Düzce University Journal of Science & Technology

Research Article

Antifungal Activity of Soil *Streptomyces* Isolates Against *Cryptococcus neoformans*

Gorkem DULGER[a,*], Basaran DULGER[b]

[a] *Department of Medical Biology, Faculty of Medicine, Düzce University, Düzce, TURKEY*
[b] *Department of Biology, Faculty of Arts and Science, DüzceUniversity, Düzce, TURKEY*
** Corresponding author's e-mail address: gorkemdulger@yandex.com*
DOI: 10.29130/dubited.862551

ABSTRACT

In this study, 26.5% of 128 different isolates of *Streptomyces* recovered from soils in Duzce province, Turkey showed antifungal activity against *Cryptococcus neoformans* ATCC 90112. Considering the diameter of the inhibition zone formed on the agar plate, isolates were divided into four sections: section 1 (5-10 mm, slightly-active); section 2 (11-15 mm, moderately-active), section 3 (16-25 mm, highly-active) and section 4 (26-35 mm, ultra-active). It is determined that 3 isolates in section 4 may be a source of novel antibiotic against Cryptococcosis.

Keywords: *Streptomyces, Antifungal Activity, Cryptococcus neoformans*

Toprak *Streptomyces* İzolatlarının *Cryptococcus neoformans* Kültürlerine Karşı Antifungal Aktivitesi

ÖZET

Bu çalışmada, Düzce ili topraklardan elde edilen 128 farklı *Streptomyces* izolatlarının % 26,5'inin *Cryptococcus neoformans* ATCC 90112 mayasına karşı antifungal aktivite gösterdiği saptanmıştır. Agar plağında oluşan inhibisyon bölgesinin çapı dikkate alınarak izolatlar dört bölüme ayrılmıştır. Buna göre, bölüm 1 (5-10 mm, hafif aktif); bölüm 2 (11-15 mm, orta derecede aktif), bölüm 3 (16-25 mm, yüksek derecede aktif) ve bölüm 4 (26-35 mm, ultra aktif) olarak belirlenmiştir. Bölüm 4'teki 3 izolatın ise kriptokokkoz hastalığına karşı yeni bir antibiyotik kaynağı olabileceği saptanmıştır.

Anahtar Kelimeler: *Streptomyces, Antifungal Aktivite, Cryptococcus neoformans*

Received: 16./01/2021, Revised: 04/02/2021, Accepted: 08/02/2021

I. INTRODUCTION

Antimicrobial compounds are produced by quite a lot of organisms (bacteria, fungi and plants), the Actinomycetes are the most capable of these groups of organisms [1]**.** Approximately 23,000 bioactive secondary metabolites manufacturing by microorganisms have been reported, and more than 10,000 of these compounds are produced by Actinomycetes, representing 45% of all bioactive microbial metabolites discovered [2]**.**

Many scientists today are looking for new antibiotics from different habitats. In addition, the investigations on Actinomycetes are quite insufficient in Turkey. Quite a few studies have so far been done to isolate and evaluate Actinomycetes. The purpose of this study is to isolate, characterize and screen antibiotic-manufacturing *Streptomyces* species from the soil samples. Besides, the purpose was to determine the antifungal effects of the isolates against *Cryptococcus neoformans* ATCC 90112.

II. MATERIALS AND METHODS

A. SAMPLING, ISOLATION AND CHARACTERIZATION

Soil sampling, collection, isolation and characterization of *Streptomyces* were done according to the procedure described by Saadoun and Al-Momani [3]**.**

B. ANTIFUNGAL ACTIVITY

Antifungal activity was performed by Bauer-Kirby method [4] against *Crytococcus neoformans* ATCC 90112. Isolates were developed on Oatmeal Agar (Oxoid) for fourteen days, then three discs (6 mm in diameter) were transplanted to Nutrient Agar (Oxoid) previously inoculated with the test microorganism and incubated at 27±0.1 °C. Inhibition zones were visually detected after 48 h. The extend of the inhibitory effect of the active isolates was divided into 4 sections according to the diameter of the inhibition zone on the agar and as follows: section 1 (5-10 mm, slightly-active); section 2 (11-15 mm, moderately-active), section 3 (16-25 mm, highly-active) and section 4 (26-35 mm, ultra-active).

III. RESULTS AND DISCUSSION

Actinomycetes are the top antimicrobial compound manufacturers [5]. The primary antibiotic manufacturing microorganisms used by the pharmaceutical industry are species belonging to the *Streptomyces* genus. In addition, these strains are responsible for over 60% of known antibiotics. In addition, 15% of it consists of species related to other Actinomycetes genera [6], [7].

The importance of antibiotics in antifungal therapy prompted us to determine the activity of these isolates against *Crytococcus neoformans* ATCC 90112. As shown in Table 1, the antifungal activity against *Cryptococcus neoformans* ATCC 90112 was shown by 26.5% of the *Streptomyces* isolates. Those isolates that shown high activity (16-35 mm inhibition zones) were distributed into 4 sections and were further characterized culturally and morphologically (Table 2). Test results revealed that most of the isolates (16 isolates, 47%) were belonged to section 1 (5-10 mm), followed by section 2 (12 isolates), (11-15 mm, %32), section 3 (4 isolates), (16-25 mm, 11.7%) and section 4 (3 isolates), (26-35 mm, 8.8%), respectively.

Although various studies have been reported on screening and identification of *Streptomyces* from all around Turkey and the other countries, reviewing provided data has demonstrated that not yet

comprehensive survey on this issue has been conducted. In the literature scanning, Eighteen percent of 116 *Streptomyces* isolates obtained from lands in the north of Jordan were found to have activity against *Candida albicans*. *Streptomyces* isolates were divided into 3 groups according to the diameter of the inhibition zone on the agar plate, and it was revealed that the group 3 (16 ± 35 mm) was quite active [3]. In previous study, 356 *Streptomyces* isolates were obtained from soil samples in the Aegean and East Black Sea regions of Turkey. 36% of these isolates were determined to be effective against *S. aureus* (20.78%), *E. coli* (2.52%), *M. luteus* (18.25%), *M. smegmatis* (22.47%) and *B. subtilis* (12.07%) [8]. In another study, 74 *Streptomyces* were isolated from the soil samples of Mugla province, Turkey. Antagonistic effect in 45.9% of the isolates was observed. 15 isolates showed potential antibacterial effects against coagulase-negative *Staphylococcus* (CoNS). In addition, it was determined that 5 isolates were found to have a strong antimicrobial effect against coagulase negative *Staphylococcus* (CoNS) and the yeast cultures (forming an inhibition zone at < 20 mm) [9]. In another study on the subject, 44 Actinomycetes isolates from sediments of Caspian Sea were isolated and their antimicrobial studies was revealed by the cross streak method against two Gram positive bacteria and four Gram negative bacteria. While MN38 isolate had shown a strong antimicrobial effect against *S. aureus* (20.0±0.5 mm), *B. subtilis* (27.0±0.2 mm), and *E. coli* (20.0±0.3 mm). MN39 isolate showed highly efficient activity against *E. coli* (23.0±0.4 mm), *B. subtilis* (23.0±0.2 mm), *K. pneumonia* (24±0.1 mm), MN3 isolate was active against *P. aeruginosa* (20.0±0.2mm) [10]. With references to the findings obtained in this research are similar to those reported in the mentioned studies. More detailed characterization researches were carried out on the section 4 isolates belonging to potential antimicrobial effect in order to determine their secondary metabolites.

Table 1. *Activity of different Streptomyces isolated against Cryptococcus neoformans ATCC 90112.*

Colour series	**Number of isolates [a]**	***Cryptococcus neoformans***
Grey	38 (29.6%)	12 (31.5%)
White	19 (14.8%)	5 (14.7%)
Yellow	21 (16.4%)	7 (20.5%)
Green	17 (13.2%)	3 (8.8%)
Red	5 (3.9%)	4 (11.7%)
Blue	2 (1.5%)	0 (0)
Variable[b]	15 (11.7%)	0 (0)
NAM[c]	11 (8.5%)	3 (8.8%)
TOTAL	128 (100%)	34 (26.5%)

[a] Numbers in parenthesis represent the percentage out of the total
[b] Variable colour: Pink, orange or violet
[c] NAM: No aerial mycelium

Cryptococcus neoformans is the agent in cryptococcal infections. It is an encapsulated yeast fungus that is common in nature. It enters the human body through the respiratory tract and causes cryptococcosis. It creates an infection in the lungs in healthy individuals that progresses with symptoms and signs similar to flu and passes spontaneously. The agent that multiplies in the lungs of immunocompromised people mixes with the blood and creates widespread infections. Although fungi can settle in all systems, it tends to settle mostly in the central nervous system (CNS). The most common clinical form is meningoencephalitis. Cryptococcosis is fatal if not treated properly. The classic drug in treatment is amphotericin B [11], [12]. The results obtained from this study indicated

that *Streptomyces* isolates especially the section 4 strains possessed significant antifungal effect against *C. neoformans* ATCC 90112. Our findings clearly indicate that the section 4 strains have strong effects against *C. neoformans* ATCC 90112.

Table 2. *Characteristics of sections 1, 2, 3 and 4 of Streptomyces isolates.*

Strain no	Cultural characters[a]				Spore chain	Antibiosis[b] *Cryptococcus neoformans*
	AM	ME	RP	SP		
A1	Gray	+	+	+	Spiral	+ (1)
A2	Gray	+	+	-	Spiral	+ (1)
A3	Gray	-	+	+	Flexous	+ (2)
A4	Gray	-	+	+	Flexous	+ (1)
A5	Gray	+	+	+	Flexous	+ (1)
A6	Gray	+	+	-	Spiral	+ (4)
A7	Gray	+	+	-	Flexous	+ (1)
A8	Gray	-	+	+	Spiral	+ (2)
A9	Gray	-	+	+	Spiral	+ (1)
A10	Gray	-	+	-	Spiral	+ (1)
A11	Gray	+	+	-	Spiral	+ (2)
A12	Gray	+	+	-	Spiral	+ (4)
B1	White	-	-	-	Flexous	+ (1)
B2	White	-	+	-	Retinaculum apertum	+ (1)
B3	White	-	-	-	Rectus	+ (1)
B4	White	-	+	-	Flexous	+ (2)
B5	White	-	-	-	Flexous	+ (1)
C1	Yellow	-	-	-	Rectus	+ (2)
C2	Yellow	+	+	-	Flexous	+ (2)
C3	Yellow	+	+	+	Spiral	+ (3)
C4	Yellow	+	+	-	Spiral	+ (1)
C5	Yellow	-	-	-	Rectus	+ (4)
C6	Yellow	-	+	-	Spiral	+ (2)
C7	Yellow	-	+	-	Flexous	+ (1)
D1	Green	-	-	-	Retinaculum apertum	+ (1)
D2	Green	-	+	-	Flexous	+ (2)
D3	Green	-	-	-	Retinaculum apertum	+ (2)
E1	Red	-	+	-	Flexous	+ (1)
E2	Red	-	+	+	Flexous	+ (3)
E3	Red	-	-	-	Spiral	+ (2)
E4	Red	-	+	-	Flexous	+ (1)
E5	Red	-	+	-	Spiral	+ (2)
F1	NAM[c]	-	-	-	NAM[c]	+ (3)
F2	NAM[c]	-	+	+	NAM[c]	+ (3)
F3	NAM[c]	-	+	+	NAM[c]	+ (2)

[a] AM: Aerial mycelium colour; ME: Melanin pigment; RP: Reverse pigment; SP: Soluble pigment

[b] Numbers in parenthesis represent the group activity to the dimeter of inhibition zone, section 1 (5-10 mm); section 2 (11-15 mm), section 3 (16-25 mm) and section 4 (26-35 mm).

[c] NAM: No aerial mycelium

IV. CONCLUSION

As can be understood from recent literature reviews, secondary metabolites obtained from Actinomycetes are in the center of attention due to their various biological effects such as antioxidant, antitumor, antifungal, antibacterial and antiviral. In this context, three isolates of section 4 (26-35 mm) may be a source of novel antibiotics. Further studies on group 4 are needed in order to determine for secondary metabolites.

V. REFERENCES

[1] G. Gebreselema, M. Feleke, S. Samuel, R. Nagappan, "Isolation and characterization of potential antibiotic producing Actinomycetes from water and sediments of Lake Tana Ethiopia," *Asian Pacific Journal of Tropical Biomedicine,* vol. 3, pp. 426-35, 2013.

[2] J. Berdy, "Bioactive microbial metabolites: a personal view," *The Journal of Antibiotics,* vol. 58, no.1, pp. 1-26, 2005.

[3] I. Saadoun, F. Al-Momani, "*Steptomycetes* from Jordan soils active against *Agrobacterium tumefaciens*," *Actinomycetes,* vol. 8, pp. 29-36, 1997.

[4] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turk, "Antibiotic susceptibility testing by a standardized single disk method," *American Journal of Clinical Pathology,* vol. 45, pp. 493-496, 1966.

[5] H.M. Atta, "Production, purification, physico-chemical characteristics and biological activities of antifungal antibiotic produced by *Streptomyces antibioticus*, AZ-Z710," *American-Eurasian Journal of Scientific Research,* vol. 5, no.1, pp. 39-49, 2010.

[6] S. Ramesh, N. Mathivanan, "Screening of marine *Actinomycetes* isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes," *World Journal of Microbiology and Biotechnology,* vol. 25, pp. 2103–2111, 2009.

[7] P. R. Jensen, P.G. Williams, D.C. Oh, L. Zeigler, W. Fenical, "Species-specific secondary metabolite production in marine Actinomycetes of the genus *Salinispora*," *Applied* and *Environmental Microbiology,* vol. 73, pp. 1146–1152, 2007.

[8] A. A. Denizci, "Ege ve Doğu Karadeniz bölgesi topraklarinden izole edilen aktinomisetlerden antibakteriyal antibiyotiklerin aranması ve üretimi üzerine bir araştırma", Doktora Tezi, Ege Üniversitesi, Fen Bilimleri Enstitisü, 1996.

[9] N. Sahin, A. Ugur, "Investigation of the antimicrobial activity of some *Streptomyces* isolates," *Turkish Journal of Biology,* vol. 27, pp. 79-84, 2003.

[10] M. Mohseni, H. Norouzi, J. Hamedi, A. Roohi, "Screening of antibacterial producing actinomycetes from sediments of the Caspian Sea," *International Journal of Molecular* and *Cellular Medicine,* vol. 2, no.2, pp. 65-71, 2013.

[11] C. Coelho, A.L. Bocca, A. Casadevall, "The tools for virulence of *Cryptococcus neoformans,*" *Advances in Applied Microbiology,* vol. 87, pp. 1-41, 2014.

[12] R. Inci, "Kriptokok infeksiyonları," *Flora,* vol. 13, no. 2, pp. 61-71, 2008.