Screening of Groundnut Plant Associated Rhizobacteria for Multiple Plant Beneficial Plant Growth Promoting Traits

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Abstract

Objective: The plant growth-promoting rhizobacteria are promising, sustainable approach for the world present food and environmental crisis. This research was devised to screen the plant growth-promoting rhizobacterial isolates in context to search for a candidate of potent bioinoculant formulation for enhanced sustainable productivity.

Methods: Previously isolated 50 different bacterial morphotypes were screened for their plant growth promoting traits of phosphate solubilization, auxin hormone production, ammonia production, HCN, chitinase production, antagonistic activity against fungal pathogens Sclerotium rolfsii and Aspergillus niger and for seed germination assay.

Results and conclusion: Among 50 selected isolates, 58.00% of the isolates were phosphate solubilizers where the isolates GSH 1 and GSB 13 were the most promising, 54% exhibited the production of auxin hormone where the three isolates GST 3, GSB 13 and GSH 1 were the most efficient. 70% isolates exhibited the production of ammonia among the strains GSL 4 and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme chitinase producer. 5 (10%) isolates exhibited the antagonistic activity for fungal pathogens Sclerotium rolfsii and Aspergillus niger, among two isolates GSB 5 (47.96% and 30.67%) and GSB 2 (45.41% and 23.85%) showed the maximum inhibitory effect against both fungal pathogens. 10% of the rhizobacterial isolates showed a significant enhancement of the seed germination rate. The study showed that the groundnut plant associated rhizobacterial isolates possessed the PGPR traits and thereby can be exploited to be used as biofertilizers.

Keywords: Multitrat Pgpr; Phosphate solubilisation; Auxin production; Ammonia production; HCN production; Antagonistic activity; Seed germination

Introduction

Recently, the biological approaches for improving crop production make the use of rhizosphere microorganisms, particularly the plant growth promoting microorganisms gaining strong approach. In this context, there is an ongoing rigorous research worldwide with greater impetus to explore a wide range of novel rhizobacterial strains possessing novel traits like biological control of phytopathogens and insects [1-3], phytohormone production [4], siderophores production [5], 1-aminocyclopropane-1-carboxylate, hydrogen cyanide (HCN), ammonia production, nitrogenase activity [6] and phosphate solubilization [4]. The bacterial population colonizing the rhizosphere and bringing the enhancement of plant growth are termed as "plant growth promoting rhizobacteria" (PGPR) [7]. Majority of credible group of PGPR were found to belonging to the genera: Acinetobacter, Agrobacterium, Arthobacter, Azotobacter, Azospirillum, Burkholderia, Bradyrhizobium, Rhizobium, Frankia, Serratia, Thioacillus, Pseudomonas, and Bacillus [8,9], Azospirillum, Azotobacter, Burkholderia, Herbaspirillum, Bacillus and Paenibacillus [10-13]. Enhanced plant growth and yield among various crops employing the PGPR have been reported under various study such as in rice [14], sunflower [15], maize [16], and wheat [17]. The multiplicity of climatic and environmental conditions cause disparity in the potentiality of PGPR- based biofertilizers [18]. To overcome these problems the selection of PGPR with multiple plant growth-promoting attributes or co-inoculation of efficient microorganisms having different plant growth promoting attributes proved to be more significant for increasing the growth and yield [19,20]. Thus keeping the above problem in view we have made an attempt to explore groundnut rhizobacteria with multiple PGPR traits for novel bio-inoculant production.

Materials and Methods

A total of 50 rhizobacterial isolates were selected to screen for their plant growth promoting traits of phosphate solubilization, auxin hormone production, ammonia production, HCN production, antagonistic activity and for seed germination assay.

Screening for plant growth-promoting attributes

Phosphate solubilization: Actively dividing test isolates was screened to solubilize phosphate by employing Pikovskaya's (PVK) agar medium [21] without and with bromophenol blue dye (0.01-0.001 mg/l) [22]. Qualitative phosphate solubilizing efficiency (% SE) was calculated using the following formula [23]:

\[ \text{Solubilizing efficiency} = \frac{Z-C}{C} \times 100 \]

where  
\[ Z = \text{Total solubilization diameter (cm)} \]
\[ C = \text{Colonies diameter (cm)} \]

Auxin hormone production: Isolates were screened for their qualitative and quantitative ability to produce the auxin hormone by adopting the method of Loper and Scooth, [24]. All isolates were grown

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in nutrient broth medium supplemented with tryptophan (0.1%), at 28 ± 2°C in incubator shaker under dark condition and measurement was done continuously for 10 days with 2 days interval. Absorbance (OD) of resultant color was recorded at 530 nm and the concentration (µg ml⁻¹ over) of auxin hormone was estimated by preparing calibration curve of Indole-3-acetic acid. The most efficient auxin hormone producers were further analyzed for the type of auxin hormone produced employing HPLC technique. HPLC analysis for the amount and presence of Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) types of indolic auxin was done at Department of Chemistry, National Facility for Drug Discovery Centre, Saurashtra University, Rajkot, and Gujarat, India.

Ammonia production: The bacterial isolates were screened for the production of ammonia, employing the method described by Cappuccino and Sherman, [25]. Actively growing culture inoculated in 10 ml peptone broth and incubated at 28 ± 2°C for 48 hours in incubator shaker (120 rpm). 0.5 ml of Nessler’s reagent was added in each culture tube, development of faint yellow to dark brown color indicated the production of ammonia.

Hydrogen cyanide production: The actively growing inoculum of isolates was streaked on NA medium amended with 4.4 g l⁻¹ glycine (Hi-media) [26]. A Whatman no. 1 filter paper impregnated with 2% sodium carbonate in 0.5% picric acid solution was placed inside the lid of inoculated plates and sealed with parafilm. Plates were incubated for 4 days at 28+2°C. Colour change in the filter paper from yellow to light yellow, dark brown and reddish brown indicated as weak, moderate and strong cyanogenic potential, respectively. Plates without bacterial inoculation were maintained as control.

Production of enzyme chitinases: Colloidal chitin was prepared from chitin adopting the method of Berger and Reynolds, [27]. Actively growing inoculums of the test isolates were spot inoculated in nutrient agar medium amended with 0.2% colloidal chitin and incubated at 30°C temperature for 72 to 96 hours. Observation was made for a clearing zone of the medium around the bacterial growth indicating the utilization of colloidal chitin.

Antagonistic activity against phytopathogens

Antagonistic activity against two groundnut fungal pathogens 'Sclerotium rolfsii' and 'Aspergillus niger' were determined employing the dual culture plate method on Sabouraud dextrose agar (SDA) medium [28]. Groundnut crop disease fungal pathogen 'Sclerotium rolfsii' and 'Aspergillus niger' were collected from 'Groundnut Research Institute' Junaghar, Gujarat, at its Plant Pathology Department. The pathogenic isolates Sclerotium rolfsii and Aspergillus niger were grown on potato dextrose agar medium at 28 ± 2°C. The bacterial isolates were streaked near the periphery on SDA agar plates and the agar discs of about 6 mm diameter with full mycelium growth of fungal pathogens were spot inoculated at perpendicular to the bacterial streak on the centre of the same plate. The media plates with spot inoculation of fungal pathogen and without bacterial inoculation kept as control. All the plates in triplicates were incubated at 28 ± 2°C for up to 7 days. Inhibition of the fungal growth by bacterial antagonistic activity was calculated using the following formula:

\[
\text{Inhibition} = \left( \frac{\text{Control} - \text{Treatment}}{\text{Control}} \right) \times 100
\]

Seed germination bioassay

Healthy groundnut seeds of variety G-20 were surface sterilized and inoculated with 50 ml of actively growing culture of each isolates and incubated at 37°C for 24 hour, along with un-inoculated media as control. Inoculated seeds were placed in 90 mm sterilized petri dishes containing wet cotton and incubated at 37°C for 7 days at 28 ± 2°C. 3 ml distilled water was sprinkled to each petri dishes every day so as to maintain the sufficient moisture for germination. After 7 days germinated seeds were counted, average lengths of radical and plumule of each treatment were also measured and the germination rate and vigor index was calculated by applying the following formula:

Germination rate (%) = (number of seeds germinated/total number of seeds) × 100

Vigour index=% germination × total plant length (mean of plumule+radicle lengths) [29].

Whole experiment was carried out in triplicate, and the result was compared with control.

Statistical analysis

Results of the measurements were subjected to analysis of variance using window SPSS 16.0, 2007 software. Mean, standard error, standard deviation and significance were calculated by comparing the mean by applying one sample t-test and Kruskal Wallis test.

Result and Discussion

Results obtained for screening of PGPR activities of 50 selected isolates presented in Table 1.

Phosphate solubilization

As per the results recorded in Table 1, the 58% (29) of the selected isolates were phosphate solubilizers where the two isolates GSH 1 and GSB 13 were exhibited the highest qualitative efficiency (289.26% and 243.36%). A significant potential to solubilize the phosphate, indicated from production of a large, clear and yellow colored halo zone of phosphate solubilization on PVK agar.
Figure 2: HCN production by the isolates: (a) GSD 67, (b) GSH 8, (c) GSB 5, (d) GSH 1, (e) GSB 13, (f) GSB 2 and (g) Control.

Figure 3: Antifungal activity of isolate against A. niger (a to e): (a) GSH 8, (b) GSB 5, (c) GSH 1, (d) GSB 13, (e) GSB 2 and against S. rolfsii (f to k): (f) GSH 8, (g) GSB 5, (h) GSB 13, (i) GSH 1, (j) GSB 2, (k) Control.
Isolates | Phosphate solubilization | Auxin | Ammonia | HCN | Isolates | Phosphate solubilization | Auxin | Ammonia | HCN
--- | --- | --- | --- | --- | --- | --- | --- | --- | ---
GSS 1 | - | - | - | - | GSL 4 | - | - | - | -
GSS 9 | - | - | - | - | GSL 5 | - | - | - | -
GSS 13 | - | - | - | - | GSL 7 | - | - | - | -
GSS 20 | - | - | - | - | GSL 11 | - | - | - | -
GSS 25 | - | - | - | - | GSH 8 | - | - | - | ++
GST 5 | - | - | - | - | GSB 3 | - | - | - | -
GST 7 | - | - | - | - | GSA 24 | - | - | - | -
GST 11 | - | - | - | - | GSH 18 | - | - | - | -
GST 14 | - | - | - | - | GSB 5 | - | - | - | +++
GST 19 | - | - | - | - | GST 3 | - | - | - | -
GSV 1 | - | - | - | - | GSB 13 | - | - | - | ++
GSV 2 | - | - | - | - | GSO 1 | - | - | - | -
GST 6 | - | - | - | - | GSO 3 | - | - | - | -
GSV 9 | - | - | - | - | GSH 1 | - | - | - | -
GSA 10 | - | - | - | - | GSP 5 | - | - | - | -
GSA 19 | - | - | - | - | GSH 3 | - | - | - | -
GSA 25 | - | - | - | - | GSB 14 | - | - | - | -
GSA 28 | - | - | - | - | GSV | - | - | - | -
GSA 29 | - | - | - | - | GSA 26 | - | - | - | -
GSP 4 | - | - | - | - | GSB 2 | - | - | - | +++
GSP 7 | - | - | - | - | GSD 2 | - | - | - | -
GSP 18 | - | - | - | - | GSV 4 | - | - | - | -
GSD 67 | - | - | - | - | GSV 3 | - | - | - | -
GSH 17 | - | - | - | - | GSL 12 | - | - | - | -
GSI 6 | - | - | - | - | GSL 1 | - | - | - | -

Note: + Positive reaction, -Negative reaction, +Weak HCN production, ++moderate HCN production, +++stronger HCN production.

Table 1: Plant growth promoting characteristics of rhizobacterial isolates.

<table>
<thead>
<tr>
<th>Auxin conc. (µg/ml) in days</th>
<th>Isolates</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSS 25</td>
<td>1.26 ± 0.02</td>
<td>2.30 ± 0.04</td>
<td>3.13 ± 0.11</td>
<td>3.0 ± 0.04</td>
<td>3.39 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>GSS 13</td>
<td>0.67 ± 0.03</td>
<td>1.10 ± 0.03</td>
<td>1.04 ± 0.003</td>
<td>1.01 ± 0.003</td>
<td>0.935 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>GSV 1</td>
<td>0.83 ± 0.02</td>
<td>0.95 ± 0.01</td>
<td>1.08 ± 0.012</td>
<td>1.04 ± 0.03</td>
<td>0.34 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>GST 6</td>
<td>0.80 ± 0.005</td>
<td>1.32 ± 0.010</td>
<td>2.57 ± 0.15</td>
<td>2.63 ± 0.03</td>
<td>0.27 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>GSA 10</td>
<td>0.60 ± 0.01</td>
<td>0.67 ± 0.02</td>
<td>0.83 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSA 19</td>
<td>0.57 ± 0.01</td>
<td>0.80 ± 0.011</td>
<td>1.01 ± 0.01</td>
<td>0.87 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>GSA 25</td>
<td>0.60 ± 0.04</td>
<td>0.83 ± 0.04</td>
<td>0.94 ± 0.03</td>
<td>0.88 ± 0.03</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSP 7</td>
<td>1.91± 0.03</td>
<td>2.27 ± 0.03</td>
<td>2.80 ± 0.010</td>
<td>3.23 ± 0.01</td>
<td>1.47 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>GSP 18</td>
<td>0.43 ± 0.04</td>
<td>0.66 ± 0.02</td>
<td>0.90 ± 0.04</td>
<td>0.73 ± 0.02</td>
<td>0.07 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSD 67</td>
<td>0.58 ± 0.03</td>
<td>0.74 ± 0.014</td>
<td>1.08 ± 0.02</td>
<td>0.67 ± 0.01</td>
<td>0.14 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>GSI 3</td>
<td>0.53 ± 0.02</td>
<td>0.78 ± 0.01</td>
<td>1.01 ± 0.013</td>
<td>1.15 ± 0.003</td>
<td>0.30 ± 0.014</td>
<td></td>
</tr>
<tr>
<td>GSI 6</td>
<td>0.83 ± 0.002</td>
<td>0.95 ± 0.014</td>
<td>1.10 ± 0.011</td>
<td>0.94 ± 0.003</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSI 6</td>
<td>0.83 ± 0.002</td>
<td>0.95 ± 0.014</td>
<td>1.10 ± 0.011</td>
<td>0.94 ± 0.003</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSI 6</td>
<td>0.83 ± 0.002</td>
<td>0.95 ± 0.014</td>
<td>1.10 ± 0.011</td>
<td>0.94 ± 0.003</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSI 6</td>
<td>0.83 ± 0.002</td>
<td>0.95 ± 0.014</td>
<td>1.10 ± 0.011</td>
<td>0.94 ± 0.003</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSI 6</td>
<td>0.83 ± 0.002</td>
<td>0.95 ± 0.014</td>
<td>1.10 ± 0.011</td>
<td>0.94 ± 0.003</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSI 6</td>
<td>0.83 ± 0.002</td>
<td>0.95 ± 0.014</td>
<td>1.10 ± 0.011</td>
<td>0.94 ± 0.003</td>
<td>0.20 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>GSA 10</td>
<td>0.23 ± 0.02</td>
<td>0.53 ± 0.01</td>
<td>1.66 ± 0.003</td>
<td>0.90 ± 0.013</td>
<td>0.29 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>GSO 1</td>
<td>0.57 ± 0.01</td>
<td>0.74 ± 0.014</td>
<td>1.10 ± 0.005</td>
<td>0.90 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>GSO 3</td>
<td>0.80 ± 0.013</td>
<td>1.38 ± 0.02</td>
<td>1.66 ± 0.02</td>
<td>0.90 ± 0.024</td>
<td>0.23 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>GSH 1</td>
<td>2.26 ± 0.002</td>
<td>3.81 ± 0.042</td>
<td>4.81 ± 0.05</td>
<td>3.31 ± 0.04</td>
<td>0.90 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>GSP 9</td>
<td>0.57 ± 0.011</td>
<td>0.80 ± 0.003</td>
<td>0.94 ± 0.01</td>
<td>0.53 ± 0.0042</td>
<td>0.22 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>GSV 1</td>
<td>0.46 ± 0.01</td>
<td>0.58 ± 0.003</td>
<td>0.60 ± 0.004</td>
<td>0.23 ± 0.004</td>
<td>0.09 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>GSB 2</td>
<td>2.91 ± 0.014</td>
<td>3.86 ± 0.01</td>
<td>4.32 ± 0.02</td>
<td>4.86 ± 0.101</td>
<td>2.42 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>GSV 4</td>
<td>1.17 ± 0.033</td>
<td>1.63 ± 0.04</td>
<td>2.57 ± 0.10</td>
<td>0.71 ± 0.01</td>
<td>0.1 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>GSV 3</td>
<td>0.16 ± 0.01</td>
<td>0.46 ± 0.012</td>
<td>4.00 ± 0.14</td>
<td>5.16 ± 0.05</td>
<td>0.50 ± 0.011</td>
<td></td>
</tr>
</tbody>
</table>

Over all
incubation at 28 ± 2°C. rhizobacterial isolates in tryptophan supplemented medium after 72 hrs of...

Table 3: HPLC analysis for the production of indolic auxin by three efficient and shoot length after 7 days of incubation.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>GST 3</th>
<th>GSB 13</th>
<th>GSH 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>3.85</td>
<td>10.5</td>
<td>5.32</td>
</tr>
<tr>
<td>IBA</td>
<td>6.68</td>
<td>30.6</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>Mean</td>
<td>5.25</td>
<td>7.08</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.01</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>SE (Mean ±)</td>
<td>1.42</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>p at 95% CI</td>
<td>0.167</td>
<td>0.364</td>
</tr>
</tbody>
</table>

Table 3: HPLC analysis for the production of indolic auxin by three efficient rhizobacterial isolates in tryptophan supplemented medium after 72 hrs of incubation at 28 ± 2°C.

<table>
<thead>
<tr>
<th>Fungal pathogen</th>
<th>A. niger</th>
<th>S. rolfsii</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH 8</td>
<td>21.94 ± 0.36</td>
<td>18.2 ± 0.80</td>
</tr>
<tr>
<td>GSB 5</td>
<td>47.96 ± 0.00</td>
<td>30.67 ± 3.21</td>
</tr>
<tr>
<td>GSB 13</td>
<td>43.37 ± 0.36</td>
<td>25.0 ± 2.41</td>
</tr>
<tr>
<td>GSH 1</td>
<td>30.6 ± 1.44</td>
<td>15.91 ± 0.00</td>
</tr>
<tr>
<td>GSB 2</td>
<td>45.41 ± 0.36</td>
<td>23.85 ± 4.82</td>
</tr>
<tr>
<td>Overall</td>
<td>Mean ± SE</td>
<td>37.86 ± 4.05</td>
</tr>
</tbody>
</table>

Table 4: Effect of five antifungal isolates on the growth of two groundnut pathogenic fungus: A. niger and S. rolfsii in terms of growth inhibition %.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination rate (%)</th>
<th>Radical length (cm)</th>
<th>Plumule length (cm)</th>
<th>Vigor index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63.2</td>
<td>0.57 ± 0.07</td>
<td>0</td>
<td>35.8</td>
</tr>
<tr>
<td>GSP 4</td>
<td>83.2</td>
<td>0.9 ± 0.06</td>
<td>1.72 ± 0.02</td>
<td>219.08</td>
</tr>
<tr>
<td>GST 3</td>
<td>93.2</td>
<td>2.17 ± 0.07</td>
<td>1.62 ± 0.09</td>
<td>354.16</td>
</tr>
<tr>
<td>GSB 13</td>
<td>96.7</td>
<td>2.06 ± 0.07</td>
<td>1.7 ± 0.06</td>
<td>415.81</td>
</tr>
<tr>
<td>GSH 1</td>
<td>96.7</td>
<td>3.57 ± 0.11</td>
<td>2.22 ± 0.09</td>
<td>560.86</td>
</tr>
<tr>
<td>GSV 3</td>
<td>76</td>
<td>1.7 ± 0.07</td>
<td>1.02 ± 0.19</td>
<td>207.72</td>
</tr>
<tr>
<td>Overall</td>
<td>Mean ± SE</td>
<td>84.82 ± 1.92</td>
<td>1.38 ± 0.38</td>
<td>298.91</td>
</tr>
</tbody>
</table>

| SD        | 13.42                | 1.11                 | 0.78                | 183.97      |
| SE (± mean)| 5.48                 | 0.44                 | 0.32                | 75.1        |
| Sig. at 95% CI | 0.955              | 1                    | 1                   | 1           |

Table 5: Effect of efficient PGPR effect on groundnut seeds germination rate, root and shoot length after 7 days of incubation.

Auxin hormone production

54% (27) of the isolates exhibited the production of auxin hormone, among isolates GST 3 was the most efficient produced 10.43 μg/ml of auxin at 8th day of incubation. All isolates exhibited the production of a varied amount of auxin hormone during the incubation period for up to ten days shown in Table 2. Gangwar et al., [30] have also found the similar observation where twenty five (69.4%) endophytic actinomycetes isolated from Emblica officinalis Gaertn were observed to produce indole-3-acetic acid ranging between 1.93±0.3 to 125.7 ± 0.6 μg/ml. All isolates showed a different time period of incubation for production of auxin to the optimum level and for most of isolate it was 6th and 8th day of their incubation. Liu et al. [31] also reported the similar observation of different optimum incubation period for the optimum production of indole acetic acid by different strains.

HPLC analysis to detect the type of auxin hormone

As per the results recorded in Table 3, all three isolates showed the production of Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) except GSH 1, which produced only IAA type indolic auxin. The quantities of IAA and IBA produced was ranged from 3.85 - 11.65 μg ml-1 and 2.53 - 6.68 μg ml-1 respectively. The isolate GSB 13 produced a significantly higher amount of IAA (11.65 μg ml-1) than IBA (2.53 μg ml-1).

During HPLC analysis comparably higher amount of auxin hormone was detected in the culture filtrate of three efficient isolates than colorimetric analysis indicated the higher specificity of HPLC based techniques for precisely detection and quantification of the auxin hormone by rhizobacterial isolates [32-34].

Ammonia production

As per the results recorded in Table 1 and interpretation of results shown in Figure 1, 70% (35) of the selected isolates exhibited the production of ammonia among the two strains GSL 4 and GST 7 was the most efficient.

Thereby a majority (70%) of rhizosphere associated bacteria possessed the trait of ammonia production, similar to a study by Agbdjato et al. [35], who have also reported that among the rhizobacteria isolated from maize (Zea mays L.) rhizosphere, all Serratia strains (100%), 80% of Bacillus and 77.77% of Pseudomonas was found to produce ammonia.

HCN production

12% (6) of the rhizobacterial isolates exhibited the HCN production among the three isolate GSB 5, GSH 1 and GSB 2 were the most efficient produced a brown color in filter paper disc during assay for HCN production, as shown in Figure 2. Only 12% of the selected rhizobacterial isolates showed the production of HCN. El-Sayed et al. [36] has also found the similar observation, where out of 531 rhizobacterial isolates only 6.45% were cyanogenic. The production of HCN by rhizobacterial isolates have been considered as a volatile secondary metabolite acting indirectly as biological agent to control various plant disease, acting by the formation of complexes with some metal [37].

Chitinase production

Only two (4%) isolates: GSH 3 and GSV 3 showed the production of enzyme chitinase.

Antagonistic activity against phytopathogen

10% (5) of the isolates exhibited a significant antagonistic activity against both fungal pathogens Sclerotium rolfsii and Aspergillus niger shown in Figure 3 and their relative value of growth inhibition of fungal pathogens was recorded in Table 4. All five antifungal isolates showed the inhibition % against pathogen A. niger ranged from 21.94%
to 47.96% and against S. rolfsii in the range of 15.91% to 30.67%. The isolate GSB 5 (47.96% and 30.67%) and GSB 2 (45.41% and 23.85%) showed the maximum inhibitory effect against both fungal pathogens. The antifungal activity is found to associate with five isolates GSH 8, GSB 5, GSB 13, GSH 1 and GSB 2 which also showed the production of HCN, ammonia and chitinase. Therefore their antagonistic activity would be related to the production of the inhibitory substances such as HCN, ammonia and chitinase. Similar observation was also obtained in various study where it have reported that production of fungal cell wall degrading enzymes (glucanase, chitinase, protease) and secondary metabolites (Siderophore and HCN) are common mechanisms associated with bacterial antifungal activities [38,39].

Seed germination bioassay

Among 50 isolates, only five isolates GSP 4, GST 3, GSB 13, GSH 1 and GSV 3 showed a comparatively significant result for enhancing the groundnut plant growth value recorded in Table 5. Two isolates GSH 1 and GSB 13 showed the maximum enhancement of germination rate of 96.70%, radical length of 3.57 cm, 2.6 cm, plumule length of 2.22 cm, 1.7 cm and vigor index of 560.86 and 415.81. Statistically there was no significant difference among all the parameter measured for seed germination across all categories of test isolates. There was normal distribution with mean and standard deviation. All of these five isolates showed a significant effect on the enhancement of seed germination as these isolates possessed the traits of phosphate solubilization, IAA production and with almost all PGPR traits we have screened. Previous study reported that the rate of seed germination also influenced by IAA which interact and cross talk with gibberellins and ethylene [40]. Therefore the secretion of IAA and various other plant growth favoring activity could be the possible reason to enhanced seed germination by these isolates.

Conclusion

Among 50 selected isolates, 58.00% of the isolates were phosphate solubilizers where the isolates GSH 1 and GSB 13 were the most promising, 54% exhibited the production of auxin hormone where the isolates GSH 1 and GSB 13 were the most efficient. 70% of the selected isolates GST 3, GSB 13, GSH 1 and GST 7 showed, the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency. Only 6 (12%) of the selected isolates were HCN producer and two (4%) isolates were enzyme and GST 7, showed the highest efficiency.

References


