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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 (R_f 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, *Actimomycetes, Streptomycetes*, 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 20^0C. The anti-microbial activity to be determined was revived by streaking on Starch-casein agar and incubated at 28^0C 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, Bacilus 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 28^0C, the plates were seeded with test organisms. The microbial interactions were analyzed by determining the inhibition zone.

Fermentation: *Streptomyces hygroscopicus* was grown in a $500cm^3$ shake flask containing $100cm^3$ of the culture medium (0.8g NaCl, 1g NH_4Cl, 0.1g KCl, 0.1g KH_2PO_4, 0.2g $MgSO_4 \cdot 7H_2O$, 0.04g $CaCl_2 \cdot 2H_2O$, 2g

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

Test Microorganisms	Isolates								
	SH100	SH101	SH102	SH103	SH104	SH105	SH106	SH107	SH108
Borthrytis cinerea	3	2	3	3	1	1	3	2	3
Staphylococcus aureus ATTC6538	–	1	–	–	–	1	3	1	3
Staphylococcus epidermis MU29	2	1	2	2	2	–	3	1	2
Bacillus anhtracis ATTC38	3	2	1	2	–	1	2	–	2
Bacillus subtilis ATTC5538	–	1	–	–	–	–	2	–	2
Enterobacter aerogenes RSKK750	2	–	–	–	–	–	–	–	2
Escherichia coli ATTC95	–	–	–	–	–	–	1	–	1
Proteus aureginosa RSKK102	3	–	1	1	–	–	–	–	–
Proteus mirabilis	1	–	1	2	–	–	–	–	1
Candida albicans ATTC10231	3	1	3	3	1	2	2	–	2
Saccharomyces cerevisiae RSKK102	3	–	3	3	–	–	1	–	3
Herpes simplex	3	1	3	3	2	1	3	2	3

* 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 (21-30mm) and highly active group (≥31 mm).

glucose, 3g yeast extract in 1dm^3 of distilled water, pH 7.3). Incubation was carried out at 28^{0}C for 120 hours under the standard condition of aeration and agitation. The formation of isolates was followed by the testing of the antibiotic activity at various times during the fermentation. For this procedure, each sample was mixed with an equal volume of methanol and the mixture was shaken for 2 hours and filtered. The filtrate was assayed by the paper disk method using *Bacillus subtilis* as a test organism.

Isolation of antibacterial metabolites: Antibacterial compound was recovered from the filtrate by solvent extraction with ethyl acetate. Ethyl acetate was added to the filtrate in the ratio 1:1 (v/v) and shaken vigorously for 1 hour for complete extraction. The ethyl acetate phase that contains an antibiotic was separated from the aqueous phase. It was evaporated to dryness in a water bath at 80^0-90^{0}C and the residue obtained was weighed. Thus obtained compound was used to determine the antimicrobial activity. Filter paper disks (6mm in diameter) were impregnated with extracted broth, dried and placed onto BSM plates previously seeded with *Bacillus subtilis*. The plates were incubated at 37^{0}C for 48h and examined for zones of inhibition and verified active substance extraction. The absorption spectrum of each active extract was determined in the UV region (200-400nm) by using a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer.

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 *Bortrytis cinerea, Herpes simplex, 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.

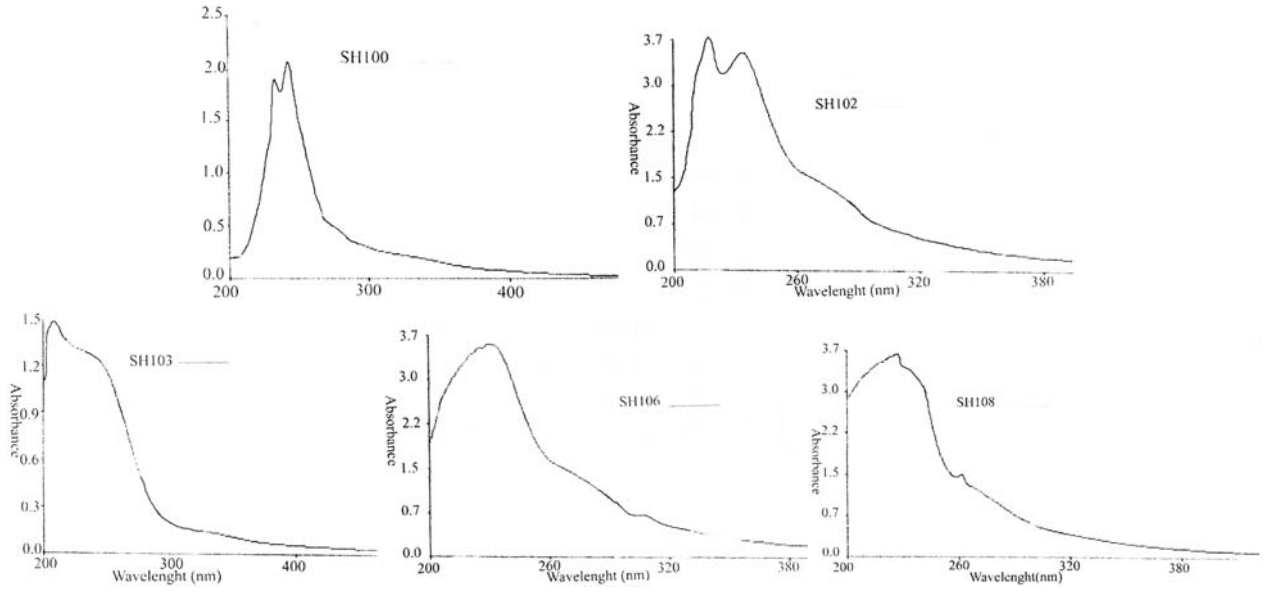

Fig. 1. UV spectra of ethyl acetate extracts of fermentation broth

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 (R_f 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

Strain	λ_{max} (nm)	Shoulder (nm)
SH100	221, 262	274
SH102	226, 246	281
SH103	216	252, 317
SH106	221	272, 313
SH108	226, 260	231

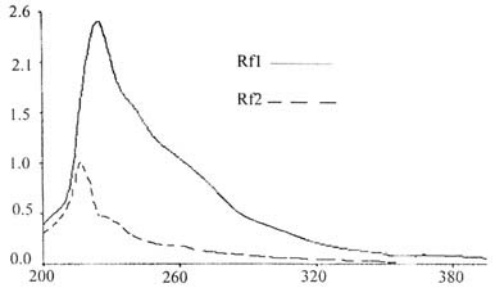

Fig. 2. The UV spectrum of the active components in methanol

Streptomyces, gained from the soils of Southeastern Serbia, show a high activity against *Borthrytis 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 biofugicides and teratogenes don't exist. The preparation gained from the investigated isolates would have a great advantage over the existing commercial preparations.

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UV/VIS ANALIZA ANTIMIKROBNA AKTIVNOST *STREPTOMYCES* IZOLATA

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

Tehnološki fakultet, Leskovac

Kratak sadržaj: *Dvadeset različitih izolata Streptomyces je dobijeno iz tla sa područja jugoistočne Srbije. Devet izolata pokazuje jaku aktivnost na Bortrytis cinerea, parazit nadjen u domaćem vinu. Ispitivana je antimikrobna aktivnost ovih izolata in vitro na Gram-pozitivne, Gram-negativne bakterije i gljive. Rezultati pokazuju da dobijeni izolati imaju veliku aktivnost na Bortrytis cinerea, Herpes simplex i Candida albicans sa zonama inhibicije ≥31 mm. Pet od ovih izolata su identifikovani kao Streptomyces hygroscopicus (SH100, SH101, SH102, SH103, SH104). UV spektri ekstrakta kulture za aktivne izolate pokazuju pik apsorbancije u oblasti izmedju 221 i 240 nm. Dva bioaktivna regiona su detektovana pomoću TLC (R_f 0,7 i 0,88). UV spektar aktivnih jedinjenja u metanolu pokazuje pikove na 217 i 221 nm.*

Ključne reči: *Antimikrobna aktivnost, antibiotici, Streptomyces*