Evaluation of the toxicity of *Streptomyces aburaviensis* (R9) extract towards various agricultural pests

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**ABSTRACT**

The dichloromethane extract of culture filtrate from *Streptomyces aburaviensis* R9 was evaluated using various rapid bioassays to determine potential inhibitory effects towards phytopathogenic fungi (*Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioids*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phomopsis viticola* and *P. obscurans*), fish bacterial pathogens (*Edwardsiella ictaluri* and *Flavobacterium columnare*), a green alga (*Selenastrum capricornutum*), plant seeds [Bent grass (*Agrostis* sp.) monocot and lettuce (*Lactuca sativa*) dicot] and 2-methylisoborneol (MIB)-producing cyanobacteria (*Planktothrix perornata* and *Pseudanabaena* sp.). The dichloromethane extract showed selective inhibition against the cyanobacterium *P. perornata*, with a lowest-complete-inhibition concentration (LCIC) of 10 mg/L and lowest-observed-effect concentration (LOEC) of 10 mg/L while LCIC and LOEC values were 100 mg/L when tested against *S. capricornutum*. This extract also showed slight meristematic cytogenic necrosis at 200 mg/L towards germinated seeds of both test plants. The compounds were not very toxic towards the channel catfish (*Ictalurus punctatus*) pathogenic bacteria *E. ictaluri* and *F. columnare*. Preliminary evaluation of the extract toward *C. acutatum*, *C. fragariae* and *C. gloeosporioids* using TLC bioautography revealed moderate activity. However, further evaluation of the extract using a microtiter plate bioassay determined that inhibition was strongest against *C. acutatum* and *C. fragariae*, though this inhibitory activity diminished at 72 hours and was moderately less active than the commercial fungicides azoxystrobin and captan when comparing 1 - 100 mg/L levels at 48 hours.

**Keywords:** Algae; Catfish; Cyanobacteria; Fungi; Pathogens; Streptomyces

**1. INTRODUCTION**

Biological methods and technologies in coordination with agricultural production [1,2] were established to solve problems of the deleterious effects of chemical fertilizers and pesticides on the environment and the appearance of organisms with resistance resulting from the high application rates of currently used agrochemicals [3-5]. Such chemicals are widely used despite the growing public concerns of several undesirable consequences of using them such as accumulation in the environment, biomagnification and excessive persistence [6].

Microorganisms are ubiquitous in nature and well-known to produce bioactive materials of particular practical value. Among these beneficial microorganisms are the actinomycetes, a group of filamentous bacteria which include many species that are characterized by the production of important extracellular bioactive compounds including antibiotics [7,8]. The majority of those isolates producing bioactive compounds belong to species within the genus *Streptomyces*, and several *Streptomyces* spp. have been advocated as promising biocontrol agents against several phytopathogenic fungi and bacteria [9-14]. In addition, members of the genus *Streptomyces* are well known for their potential to produce herbicides [5,15]. Natural compounds, such as the secondary metabolites of actinomycetes and particularly streptomycetes, have been demonstrated to be bioherbicides and include anisomycin, bialaphos, herbicidans A and B [5]. Therefore, attempts have been made to isolate streptomycetes with bioherbicidal properties.

Several studies have previously been conducted with soil streptomycetes isolated in Jordan for their potential to produce antibiotics [16-18]. In a recent study by
Bataineh et al. [19], the herbicidal activity of streptomycetes isolated from different habitats in Jordan were evaluated for their phytotoxic potential against common broad leaf and grass. In addition, the optimal medium suitable for higher phytotoxic activity in the culture filtrate of isolates and the method for extraction of potential phytotoxic compounds were investigated.

The present study was conducted to extend that investigation by determining the activity of the cell-free culture extract of one streptomycete isolate (Streptomyces aburaviensis R9) against various agricultural pests. In addition to potential herbicidal activity, the extract was evaluated using rapid bioassays for activity against off-flavor compound-producing species of cyanobacteria (blue-green algae), bacterial pathogens of catfish, and fungal phytopathogens in order to determine if other useful bioactive compounds (toxins) were produced by S. aburaviensis R9.

2. MATERIALS AND METHODS

2.1. Extract Preparation

Extracts from S. aburaviensis R9 were obtained according to Mallik [15]. Isolate R9 (7-day growth) from an agar plate was used to inoculate a flask containing 25 mL of GPM broth (10 g/L glucose, 5.0 g/L peptone and 20.0 g/L molasses). This primary culture was incubated at 28°C with shaking at 110 rpm for 5 days inside an orbital shaker incubator (TEQ, Portugal), after which the primary culture was used to inoculate a 500-mL mass culture of GPM broth. The mass culture was incubated for 7 days under the same conditions used for the primary culture. Mass culture cell-free filtrate was extracted with 100% dichloromethane (1:3 v/v) (Acros Organics, USA). The solvent was evaporated using a rotary evaporator (Heidolph, Germany) at 29°C. The residues were then dissolved in 4 mL 100% dichloromethane (HPLC grade) to provide stock solutions of 2.0, 20.0, 200.0, and 2000.0 mg/L. Absorbance measurements of microplate wells were made every 24 h for 4 days using a Packard model SpectraCount microplate photometer (Packard Instrument Co., Downers Grove, Illinois, USA) at 650 nm. Three replications were used for each extract and control concentration and experiments were repeated. Mean absorbance measurements were calculated and graphed to determine the lowest-observed-effect concentration (LOEC) and lowest-complete-inhibition concentration (LCIC).

2.2. Herbicide Bioassay

A standardized 24-well microtiter plate assay [20] was used to determine the R9 extract activity towards monocot (Agrostis stolonifera L. bentgrass) and dicot (Lactuca sativa L. lettuce) representative plant types. The wells in the microplate were prepared by adding 20 μL of each of the test solutions of the extract to filter paper, allowing the filter paper to dry and then adding 200 μL of water. Final exposure concentrations were 0.2, 2.0, 20.0, and 200.0 mg/L.

2.3. Algaecide Bioassay

The methods outlined in Schrader et al. [21] were used to evaluate the crude extract for selective toxicity towards the 2-methylisoborneol (MIB)-producing-cyanobacteria [Planktothrix perornata [Skuja] Anagnostidis and Komárek (syn. Oscillatoria perornata)], isolated from a Mississippi, USA, catfish pond, and Pseudanabaena sp. (strain LW397) [Skuja] Anagnostidis and Komárek, isolated from a municipal drinking water reservoir in Virginia, USA. The green alga (Selenastrum capricornutum) [Printz] was included as a representative of division Chlorophyta and to determine selective toxicity of the test extract.

The crude extract of S. aburaviensis R9 and the extract of the media (control) were dissolved separately in 100% dichloromethane (HPLC grade) to provide stock solutions of 2.0, 20.0, 200.0, and 2000.0 mg/L. Final test concentrations in microplate wells were 0.1, 1.0, 10.0, and 100.0 mg/L. Absorbance measurements of microplate wells were made every 24 h for 4 days using a Packard model SpectraCount microplate photometer (Packard Instrument Co., Downers Grove, Illinois, USA) at 650 nm. Three replications were used for each extract and control concentration and experiments were repeated. Mean absorbance measurements were calculated and graphed to determine the lowest-observed-effect concentration (LOEC) and lowest-complete-inhibition concentration (LCIC).

2.4. Bactericide Bioassay

The same procedures used by Schrader and Harries [22] were used to evaluate the crude extract of S. aburaviensis R9 for antibacterial activity towards the catfish pathogenic bacteria Edwardsiella ictaluri and Flavobacterium columnare isolate 1016, except that a 96-well quartz microplate (Hellma Cells, Inc., Forest Hills, New York, USA) was used to perform the bioassay because the dichloromethane loading solvent is incompatible with polystyrene microplates. Stock solutions and final concentrations of the crude extract and media extract (control) were the same as those used in the algaecide bioassay.

2.5. Fungicide Bioassay

Isolates of Colletotrichum acutatum Simmonds, Colletotrichum fragariae Brooks, and Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. in Penz. were obtained from Barabara J. Smith, USDA, ARS, Small Fruit Research Station, Poplarville, Mississippi, USA. Colletotrichum fragariae (isolate CF63), C. acutatum (isolate CAGoff), and C. gloeosporioides (isolate CG162) were used for the pathogen and bioautography studies. Isolate
CF63 is one of the most virulent isolates that infects strawberry plants and induces both crown and fruit rot [23]. CF63, CAGoff, and CG162 were used as standard test isolates because of our extensive knowledge of these isolates and their known fungicide sensitivity profiles in both bioautography and microtiter formats. The three Colletotrichum species were isolated from strawberry (Fragaria × ananassa Duchesne). Botrytis cinerea Pers.:Fr, was isolated from commercial grape (Vitis vinifera L.) and Fusarium oxysporum Schlechtend:Fr from orchid (Cynoches sp.) by D. E. Wedge, USDA ARS Natural Products Utilization Research Unit, University, Mississippi, USA. Phomopsis viticola (Sacc.) and P. obscurans (Ellis and Everh) Sutton were obtained from Mike A. Ellis, Ohio State University, Wooster, Ohio, USA.

Photoperiod under 60°C ± 5 µmols·sec−1 light) with 12 h photoperiod and under cool-white fluorescent lights (55 ± 5 µmols·m−2·sec−1 light) with 12 h photoperiod. Conidia of Colletotrichum fragariae and C. gloeosporioides were isolated from strawberry plants and induces both crown and fruit rot [23]. CF63, CAGoff, and CG162 were used as standard test isolates because of our extensive knowledge of these isolates and their known fungicide sensitivity profiles in both bioautography and microtiter formats. The three Colletotrichum species were isolated from strawberry (Fragaria × ananassa Duchesne). Botrytis cinerea Pers.:Fr, was isolated from commercial grape (Vitis vinifera L.) and Fusarium oxysporum Schlechtend:Fr from orchid (Cynoches sp.) by D. E. Wedge, USDA ARS Natural Products Utilization Research Unit, University, Mississippi, USA. Phomopsis viticola (Sacc.) and P. obscurans (Ellis and Everh) Sutton were obtained from Mike A. Ellis, Ohio State University, Wooster, Ohio, USA. Fungi were grown on potato-dextrose agar (PDA, Difco, Detroit MI) in 9 cm petri dishes and incubated in a growth chamber at 24°C ± 2°C and under cool-white fluorescent lights (55 ± 5 µmols·m−2·sec−1 light) with 12 h photoperiod. Conidia were harvested from 7 - 10 day-old cultures by flooding plates with 5 mL of sterile distilled water and dislodging conidia by softly brushing the colonies with an L-shaped glass rod. Conidial suspensions were filtered through sterile miracloth (Calbiochem-Novabiochem Corp., La Jolla, California, USA) to remove mycelia. Conidia concentrations were determined photometrically, from a standard curve based on the absorbance at 625 nm and suspensions were then adjusted with sterile distilled water to a concentration of 1.0 × 10^6 conidia/mL.

Bioautography procedures of Meeaza et al. [24] and Tabanca et al. [25] for detection of naturally occurring antifungal agents were used to evaluate antifungal activity of the dried dichloromethane cell-free culture extract of R9 and GPM broth. Samples were prepared in dichloromethane at a concentration of 2000 mg/L and 4 and 8 µL were used in a dose-response to evaluate each sample. Conidia of Colletotrichum fragariae, C. acutatum and C. gloeosporioides suspensions were adjusted to 3.0 × 10^5 conidia/mL with liquid potato-dextrose broth (PDB, Difco, Detroit, Michigan, USA) and 0.1% Tween-80. Using a sterile 50-mL chromatographic sprayer, each glass silica-gel thin-layer chromatography (TLC) plate with fluorescent indicator (250 mm, silica gel GF Uniplate, Analtech, Inc., Newark, Delaware, USA) was sprayed lightly (to dampness) three times with the conidial suspension. Inoculated plates were placed in a 30 × 13 × 7.5 cm moisture chamber (100% RH, 398-C, Pioneer Plastics, Inc., Dixon, Kentucky, USA) and incubated in a growth chamber at 24°C ± 1°C and 12 h photoperiod under 60 ± 5 µmols·m−2·sec−1 light. Inhibition of fungal growth was measured 4 d after treatment.

Sensitivity of each fungal species to each test compound was determined by comparing size of inhibitory zones. Means and standard deviations of inhibitory zone size were used to evaluate antifungal activity of essential oils, solvent fractions, and pure compounds. Bioautography experiments were performed multiple times using both dose- and non-dose-response formats. Fungicide technical grade standards benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc., West Chester, Pennsylvania, USA) were used as controls at 2.0 mM in 2.0 µL of ethanol.

A standardized 96-well microtiter plate assay developed for discovery of natural product fungicidal agents [26,27] was used to determine sensitivity of B. cinerea, C. acutatum, C. fragariae, C. gloeosporioides, F. oxysporum, Phomopsis viticola, and P. obscurans to the various antifungal compounds in comparison with known fungicidal standards. Captan and azoxystrobin were used as standards in this experiment. Each fungus was challenged in a 6-point dose-response format using test compounds where the final treatment concentrations were 0.01, 0.1, 1.0, 10.0 and 100.0 mg/L. Microtiter 96-well quartz plates (Hellma Cells, Inc., Forest Hills, New York, USA) were covered with a plastic lid and incubated in a growth chamber as described previously for fungal growth. Growth was then evaluated by measuring absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count).

Chemical sensitivity of each fungus was evaluated using 96-well plate microbioassay format. Each chemical was evaluated in duplicate at each dose (0.01, 0.1, 1.0, 10.0 and 100.0 mg/L). Mean absorbance values and standard errors were used to evaluate fungal growth at 48 h and 72 h, except for P. obscurans and P. viticola the data were recorded at 120 h. Analysis of variance of means for percent inhibition of each fungus at each dose of test compound (n = 4) relative to the untreated positive growth controls (n = 32) were used to evaluate fungal growth inhibition. Each test fungicide was run in duplicate at each concentration, and the experiment was repeated three times.

3. RESULTS AND DISCUSSION

3.1. Extract Yields from Cultures of Streptomyces aburaviensis R9

Table 1 shows the yield of the concentrated dichloromethane cell-free extracted broth (mg) of S. aburaviensis R9 culture from different batches. An average of 138 mg of dried cell-free dichloromethane crude extract was obtained per 500 mL of GPM culture broth. However, the media extract (control) yielded an average 120 mg of dried extract per the same volume. This difference in the
3.2. Herbicide Bioassay

In the study by Bataineh et al. [19], a total of 231 different soil Streptomyces isolates were assessed for their phytotoxic activity on seeds of cucumber and ryegrass on the basis of suppressed seed germination, discoloration of the root tip, reduced root and shoot growth and eventual death of the root. The phytotoxicity symptoms observed in the study by Bataineh et al. [19] were represented by discoloration and death of the root tips, and these symptoms were profoundly evident when the same isolate used in the current study, S. aburaviensis R9, was evaluated for activity. The results suggested that the phytotoxic effect is cytotoxic and affects the meristematic cells due to production of an extracellular agent(s) or toxin(s) by the bacterium.

The results of the current investigation determined that there was phytotoxic activity of the dichloromethane culture filtrate crude extract at 200 mg/L towards the germinated seeds of both test-plant types [A. stolonifera (monocot) and L. sativa (dicot)] (Table 2). None of the lower test concentrations of the S. aburaviensis R9 extract were phytotoxic. In accordance with the bioassay protocol, test results were reported in tenfold dilutions of the test material (e.g., extract). Overall, the results from this bioassay indicate that the S. aburaviensis R9 extract was weakly phytotoxic activity towards both the monocot and dicot plants tested because only the highest test concentration inhibited growth.

3.3. Algaecide Bioassay

The dichloromethane extract showed selective toxicity against P. perornata with a lowest-complete-inhibition concentration (LCIC) of 10 mg/L and lowest-observed-effect concentration (LOEC) of 10 mg/L (Table 3). For S. capricornutum, the LCIC and LOEC values for the R9 extract were 100 mg/L, an order of magnitude less toxic which exemplifies the selective toxicity of the extract. It is interesting to note that the Pseudanabaena sp. was inhibited by 10 mg/L of the GPM broth medium extract (without S. aburaviensis-R9 culture). Therefore, an ingredient in this media appears to be toxic towards this species of cyanobacteria. However, such effect against the green alga S. capricornutum was only at 100 mg/L as indicated by the LCIC and LOEC results. In accordance with the bioassay protocol, test results were reported in tenfold dilutions of the test material (e.g., extract).

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Table 1. Yield of the concentrated dichloromethane cell-free extracted broth (mg) of S. aburaviensis R9 culture from different batches.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Average weight</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126</td>
<td>154</td>
<td>141</td>
<td>140 ± 14</td>
<td>110</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>114</td>
<td>107</td>
<td>136 ± 44</td>
<td>130</td>
</tr>
</tbody>
</table>

*The control and tests volumes were 500 mL; †The average ± standard deviation were calculated from the tests of the same batch.

Table 2. Growth observation of monocot and dicot plants at different concentrations of the concentrated dichloromethane cell-free extracted broth of S. aburaviensis R9 culture.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>R9 culture filtrate extract</th>
<th>GPM broth medium extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactuca sativa L (lettuce, dicot)</td>
<td>Agrostis stolonifera L (bentgrass, monocot)</td>
</tr>
<tr>
<td>0.2</td>
<td>0†</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>200.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†0: No effect, 1: Complete inhibition of growth; Growth was observed after 7 days.

Table 3. Activity of S. aburaviensis R9 culture filtrate extract on cyanobacteria and green alga as compared to the broth medium extract.

<table>
<thead>
<tr>
<th>Planktothrix perornata</th>
<th>Selenastrum capricornutum</th>
<th>Pseudanabaena LW397</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOEC†</td>
<td>LCIC‡</td>
<td>LOEC†</td>
</tr>
<tr>
<td>R9 culture filtrate extract</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>GPM broth medium extract</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

†LOEC: lowest-observed-effect concentration; ‡LCIC: lowest-complete-inhibition concentration.
3.4. Bactericide Bioassay

The extracts were not very toxic at the concentrations tested towards the catfish pathogenic bacteria *Edwardsiella ictaluri* and *Flavobacterium columnare* isolate 1016 (data not shown). For this bioassay, only activity below 100 mg/L for IC50 and MIC values is considered significant.

3.5. Fungicide Bioassay

Results of the preliminary evaluation of the *S. aburaviensis* R9 extract using TLC plates on *Colletotrichum acutatum* (CaGoff); *C. fragariae* (Cf63) and *C. gloeosporioides* (CG162) showed strong activity against these fungal isolates. However, results from the microtiter-plate bioassay found the most significant activity was towards *C. acutatum* and *C. fragariae* (Table 4). Generally, as the test concentration of culture extract increased, the percentage of growth inhibition of *C. acutatum* and *C. fragariae* increased. Also, the culture extract was moderately less active than the commercial fungicides azoxystrobin and captan when comparing 1, 10 and 100 mg/L treatments at 48 h. There was less inhibition of *C. acutatum* and *C. fragariae* by the culture extract at 1, 10, and 100 mg/L at 72 h compared to 48 h results (Table 4). This loss of activity could be due to hydrolyzation of the active compound by the fungal species, thereby making it less active. This can occur with many commercial fungicides which actually make them more fungistatic than fungicidal at the concentrations that they are applied. However, it is somewhat difficult to infer a direct comparison since we are comparing a crude extract to pure compounds. The variations of percent growth inhibition observed for the different dilutions of the control broth were attributed to incomplete solubilization of the control broth extract that was encountered when conducting the fungicide bioassay. It is interesting that the broth medium extract also inhibited the cyanobacterium *Pseudanabaena* sp. used in this study.

Table 4. Evaluation of the crude extract of *S. aburaviensis* R9 culture fractions for fungicidal activity towards different fungal phytopathogens at different concentrations as compared to known fungicidal standards, azoxystrobin and captan.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Sample</th>
<th>% Inhibitiona,b</th>
<th>C. acutatum</th>
<th>C. fragariae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture extract</td>
<td>4.5 (0)</td>
<td>1.0 (1.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control broth</td>
<td>10.0 (7.2)</td>
<td>12.4 (8.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin*</td>
<td>5.3 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Captan*</td>
<td>2.7 (0)</td>
<td>4.5 (7.3)</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>Culture extract</td>
<td>17.4 (18.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control broth</td>
<td>0 (0)</td>
<td>22.3 (20.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin</td>
<td>37.6 (6.7)</td>
<td>47.5 (36.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td>5.1 (0)</td>
<td>19.3 (14.8)</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>Culture extract</td>
<td>5.1 (0)</td>
<td>21.0 (15.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control broth</td>
<td>5.9 (14.3)</td>
<td>0 (3.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin</td>
<td>75.2 (26.9)</td>
<td>77.2 (58.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td>12.2 (2.2)</td>
<td>26.6 (21.8)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Culture extract</td>
<td>13.8 (0.06)</td>
<td>53.9 (30.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control broth</td>
<td>0 (0)</td>
<td>25.3 (25.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin</td>
<td>77.9 (39)</td>
<td>76.98 (63.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td>72.5 (43.9)</td>
<td>59.4 (9.5)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Culture extract</td>
<td>45.5 (0)</td>
<td>66.4 (14.0)</td>
<td></td>
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<tr>
<td></td>
<td>Control broth</td>
<td>0 (0)</td>
<td>21.3 (22.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin</td>
<td>86.6 (64.7)</td>
<td>82.0 (68.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td>100.0 (95.6)</td>
<td>100.0 (100.0)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Culture extract</td>
<td>78.7 (27.4)</td>
<td>91.6 (20.8)</td>
<td></td>
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<tr>
<td></td>
<td>Control broth</td>
<td>12.3 (15.0)</td>
<td>17.0 (24.3)</td>
<td></td>
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<tr>
<td></td>
<td>Azoxystrobin</td>
<td>88.7 (78.0)</td>
<td>85.9 (73.5)</td>
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</tr>
<tr>
<td></td>
<td>Captan</td>
<td>98.1 (98.0)</td>
<td>99.2 (99.8)</td>
<td></td>
</tr>
</tbody>
</table>

aSample results only indicate inhibition. Zero (0) does not indicate the degree of stimulation, only that there was no inhibition. bNumbers not enclosed in parentheses = 48 h results; numbers enclosed in parentheses = 72 h results. cInternal standard compound(s) utilized in the 96-well assay.
The antifungal activity of the culture extract is likely
due to the production of an antibiotic, though such a
metabolite produced by S. aburaviensis R9 appears to
not be a broad-spectrum antibiotic, but more specific
towards certain species of fungi. While growth inhibition
of some of the test species of fungi (e.g., Colletotrichum
acutatum (CaGoff); C. fragariae (Cf63) and C. gloeo-
sporioides (CG162) occurred in the presence of the cul-
ture extract, there was little to no activity towards the
Gram-negative bacteria fish pathogens used in this study
(Edwardsiella ictaluri and Flavobacterium columnare
isolate 1016). A previous study by Raytapadar and Paul
[28] determined that another isolate of Streptomyces
aburaviensis var. ablastmyceticus (MTCC 2469). An-
other study by Thumar et al. [29] identified antibiotic
production by a halotolerant alkaliphilic Streptomyces
aburaviensis strain Kut-8 that inhibited the growth of the
Gram-positive bacterium Bacillus subtilis. At present, it
is unknown if the antibiotic production of S. aburavien-
sis R9 is similar or identical to those cited in the previ-
ous studies above. Future isolation and characterization
of the active antifungal metabolite(s) would determine
these properties. Additional studies would also aid in
determining if the active antifungal metabolite(s) pro-
duced by S. aburaviensis R9 is also responsible for the
phytotoxic activity observed towards the plants A. stolo-
nifera and L. sativa and the cyanobacterium P. perornata.

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REFERENCES

able agriculture. Scientific American, 262, 112-120.
doi:10.1038/scientificamerican0690-112
amendments: A historical perspective. American Journal of
Alternative Agriculture, 7, 181-189.
doi:10.1017/S0889189300004781
the control of oomycetes. Annual Review of Phytopath-
ology, 24, 311-338.
Natural products as pesticides: Two examples of strep-rose-
doi:10.1002/(SICI)1096-9063(199601)46:1<37::AID-PS
339>3.0.CO;2-K
of soil microorganisms for metabolites with herbicidal
doi:10.1300/J144v04n02_07
ty of the antibiotics geldanamycin and nigericin. Journal
of Plant Growth Regulation, 9, 19-25.
doi:10.1007/BF02041937
for mankind. In: Parr, J.F., Hornick, S.B. and Whitman,
S.E., Eds., Proceedings of the 1st International Conference
on Kyusei Nature Farming, USDA, Washington, DC,
8-14.
producing Streptomyces. Trends in Microbiology, 6,
76-83. doi:10.1016/S0966-842X(97)01161-X
[9] El-Trabili, K.A., St. J. Hardy, G.E., Sivasithamparam, K.,
for the biological control of cavity-spot disease of carrots,
caused by Pythium coloratum, by streptomycete and
non-streptomycete actinomycines. New Phytopathologist,
137, 495-507. doi:10.1046/j.1469-8137.1997.00856.x
[10] El-Tarabily, K., Soliman, M., Nassar, A., Al-Hassani, H.,
Sivasithamparam, K., McKenna, F. and St. J. Hardy, G.E.
(2000) Biological control of Sclerotinia minor using a
chitinolytic bacterium and actinomycines. Plant Pathology,
49, 573-583. doi:10.1046/j.1365-3059.2000.00494.x
from Jordan soils active against Agrobacterium tumefa-
ciens. Actinomycines, 8, 29-36.
[12] Saadoun, I., Hameed, K., Al-Momani, F., Malkawi, H.,
Meqdam, M. and Mohammad, M.J. (2000) Characteriza-
tion and analysis of antifungal activity of soil streptomy-
cetes isolated from North Jordan. Egyptian Journal of
Microbiology, 35, 463-471.
[13] Seto, H., Fujioka, T., Furihatha, K., Kaneko, J. and Ta-
kakashi, S. (1989) Structure of complestan a very
strong inhibitor of protease activity of complement in the
human complement system. Tetrahedron Letters, 37, 4987-
4990. doi:10.1016/S0040-4039(01)80562-1
(2006) Biological control of Sclerotinia sclerotiorum
using indigenous chitinolytic actinomyctes in Jordan. The
Plant Pathology Journal, 22, 107-114.
with potential for phytotoxin production. Journal of
Chemical Ecology, 23, 2683-2693.
doi:10.1023/A:10225027224711
[16] Saadoun, I., Al-Momani, F., Malkawi, H. and Mohammad,
M.J. (1999) Isolation, identification and analysis of anti-
bacterial activity of soil streptomyctes isolates from
North Jordan. Microbios, 100, 41-46.
flora of Badia region of Jordan and its potential as a
source of antibiotics active against antibiotic-resistant
[18] Saadoun, I., Wahiby, L., Ababneh, Q., Jaradat, Z., Mas-
streptomyctes from arid habitats in Jordan and their po-
tential to inhibit multi-drug resistant Pseudomonas aer-
ginosa pathogens. World Journal of Microbiology and
Biotechnology, 24, 157-162.


