Turkish Journal of Biology

Volume 27 | Number 2 Article 4

1-1-2003

Investigation of the Antimicrobial Activity of Some Streptomyces Isolates

NURETTİN ŞAHİN

AYSEL UĞUR

Turk J Biol
27 (2003) 79-84

Investigation of the Antimicrobial Activity of Some *Streptomyces* Isolates

Nurettin ŞAHİN*
Biology Section, Faculty of Education, Muğla University, 48170, Kötekli, Muğla - TURKEY
Aysel UĞUR
Department of Biology, Faculty of Arts and Sciences, Muğla University, 48170, Kötekli, Muğla - TURKEY

Received: 21.10.2002

Abstract: Seventy-four different *Streptomyces* isolates were obtained from the soils of Muğla province. Antimicrobial activity was determined in 45.9% of the isolates. Fifteen isolates showed strong activity against coagulase-negative *Staphylococcus* (CoNS). These isolates were extensively studied for their in vitro antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts. The results indicated that five isolates were highly active against CoNS and yeast with an inhibition zone at ≥ 20 mm. Three of these isolates were identified as *Streptomyces antibioticus* (MU106, MU107) and *S. rimosus* (MU114). The UV spectra of the culture extracts for the active isolates showed absorbance peaks ranging between 212 and 260 nm. Two bioactive regions were detected on the TLC plate (R_f 0.60 and 0.80). The UV spectrum of the active compounds in methanol showed peaks at 211 and 215 nm.

Key Words: Antimicrobial activity, Antibiotics, *Streptomyces*, *Actinomycetes*

Bazı *Streptomyces* İzolatlarının Antimikrobiyal Aktivitelerinin Araştırılması

Özet: Muğla yöresi topraklarından 74 farklı *Streptomyces* izolatı elde edildi. İzolatların % 45.9'unda antimikrobiyal aktivite belirlendi. Onbeş izolat koagulaz-negatif Stafilokoklara (CoNS) karşı güçlü antimikrobiyal aktivite gösterdi. Bu izolatların Gram-pozitif, Gram-negatif bakteriler ve mayalara karşı in vitro antimikrobiyal aktiviteleri kapsamlı olarak çalışıldı. Sonuçlar göstermiştir ki, 5 izolat ≥ 20 mm inhibisyon zonuyla CoNS ve mayalara karşı yüksek derecede aktiftir. Bu izolatların üçü, *Streptomyces antibioticus* (MU106, MU107) ve *S. rimosus* (MU114) olarak tanımlanmıştır. Aktif izolatların kültür ekstraktlarının UV spektrumları 212 ve 260 nm arasında değişen absorbans pikleri göstermiştir. TLC plakaları üzerinde iki bioaktif bölge (R_f 0.60 ve 0.80) tespit edildi. Aktif bileşiklerin metanoldeki UV spektrumları 211 ve 215 nm de pikler gösterdi.

Anahtar Sözcükler: Antmikrobiyal aktivite, Antibiyotikler, *Streptomyces*, *Actinomycetes*

Introduction

Streptomyces is the largest antibiotic-producing genus in the microbial world discovered so far. The number of antimicrobial compounds reported from the species of this genus per year has increased almost exponentially for about two decades. Recent reports show that this group of microorganisms still remains an important source of antibiotics (1). As a result of the increasing prevalence of antibiotic-resistant pathogens and the pharmacological limitations of antibiotics, there is an exigency for new antimicrobial substances. The results of extensive screening have been the discovery of about 4000 antibiotic substances from bacteria and fungi, many of which have found applications in medicine; most of them are produced by *Streptomyces* (2). Most *Streptomyces* and other *Actinomycetes* produce a diverse array of antibiotics including aminoglycosides, anthracyclins, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyethers and tetracyclines (3).

With the seemingly exponential emergence of microorganisms becoming resistant to the clinically available antibiotics already marketed, the need for

* Correspondence author: e-mail: nsahin@mu.edu.tr

discovering novel drugs is real. For example, the occurrence of methicillin-resistant *S. aureus* in hospitals has risen from less than 3% in the early 1980s to as much as 40% now. It has been reported that coagulase-negative *Staphylococcus* spp. (CoNS) are becoming increasingly important in nosocominal infections and that they may cause serious infections (4). Therapeutic options for CoNS infections caused by methicillin-oxacillin-resistant strains are limited to vancomycin-based regimens. However, vancomycin therapy of staphylococcal infections has been associated with a slow and inadequate response in many instances (5). Effective treatment of the infections caused by these organisms is yet to be established. Thus, the need for the discovery and development of new and effective antibiotics is a priority. Presently, there is little documented information on the occurrence in Turkey with the *Streptomyces* spp. of potential to produce antimicrobial compounds (6,7).

In the present study, the isolation and characterization as well as the inhibitory effects of local *Streptomyces* isolates tested against various bacteria and yeasts were reported.

Materials and Methods

Isolation of microorganisms: Soil samples were collected from various locations in Muğla province from 1998 to 2000. Several diverse habitats in different areas were selected for the isolation of *Streptomyces* strains. These habitats included the rhizosphere of plants, agricultural soil, preserved areas and forest soils. The samples were taken from up to 20 cm depth, after removing approximately 3 cm of the soil surface. The samples were placed in polyethylene bags, closed tightly and stored in a refrigerator.

The following screening procedure was adopted for the isolation of *Streptomyces* (2). The soil was pretreated with $CaCO_3$ (10:1 w/w) and incubated at 37 °C for 4 days. It was then suspended in sterile Ringer solution (1/4 strength). Test tubes containing a 10^{-2} dilution of samples were placed in a water bath at 45 °C for 16 h so that the spores would separate from vegetative cells and the dilutions were inoculated on the surface of Actinomycete Isolation agar (Difco 0957) plates. The plates were incubated at 28 °C until the sporulation of *Streptomyces* colonies occurred. *Streptomyces* colonies (where the mycelium remained intact and the aerial mycelium and long spore chains were abundant) were then picked up and transferred to yeast extract-malt extract agar (ISP2) slants. Pure cultures were obtained from selected colonies for repeated subculturing. After antimicrobial activity screening, the isolated *Streptomyces* strains were maintained as suspensions of spores and mycelial fragments in 10% glycerol (v/v) at –20 ºC in the Muğla University Collection of Microorganisms (MU).

Characterization of the isolates: All strains were cultivated on ISP 2 medium. Some diagnostic characters of highly active *Streptomyces* strains were determined following the directions given in the probabilistic identification matrix of Williams (8) and *Bergey's Manual of Systematic Bacteriology* (9). A Willcox probability matrix was used to assign and identify isolates where scores of 0.8 and above indicated a positive identification (10).

Test microorganisms: Nine bacteria, including six Gram-positive and three Gram-negatives and three yeasts were used to determine the antimicrobial activity of the isolated *Streptomyces* strains (Table 1). All these microorganisms were obtained from the Refik Saydam National Type Culture Collection (RSKK) in Ankara, and Muğla University Collection of Microorganisms (MU) in Muğla-Turkey.

In vitro screening of isolates for antagonism: Balanced sensivity 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 by a single streak at a 90° angle to *Streptomyces* strains. The microbial interactions were analyzed by the determination of the size of the inhibition zone (11).

Fermentation and extraction: Isolates that showed activity against test microorganisms were grown in submerged culture in 250 ml flasks containing 50 ml of liquid medium (NaCl 0.8 g, NH_4Cl 1 g, KCl 0.1 g, KH_2PO_4 0.1 g, $MgSO_4.7H_2O$ 0.2 g, $CaCl_2.2H_2O$ 0.04 g, glucose 2 g, yeast extract 3 g, and distilled water 1 l. pH: 7.3). The flasks were inoculated with 1 ml of active *Streptomyces* culture and incubated at 28 °C for 120 h with shaking at 105 t/min. After growth, the contents of each flask were extracted twice with *n*-butanol (1-2.5 v/v). Filter paper disks (6 mm in diameter) were impregnated with extracted broth, dried and placed onto BSM plates

Table 1. Antimicrobial activity of *Streptomyces* isolates. *

Test Microorganisms	Isolates														
	MU106	MU107	MU108	MU113	MU114	MU115	MU116	MU117	MU118	MU120	MU123	MU124	MU125	MU126	MU128
Gram-positive bacteria															
Staphylococcus xylosus															
(MU29)	3	3	3	1	3	-	1	1	2	1	2	1	1	2	3
S. capitis (MU27)	3	3	2	1	3	2	2	1	2	-	2	1	1	2	2
S. epidermidis (MU30)	1	2	2	1	3	-	-	1	2	-	1	-	1	2	-
S. aureus															
(ATCC 6538/P)	-	-	2	1	2	-	-	1	1	-	2	1	-	2	2
S. aureus (MU38)	-	-	2	1	2	-	-	1	2	-	2	2	-	2	2
Bacillus subtilis															
(ATCC 6633)	-	-	2	-	2	-	-	-	2	-	2	3	-	-	2
Gram-negative bacteria															
Enterobacter aerogenes															
(RSKK 720)	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2
Pseudomonas aeruginosa															
(ATCC 29212)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli															
(ATCC 11230)	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Yeasts															
Candida albicans															
(ATCC 10239)	3	1	3	1	3	3	1	2	1	1	1	3	-	-	1
C. tropicalis (RSKK 665)	3	1	3	1	3	3	-	1	-	-	3	-	-	-	3
Saccharomyces carlsbergensis															
(RSKK 9080)	3	-	3	-	3	3	2	1	-	-	3	3	-	2	3

* The inhibitory effect of the strains was divided into four groups according to the size of the inhibition zone and as follows: - (passive group, ≤ 10 mm); group 1 (11-20 mm, slightly active); group 2 (21-30 mm, moderately active); and group 3 (≥ 31 mm, highly active).

previously seeded with *S. aureus* ATCC 6538P. The plates were incubated at 37 °C for 48 h and examined for zones of inhibition and verified active substance extraction. The absorption spectrum of each active extract was determined in the UV region (200-400 nm) by using a Shimadzu 1601 UV-visible spectrophotometer.

The butanol extracts containing the bioactive components were concentrated in vacuo and fractionated using thin layer chromatography (TLC) on a 3 x 8 cm silica gel plate (60 F_{254}, 0.2 mm, Merck) and developed with a butanol-acetic acid-water (4:1:2) solvent system. Afterwards, the TLC plates were air-dried. Bands were scraped from the plates with a spatula under UV light, extracted with methanol and filtered through Whatman No. 5 paper. Each band was bioassayed using *S. aureus* ATCC 6538P and the active bands were purified again on TLC using the same solvent system and visualized using UV light or anisaldehyde-sulfuric acid color reaction (12). The R_f for each band was measured. Each isolated band was also dissolved in methanol, and its UV absorption spectrum was measured with a Shimadzu 1601 spectrophotometer to determine the λ maximum of the band.

Results and Discussion

A total of 74 different *Streptomyces* isolates were recovered from 46 soil samples. According to some morphological and cultural characteristics, these strains belonged to four different cluster groups (data not shown). Antibacterial activity was exhibited by 45.9% of the isolates. The lowest activity was exhibited against Gram-negative isolates (5.9%). Fifteen of the isolates were active against one or more of the tested CoNS and yeasts (Table 1). The antibacterial activity of *Streptomyces* strains against *S. xylosus* and *S. capitis* was almost equal (41.2%), while 29.4% were active against *S. epidermidis*. *S. epidermidis* is considered to be the most important of the CoNS (4). In a previous study (7),

356 *Streptomyces* isolates were obtained from soils in the Aegean and East Black Sea regions of Turkey and 36% of the isolates were found to be active against tested microorganisms; they are active against *S. aureus* (20.78%), *E. coli* (2.52%), *Micrococcus luteus* (18.25%), *Mycobacterium smegmatis* (22.47%) and *B. subtilis* (12.07%).

With reference to the results obtained, more detailed characterization studies were carried out on the five strains belonging to high antimicrobial activity group 2 and 3 (≥ 20 mm inhibition zone) in order to determine their taxonomic status (Table 2). Three of these isolates were identified as *Streptomyces antibioticus* (MU106, MU107) and *S. rimosus* (MU114) with Willcox probability scores of 0.8 and above.

The UV spectral data for the *n*-butanol extract of selected active fermented broth are shown in Table 3. Maximum absorbance peaks range between 212 and 260 nm and the characteristics of the absorption peaks indicate a mostly polyene nature (Fig. 1). The spectral

Table 3. Characteristics of UV absorption spectra of *n*-butanol extract of fermentation broth.

Strain	Maxima (nm)	Shoulder (nm)
MU106	212	(249), (274)
MU107	216	(221), (266), (307), (321)
MU108	224	(214), (266), (304), (320)
MU114	226	(218), (272), (304), (318)
MU115	222	(264)
MU117	222	(216), (273), (304), (318)
MU118	216, 252	-
MU123	226, 260	(214), (220)
MU124	220, 260	(266), (274)

Table 2. Some taxonomical properties of the highly active (≥ 30 mm) Streptomyces strains.

Character	MU106	MU107	MU108	MU114	MU128
Spore chain *Rectiflexibilis*	+	+	+	+	-
Spore chains *Spirales*	-	-	-	-	+
Spore mass gray	+	-	-	-	+
Diffusible pigment produced	-	-	-	-	+
Melanin on pepton/yeast/iron agar	+	+	+	-	-
Growth at 45 °C	+	+	+	-	+
DNase	+	+	+	+	+
Nitrate reduction	+	+	+	-	-
Growth with (% w/v)					
NaCl (7.0)	-	+	+	+	+
Sodium azide (0.01)	+	+	-	-	nt
Phenol (0.1)	+	-	+	-	nt
Utilization of:					
Citrate	-	-	-	-	-
Malonate	-	-	-	-	-
Tartrate	-	-	-	-	-
Oxalate	-	-	-	-	-
Antibiosis against					
Bacillus subtilis ATCC 6633	-	-	+	+	+
Staphylococcus aureus ATCC 6538/P	-	-	+	+	+
Candida albicans ATCC 10239	+	+	+	+	+
Escherichia coli ATCC 11230	-	-	-	-	+
Willcox Probability	0.980	0.925	0.407	0.838	0.651
Most likely species	*S. ant.*	*S. ant.*	*S. rim.*	*S. rim.*	*S. grisv.*

+, positive; -, negative; nt, not tested

S. ant., *Streptomyces antibioticus*; S. rim., *Streptomyces rimosus*; S. grisv., *Streptomyces griseoviridis*

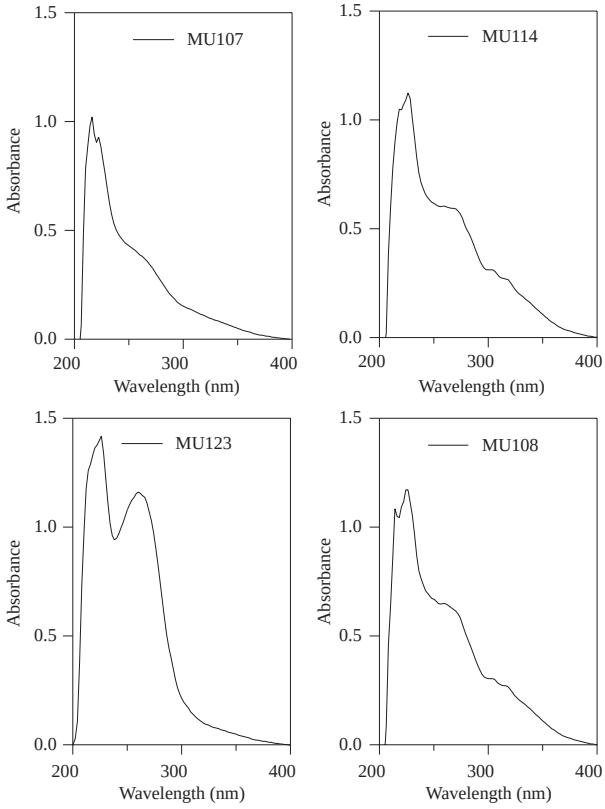

Figure 1. UV spectra of n-butanol extract of fermentation broth.

data are in agreement with those obtained by Swaadoun et al. (13). Two bioactive regions appeared on the chromatogram. Both regions were inhibitory to *S. aureus*

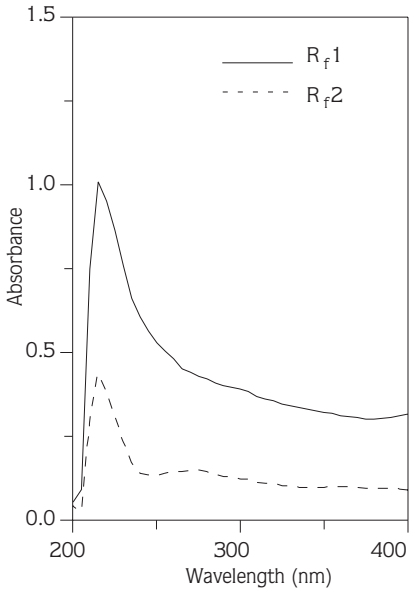

Figure 2. The UV spectrum of the active compounds in methanol.

ATCC 6538P in the position of R_f 0.60 and 0.80. The bioactive compound exhibited UV absorption maxima at 211 and 215 nm in methanol (Fig. 2). These strains produced either a broad-spectrum antimicrobial compound or several compounds with different activities. Further investigations are needed in order to determine whether the metabolites responsible for these absorbance peaks are active substances or not.

References

1. Watve, M.G., Tickoo, R., Jog., M.M., Bhole, B.D. How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol, 176: 386-390, 2001.
2. Korn-Wendisch, F., Kutzner, H.J., The Family *Streptomycetaceae*. In: *The Prokaryotes. A handbook on the biology of bacteria: Ecophysiology, isolation, identification, applications*. 2nd ed. (eds.) Balows, A., Trüper, H.G., Dworkin, M. Harder, W., Schleifer, K-H. Springer-Verlag, Berlin, Heidelberg, New York; pp 921-995, 1992.
3. Goodfellow, M., Williams, S.T., Mordarski, M., Actinomycetes in biotechnology. London. Academic Press; 1988.
4. Kloos, W.E., Bannermann, T.L., Update on the clinical significance of coagulase negative *Staphylococcus*. J Clin Microbiol, 33: 1060-1063, 1994.
5. Levine, D.P., Fromm, B.S., Reddy, B.R., Slow response to vancomycin or vancomycin plus rifampin in therapy among patients with methicillin-resistant *Staphylococcus aureus* endocarditis. Ann Int Med, 115: 674-680, 1991.
6. Aslan, B. *Streptomyces* türlerinin izolasyonu, karakterizasyonu ve antibiyotik üretimi üzerine çalışmalar. Doctoral thesis, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, 1999.
7. Denizci, A.A., Ege ve Doğu Karadeniz bölgesi topraklarinden izole edilen aktinomisetlerden antibakteriyal antibiyotiklerin aranması ve üretimi üzerine bir araştırma. Doctoral thesis, Ege Üniversitesi, Fen Bilimleri Enstitüsü, 1996.
8. Williams, S.T., Goodfellow, M., Wellington, E.M.H., Vickers, J.C., Alderson, G., Sneath, P.H.A., Sackin, M.J., Mortimer, A.M., A probability matrix for identification of some Streptomycetes. J Gen Microbiol, 129: 1815-1830, 1983.

9. Williams, S.T., Goodfellow, M., Alderson., G.: Genus *Streptomyces* (Waksman & Hanrici 1943) 339^{AL}. In: *Bergey's Manual of Systematic Bacteriology,* Edited by Williams, S.T., Sharpe, M.E., Holt, J.G. vol. 4, Williams & Wilkins. Baltimore; pp. 2452-2492, 1989.

10. Willcox, W.R., Lapage, S.P., Bascomb, S., Curtis, M.A., Identification of bacteria by computer: theory and programming. J Gen Microbiol, 77: 317-330, 1973.

11. Madigan, M.T., Martiko, J.M., Parker, J., Antibiotics: Isolation and characterization, In: Brock Biology of Microorganisms, 8th edn. Prentice-Hall International Inc. New Jersey; pp: 440-442, 1997.

12. Hwang, B.K., Ahn, S.J., Moon, S.S. Production, purification and antifungal activity of the antibiotic nucleoside, tubericidine, produced by *Streptomyces violaceoniger*. Can J Bot, 72: 480-485, 1994.

13. Saadoun, I., Hameed, K.M., Moussauui, A., Characterization and analysis of antibiotic activity of some aquatic actinomycetes. Microbios, 99: 173-179, 1999.