Antagonistic Bioactivity of Endophytic Actinomycetes Isolated from Medicinal Plants

M. Gangwar¹*, S. Dogra² and N. Sharma³

¹,²*Department of Microbiology, Punjab Agricultural University, Ludhiana-141004, India.
³Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141004, India.

Abstract: Endophytic actinomycetes are promising biocontrol agents for use in agriculture and have been isolated from various plant species. In the present study, 40 endophytic actinomycetes were isolated from roots, stems and leaves of three medicinal plants viz. Aloe vera, Mentha arvensis and Ocimum sanctum. The identification revealed that the majority of the isolates were Streptomyces spp. and the rest were identified as Saccharopolyspora spp., Micromonospora spp. and Actinopolyspora spp. The dual tests revealed that nine endophytic actinomycete isolates displayed a wide spectrum activity against nine fungal phytopathogens. Out of 8 isolates, 90% inhibited the growth of at least one or more phytopathogenic fungi and Saccharopolyspora 0-9 (Out of 8 isolates, 90% inhibited the growth of at least one or more phytopathogenic fungi and Saccharopolyspora 0-9 exhibited antagonistic activity against Aspergillus niger, Aspergillus flavus, Alternaria brassicicola, Botrytis cinerea, Penicillium digitatum, Fusarium oxysporum, Penicillium pinophilum, Phytophthora dresclea and Colletotrichum falcatus.

Keywords: Endophytic actinomycetes, Phytopathogenic fungi, Biocontrol agents, Antagonistic activity.

1. Introduction

Actinomycetes are known as producers of antibiotics and other biologically active substances with high commercial value such as vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors (Tanaka and Omura, 1993). Approximately two-thirds of naturally occurring antibiotics, including some of agricultural importance, have also been isolated from these soil microorganisms. Evidence indicated that actinomycetes are important in the rhizosphere because they can influence plant growth and protect plant roots against invasion by root pathogenic fungi (Crawford et al., 1993; Tokala et al., 2002). Actinomycetes are found also as endophytes that colonize the plant tissues. Actinomycetes have been isolated from surface sterilized roots of Italian native plants (Sardi et al., 1992), from roots and leaves of maize (De-Araujo et al., 2000), from roots and leaves of banana plants (Cao et al., 2004) and from surface sterilized wheat roots (Coombs and Franco, 2003). In vitro and in vivo antagonistic activities of endophytic actinomycetes against plant pathogens have been reported (Cao et al., 2005; Taechowisan et al., 2003; Tian et al., 2004). The introduction of endophytic actinomycetes into plants with the ability to colonize the internal tissue would further enhance the stability and increase their potential effectiveness as biocontrol agents (Coombs et al., 2004).

In order to find effective biocontrol agents for fungal plant pathogens, endophytic actinomycetes were isolated from surface-sterilized roots, stems and leaves of medicinal plants. The present study was undertaken with a view to testing the potential of endophytic actinomycetes as biocontrol agents against various phytopathogenic fungi.
2. Materials and Methods

2.1 In vitro antagonistic bioassay

The actinomycete isolates were evaluated for their activity towards nine pathogenic fungi: Aspergillus niger, Aspergillus flavus, Alternaria brassicicola, Botrytis cinerea, Penicillium digitatum, Fusarium oxysporum, Penicillium pinophilum, Phytophthora dresclea and Colletotrichum falcatum by dual-culture in vitro assay. Fungal discs (8mm in diameter), 5 days old on PDA at 28°C were placed at the center of PDA plates. Two actinomycete discs (8mm) 5 days old, grown on yeast malt extract (YM) incubated at 28°C were placed on opposite sides of the plates, 3cm away from fungal disc. Plates without the actinomycete disc serve as controls. All the plates were incubated at 28°C for 14 days and colony growth inhibition (%) was calculated by using the formula: C – T/C x 100, where C is the colony growth of pathogen and actinomycete isolates (Khamna et al., 2009).

3. Results and Discussion

3.1 Antifungal activities

Eight (20%) of actinomycete isolates were active against at least one of the nine phytopathogenic fungi (Table 1).

Different isolates of Streptomyces spp. displayed an array of activity against pathogenic fungi, particularly S. albosporus A4, an Aloe vera isolate and O-11, an Ocimum sanctum isolate. This is in conformity with the results of several studies carried out by other investigators (Crawford et al., 1993; Taechowisan and Lumyong, 2003; Tian et al., 2004; Verma et al., 2009). Saccharopolyspora O-9 from Ocimum sanctum strongly inhibited all of the pathogenic fungi (Fig. 1) with maximum percent inhibition was observed against the fungus Penicillium digitatum (71.4%).

Actinomycetes-fungus antagonism has been demonstrated for a variety of plant pathogens such as Alternaria, Rhizoctonia, Verticillium, Fusarium, Phytophthora and Pythium spp. (Yuan and Crawford, 1995; Aghighi et al., 2004). The ability of isolates to inhibit the growth of fungal pathogens is implication of the volatile secondary metabolites secreted by actinomycetes. So, in the present study, the potential of endophytic actinomycetes to inhibit the growth of pathogens has been studied. Dual-culture assays showed that some actinomycetes isolated can be developed as potential biocontrol agents. Therefore, further studies are necessary to assess the ability of the isolates to confer protection against pathogens and their role in enhancing growth and yield of plants under field conditions.

In conclusion, all but one of our Streptomyces isolates tested displayed antifungal activity. In addition, to Streptomyces, the medicinal plants carried rare actinomycetes all of which displayed antifungal activity with Saccharopolyspora being the potent antagonist. Our survey suggested that medicinal plants are a potent source of endophytic actinomycetes with wide biological activity against pathogenic fungi.

Table 1. Antifungal activity of actinomycete isolates.

<table>
<thead>
<tr>
<th>Actinomycete isolates</th>
<th>Aspergillus niger</th>
<th>Colletotrichum falcatum</th>
<th>Aspergillus flavus</th>
<th>Alternaria brassicicola</th>
<th>Penicillium digitatum</th>
<th>Fusarium oxysporum</th>
<th>Penicillium pinophilum</th>
<th>Phytophthora dresclea</th>
<th>Botrytis cinerea</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micromonaspora O-14</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>62.2 ± 0.1</td>
<td>19.5 ± 0.1</td>
<td>12.6 ± 0.1</td>
<td>0</td>
<td>16 ± 0.1</td>
<td>16.1 ± 0.1</td>
</tr>
<tr>
<td>S. viridis A3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39.6 ± 0.2</td>
<td>22.8 ± 0.1</td>
<td>45.8 ± 0.1</td>
<td>0</td>
<td>17.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td><em>S. albosporus A4</em></td>
<td>11.4 ± 0.2</td>
<td>14.5 ± 0.1</td>
<td>0</td>
<td>63.5 ± 0.1</td>
<td>60.6 ± 0.1</td>
<td>19.8 ± 0.2</td>
<td>0</td>
<td>69.3 ± 0.3</td>
<td>57.9 ± 0.1</td>
</tr>
<tr>
<td>S. cinereus A6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42.4 ± 0.08</td>
<td>17.6 ± 0.1</td>
<td>34.5 ± 0.2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Micromonaspora A9</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40.3 ± 0.2</td>
<td>48.5 ± 0.2</td>
<td>13.9 ± 0.1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>S. cinereus O-1</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.3 ± 0.1</td>
<td>15.2 ± 0.1</td>
<td>0</td>
<td>0</td>
<td>42.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td><em>Saccharopolyspora O-9</em></td>
<td>13.2 ± 0.1</td>
<td>28.6 ± 0.1</td>
<td>26.5 ± 0.2</td>
<td>30.3 ± 0.1</td>
<td>71.4 ± 0.2</td>
<td>51.4 ± 0.1</td>
<td>17.8 ± 0.1</td>
<td>56.4 ± 0.1</td>
<td>49.9 ± 0.1</td>
</tr>
<tr>
<td><em>S. albosporus O-11</em></td>
<td>16.7 ± 0.1</td>
<td>17.5 ± 0.2</td>
<td>0</td>
<td>44.6 ± 0.2</td>
<td>18.6 ± 0.1</td>
<td>59.5 ± 0.2</td>
<td>0</td>
<td>53.5 ± 0.2</td>
<td>53.4 ± 0.2</td>
</tr>
</tbody>
</table>

*Average ± standard error from triplicate samples*
Antagonistic Bioactivity of Endophytic Actinomycetes

Fig. 1. Inhibition effect of Saccharopolyspora O-9 against Penicillium digitatum (A), Fusarium oxysporum (B), Aspergillus niger (C), Colletotrichum falcatus (D), Botrytis cinerea (E), Aspergillus flavus (F), Phytophthora dresciea (G), Alternaria brassicicola (H) and Penicillium pinophilum (I), Penicillium digitatum

References


