Isolation and characterization of plant growth promoting *Burkholderia* spp

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**ABSTRACT**

In this study, a total of eight *Burkholderia* isolates obtained from rhizosphere soil of maize, cotton, rice, sugarcane, groundnut and soybean and tested for the N fixation, phosphate solubilisation and antagonistic activity against *Rhizoctonia solani*. All the eight isolates produced the band for the *nifH* gene in the polymerase chain reaction confirming the presence of *nifH* gene. Among the 8 isolates, RMS1 produced clear halo zone around 7 mm on surface of HAP medium. CMS1, RMS1 and SMS1 are inhibited the growth of *Rhizoctonia solani* for 43, 40 and 35 per cent, respectively. An isolate RMS1 possessed all the three plant growth promoting traits *viz.*, higher phosphate solubilizing ability, antagonistic activity and presence of *nifH* gene selected for molecular characterization with amplifying and sequencing of 1.46 kb 16S rRNA gene. Based on the BLASTn homology it was found that 16S rRNA sequence of isolate RMS1 having 93 per cent identity with showed 97 per cent homology with *Burkholderia thailandensis*.


**KEY WORDS:** Plant growth promoting rhizobacteria, *Burkholderia* sp., Polymerase chain reaction
are called as rhizobacteria. This group of bacteria has been termed Plant Growth Promoting Rhizobacteria (PGPR) and among them are strains from genera such as Alcaligenes, Acinetobacter, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Paenibacillus, Pseudomonas, Rhizobium and Serratia. The important traits of PGPRs include fixation of atmospheric nitrogen, solubilization of insoluble inorganic phosphates, production of plant hormones, siderophores, bacteriocins etc. These organisms also provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms. Bioinoculant preparations containing these organisms are very cost effective, pollution free and a potentially renewable source of plant nutrients, making an ideal partner and an excellent supplement to chemical fertilizers (Estrada-de los Santos et al., 2001).

Over the past two decades, research on Burkholderia species has been steadily expanding. Members of the genus Burkholderia are very abundant, occupying diverse ecological niches, including soil (Janssen, 2006). It can be free-living in the rhizosphere as well as epiphytic and endophytic (Compant et al., 2008). Burkholderia sp. also having many beneficial plant growth promoting activities like controlling the plant pathogens (biocontrol), production of IAA and siderosporides for promoting the crop growth (Pandey et al., 2008). These findings have stimulated a growing interest in using Burkholderia sp. isolates in agriculture.

**MATERIAL AND METHODS**

**Isolation and morphological characterization of the bacterial isolates:**

Soil samples were collected from the rhizospheres of maize, cotton, rice, sugarcane, groundnut and soybean plants from various field location of Tamil Nadu Agricultural University, Coimbatore. A total of 25 composite soil samples were used to isolate phosphate solubilizing Burkholderia sp. by plating on Burkholderia selective BAZ medium with ammonium sulphate as N source. Spread plate method was followed to isolate Burkholderia on BAZ agar medium at pH 7.0. The soil samples were serially diluted up to 10⁴ dilution and one ml of 10⁻⁴ dilution was transferred to sterile Petriplates and BAZ agar medium was poured and mixed thoroughly. Colonies were observed on the surface of agar plates after 96 hours of incubation.

Cultural characteristics of the isolates were studied by streaking it on BAZ agar medium in Petriplates. Colony characters like size, margin, elevation and optical characteristics were observed after 48 hours. The morphological characterizations of isolates were made after staining the cultures by gram staining. After staining slides were observed under microscope to visualise the cell morphology (Sivaji et al., 2013).

**Screening of atmospheric N fixing Burkholderia sp.:**

Genomic DNA isolation was carried out from the isolates by following the protocol of Sambrook and Russell (2001). The total genomic DNA isolated from Burkholderia isolates was amplified by Polymerase Chain reaction (PCR) with nifH gene primers, which was performed using the Eppendorf Master Cycler, Gradient (Eppendorf, Germany) (Suga et al., 1995). The details of primers used and temperature profile, time duration of each step for amplification of nifH gene are given in Table A and B.

**Screening of phosphate solubilising bacteria Burkholderia sp.:**

Colonies were taken from the BAZ agar medium and streaked on the Hydroxy apatite (HAP) medium. The pH of the medium was 7.0. The plates were incubated at 30⁰C and maintained for 3 days in HAP medium to observe the halo zone produced by the isolates in the medium. The isolates with ‘P’ solubilising ability formed halo zones after 48 to 72 hours incubation. The diameter of the zone of solubilization was measured and expressed in millimetre. The results are expressed as P solubilization (PS) (Nguyen et al., 1992).

\[ PS = \text{Solubilization diameter} - \text{Growth diameter} \]

<table>
<thead>
<tr>
<th>Table A : Details of primers used for amplification of nifH gene</th>
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<tr>
<td>Target gene</td>
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Screening of antagonistic ability of *Burkholderia* isolates:

The antagonistic activity of the isolates was determined by dual culture method, in which two media PDA and BAZ are mixed together to form dual medium. In this test the *Burkholderia* was streaked on one side and plant pathogenic fungi *Rhizoctonia solani* was kept on another side of plate to check antagonistic activity of *Burkholderia* against the same fungi (Dennis and Webster, 1971). Plates inoculated only with pathogen were maintained as control. The zone of inhibition was recorded as the distance between the fungal pathogen and the area of antagonist growth after 72 h.

\[ PI = \frac{C - T}{C} \times 100 \]

where,

- \( PI \) = Per cent inhibition of mycelial growth
- \( C \) = Radial growth of pathogen in control plates (mm)
- \( T \) = Radial growth of pathogen in dual culture (mm)

Molecular characterization of the isolate:

PCR amplification was carried out using 16S rRNA universal primer (Marchesi et al., 1998). The details of primers used to amplify 16S ribosomal RNA (16S rRNA) gene are given in Table C. Steps followed in PCR, temperature profile and time duration of each step for amplification of 16S rRNA are given in Table D. The amplified 16S rRNA gene PCR product of RMS1 isolate were sent for sequencing to SciGenome Labs Pvt Ltd., Cochin and sequenced through single pass analysis from forward and reverse direction. Sequencing was done by using Automated sequencer.

**RESULTS AND DISCUSSION**

The findings of the present study as well as relevant discussion have been presented under the following heads:

**Isolation, cultural and morphological characterization *Burkholderia* sp**:

Among the 25 soil samples used to isolate...
**Burkholderia**, only 8 samples exhibited colonies on BAZ agar medium after 4 days of incubation. Details of the isolates is given in Table 1. Colonies were, Small (1 to 2 mm), circular, pale yellowish brown and translucent at the margins (Fig. 1). All these isolates were found to be rod shaped and Gram negative which showed the possibility of these isolates being *Burkholderia* sp. Similar morphology of *Burkholderia* and Gram reaction were reported by Linu et al. (2009).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Crops</th>
<th>Isolate name</th>
<th>Sr. No.</th>
<th>Crops</th>
<th>Isolate name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Maize</td>
<td>MMS1</td>
<td>5.</td>
<td>Sugarcane</td>
<td>SMS1</td>
</tr>
<tr>
<td>2.</td>
<td>Cotton</td>
<td>CMS1</td>
<td>6.</td>
<td>Sugarcane</td>
<td>SMS2</td>
</tr>
<tr>
<td>3.</td>
<td>Rice</td>
<td>RMS1</td>
<td>7.</td>
<td>Groundnut</td>
<td>GMS1</td>
</tr>
<tr>
<td>4.</td>
<td>Rice</td>
<td>RMS2</td>
<td>8.</td>
<td>Soybean</td>
<td>SBMS1</td>
</tr>
</tbody>
</table>

**Table 1 : List of Burkholderia isolates obtained in this study**

‘P’ solubilizing *Burkholderia* sp. :
Isolates having phosphate solubilising ability produced halo zone due to solubilisation of insoluble mineral phosphate on HAP after 3 days incubation. Out of 8 *Burkholderia* isolates only three isolates viz., RMS1, GMS1 and SMS2 produced clear halo zone (7, 5 and 4 mm, respectively) (Fig. 3) on surface of HAP medium.

Amplification of *nifH* gene primers :
All the eight isolates produced the band for the *nifH* gene in the polymerase chain reaction confirmed the presence of *nifH* gene. It was confirmed by run the PCR product on 1 per cent agarose gel (Fig. 2). Estrada-de los Santos et al. (2001) reported that genus *Burkholderia* comprises 19 species, including *Burkholderia vietnamiensis*, which is the only known N₂-fixing species of this bacterial genus and in their investigation most of the N₂-fixing isolates were recovered from the environment of field-grown maize and coffee plants. These reports found to be similar with our findings. This report confirms the *nif* gene presence in *Burkholderia* sp. during present investigation.

It is generally accepted that the mechanism of mineral phosphate solubilisation by PSB strains is associated with the release of low molecular weight...
organic acids (Goldstein, 1995 and Halder et al., 1990) which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabai, 1994).

Linu et al. (2009) reported high ‘P’ solubilizing ability in cowpea rhizosphere. This indicates efficacy of ‘P’ solubilisation by Burkholderia varies from crop to crop. The presence of Burkholderia sp. in the rhizosphere of maize (Paulina et al., 2002) and sugarcane (Danice et al., 2010) was already reported.

Screening of antagonistic ability of Burkholderia isolates:
Antagonistic activity by Burkholderia:
Antagonistic activity of Burkholderia was tested by dual culture method. Among the 8 isolates CMS1, RMS1 and SMS1 are produced inhibition zone of 43, 40 and 35 per cent, respectively against Rhizoctonia solani. No inhibition was found for other isolates (Fig. 4). Sfalanga et al. (1999) found new antagonistic Burkholderia strain from the rhizosphere of healthy tomato plants that was resistant to several antibiotic substances and suppress the growth of important bacterial and fungal phytopathogens.

Ganyu et al. (2009) reported Burkholderia bacteria are frequently isolated from the rhizosphere of crops, and are involved in growth promotion of plants and suppression of plant diseases. Burkholderia contaminans strain MS14 has a broad range of antifungal activities to plant and human pathogens especially for Geotrichum candidum. These reports are in conformation with the findings of present investigation for antagonistic properties of Burkholderia against different plant pathogens.

![Fig. 4: Inhibition zone of Burkholderia isolates against R. solani](image)

![Fig. 5: Amplification of 16S rRNA from all Trichoderma viridi (fungi)](image)
Molecular characterisation of isolates:

The genomic DNA of *Burkholderia* isolates were amplified by PCR using 16S rRNA primers, they yielded 1.46 kb band which was notified by running the PCR product on 1.2 per cent agarase gel (Fig. 5). Nucleotide sequence of the 16S rRNA region of the genome of the isolate RMS1 was sequenced by using automated sequencer. The sequences were blasted against NCBI database. Based on the BLASTn homology it was found that isolate RMS1 having 93 per cent identity with showed 97 per cent homology with *Burkholderia thailandensis* (Govindarajan et al., 2007 and Salles et al., 2002)

Conclusion :

Identification a new effective plant growth-promoting bacteria and characterization of its PGPR traits is an important process to develop the new multifunctional biofertilizer. This will lead the higher crop productivity and also help to reduce the usage of chemical fertilizers, pesticides and environment polluting substances. In this study, a total of 8 *Burkholderia* isolates obtained from rhizosphere soil of maize, cotton, rice, sugarcane, groundnut and soybean plants. Isolate RMS1 has multiple PGPR traits like N fixation, phosphate solubilisation and biocontrol activity against plant pathogens. This can be exploited as a new bioinoculant that have multiple benefits for the agricultural crops.

REFERENCES


method to assess the diversity of *Burkholderia* species in environmental samples. *Appl. & Environ. Microbiol.*, **68**: 1595-1603.


