Bio-efficacy of endophytic actinomycetes for plant growth promotion and management of bacterial wilt in tomato

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ABSTRACT: Antagonistic effect of endophytic actinomycetes isolated from tomato plants collected from different locations in Kerala was evaluated against bacterial wilt pathogen under in vivo conditions. Maximum inhibition (44.63%) of the pathogen was observed with Cherumkuzhy isolate (EACK) which was on par with Ozhalapathy isolate (EAOP) (42.59%). All the isolates belonged to genus Streptomyces sp. Evaluation of endophytic actinomycetes against bacterial wilt pathogen under pot culture conditions revealed that minimum per cent wilt incidence (37.03%) was shown by plants treated with EAOP isolate. Observations on biometric characters showed that Ozhalapathy (EAOP) and Eruthenapathy (EAET) isolates were the most promising in plant growth promotion. It was concluded from the study that the Ozhalapathy isolate (EAOP) (Streptomyces sp.) was the best among the five endophytic actinomycetes isolates in plant growth promotion as well as in the management of bacterial wilt in tomato.

Keywords: Bacterial wilt, endophytic actinomycetes, Ralstonia solanacearum, tomato

INTRODUCTION

Tomato (Solanum lycopersicum Mill.) is one of the most popular protective foods because of its high lycopene content and is a widely grown vegetable in the world. Being versatile for culinary purposes, it is also one of the most commonly grown vegetable in the kitchen garden (Rana, 2008). Among the various diseases affecting the crop, bacterial wilt caused by Ralstonia solanacearum is the major one, and cent per cent yield loss has been reported among the susceptible varieties. Indiscriminate use of chemicals for the management of the disease may result in the development of resistant strains of pathogen as well as residual effect on the produce. So, biological control of disease is an alternative measure, because of its eco-friendly nature, cost effectiveness and long lasting effects. Endophytes are microorganisms that inhabit at least for a period of their lifecycle inside plant tissues without causing any apparent harm to the hosts. The use of endophytic actinomycetes as bioagents of soil borne root diseases is of interest through their ability to colonize healthy plant tissues and produce antibiotics in situ. de-Oliveira (2010) observed that Streptomyces pluricolorescens, an endophytic actinomycete isolated from tomato plants in Brazil showed (86.6%) antimicrobial activity against at least one pathogen in tomato. As plant growth promoters, endophytic actinomycetes enhance growth of plants including the formation of increased number of lateral roots and root hairs (Pillay and Nowak, 1997) in addition to an increase in plant height, shoot weight and shoot diameter (Yates et al., 1997). In this context, the present study was taken up to know more about the bioefficacy of endophytic actinomycetes on plant growth promotion and management of bacterial wilt in tomato.

MATERIALS AND METHODS

The present study was carried out at the Department of Plant Pathology, College of Horticulture, Thrissur, Kerala (India) during the years 2009-11. Endophytic actinomycetes were isolated from healthy tomato plants collected from high wilt incidence areas of Vellanikkara, Cherumkuzhy and Elanad (Thrissur district, Kerala, India) and low wilt incidence areas of Ozhalapathy and Eruthenpathy (Palakkad district, Kerala, India). Local cultivars of both young (less than two weeks) and old plants (more than two weeks) were obtained from different locations of the above mentioned areas. The pathogen, Ralstonia solanacearum causing bacterial wilt in tomato was isolated from the naturally infected tomato plants using Triphenyl Tetrazolium Chloride (TZC) agar medium. An in vitro experiment was conducted to find out the antagonistic effect of the isolated endophytic actinomycetes against the pathogen by dual culture method and the per cent inhibition was calculated by PI = (C – T)/C x 100 where PI = Per cent inhibition; C = Growth of the pathogen in control plates (cm); T = Growth of the pathogen in dual culture plates (cm).- It was statistically analysed using MSTAT software followed by Duncans Multiple Range Test (DMRT). The
morphological and cultural characters of endophytic actinomycetes were studied based on standard keys given by Locci (1989).

Two pot culture experiments were conducted to find out the efficacy of endophytic actinomycetes isolates on plant growth promotion and management of bacterial wilt in tomato. Sterilized potting mixture consisting of sand: soil: cow dung at the rate of 1:1:1 was filled in earthen pots of size 12”x12”. Bacterial wilt susceptible variety Pusa Ruby was used for the study. All agronomic practices were performed as envisaged in the package of practices recommendations of the Kerala Agricultural University (KAU, 2007). Experiment I was laid out in CRD with 8 treatments and three replications, to find out the efficacy of endophytic actinomycetes in the management of bacterial wilt in tomato. Nine plants were maintained for each treatment. The application of treatments were started at the time of planting of seedlings, 45 days after planting and 60 days after planting as soil drenching. Challenge inoculation of pathogen (10^5 cfu/ml) was done at 30 DAP using fresh bacterial suspension (at the rate of 10 ml/plant). Experiment II was also laid out in CRD with 6 treatments and three replications, to find out the efficacy of endophytic actinomycetes in plant growth promotion without giving the challenge inoculation of the pathogen. Observations on per cent wilt incidence, days to flowering, days to first harvest, number of fruits per plant, per fruit weight and yield per plant were recorded and statistically analysed using MSTAT software followed by Duncans Multiple Range Test (DMRT).

After the evaluation of endophytic actinomycetes isolates against Ralstonia solanacearum under pot experiments, the most promising one was selected and 16S rRNA sequence analysis was carried out based on the amplification of 16S rRNA gene using Polymerase Chain Reaction (PCR) as described by Cook and Meyers (2003). The quality of isolated DNA was evaluated through agarose gel electrophoresis. The DNA bands separated by electrophoresis were views and photographed using Vision Works LS software and UVP GelDoc- IT™ imaging system. 20μl of the PCR product was purified using PCR purification kit (Bioserve) and the purified product was sequenced at Seigenome Pvt. Ltd. Cochin using the primers 27f and 1492r. The Blastn programme (http://www.ncbi.nlm.nih.gov/blast/) was used to find out the homology of the nucleotide sequences.

RESULTS AND DISCUSSION
Isolation of endophytic actinomycetes

The endophytic actinomycetes isolated from healthy tomato plants collected from five different locations were designated as EAVK (Vellanikkara), EACK (Cherumkuzhy), EAEN (Elanad), EAOOP (Ozhalapathy) and EAET (Eruthenpathy). Only a single type of actinomycete isolate was obtained from each location. The maximum population was obtained from the roots of tomato plant collected from Cherumkuzhy (EACK) (8x10^1 cfu/g), followed by Eruthenpathy (EAET) (3x10^1 cfu/g). The least count was obtained from Vellanikkara (EAVK), Elanad (EAEN) and Ozhalapathy (EAOOP) (1x10^1 cfu/g). More number of actinomycetes was obtained from roots than from stem of tomato plants and it was in accordance with the results obtained by Shimizu et al. (2000).

In vitro evaluation of antagonistic effect of endophytic actinomycetes on Ralstonia solanacearum

Of the five isolates, maximum inhibition (44.63%) of pathogen was recorded with the Cherumkuzhy isolate (EACK) which was on par with the Ozhalapathy isolate (EAOOP) (42.59%) (Table 1). Moura et al. (1998) observed that endophytic actinomycetes isolated from the root tissues of tomato showed cent percent control of the pathogen. Similarly, Moura and de-Romeiro (1999) noted that endophytic actinomycetes isolated from various hosts showed a high inhibitory activity against R. solanacearum.

Identification of endophytic actinomycetes

The colony size of all the isolates was medium. On the solid agar substratum, the branching network of hyphae developed by all the isolates grew both on the surface of the substratum and inside it forming a substrate mycelium. But chain of spores and sporangia were absent in the substrate mycelium of all the isolates. Fragmentation of substrate mycelium and motility of spores were absent for all the isolates. Later the colonies of all the isolates became covered with aerial mycelium bearing chain of arthrospores, which was initially white but assumed a range of colors when spore formation started. Color of the spore mass for all the isolates except Cherumkuzhy isolate was gray. Spiral spore chain morphology was observed for all the isolates and the spores were smooth surfaced. Red-orange colored, diffusible pigments were produced by Vellanikkara and Cherumkuzhy isolates which were absent for the other three isolates. The isolates from Vellanikkara and Cherumkuzhy produced red-orange colored pigmentation for the substrate mycelium whereas for the other three isolates distinctive pigmentation was absent. Based on these morphological and cultural characters, the isolates were tentatively identified as genus Streptomyces. This
was in agreement with the results of identification of endophytic actinomycetes from wheat roots from a range of sites across South Australia (Coombs and Franco, 2003) and tomato roots in South China (Cao, et al., 2004).

Evaluation of endophytic actinomycetes on plant growth promotion and management of bacterial wilt in tomato under pot culture conditions

Experiment I

Evaluation of endophytic actinomycetes against bacterial wilt pathogen under pot culture conditions revealed that minimum per cent wilt incidence was shown by plants in pots drenched with urea (@ 44 g m$^{-2}$) and lime (@ 500 g m$^{-2}$) mixture [29.63%] and it differed significantly from the other treatments. The results were in accordance with the findings of Mathew (2004), who reported the effectiveness of urea and lime mixture for the management of *R. solanacearum* infecting solanaceous vegetables. Among the endophytes, the plants treated with EAOP isolate showed minimum per cent wilt incidence [37.03%] (Table 1). Consequently, the study confirmed that application of either a mixture of urea and lime or EAOP isolate were the best treatments in the management of bacterial wilt in tomato.

Experiment II

With respect to yield parameters such as days to flowering, days to first harvest, number of fruits per plant, per fruit weight and yield, plants treated with the endophytic actinomycetes were superior in performance when compared to the control (Table 2). The number of days taken for the emergence of first flower and also the number of days taken from fruit set to harvest should be less so that the total crop duration is not extended unduly. In the present study the plants treated with EAEN isolate recorded the minimum number of days (34.83) for flowering and the plants treated with EAVK isolate took the minimum number of days (81.66) to first harvest. Numbers of fruits per plant and per fruit weight are the two parameters having a direct effect on crop yield. In the present study, the plants treated with EAET isolate produced the maximum number of fruits per plant (17.57) followed by EAOP isolate (16.25) and they were found to be statistically on par. The data regarding per fruit weight indicated that the plants treated with EAOP isolate produced fruits with maximum weight (20.30 g) which was on par with EAET isolate (19.33 g) and was statistically superior to all other treatments. Highest yield (332.02 g) was recorded for the plants treated with EAET isolate followed by EAOP isolate (316.81 g). Hence, the observations on biometric characters revealed that Ozhalapathy (EAOP) and Eruthenpathy (EAET) isolates were the most promising in plant growth promotion. Hence, it is concluded from the study that the endophytic actinomycete *Streptomyces* sp. from Ozhalapathy (EAOP) showed superior performance among the five isolates in plant growth promotion as well as in the management of bacterial wilt in tomato. This promising endophytic actinomycete has a potential to be developed as a biocontrol agent for the management of bacterial wilt in tomato.

16S rRNA sequence analysis of the promising endophytic actinomycete isolate

Based on the results of pot experiments, the efficient endophytic actinomycete isolate, EAOP was identified by 16S rRNA sequence analysis using Polymerase Chain

Table 1. Effect of various treatments on bacterial wilt in tomato

<table>
<thead>
<tr>
<th>Isolate/Treatment</th>
<th>Per cent inhibition (<em>in vitro</em>)</th>
<th>Per cent wilt incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAVK</td>
<td>27.22b</td>
<td>66.67 (0.96)$^b$</td>
</tr>
<tr>
<td>EACK</td>
<td>44.63a</td>
<td>55.56 (0.84)$^{bc}$</td>
</tr>
<tr>
<td>EAEN</td>
<td>26.11b</td>
<td>66.67 (0.96)$^b$</td>
</tr>
<tr>
<td>EAOP</td>
<td>42.59a</td>
<td>37.03 (0.65)$^{cd}$</td>
</tr>
<tr>
<td>EAET</td>
<td>31.11b</td>
<td>48.15 (0.77)$^{bcd}$</td>
</tr>
<tr>
<td>Urea + lime mixture</td>
<td>–</td>
<td>29.63 (0.57)$^d$</td>
</tr>
<tr>
<td>Copper hydroxide</td>
<td>–</td>
<td>40.74 (0.69)$^{cd}$</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>87.96 (1.24)$^a$</td>
</tr>
<tr>
<td>CD (p=0.05)</td>
<td>6.10</td>
<td>16.6</td>
</tr>
</tbody>
</table>

Figures in parenthesis are arc- sine transformed values.

Means followed by a common letter in a column are not significantly different at 5 % level by DMRT

Mean of 3 replications
Only one amplicon of about 1500bp was obtained. Since only a single band was obtained, PCR product was directly purified and sequenced. Homology search of nucleotide sequences obtained from the isolate with other reported 16S rRNA gene sequences revealed that EAOP showed homology with \textit{Streptomyces thermodiastaticus} (95\% query coverage and 97\% identity). Consequently, it was concluded from the study that the endophytic actinomycete \textit{Streptomyces thermodiastaticus} from Ozhalapathy (EAOP) showed superior performance among the five isolates in plant growth promotion as well as in the management of bacterial wilt in tomato. This promising endophytic actinomycete has a potential to be developed as a biocontrol agent for the management of bacterial wilt in tomato.

\textbf{REFERENCES}


\textbf{Table 2. Effect of endophytic actinomycetes isolates on yield parameters of tomato}

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Days to flowering</th>
<th>Days to first harvest</th>
<th>No. of fruits</th>
<th>Per fruit weight (g)</th>
<th>Fruit yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAVK</td>
<td>35.50\textsuperscript{ab}</td>
<td>81.66\textsuperscript{b}</td>
<td>11.26\textsuperscript{b}</td>
<td>15.46\textsuperscript{c}</td>
<td>161.65\textsuperscript{c}</td>
</tr>
<tr>
<td>EACK</td>
<td>40.20\textsuperscript{ab}</td>
<td>83.93\textsuperscript{ab}</td>
<td>13.75\textsuperscript{ab}</td>
<td>18.21\textsuperscript{ab}</td>
<td>226.65\textsuperscript{bc}</td>
</tr>
<tr>
<td>EAEN</td>
<td>34.83\textsuperscript{c}</td>
<td>83.73\textsuperscript{ab}</td>
<td>13.57\textsuperscript{ab}</td>
<td>14.49\textsuperscript{ab}</td>
<td>182.67\textsuperscript{c}</td>
</tr>
<tr>
<td>EAOP</td>
<td>39.39\textsuperscript{ab}</td>
<td>85.01\textsuperscript{ab}</td>
<td>16.25\textsuperscript{ab}</td>
<td>20.30\textsuperscript{ab}</td>
<td>316.81\textsuperscript{b}</td>
</tr>
<tr>
<td>EAET</td>
<td>36.77\textsuperscript{ab}</td>
<td>82.45\textsuperscript{ab}</td>
<td>17.57\textsuperscript{a}</td>
<td>19.33\textsuperscript{ab}</td>
<td>332.02\textsuperscript{a}</td>
</tr>
<tr>
<td>Control</td>
<td>40.39\textsuperscript{a}</td>
<td>86.38\textsuperscript{a}</td>
<td>11.48\textsuperscript{c}</td>
<td>11.96\textsuperscript{c}</td>
<td>131.75\textsuperscript{c}</td>
</tr>
<tr>
<td>CD (p=0.05)</td>
<td>4.6</td>
<td>3.0</td>
<td>4.3</td>
<td>9.0</td>
<td>85.5</td>
</tr>
</tbody>
</table>

DAP - Days after transplanting
Means followed by a common letter in a column are not significantly different at 5 \% level by DMRT
Mean of 3 replications

\textbf{REFERENCES}


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