UV/VIS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF STREPTOMYCES ISOLATES

Slavica B. Ilić, Sandra S. Konstantinović, Zoran B. Todorović

Faculty of Technology, University of Niš, Leskovac, Serbia and Montenegro
E-mail: ilicslavica@yahoo.com

Summary. Twenty different Streptomyces isolates were gained from the soils of Southeastern Serbia. Nine isolates showed a strong activity against Bortrytis cinerea, a parasite found in domestic vine. These isolates where extensively studied for their in vitro anti-microbial activity against Gram-positive and Gram-negative bacteria and yeasts. The results indicated that obtained isolates were highly active against Bortrytis cinerea, Herpes simplex and Candida albicans with an inhibition zone at ≥ 31 mm. Five of these isolates were identified as Streptomyces hygroscopicus (SH100, SH101, SH102, SH103, SH104). The UV spectra of the culture extracts for the active isolates showed absorbance peaks ranging between 221 and 240 nm. Two bioactive regions were detected on the TLC plate (Rf 0.70 and 0.88). The UV spectra of the active compounds in methanol showed peaks at 217 and 221 nm.

Key words: Anti-microbial activity, antibiotics, Streptomyces

Introduction

Streptomycetes, the gram (+) filamentous bacteria, are widely distributed in a variety of natural and man-made environments, constituting a significant component of the microbial population in most soils (1). The results of extensive screenings have led to the discovery of about 4,000 antibiotic substances from bacteria and fungi, many of which have been applied in human medicine, veterinary and agriculture. Most of them are produced from Streptomyces (2). Most Streptomyces and Actinomycetes are used in the production of a diverse array of antibiotics including aminoglycosides, macrolides, β-lactams, peptides, polyenes, polyether, tetracyclines, etc. In searching for new antibiotics, over 1,000 different bacteria, Actinomycetes, Streptomyces, fungi and algae have been investigated. To prevent exponential emergence of microorganisms from becoming resistant to the clinically available antibiotics already marketed, a periodic replace of the existing antibiotics is necessary. In the present study, the isolation and characterization, as well as the anti-microbial activity of local Streptomyces isolates, were studied.

Materials and Methods

The Streptomyces used in this study were isolated from the soils of Southeaster Serbian region. Soil from different places of Southeastern Serbia was brought to the laboratory in aseptic condition. Streptomyces from the soil had been isolated by pour plate technique on Starch-casein agar and Glycerol-arginine agar after serial dilution in distilled water. Dry colonies of Streptomyces were selected and isolated. Thus isolated colonies were preserved in Glycerol based media and stored at 20°C. The anti-microbial activity to be determined was revived by streaking on Starch-casein agar and incubated at 28°C for 7 days.

Characterization of the isolates: All strains were cultivated on the ISP 2 medium. Some diagnostic properties of highly active Streptomyces strains were determined following the directions given in the probabilistic identification matrix of Williams and Bergey’s Manual of Systematic Bacteriology (3). A Willcox probability matrix was used to assign and identify isolates where the scores of 0.8 and above indicated a positive identification (4).

Test microorganisms: Staphylococcus aureus, Staphylococcus epidermis, Bacillus anthracis, Bacillus subtilis, Proteus mirabilis, Proteus aureginosa, Escherichia coli, Enterococcus group D, Borthrytis cinerea, Staphylococcus lutea, Sacharomyces cerevisiae, Herpes simplex, and Candida albicans were used to determine the anti-microbial activity of isolated Streptomyces strains (Table 1). All these microorganisms were obtained from the Institute of Health Protection.

In vitro screening of isolates for antagonisms: Balanced sensitivity medium (BSM, Difco 1863) plates were prepared and inoculated with Streptomyces isolate by a single streak of inoculum in the center of the petri dish. After 4 days of incubation at 28°C, the plates were seeded with test organisms. The microbial interactions were analyzed by determining the inhibition zone.

Fermentation: Streptomyces hygroscopicus was grown in a 500cm³ shake flask containing 100cm³ of the culture medium (0.8g NaCl, 1g NH₄Cl, 0.1g KCl, 0.1g KH₂PO₄, 0.2g MgSO₄ •7H₂O, 0.04g CaCl₂ •2H₂O, 2g
UV/VIS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *STREPTOMYCES* ISOLATES

Table 1. Anti-microbial activity of *Streptomyces* isolates

<table>
<thead>
<tr>
<th>Test Microorganisms</th>
<th>SH100</th>
<th>SH101</th>
<th>SH102</th>
<th>SH103</th>
<th>SH104</th>
<th>SH105</th>
<th>SH106</th>
<th>SH107</th>
<th>SH108</th>
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</thead>
<tbody>
<tr>
<td>Botrytis cinerea</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus aureus ATTC6538</td>
<td>−</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>Staphylococcus epidermis MU29</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>−</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bacillus anthracis ATTC38</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>−</td>
<td>1</td>
<td>2</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Bacillus subtilis ATTC5538</td>
<td>−</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>2</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter aerogenes RSKK750</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli ATCC95</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>Proteus aureginosa RSKK102</td>
<td>3</td>
<td>−</td>
<td>1</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
<td>−</td>
<td>1</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Candida albicans ATTC10231</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>−</td>
<td>2</td>
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<tr>
<td>Saccharomyces cerevisiae RSKK102</td>
<td>3</td>
<td>−</td>
<td>3</td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>1</td>
<td>−</td>
<td>3</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
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</table>

*The inhibitory effect of the strains was divided into four groups according to the size of the inhibition zone as follows: passive group (≤10 mm); slightly active group (11-20 mm); moderately active group (21-30 mm) and highly active group (≥31 mm).

Results and Discussion

A total of 20 different *Streptomyces* isolates were recovered from 33 soil samples. Antibacterial activity was exhibited in 44.5% of all isolates, while nine isolates exhibited a very strong activity, especially against Botrytis cinerea, Bacillus subtilis, Candida albicans and Staphylococcus epidermis. Among these nine isolates, five of them (SH100, SH102, SH103, SH106, SH108) showed the best activity against selective microorganisms.

The UV spectral data for the ethyl acetate extract of selected active fermented broth are shown in Table 2.

Fig. 1. UV spectra of ethyl acetate extracts of fermentation broth
Maximum absorbance peaks range between 215-270nm and the characteristics of absorption peaks indicate a highly polyene nature (Figure 1) The spectral data are consistent with those obtained by Swaadoun et al. (5). Two bioactive regions were detected on the TLC plate (Rf 0.70 and 0.88). The bioactive compound exhibited a maximum UV absorption at 217 and 221nm in methanol (Figure 2). These strains produced either a broad-spectrum anti-microbial compound or several compounds with different activities.

Table 2. UV spectral data of ethyl acetate extract of fermentation broth

<table>
<thead>
<tr>
<th>Strain</th>
<th>λ_max (nm)</th>
<th>Shoulder (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH100</td>
<td>221, 262</td>
<td>274</td>
</tr>
<tr>
<td>SH102</td>
<td>226, 246</td>
<td>281</td>
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<tr>
<td>SH103</td>
<td>216</td>
<td>252, 317</td>
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<tr>
<td>SH106</td>
<td>221</td>
<td>272, 313</td>
</tr>
<tr>
<td>SH108</td>
<td>226, 260</td>
<td>231</td>
</tr>
</tbody>
</table>

Fig. 2. The UV spectrum of the active components in methanol

Streptomyces, gained from the soils of Southeastern Serbia, show a high activity against Bortrytis cinerea, a parasite found in vine and Herpes simplex.

Further investigation is needed in order to determine the structure of active components. On domestic market, not toxic biofungicides and teratogenes don’t exist. The preparation gained from the investigated isolates would have a great advantage over the existing commercial preparations.

References