Management of bacterial blight in rice (Oryza sativa) through combined application of endophytes and rhizosphere antagonist

KAKUMONI SAIKI1, L C BORA2, POBY BORA3* and HRISHIKESH HAZARIKA4

Assam Agricultural University, Jorhat, Assam, India

Received: 13 November 2019; Accepted: 8 November 2020

ABSTRACT

Biocontrol agents and plant growth promoting microbes have emerged as an effective alternative for chemical management of plant diseases in addition to realising an increase in the crop yield. Present study was made to explore endophytic microbes and rhizospheric Streptomyces of rice plant to develop biocontrol strategy for the management of bacterial blight (BB) of rice (Oryza sativa L.) caused by Xanthomonas oryzae pv. oryzae (Xoo). In-vitro studies revealed that few promising endophytic microbes (E1 and E2) and rhizospheric Streptomyces (S1 and S2) could suppress Xoo effectively in dual culture assay in vitro. The combination of antagonistic microbes (E1+S1+S2) showed highest (58.71%) inhibition of BB pathogen. Pot experiments were conducted to study the effect of the promising endophytic microbes on disease reduction, yield and yield attributing characteristics of rice. Results revealed the lowest disease incidence in plants treated with combination of E1+S1+S2 (10.29 %) compared to other treatments. Similarly, the highest yield (50.06g per hill) and other yield attributing characters of rice plants were recorded with microbial treatment E1+S1+S2. These observations suggested far better superiority of rhizosphere antagonists plus endophytes than either of the two alone.

Key words: Bacterial blight, Endophytes, Streptomyces, Yield

The use of biocontrol agents having suppressing effect on disease-causing agents and to increase the production of major crops, provide a strategically viable option for the host plant resistance vis-a-vis disease management (Bora et al. 2019). Bacterial blight (BB) caused by Xanthomonas oryzae pv. oryzae (Xoo) is one of the most destructive diseases of rice causing severe losses during conducive conditions (Ou 1985). Traditional methods employed to minimize the pathogen-borne losses have limitations. Therefore, viable, economic and eco-friendly disease management tools need to be developed. Biocontrol agents and Plant Growth Promoting Microbes (PGPM) are known to enhance crop productivity in a sustainable manner (Sharma et al. 2013, Beneduzi et al. 2012, Bora et al. 2020).

The bacterial community inhabiting rhizosphere is usually considered as a source of formation of the community of endophytic bacteria. At the same time, it is known that the mechanisms used by endophytic bacteria to improve plant growth are similar to those of rhizospheric bacteria. However, endophytes have been used to enhance plant growth and yield, besides protection against plant diseases via several mechanisms (Kumar et al. 2015, Bora et al. 2019). Ability of Streptomyces to suppress some plant pathogens is also well documented (Rizk et al. 2007, Park et al. 2011). Our study aimed to manage BB of rice as well as increase yield of rice using endophytes and rhizospheric Streptomyces of rice ecosystem. The major objectives of our study included isolation and characterization of various endophytic microbes and rhizosphere-based Streptomyces of rice ecosystem, and later combining them for management of BB of rice with emphasis on enhancement in yield and yield attributing characters.

MATERIALS AND METHODS

The above study was carried out under laboratory conditions at Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam (Replicated seasons of 2017-18). Diseased leaves showing typical bacterial blight symptoms were collected from rice fields of Regional Agricultural Research Station (RARS), Titabar. The pathogen, Xoo was isolated using Sucrose Peptone Agar (SPA) and Modified Wakimoto’s media. Pure culture of Xoo was preserved on Nutrient Agar (NA) slants at 4°C for future use. Pathogenicity test was conducted by clip inoculating...
10^7-10^8 cfu/ml of bacterial inoculums on 1 month old rice seedlings (Kauffman et al. 1973).

Isolation of endophytic and rhizospheric antagonists

Endophytes were isolated from the healthy leaf, stem and root samples of rice plants using the protocol suggested by McInroy and Kloeppe(r 1995). Rhizospheric Streptomyces were isolated from the rice rhizosphere using the protocol suggested by Maleki et al. (2013).

In-vitro efficacy of microbial antagonists against Xoo

The inhibitory effect of the isolated endophytes and Rhizospheric Streptomyces isolates against Xoo were tested in vitro by using a modified dual culture assay method (Aspiraz and Craz 1985). The per cent inhibition was calculated following the formula described by Myee and Datar (1986) as: 

\[ \text{Per cent inhibition} = \frac{\text{Mean of inhibition zone (mm)}}{90} \times 100 \]

On the basis of its percentage inhibition, 2 endophytes and 2 rhizospheric Streptomyces were further selected for compatibility test in-vitro using NA and PDA as basal media. Various morphological, cultural, biochemical and molecular characterization schemes were adopted to identify the isolates.

In-vivo efficacy for the management of BB of rice

Selected combination of endophytic microbes and Rhizospheric Streptomyces showing highest inhibition (%) against Xoo in-vitro were evaluated for suppression of BB in pot grown rice plants under green shade net condition (cv. TN1). Five replications were maintained for each treatment following completely randomised design. Selected endophytic microbes and Rhizospheric Streptomyces were applied as seed treatment (100 seeds/20ml of solution), root treatment for 1 hour prior to transplanting (100 seedlings/100 ml of solution), soil treatment was done 30 days after transplanting (100 ml/ plant) and spray application (2% solution), 30 days after transplanting with subsequent sprays undertaken at 45, 60 and 75 DAT.

Per cent disease incidence (PDI) and per cent disease reduction were assayed in treated rice plants. Disease severity percentage was calculated based on 0-9 scale of Standard Evaluation System for rice (IRRI 2002). Moreover, yield and plant attributing characters were recorded.

Statistical analysis

Data from in-vitro and in-planta experiments were analyzed and subjected to Analysis of Variance (ANOVA). Critical differences were estimated to compare different treatments and the per cent values were transformed by angular transformation.

RESULTS AND DISCUSSION

The bacterial blight (BB) pathogen (Xoo) was isolated from symptomatic rice leaves and ooze test was done to confirm the bacterial presence in the diseased tissues. Light yellow, circular, convex, opaque and smooth colonies typical to Xoo were observed on Modified Wakimoto’s and SPA plates. Morphologically the bacterium was rod shaped and retained red colour when counter-stained with safranin revealing its gram negative reaction. Biochemically it showed positive reaction to KOH test, Citrate utilization, Gelatin liquefication, Catalase test, Levan production test. Similar results of biochemical tests were also obtained by Arshad et al. (2015) during identification of Xoo. The bacterium appeared to be rod shaped under a Field Emission Scanning Electron Microscope (FESEM).16S rRNA sequencing results of the bacterium showed maximum homology (~97%) with Xanthomonas oryzae pv. oryzae. Pathogenicity test of Xoo was carried out on healthy rice plants (var. TN1) which revealed highly virulent reaction, producing typical disease symptoms in the inoculated plants within 10 days. Similar results were obtained by Kauffman et al. (1973), wherein successful inoculation of the Xoo was obtained by leaf clipping method.

Isolation and of endophytes and rhizosphere antagonists characterisation

As many 20 bacterial and 6 fungal endophytes were isolated and identified based on various cultural and micro-morphological studies. Moreover, 16 rhizospheric Streptomyces were isolated from different locations. In-vitro tests were conducted with the endophytic microbes and rhizospheric Streptomyces against Xoo. Among all the isolates, 2 endophytes (E_1 and E_2) and 2 rhizospheric Streptomyces (S_1 and S_2) were found to be most effective in inhibiting Xoo which were further characterised.

The gram staining revealed that the endophytic bacterium E_1 was gram negative and rod shaped. In King’s B medium it showed fast growing greenish yellow colony which were opaque, smooth edged and convex. Biochemical characterization of E_1 revealed positive reactions to Gelatin liquefication test, Catalase test, Oxidase test, Citrate utilization test, Nitrate reduction test, Arginine dihydrolase test, KOH test and Levan production test. Molecular characterization using 16S rRNA sequencing of the endophyte exhibited maximum homology (95%) with Pseudomonas putida. These results of biochemical tests are in conformity with the results of tests conducted by Kumar et al. (2016) for identification of P. putida.

Cultural characteristics of E2 on PDA media was found to have Conidia aseptate, cylindrical to ovoid, formed in chains, pale to bright green to yellow-green or olivaceous in colour. The length of the conidia varied from 5.5 to 7.15 μm and the width varied from 1.8 to 2.87 μm. 16S rRNA sequencing results showed maximum homology (~97%) with Metarhizium anisopliae. Sasen and Bidoehka (2013) reported that M. robertsi can endophytically colonize roots of switch grass and haricot beans.

Similarly, Gram staining of S_1 and S_2 showed both the bacteria as gram positive filamentous strands. Colony size varied from small to medium, rough edges and
In-vitro evaluation of antagonistic ability of endophytes and rhizospheric Streptomyces

Both the endophytes and rhizospheric microbes either alone or in combination were found to have inhibited Xoo under in vitro and also exhibited positive compatibility for E1 + E2, S1 + S2, E1 + S1, E1 + S2, E1 + S1 + S2, S1 + S2, E1 + S2 and E1 + S1 + S2. Highest inhibition was shown by combination of E1+S1+S2 (58.71%) followed by E2 + S1+S2 (56.62%) after 48 hr of inoculation (Table 1). P. putida (E1) has been recognized as an efficient BCA against many phytopathogens and antagonism is attributed to siderophore production, active colonization and antibiotic production (Bora et al. 2018). Fungal endophyte M. anisoploea (E2) produces secondary metabolites which have antimicrobial activity (Ravindra et al. 2014). Endophytic Streptomyces spp. and their metabolites are promising option in controlling various fungal and bacterial phytopathogens (Vurukunda et al. 2018).

Table 1 Inhibition (%) of Xoo by effective endophytic microbes (E1 and E2) and rhizospheric Streptomyces (S1 and S2)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Zone of inhibition (mm dia.)</th>
<th>Per cent inhibition</th>
<th>24 hr</th>
<th>48 hr</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>23.53</td>
<td>38.97</td>
<td>26.14 (30.72)*</td>
<td>43.3 (41.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>17.69</td>
<td>24.90</td>
<td>19.65 (26.28)</td>
<td>27.67 (31.69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 + E2</td>
<td>34.35</td>
<td>42.62</td>
<td>38.37 (38.23)</td>
<td>47.36 (43.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>17.40</td>
<td>24.54</td>
<td>19.33 (26.06)</td>
<td>27.27 (31.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>21.00</td>
<td>28.00</td>
<td>23.33 (28.86)</td>
<td>31.10 (33.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1 + S2</td>
<td>32.07</td>
<td>39.35</td>
<td>35.63 (36.63)</td>
<td>43.72 (41.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 + S1</td>
<td>40.60</td>
<td>49.42</td>
<td>45.11 (42.19)</td>
<td>54.91 (47.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 + S2</td>
<td>43.46</td>
<td>50.60</td>
<td>48.29 (43.97)</td>
<td>56.22 (48.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 + S1 + S2</td>
<td>47.32</td>
<td>52.84</td>
<td>52.58 (46.43)</td>
<td>58.71 (50.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 + S1</td>
<td>31.92</td>
<td>40.80</td>
<td>35.46 (36.51)</td>
<td>45.33 (42.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 + S2</td>
<td>32.65</td>
<td>41.93</td>
<td>36.28 (36.99)</td>
<td>46.58 (42.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 + S1 + S2</td>
<td>42.84</td>
<td>50.96</td>
<td>47.60 (43.62)</td>
<td>56.62 (48.79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>No inhibition</td>
<td></td>
<td>0 (0.57)</td>
<td>0 (0.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEd ±</td>
<td>-</td>
<td>-</td>
<td>1.82</td>
<td>1.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td></td>
<td>-</td>
<td>3.78</td>
<td>3.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values in parentheses are arcsine-transformed values.

Table 2 Effects of different treatments on BB incidence (%) and severity (%) in pot grown rice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PDI</th>
<th>Disease reduction (%)</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>74.99 (59.93)</td>
<td>0.00 (0.00)</td>
<td>70.73 (57.23)*</td>
</tr>
<tr>
<td>T2</td>
<td>13.14 (21.22)</td>
<td>80.06 (63.43)</td>
<td>11.63 (19.91)</td>
</tr>
<tr>
<td>T3</td>
<td>17.57 (24.73)</td>
<td>76.57 (61.00)</td>
<td>24.30 (29.53)</td>
</tr>
<tr>
<td>T4</td>
<td>25.47 (30.26)</td>
<td>66.03 (54.33)</td>
<td>29.71 (33.02)</td>
</tr>
<tr>
<td>T5</td>
<td>16.59 (23.97)</td>
<td>77.87 (61.89)</td>
<td>23.23 (28.79)</td>
</tr>
<tr>
<td>T6</td>
<td>16.08 (23.58)</td>
<td>78.55 (62.38)</td>
<td>22.73 (28.45)</td>
</tr>
<tr>
<td>T7</td>
<td>10.29 (18.63)</td>
<td>86.27 (68.19)</td>
<td>9.73 (18.15)</td>
</tr>
<tr>
<td>T8</td>
<td>23.99 (29.27)</td>
<td>68.00 (55.55)</td>
<td>27.33 (31.5)</td>
</tr>
<tr>
<td>T9</td>
<td>19.62 (26.28)</td>
<td>73.83 (59.21)</td>
<td>24.83 (29.87)</td>
</tr>
<tr>
<td>T10</td>
<td>14.95 (22.71)</td>
<td>82.47 (65.20)</td>
<td>18.16 (25.18)</td>
</tr>
<tr>
<td>SEd ±</td>
<td>1.32</td>
<td>-</td>
<td>1.87</td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td>2.73</td>
<td>-</td>
<td>3.89</td>
</tr>
</tbody>
</table>

*Values in parentheses are arcsine-transformed values. Data are mean of three replications. T1 = Inoculated control; T2 = Uninoculated control; T3 = E1+S1+S2; T4 = E2+S1+S2; T5 = E1+S1; T6 = E1+S2; T7 = E2+S1; T8 = E2+S2; T9 = E2+S1+S2; T10 = E2+S1+S2.
of diseases on account of multiple functions and synergistic effect of more number of other micro-organisms, collectively resulted in reduction of BB of rice. Bora et al. (2013) reported that combined application of more than one compatible BCA produced a greater control of bacterial wilt disease of different crops under varied field conditions.

**Effect on the yield and yield attributing characteristics**

The application of effective endophytic microbes and rhizospheric *Streptomyces* in combinations has significant effect on rice yield and yield attributing characteristics (Table 3). Significant difference in all the yield attributing characters was observed between inoculated control and treated plants. The highest number of panicles in rice plant was observed in T7 (20.66) with highest panicle length (28.7 cm) and weight (29.16 g). Lowest was recorded in T1. Data showed that T5 also recorded highest root dry weight (46.54g) and lowest root weight was recorded in T1 (11.8g). The field study revealed that T7 exhibited highest shoot weight, root-shoot ratio, root length, test weight leading to the highest yield (g per hill) in plants inoculated with T7 (50.06 g).

Kaur et al. (2017) exploited the endophytic *Pseudomonas* sp. for improving growth and productivity in rice under sustainable management system. Anwar et al. (2016) demonstrated the ability of rhizospheric *Streptomyces* to enhance the plant growth which results in yield enhancement of wheat crop. Actinobacteria, such as *Streptomyces* spp., produce siderophores and plays significant role in solubilizing phosphate, and also produces array of enzymes, viz. amylase, chitinase, cellulase, invertase, lipase, pectinase, protease, phytase, and xylanase which make the complex nutrients into simple mineral forms (Vurukonda et al. 2018). Various microbes involved in this study, showed promising efficacy against *Xanthomonas oryzae* pv. *oryzae* (Xoo) both in-vitro and in-vivo.

In our study, endophytic *P. putida*, *M. anisopleae* and rhizospheric *Streptomyces* showed satisfactory control over BB pathogen *Xoo* both in vitro and in vivo condition. they were also found to be promising in enhancing plant growth parameters and increasing rice yield. The synergistic biochemistry amongst bioactive compounds released in vivo by different microorganisms, possibly render them more effective towards growth and development as observed in our rice plants treated with combination of divergent microbes. New research advances in plant–bacteria interactions would decipher the plants ability to shape their rhizosphere and endorhiza microbiome in a synergised sequence to safeguard soil health as well as plant health.

Data are mean of three replications. T1= Inoculated control; T2= Uninoculated control; T3= E1+S1; T4= S1+S2; T5= E1+S1+S2; T6= E1+S2; T7= E1+S1+S2; T8= E2+S1; T9= E2+S2; T10= E2+S1+S2.

ACKNOWLEDGEMENT

The authors are highly grateful to Assam Agricultural University, Jorhat, Assam, India for rendering necessary financial support during the tenure of the investigation.

**REFERENCES**


