Medical Journal of Babylon
Vol. 13- No. 1:258 - 263 , 2016
http://www.medicaljb.com
ISSN 2312-6760©2016 University of Babylon

Short Communication

Screening for Antibacterial Activity of *Streptomyces* Spp. Isolated in Babylon, Iraq

Alaa Hani Al-Charrakh* Mohammed Sabri Abdul-Razzak
Furqan Mohammed Al-Asady
College of Medicine, Babylon University, Hilla, IRAQ

*E-mail: ahani67@gmail.com

Accepted 28 December, 2015

Abstract

Streptomyces are the biggest source for antibiotics production, they constitute about more than 75% in antibiotic productivity, and these antibiotics have a significant application in the commercial and therapeutic fields. The study included screening on the most potent antibacterial producing *Streptomyces* isolates. 41 *Streptomyces* isolates were primarily screened for antibacterial activity toward Gram positive bacteria (*S. aureus*) and Gram negative bacteria (*E. coli*), six isolates were selected (SMI-02, SMI-03, SMI-04, SMI-05, SMI-10 and SMI-16) which showed more antibacterial effect as related to other. Secondary screening revealed that two isolates (*Streptomyces* isolates SMI-04 and SMI-10) exhibited the potent and broadest antibacterial activity toward two G-positive (*S. aureus* and *S. epidermidis*) and three G-negative (*E. coli, P. aeruginosa* and *K. pneumoniae*).

Key words: Streptomyces, Antibacterial Activity, Secondary metabolites, Antibiotics, Iraq

الخلاصة

تعتبر بكتريا الستربتومايسيس من اكبر المصادر في انتاج المضادات الحيوية, حيث تشكل اكثر من ٧٥% في انتاج المضادات التي لها تطبيقات مهمة في المجال العلاجي والاقتصادي. تضمنت الدراسة اجراء المسح الاولي لاختبار ٤١ عزلة *Streptomyces* للبحث عن الفعالية الضد بكتيرية للبكتيريا الموجبة لصبغة غرام (*S. aureus*) والسالبة لصبغة غرام (*E. coli*), اظهرت الدراسة ان ست من هذه العزلات (SMI-02, SMI-03, SMI-04, SMI-05, SMI-10 and SMI-16) اعطت فعالية جيدة ضد البكتيريا الموجبة والسالبة لصبغة غرام (المكورات العنقودية الذهبية وبكتريا القولون)، فيما أظهر المسح الثانوي إمتلاك عزلتين من الستربتومايسيس اعلى طيف فعالية ضد بكتيرية للبكتريا الموجبة لصبغة غرام (*S. aureus* , *S. epidermidis*) والسالبة لصبغة غرام (*E. coli, P. aeruginosa,* ، *peumoniae*.).

الكلمات المفتاحية: بكتريا الستربتومايسيس, الفعالية الضد بكتيرية, مركبات الايض الثانوي, المضادات الحيوية, العراق.

Introduction

Actinomycetes are a group of Gram positive bacteria with high G+C ratio (more than 69-78%) in their DNA. Most actinomycetes are free livening, saprophytic. They spread in soil, water and settled plants [1]. *Streptomyces* is highly similar to the filamentous fungi: both develop as branching hyphae that produce a vegetative mycelium and spread through spores that produce specialized reproductive structures called aerial hyphae, which originate from the colony surface into the air. This resemble may be the result of adaptations to the same ecological niches, in spite that the beyond procedures have different evolutionary origins. Similar to fungi, most streptomycetes survive as saprophytes in the soil, despite that Streptomyces spp. have also successfully inhabit a wide range of other niches, both terrestrial and aquatic are plant and animal pathogens [2]. Different secondary metabolism can be conducted by

Streptomyces and this will impart such organisms the criteria to be strong providers of antibiotics and other bioactive compounds, and the production of these molecules is scheduled with the *Streptomyces* evolutionary program [3]. Secondary metabolites are small biomolecules described to be not-essential for the life of the producing organism *Streptomyces* antibiotics are produced by microbial fermentation. The onset of biosynthesis is related to environmental factors involving phosphate ions, oxygen concentration, the nature and level of nitrogen and carbon sources (in addition to typical variables like pH, light and temperature). Many regulatory mechanisms are included in the onset, maintenance and conclusion of antibiotic production. Of these mechanisms, regulation of carbon sources is considered to be one of the important factors that required managing secondary metabolism [4]. Antibiotic production usually happens near the late of growth phases of microorganism (stationary phase). The transitory nature of their production is surely genetics, but their expression can be affected largely by environmental manipulations. As a result, formation of antibiotics is usually attracted on by consumption of nutrients, presence of inducer and/or by a reduction in the growth rate [5]. The present study was aimed for determination of antibacterial activities of *Streptomyces* isolates and selects the isolates with broad spectrum activity.

Materials and Methods

Streptomyces isolates

The current study involved testing 41 *Streptomyces* isolates, these isolates were obtained from previous study (unpublished data) from soil samples. All these isolates were tested for their ability to produce secondary antibacterial metabolites (antibiotics) through primary screening test. The most potent antibiotic-producing isolates were selected through secondary screening test.

Test Microorganism

The test bacterial isolates (indicator bacteria) used were isolated from patients suffering from otitis media (all the test bacterial isolates were considered to be pathogenic bacteria). These are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyrogenes, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. All six isolates were kept in deep freezer (for long time storage). The isolates were subcultured on nutrient agar to be active before each test.

Agar well diffusion method

Mueller Hinton agar was prepared, autoclaved, poured in sterile plates and left to solidify. Suspensions of test bacterial isolates were prepared and their turbidity were compared with 0.5 McFarland turbidity, then each plate was swabbed with one test bacteria. After that, plates were left a side to dry for about 15 minutes and wells of 6 mm diameter were made by sterile Cork borer (maximum 6 wells per each plate). Subsequently, the prepared wells were filled with the cell-free filtrate of *Streptomyces* spp. (which obtained after centrifugation of *Streptomyces spp.* broth culture) that may contain the active metabolites. After that, the plates were placed in the refrigerator for about two hours and then incubated at 37°C for 18-20 hours [6].

Cell-free filtrate preparation

Following the incubation of each of productivity broth medium, each broth culture was centrifuged in cooling centrifuge for 20 minutes at 4000 rpm and 5°C; then, the cell-free filtrate was collected from each *Streptomyces* broth culture and tested for their antibacterial activity by using agar well diffusion technique [6]. After incubation of plates for 18-20 hours, the inhibition zones (in mm), if present, were recorded.

Results and Discussion

The results that obtained from this primary test were recorded as zones of inhibition (in mm). As illustrated in table (1), the antibacterial activity of each *Streptomyces* isolate was tested toward two indicator bacterial isolates; *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) through measuring the inhibition

zones (if present) for each isolate and recorded. In addition to table (1), figure (1) also demonstrates the primary screening test to determine the antibacterial activity of *Streptomyces* isolates that exhibited the highest antibacterial activity among the others against *S. aureus*.

Both table (1) and figure (1) indicate that among the 41 isolates tested, only six isolates gave considerable antibacterial potential as compared with the other isolates under the investigation in the primary screening test and these six isolates would be subjected to the secondary screening test. These isolate of *Streptomyces* isolate are SMI-02, SMI-03, SMI-04, SMI-05, SMI-10 and SMI-16. The antibacterial behavior of the six chosen isolates related to the fact that several *Streptomyces* species are considered as unlimited source of natural secondary metabolites (one of these secondary metabolites is the antibacterial agents, antibiotics); many types of which are utilized as agrochemical and pharmaceutical products; the antibacterial compounds that produced by *Streptomyces* have a wide variety of chemical structures, molecular weight and different physicochemical properties [7] [8].

The study included that the selected six *Streptomyces* isolates (*Streptomyces* isolate SMI-02, SMI-03, SMI-04, SMI-05, SMI-10 and SMI-16) were subjected for secondary screening test against six indicator bacteria (Table 2).

Table 1 : Primary screening of antibacterial activity of *Streptomyces* spp. Isolates

Isolate No.	Zones of inhibition (mm)		Isolate No.	Zones of inhibition (mm)	
	S. aureus	*E. coli*		*S. aureus*	*E. coli*
SMI-01	0	0	SMI-22	0	0
SMI-02	18	0	SMI-23	0	0
SMI-03	16	0	SMI-24	10	0
SMI-04	20	13	SMI-25	11	10
SMI-05	19	11	SMI-26	8	7
SMI-06	0	0	SMI-27	0	0
SMI-07	8	0	SMI-28	0	0
SMI-08	0	7	SMI-29	9	0
SMI-09	0	0	SMI-30	12	10
SMI-10	19	13	SMU-31	0	0
SMI-11	0	0	SMU-32	0	0
SMI-12	0	0	SMU-33	0	0
SMI-13	10	0	SMU-34	0	0
SMI-14	0	0	SMU-35	9	8
SMI-15	0	7	SMU-36	0	0
SMI-16	15	0	SMU-37	0	0
SMI-17	8	10	SMU-38	0	0
SMI-18	12	0	SMU-39	12	9

SMI-19	0	0	SMU-40	11	8
SMI-20	11	0	SSA-41	0	0
SMI-21	0	0			

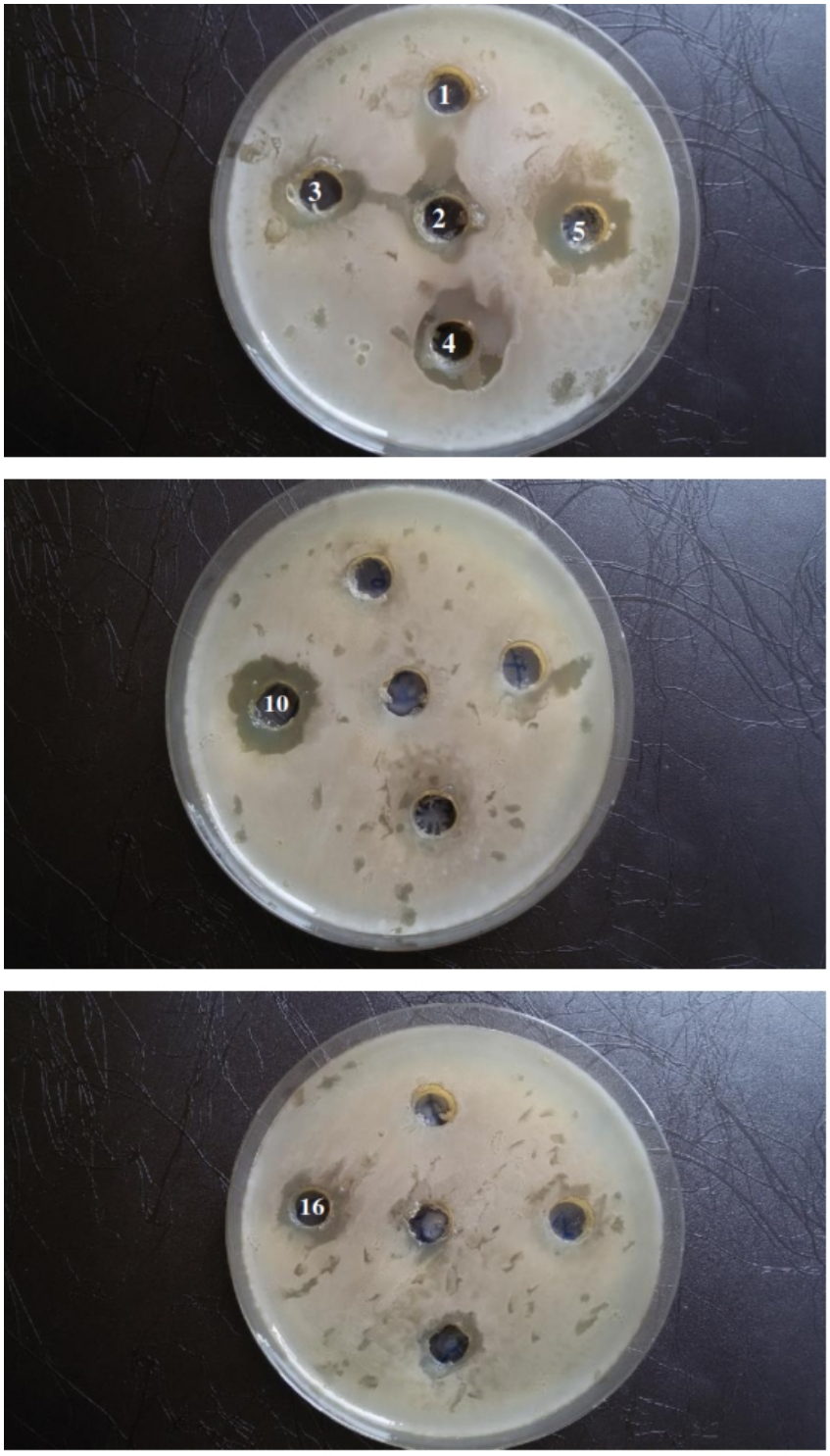

Figure 1: Primary screening test of some powerful *Streptomyces* isolates on *S. aureus*
****1:** SMI-01; **2:** SMI-02; **3:** SMI-03; **4:** SMI-04; **5:** SMI-05; **10:** SMI-10; **16:** SMI-16

Table 2: Secondary screening for selection of broad spectrum *Streptomyces* isolates

Test bacterial isolates	*Streptomyces* Isolates						
	SMI-02	SMI-03	SMI-04	SMI-05	SMI-10	SMI-16	
S. aureus	20	16	18	18	17	13	Inhibition Zones (mm)
S. epidermidis	0	8	13	0	15	0	
S. pyrogenes	0	0	0	0	0	0	
E. coli	0	0	12	13	13	0	
P. aeruginosa	0	0	11	11	13	0	
*K. pneumonia*e	0	0	13	12	13	0	

Table (2) clarifies that *S. pyrogenes* is completely resistant to all *Streptomyces* isolates (six isolates) under investigation. This can be attributed to that *S. pyrogenes* was isolated from patient with otitis media and this isolate have high level of resistant to antibiotic through possession of different mechanisms of antibiotic resistant. On the other hand, the susceptibility of the remaining five test bacterial isolates was variable widely according to the *Streptomyces* isolates. Table (2) reveals that among the six *Streptomyces* isolates tested, two *Streptomyces* isolates (isolate SMI-04 and isolate SMI-10) showed the most potent and had broadest antibacterial activity. *Streptomyces* isolate SMI-04 and isolate SMI-10 exhibited antibacterial activities toward two Gram-positive bacteria (*S. aureus* and *S. epidermidis*) and three Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumonia*e); this means that both isolates have broad spectrum antibacterial potential. This result was conducted with that recorded by other researchers [9]. This finding was in accordance with a related study [10], who stated that 20 *Streptomyces* isolates were tested for their antibacterial activities toward some test bacteria (*S. aureus*, *E. coli* and *P. aeruginosa*) and found that only one isolate (*Streptomyces* isolate SAM 10) expressed the highest antibacterial activities against Gram-positive bacteria (*S. aureus*), followed by *E. coli* and the lowest was *P. aeruginosa*.

References

1- Patzer SI, Volkmar B. Gene cluster involved in the biosynthesis of griseobactin, a catechol-peptide siderophore of *Streptomyces* spp. J. Bacteriol., 2010; 192: 426-345.

2- Hopwood DA. *Streptomyces* in Nature and Medicine. An excellent account of the history of *Streptomyces* research by the founder of *S. coelicolor* genetics. Oxford Univ. 2007; Press, New York.

3- Mohanraj G, Sekar T. Isolation and screening of actinomycetes from marine sediments for their potential to produce antimicrobials. Int. J. Life Sci. Bt. Pharm. Res., 2013; 2 (3): 115-126.

4- Sanchez S, Demain AL. Regulation of fermentation processes. Enz. Microb. Technol., 2002; 31: 895-906.

5- Bibb MJ. Regulation of secondary metabolism in Streptomycetes. Curr. Opin. Microbiol., 2005; 8 (2): 208-215.

6- Murry PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR. Manual of clinical Microbiology, 6 th edition. ASM Press, Washington, DC., 1995; 15-18.

7- Pamboukain CR, Facciotti MC. Production of antitumeral retamycin during continuous fermentations of *Streptomyces olindensis*. Process Biochem., 2004; 39: 2249-2255.
8- Ben-Fguira LF, Fosto S, Mehdi RB, Mellouli L, Laatsch H. Purification and structure elucidation of antifungal and antibacterial activities of newly isolated *Streptomyces* sp. strain US80. Microbiol. Res., 2005; 156: 341-347.
9- El-Nagger MY, El-Assar SA, Abdul-Gawad M. Meroparamycin production by newly isolated *Streptomyces* sp. strain MAR01: Taxonomy, Fermentation, purification and Structural elucidation. J. Microbiol., 2006; 44 (4): 432-438.
10- Al-Hulu SM, Al-Charrakh AH, Jarallah EM. Antibacterial activity of *Streptomyces gelaticus* isolated from Iraqi soil. Med. J. Babylon, 2011; 8 (3): 404-411.