Aspergillusniger* were placed in the middle of the plates and incubated at 28°C for 5 days (Noori and Saud, 2012).

**3. Extra Cellular Enzyme Activities**

*3.1. Lipase Production*

Lipase production was detected by using TWEEN 20 agar plates. All the 48 isolates were streaked on TWEEN 20 agar plates at 37°C for 48 hours.Lipase producing isolates had shown the zone of hydrolysis which is considered as positive result (Cappuccino and Sherman, 1992).

*3.2. Pectinase Production*

The screenings of pectinase producing bacteria were detected by using 0.2% CTAB solution on a Pectin agar plates (2% pectin). Zone of hydrolysis is considered as positive test (Cappuccino and Sherman, 1992).

*3.3. Protease Production*

Incubate the test culture on the plate as spot a line and incubate at 37ºC for 24-48 hrs. Observe for a clear zone. Zone is casein solubilization surrounding the growth of organization.

*3.4. Catalase Test*

The isolates were cultured in 10ml N-broth per isolate and kept at 37ºC for overnight and next day 3% hydrogen peroxide was added (3 ml $H_2O_2$ in 100 ml D/W) to observe the bubbles or effervescence which shows the positive result (Schaad NW, 1992).

*3.5. Amylase Test*
The bacterial isolates were inoculated on starch agar plates at 30ºC for 48 hours. After the incubation plates were flooded with iodine solution, kept for a minute and drain the excess iodine. The reaction of iodine with starch display blue color. This blue color fades rapidly. So the colorless zone surrounding colonies indicates the production of amylase(Collins, C. H 1995).

*3.6. Cellulase Test*
All the PGPR isolates were streaked on CMC (Carboxy Methyl Cellulose) agar plates containing (g/l) KH2PO4 1, MgSO4.7H2O 0.5, NaCl 0.5, FeSO4.7H2O 0.01, MnSO4.H2O 0.01, NH4NO3 0.3, CMC 10 and Agar 15. If the pH is not 7 then it is adjusted by 1M NAOH. Incubate the streaked plates 30°C for 5 days. After the incubation, agar medium was flooded with an aqueous solution of Congo red (1% w/v). here the zone of clearance indicates the positive result.

*3.7. Nitrate Reductase*
Isolates were inoculated in PNB(phoshphate nitrate broth) and incubated at 37ºC for 24 hrs and next day 0.5 ml of α- naphthylamine and 0.5ml of sulphanilic acid and development of red colour was observed within 30 seconds.

**4. Biochemical Characterization of Isolated Halotolerants**

*4.1. Methyl Red Test*
Culture was inoculated in GPB (glucose phoshphate broth) at 37°c for 24 hrs. After incubation add about 5-6 drops of methyl red indicator to the medium. Observe for the development of red color.

*4.2. Voges-Proskauer's Test*
Culture was inoculated in GPB broth at 37°C for 24 hrs. After incubation add 0.6ml of α-naphthol and 0.2ml of KOH solution per ml of culture broth (reagent should be added in this order only because of α-naphthol exerts catalytic effects only if added before KOH). Shake well after addition of each reagent and slop the tube to increase the aeration. Read results after 15-60 min.

*4.3. $H_2S$ Production*
Culture was inoculated in N-broth and lead acetate strip was added and incubated it at 37°C for 24 hrs to observe black dot on lead acetate strip.

*4.4. Sugar Test*
Three different sugars were taken- sucrose,glucose and lactose which were added along with GPB broth and culture was added and incubated at 37°C for 24 hrs& next day pink colour was allowed to observe as positive test.

*4.5. Motility Test*
Culture was stabbed in soft agar (3% N-agar, 3g in 100ml D/W) and was allowed for incubation at 37°C for 24 hrs n next day motility was observed.

*4.6. Simon-Citrate Test*
**C**ulture was streaked on simon-citrate slant and allowd to incubated at 37°C for 24 hrs. Next day colour change of slant from green to bluish green or yellow was allow to observe as positive test.

*4.7. Urea Hydrolysis Test*
Culture was inoculated in stuart's broth and allowed to incubate at 37°C for 24 hrs to observe the colour change of broth from yellow to purple red colour.

**5. Result and Discussion**

*5.1. Isolation and Characterization of Bacterial Isolates*
48 isolates have been isolated from the rhizosphere of the groundnut crop of the jodiya region of the Jamnagar district of saurahtra, Gujarat. These isolates were evaluated for their antagonistic and plant growth-promoting traits. Among that 48 isolates gram positive isolates were 22 and the remaining 26 were gram negative isolates.

*5.2. Halotolerancy*
To check the halotolerancy of microorganisms, the isolates were grown on different salt concentration such as 4%,6%,8%,10% and 12% which were streaked on N-agar plate & incubated at 37ºC for 24 hrs. [table 1]

*5.3. Morphological Characteristics and Microscopic Observation of PGPR Isolates.*
The morphological characteristics of 48 isolates were varied widely. All the isolates have been observed under microscope to investigate the characteristics of the pgpr such as shape, size, gram reaction and motily.

*5.4. Plant Growth Promoting Activities of PGPR Isolates*

The isolates showed varied levels of PGPR traits such as Nitrogen fixation, phosphate solubilization, IAA, ammonia, HCN production and Antifungal activity [Table 2].

5.4.1. Phosphate Solubilizing

Twenty four out of fourty eight isolates exerted the ability for phosphate solubilizing on NBRIP medium. The phosphate solubilizing activity characterizes the microorganisms with ability to produce and release metabolites such as organic acids that chelate the cations bound to phosphate, converting them into soluble forms.

5.4.2. IAA Production

Auxin is the most investigated hormone among plant growth regulators. The most common, best characterized and physiologically most active auxin in plant is indole-3-acetic acid (IAA). IAA is known to stimulate both a rapid response (e.g. increased cell elongation) and a longterm response (e.g. cell division and differentiation) in plants**.** In our study, all bacterial isolates were able to produce indole-3-acetic acid (IAA) growing in medium containing tryptophan. Maximum IAA production was recorded as 62.18 $\mu g/ml$.

5.4.3. HCN Production

Ability of cyanide synthesis was observed for isolates. All the isolates have shown the negative results. The increased production of HCN by the efficient strain contributes effective inhibition of mycelial growth of and appears to be a major factor in control of soil-borne disease.

5.4.4. Ammonia Production

The ammonia is useful for plant as directly or indirectly. Ammonia production by the plant growth promoting bacteria helps influence plant growth indirectly. The production of ammonia production was observe and 17 islolates were positive.

5.4.5. Nitrogen Fixation

Jensen Medium is recommended for detection and cultivation of nitrogen fixing bacteria. Freshly inoculated overnight grown culture in jensen's broth was pelleted down washed with saline and then spotted on the jensen medium plate and the colonies developed were observed.

5.4.6. Antifungal activity

The antifungal activity has been observed of the isolates. Out of fourty eight isolates only four isolates have shown the positive results as shown in figure.

**6. Extra Cellular Enzyme Activities**

Here several enzymatic tests were performed such as catalase, protease, lipase, amylase, cellulose, pectinase, nitrogen reductase results are shown in [table 3]

*6.1. Catalase Activity*

Catalase test was performed by taking a 3-4 drops of hydrogen peroxide (H2O2) was added to 48 hour old bacterial colony which is grown on N-broth liquid medium. The effervescence indicated catalase activity (Schaad NW, 1992).

*6.2. Protease Activity*

The qualitative assay for detection of protease production was performed on sterile 1% skimmed milk agar plates. Zone of protein hydrolysis around the colony indicating the enzymatic degradation of protease (Chaiharn 2008).

*6.3. Lipase Activity*

Lipase production was detected by using TWEEN 20 agar plates. Zone of hydrolysis is considered as positive test (Cappuccino and Sherman, 1992).

*6.4. Amylase (Starch Hydrolysis) Activity*

Isolates were streaked on starch agar plate and incubated at 37ºC for 24 hrs and Hence the colour less zone surrounding colonies indicates the production of amylase. (Collins, C. H 1995)

*6.5. Cellulase Activity*

Isolates were streaked on cellulose plate which was prepared by adding CMC(CM cellulose) in N-agar and incubated at 37ºc for 24 hrs and next day 1% congo red was added in plate for 10 min and extra congo red was drain off and then NaOH was flooded in plate for 20 min and after that zone of clearance was observed.

*6.6. Nitrate Reductase*

Isolates were inoculated in PNB (phoshphate nitrate broth) and incubated at 37ºC for 24 hrs and next day 0.5 ml of α- naphthylamine and 0.5ml of sulphanilic acid and development of red colour was observed within 30 seconds

*6.7. Pectinase Production*

Pectinase production was detected by using 0.2% CTAB solution on a Pectin agar plates(2% pectin). Zone of hydrolysis is considered as positive test (Cappuccino and Sherman, 1992).

**7. Biochemical Characterization of Plant Growth Promoting Rhizobacteria**

Biochemical characterization of isolated halotolerants includes following tests:

Methyl red test, Voges-proskauer's test ,$H_2S$ test, Sugar test( Sucrose,Glucose,Lactose), Motility test, Urea hydrolysis, Simmon Citratre test as shown in [figure 1]

| NAME OF ORGANISM | 2% SALT | 4% SALT | 6% SALT | 8% SALT | 10% SALT | 12% SALT | 14% SALT |
|---|---|---|---|---|---|---|---|
| JG1 | ++++ | ++++ | ++++ | ++ | + | + | - |
| JG 2 | ++++ | ++++ | ++++ | +++ | ++ | + | - |
| JG 3 | ++++ | ++++ | ++++ | +++ | ++ | ++ | - |
| JG 4 | ++++ | ++++ | ++++ | +++ | ++ | ++ | - |
| JG 5 | ++++ | ++++ | ++++ | + | + | + | - |
| JG6 | ++++ | ++++ | ++++ | ++ | ++ | + | - |
| JG 7 | ++++ | ++++ | ++++ | ++ | + | + | - |
| JG 8 | ++++ | ++++ | ++++ | + | - | - | - |
| JG 9 | ++++ | ++++ | ++++ | + | + | + | - |
| JG 10 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 11 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 12 | ++++ | ++++ | ++++ | + | + | ++ | - |
| JG 13 | ++++ | ++++ | ++++ | + | ++ | - | - |
| JG 14 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG15 | ++++ | ++++ | ++++ | + | ++ | ++ | - |
| JG 16 | ++++ | ++++ | ++++ | +++ | ++ | ++ | - |
| JG 17 | ++++ | ++++ | ++++ | +++ | ++ | ++ | - |
| JG 18 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 19 | ++++ | ++++ | ++++ | ++ | ++ | + | - |
| JG 20 | ++++ | ++++ | ++++ | + | ++ | ++ | - |
| JG 21 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 22 | ++++ | ++++ | ++++ | + | ++ | ++ | - |
| JG 23 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 24 | ++++ | ++++ | ++++ | ++ | ++ | + | - |
| JG 25 | ++++ | ++++ | ++++ | ++ | ++ | + | - |
| JG 26 | ++++ | ++++ | ++++ | + | ++ | + | - |
| JG27 | ++++ | ++++ | ++++ | + | ++ | ++ | - |
| JG 28 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 29 | ++++ | ++++ | ++++ | + | ++ | ++ | - |
| JG 30 | ++++ | ++++ | ++++ | + | ++ | ++ | - |
| JG 31 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
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| JG 40 | ++++ | ++++ | ++++ | +++ | ++ | + | - |
| JG 41 | ++++ | ++++ | ++++ | +++ | ++ | ++ | - |
| JG 42 | ++++ | ++++ | ++++ | + | + | ++ | - |
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| JG 44 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 45 | ++++ | ++++ | ++++ | +++ | ++ | ++ | - |
| JG 46 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 47 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |
| JG 48 | ++++ | ++++ | ++++ | ++ | ++ | ++ | - |

*Table 1: isolation and screening of halotolerant bacteria*

| Sr.no | Phosphate | $N_2$- fixation | Ammonia | HCN | IAA | Anti-fungal activity |
|---|---|---|---|---|---|---|
| JG1 | - | + | + | - | + | - |
| JG 2 | - | + | - | - | + | - |
| JG 3 | - | + | + | - | + | - |
| JG 4 | - | + | + | - | + | - |
| JG 5 | + | + | - | - | + | - |
| JG6 | + | + | - | - | + | - |
| JG 7 | - | + | + | - | + | - |
| JG 8 | - | + | - | - | + | - |
| JG 9 | - | + | + | - | + | - |
| JG 10 | + | + | - | - | + | - |
| JG 11 | - | + | - | - | + | - |
| JG 12 | - | + | - | - | + | - |
| JG 13 | - | + | - | - | + | - |
| JG 14 | + | + | - | - | + | - |
| JG15 | + | + | - | - | + | - |
| JG 16 | + | + | - | - | + | - |
| JG 17 | - | + | + | - | + | - |
| JG 18 | + | + | + | - | + | - |
| JG 19 | + | + | - | - | + | - |
| JG 20 | + | + | - | - | + | + |
| JG 21 | - | + | + | - | + | - |
| JG 22 | + | + | - | - | + | - |
| JG 23 | + | + | - | - | + | - |
| JG 24 | - | + | - | - | + | - |
| JG 25 | - | + | - | - | + | - |
| JG 26 | - | + | + | - | + | - |
| JG27 | + | + | + | - | + | - |
| JG 28 | + | + | - | - | + | - |
| JG 29 | + | + | + | - | + | - |
| JG 30 | + | + | + | - | + | + |
| JG 31 | - | + | - | - | + | + |
| JG 32 | - | + | - | - | + | - |
| JG 33 | + | + | - | - | + | - |
| JG 34 | + | + | + | - | + | - |
| JG 35 | - | + | + | - | + | - |
| JG 36 | - | + | + | - | + | - |
| JG 37 | + | + | - | - | + | - |
| JG 38 | - | + | - | - | + | - |
| JG 39 | + | + | - | - | + | - |
| JG 40 | + | + | + | - | + | + |
| JG 41 | + | + | - | - | + | - |
| JG 42 | + | + | - | - | + | - |
| JG 43 | + | + | - | - | + | - |
| JG 44 | - | + | + | - | + | - |
| JG 45 | - | + | - | - | + | - |
| JG 46 | + | + | - | - | + | - |
| JG 47 | - | + | - | - | + | - |
| JG 48 | - | + | - | - | + | - |

*Table 2: Screening of PGPR by analysing their Plant Growth Promoting Traits*

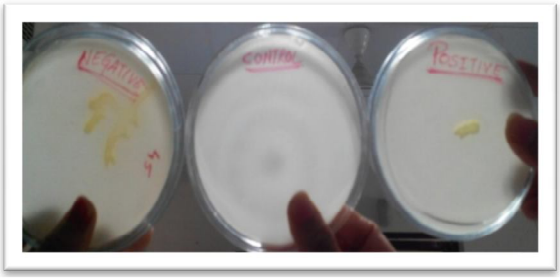


*Figure 2: Phosphate Solubilising Test*

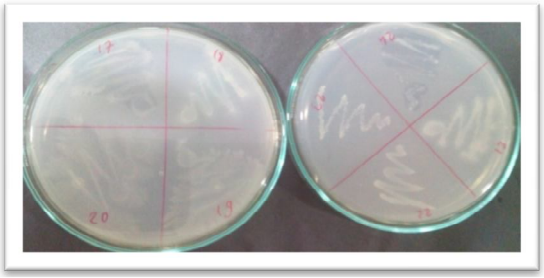

*Figure 3: Nitrogen Fixation*

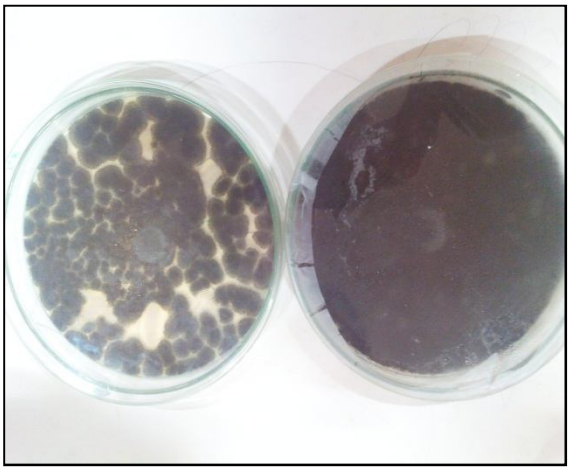

*Figure 4: Anti Fungal Activity*

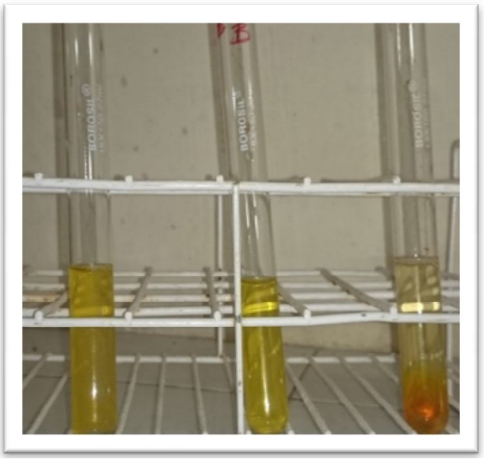

*Figure 5: Ammonia Test*

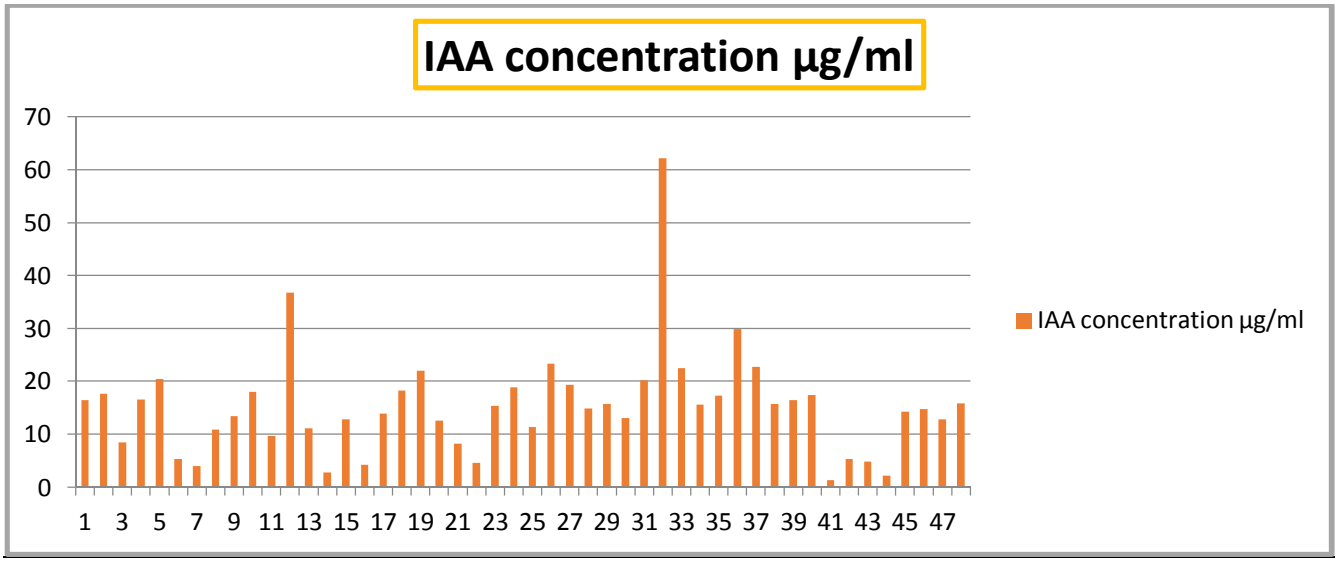


*Figure 6: Concentration Graph Of IAA*

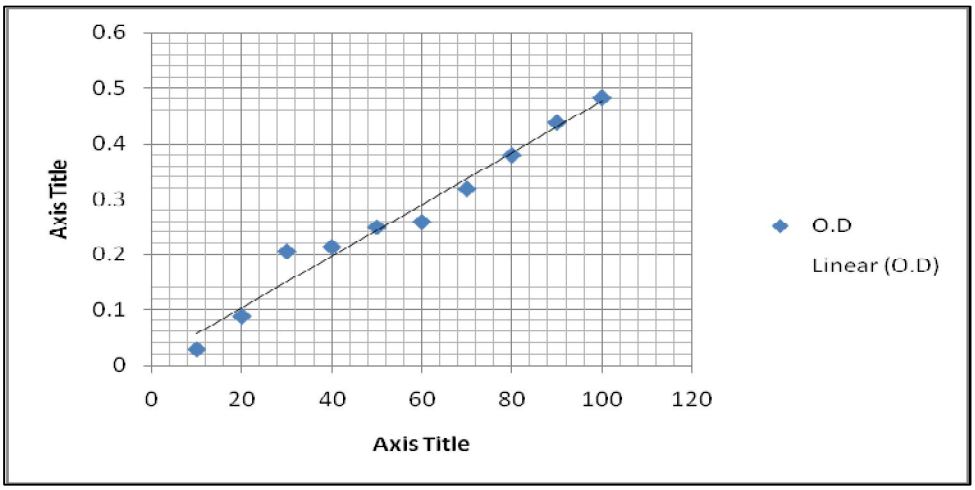


*Figure 7: Std. graph of IAA*

| Sr.no | Catalase | Amylase | Protease | Cellulose | Lipase |
|---|---|---|---|---|---|
| JP1 | + | + | + | + | - |
| JP 2 | + | + | + | + | - |
| JP3 | + | + | - | + | - |
| JP4 | + | + | + | - | - |
| JP5 | + | + | + | - | - |
| JP6 | + | - | + | - | - |
| JP7 | + | + | + | + | - |
| JP8 | + | + | + | + | - |
| JP9 | + | + | - | + | - |
| JP10 | + | - | + | - | - |
| JP11 | + | + | + | + | - |
| JP12 | + | + | - | + | - |
| JP13 | - | + | - | + | - |
| JP14 | + | + | - | + | - |
| JP15 | + | + | + | + | - |
| JP16 | + | + | + | + | - |
| JP17 | + | + | + | + | - |
| JP18 | + | + | + | + | - |
| JP19 | + | + | + | + | - |
| JP20 | + | + | + | + | - |
| JP21 | + | - | - | + | - |
| JP22 | + | + | + | + | - |
| JP23 | + | + | + | + | - |
| JP24 | + | + | + | + | - |
| JP25 | + | + | + | + | - |
| JP26 | + | + | - | + | - |
| JP27 | + | + | - | + | - |
| JP28 | + | + | - | + | - |
| JP29 | + | - | + | - | - |
| JP30 | + | + | - | + | - |
| JP31 | + | + | + | + | - |
| JP32 | + | + | + | + | - |
| JP33 | + | + | + | + | - |
| JP34 | + | + | + | - | - |
| JP35 | + | + | + | - | - |
| JP36 | + | + | + | + | - |
| JP37 | + | + | + | + | - |
| JP38 | + | + | - | - | + |
| JP39 | - | + | + | + | - |
| JP40 | + | + | + | + | - |
| JP41 | + | - | + | + | - |
| JP42 | + | + | + | - | - |
| JP43 | + | + | + | + | - |
| JP44 | + | + | + | - | - |
| JP45 | + | + | - | - | - |
| JP46 | + | + | - | + | - |
| JP47 | + | + | + | + | - |
| JP48 | + | + | + | + | - |

*Table 3: Primary screening of enzymes*

| Sr.no | MR | VP | $H_2S$ | Glu | Suc | Lac | $N_2$ reduction | Motility | Simmon citrate test |
|---|---|---|---|---|---|---|---|---|---|
| JG1 | - | + | - | + | + | + | - | + | - |
| JG 2 | - | + | - | + | + | + | + | + | + |
| JG 3 | - | + | - | + | + | + | + | + | + |
| JG 4 | - | + | - | + | + | + | + | - | - |
| JG 5 | - | + | - | + | + | + | + | + | - |
| JG 6 | - | + | - | + | + | + | + | + | - |
| JG 7 | + | - | - | + | + | + | + | + | + |
| JG 8 | + | - | - | + | + | + | + | + | - |
| JG 9 | + | - | + | + | + | + | + | + | - |
| JG 10 | + | - | - | + | + | + | + | + | - |
| JG 11 | - | + | - | + | + | + | + | + | + |
| JG 12 | - | + | - | + | + | + | + | + | - |
| JG 13 | - | + | - | + | - | + | - | + | - |
| JG 14 | - | + | - | + | + | + | + | + | - |
| JG 15 | + | - | + | + | + | + | + | + | - |
| JG 16 | + | - | + | + | + | + | + | + | - |
| JG 17 | + | - | - | - | + | + | + | + | - |
| JG 18 | + | - | - | + | + | + | - | + | - |
| JG 19 | + | - | - | + | + | + | + | + | - |
| JG20 | + | - | + | + | - | + | - | + | - |
| JG 21 | - | + | + | + | + | + | + | + | - |
| JG 22 | - | + | - | + | + | + | + | + | - |
| JG 23 | + | - | - | + | + | + | + | + | + |
| JG 24 | + | - | - | + | + | + | + | + | - |
| JG 25 | - | + | - | + | + | + | + | - | + |
| JG 26 | - | + | - | + | + | + | + | + | + |
| JG 27 | - | + | + | + | - | + | + | + | + |
| JG 28 | - | + | - | + | + | + | + | + | + |
| JG 29 | - | + | - | + | + | + | + | + | - |
| JG 30 | - | + | - | + | - | + | + | + | + |
| JG 31 | - | + | - | + | - | + | + | + | - |
| JG 32 | - | + | - | + | + | + | - | + | - |
| JG 33 | - | + | + | + | + | + | + | + | + |
| JG 34 | - | + | - | + | + | + | + | + | - |
| JG 35 | - | + | - | + | + | + | + | + | - |
| JG 36 | - | + | - | + | + | + | + | + | + |
| JG 37 | - | + | - | + | + | + | - | + | - |
| JG 38 | + | - | - | + | + | + | + | + | + |
| JG 39 | + | - | - | + | + | + | + | + | + |
| JG 40 | - | + | - | + | + | + | + | + | + |
| JG 41 | - | + | - | + | + | + | + | + | - |
| JG 42 | + | - | - | + | + | + | + | + | - |
| JG 43 | - | + | - | + | + | + | + | + | + |
| JG 44 | + | - | - | + | + | + | - | + | - |
| JG 45 | + | - | - | + | + | + | + | + | + |
| JG 46 | + | - | - | + | + | + | + | + | - |
| JG 47 | + | - | - | + | + | + | + | + | - |
| JG 48 | + | - | - | + | + | + | + | + | - |

*Table 4:-Biochemical characterization of isolated halotolents*

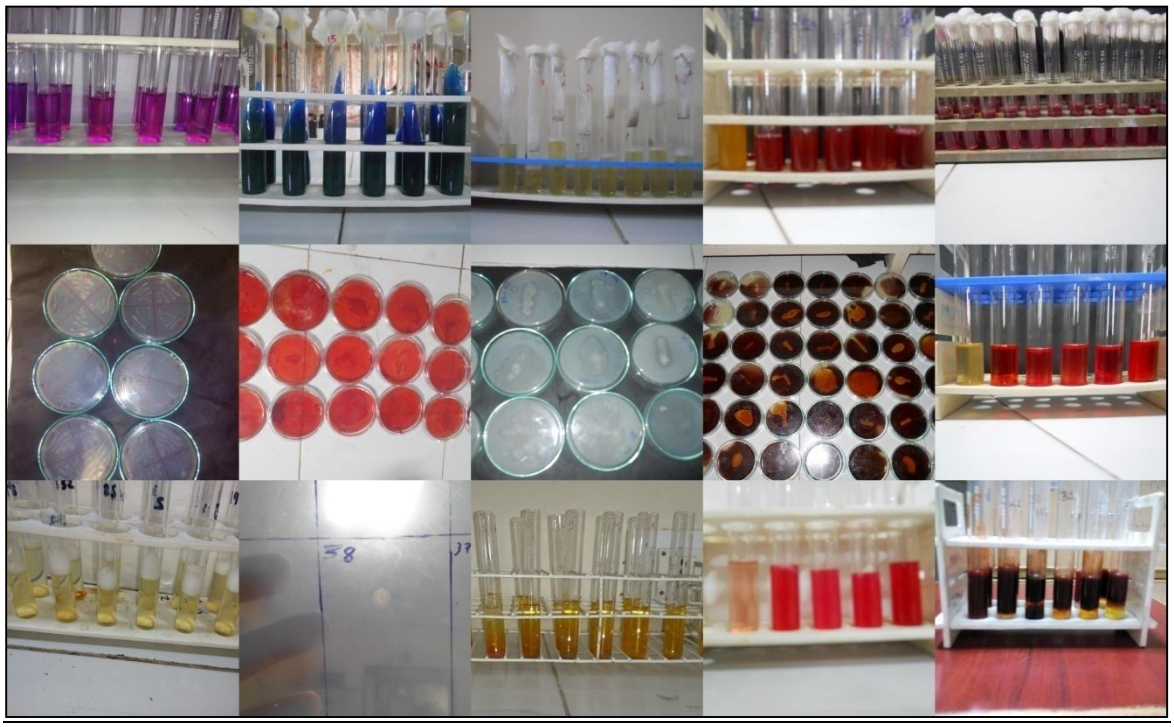

*Figure 8: All Biochemical Positive Test Result*

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